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REVIEW

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ORIGINAL ARTICLES IN DENTAL HEALTH

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CASE REPORTS

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The proximal acute aortic dissection in Marfan syndrome. Medinform synopsis

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The Marfan syndrome (MFS) is an autosomal dominant disorder of the connective tissue. The acute dissection of the ascendent aorta and aortic arch in young patients is usually the predominant cause of death, with a high mortality rate up to 80%. The syndrome is caused by the mutation of fibrillin-1 gene that builds up elastic fibers. The elastic laminae in the aortic wall rupture due to degenerative necrosis. In cases of intramural haematom, wall rupture can cause acute pericardial or mediastinal bleeding. Operation is usually performed following the clinical and imagistic diagnosis. Extracorporeal circulation and selective cerebral perfusion through right axillary artery cannulation and anterograde perfusion has acknowledged grounds nowadays. During the surgical procedure, the dissected aortic segment is replaced with vascular prosthesis. If the aortic valve is also affected by the dissection, then the solution can be a composite graft. The frequency of recidivation and reoperation is 15%, the 10 years of post operative survival rate is 50%. With optimal clinical management of patients with Marfan syndrome, life expectancy may be improved substantially. The indications for preventive elective surgery in Marfan syndromes are progressive aortic dilatation, sinobulbar insufficiency and regurgitation.

Key words: aortic dissection, Marfan syndrome, treatment

Marfan syndrome is named after it's first describer, who published the symptomatology of the disease about the build-up and skeletal disorders of a 5 year old child.[27]

Characteristic features of this genetic disease include high physique, long limbs, arachnodactylia, loose joints, dolichocephaly, gothic palate, vertebrothoracal disorders like pectus carinatum, pectus excavatum, kyphoscoliosis and the dislocation or subluxation of the crystalline lens may be present. During Marfan syndrome the most severely affected is the circulatory system which results in a fatal aortic dissection at younger ages. Lack of timely surgical treatment of this complication would result in a mortality rate up to 80%. Other associated cardiovascular symptoms such as aortic valve insufficiency and regurgitation, mitral insufficiency usually with a prolapsing valve are also common.[5,17]

According to the data of epidemiological studies and the International Registry of Acute Aortic Dissection (IRAD) the incidence of aortic dissection (AD) is estimated to be 3 cases per 100000 people per year. Causes of AD in young patients under 40 are mostly congenital, commonly Marfan syndrome, in the elders primarily acquired conditions play a role developing the dissection such as high blood pressure, atherosclerosis, aortic aneurysm.[4,14,32,39]

In Marfan syndrome the dissection of the proximal aorta appears in 65-75% of the cases and is called according to the Stanford classification type A (AAD) or named after DeBakey I-II dissection when it is originated from the root, ascendent part and arch of the aorta. If it affects primarily the distal descendent part of the aorta it is called Stanford type B or DeBakey III AD.[21]

The etiopathogenesis of AD in Marfan syndrome is partly known, it is due to the autosomal inherited degeneration of the elastic media wall of the great artery.[23,40] In the next chapters of the article the clinicopathological, cardiac surgical considerations and the prophylactic preventive treatment is reviewed of the AAD with an emphasis on Marfan syndrome.

Etiopathogenesis

The etiology of MFS is not always clear. It can be a genetically transmittable autosomal dominant disease, although familiary hereditary factors cannot be proved in all of the cases. In 25% the congenital genetic defect is a result of one from the variety of de-novo mutations. The genetic mutation manifests firstly in the components of the elastic tissue. Induced anomalies occur in the products of the genes fibrillin-1 and -2, subsequentially in the elastin and other structural proteins (fibulin, emilin-1), trophoelastin sequences and in the microfibrils. The pathological fibrillin-1 behaves in a dominantly negative way inactivating the structural

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proteins synthesized from the normal allele. The natural build-up of the microfibrils, the arrangement of the extracellular fibre matrix three-dimensional morphology, the remodeling, the stability maintained are attributable to connecting proteins that presumably modulate the Transforming Growth Factor beta superfamily's components.[1,17,23,26,42]

The elastic media wall layer of the aorta shows lamellar structure. To the middle coat of the aorta the intimal endothelium is firmly attached by the subendothelial membrane and the adventitia is loosely fixed to the external surface of it. The laminae consist of lamellé built up by mainly circular (transversal) elastic fibers' network in regular conformation that corresponds to parallel orientation with the lumen of the vessel. The number of the lamellé is usually 50 and between two lamellé there is interlamellar adhesive matrix, together they form lamellar units with a thickness of 11 millimicrons of each unit. In the matrix between the lamellé smooth muscle and fibroblast cells are found.[41]

In aortic dissection fragmentation of the elastic lamellé in the media and intramural haematoma occur, the elastic wall splits (clivation) to an internal part composed of the intima and inner medial elastic elements and to an external part comprising the outer medial elastic layer and adventitia. (Figure 1)

Figure 1. Histopathological picture of ruptured aortic wall, HE, 20x objective (Dept. of Pathology, UMFTGM)

Due to the clivation resulted by the high blood pressure in the aorta, the dissection propagates down- and/or upstream creating a false lumen next to the real lumen resembling to two elastic tubes joined together. As a consequence of the dissection the arterial wall's elasticity - that helps sustaining the vascular homeostasis - becomes reduced. Subsequent dilatation causes a pseudoaneurysm, the tensile strength substantially drops predisposing rupture. [46]

The rupture of the external part of the arterial wall is more common in AAD causing catastrophic bleeding, cardiac tamponade, mediastinal compression. The false lumen is separated from the true lumen by a layer of intimal and medial elastic tissue known as the intimal flap (internal part of the dissected arterial wall). If the intimal tear occurs circumferentially with intususception of the intimal flap, it may results in the obstruction of the coronary arteries, great supra-aortic vessels. Further possible complications are due to the space consuming and real lumen narrowing property of the false lumen: compression of the aortic lumen, the ostia of the coronary arteries and supra-aortic branches.

In 75% of the Marfan cases the aortic root dilatation and anuloectasia occur jointly. The most frequent location of the dissection is the bulb of the aorta. The severity varies, starting from a discreet grade the AD can expand to wall rupture depending on the extent of intramural cleavage that may be continuous or fractional intraluminally and/or in the adventitial coatin.[5]

Intimal tear, fissuration can be found in 70-80% of the AD-s. As a result of this, the medial coat is split because of bleeding in from the aorta and creating a false lumen. With a second intimal tear the false lumen is fenestrated initiating parallel blood flow in the aorta. The double-barreled aorta is an aorta with a second vascular lumen formed in the media of the aortic wall, which connects the proximal and distal intimal tears in an aorta with a dissecting aneurysm. No intimal injury can be found in smaller portions of the AD-s when the endothelial surface remains unbroken and the aortic wall thickness is optimal as well (5 mm). It is still under debate whether the AD can be originated from intramural haematom caused by the vasa vasorum found in the adventitia.[20,34,37]

The pathohistological picture in Marfan syndrome is similar to those found in other congenital and acquired aortic dissections. The fibers of the elastic media wall get fractured and fragmented, due to degenerative and necrotic lesions cysts filled with mucoid matter arise. This phenomenon is called Erdheim's cystic medial necrosis (or degeneration) and is usually considered to be typical of AD.[21]

The molecular diagnosis is based on the fibrillin gene's alteration analysis and needs numerous sequence parsing which can play a role only in prevention and the genetic provement of MFS. The proper diagnosis of the disease and it's complications is primarily established on clinical findings and along with therapeutic urgency is life saving.

Clinical features and diagnostic considerations

Clinical variants of the AD can be distinguished according to the course based on the time factor. The most frequent and grave is the acute form counted from the onset of the symptoms until 14 days. Certain types (AAD) of dissections, if left untreated, would result in the death of 33% of patients within the first 24 hours, 50% of patients die within 48 hours, 80% of patients die within 2 weeks. The chronic form, after 2 months of persistence, can heal spontaneously, but the outcome is doubtful, the danger of redissection and rupture still

remains a concern. The term between the two intervals is called subacute AD.[1]

The main symptoms of acute type A aortic dissection contribute to the rapid diagnosis. 93% of the patients complain of sharp, splitting chest pain that begins retrosternally and radiates to jugular and scapulo-vertebral regions. Depending on the grade of the dissection and bleedings syncope and state of shock may develop between the prodromal picture to the terminal fudrovans phase. The relevant signs of ascending aortic dissection include angina-like chest pain, marks of aortic valve insufficiency. For the dissection of the transversal aorta the features of aortic arch syndrome dominate, in consequence of the affected supra-aortic trunks neurovascular focal signs, neurocognitive disturbances and ischaemic symptoms of the upper limb occur.

The complications of the AAD increase the severity of the symptoms that are primarily induced by bleeding and the consequences of the external wall rupture. The rupture of the intrapericardial ascending aorta can cause pericardial tamponade and the occlusion of the coronary arteries. The dilatation of the aortic arch and bleeding result in the compressional mediastinal syndrome, embolization, the blockage of supra-aortic arterial trunks followed by neurovascular complications. The pleuro-pulmonary breaking in causes respiratory symptoms. Aortic insufficiency is caused by flap prolapse, direct dissection to the valve or anuloectasia. The arteries debranching from the aorta can be compressed directly by the dissection its self when spread along the intimal-medial lining inside the subiected vessel.

The early diagnosis and differential diagnosis are preconditions for the urgent intervention. The procedure is done by symptomatology analysis and imagistic methods, in the first round usually with the help of transthoracal (TTE) and transesophageal echocardiography (TEE), digital subtraction angiography (DSA), magnetic resonance imaging (MRI) and computer tomography (CT) can be used for planning the operation also. (Figure 2) Through this instruments the data acquired is pathognomistic in almost all the cases. The chest x-ray shows no specific trait in 37,4% of the cases.[4,5,6,7] Intimal flap, thrombus formation, endoluminal obturation can be detected by MRI, CT and ultrasonography. The aneurysmatic dilatation of the ascending aorta is usually characteristic in MFS and easily can be visualized by ultrasonography. In men and women the normal aortic root diameter is less than 3,7 cm. It can reach up to 5 cm in case of dilatation. From 4,5 cm in its diameter cracks can develop on the vessel. The rate of the sinotubular junction and annulus diameter is 1,1±0,1 and the rate of the sinus Valsalvae and annulus diameter is 1,3±0,1 normally. Dilatation of the ascending aorta is considered from at twice of the normal values of these rates.

The differential diagnosis of AAD may be difficult due to the complexity and variability of the clinical picture but differentiation of the real from a false

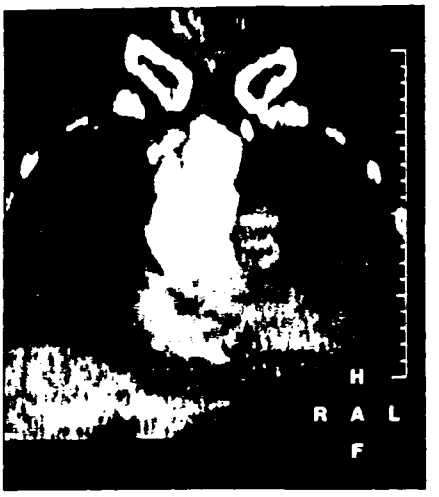

Figure 2. 3D reconstruction of CT slides of AAD (Dept. of Radiology, UMFTGM)

aneurysm as a possible complication in this case is not fundamental, because the surgical treatment in both conditions is similar. The following diseases should be excluded when suspecting an AAD: acute coronary syndrome (ACS), that can be caused by the annulovalvular lesion because of the dissection, pulmonary embolism (PE), pneumothorax (PTX), esophageal rupture and acute infectional complications of the pericardium. Physical findings include systolic difference of blood pressure between the upper limbs, systolic murmur on the arteries (carotid artery) and severe limb ischaemia may occur.[5]

The etiologic diagnosis of young patients if supposing MFS can be identified by the clinical picture. The criteria for MFS comprise the possible confirmed positive family anamnesis – although it is not always contributory, the young age, the marfanoid body constitution and the presence of various major conditions should be taken in to consideration. These major criteria manifest in the skeletal, ocular (lens dislocation) and cardiovascular (dilatation of the ascending aorta, with or without aortic regurgitation, and involving at least the sinuses of Valsalva, dissection of the ascending aorta) systems.[8]

The genetic diagnosis, the record in national registers of people with suspected or proven MFS has primarily preventive and check-up purposes. The clinical picture basically facilitates the evaluation process - done with instrumental help - of the affected cardiovascular system (e.g.: the progression of the aortic root dilatation and/or regurgitation) in the timing to perform the elective surgery preventing dissection. Secondary requirement is the differential diagnosis from other congenital genetic connective tissue disorders such as Ehlers-Danlos, Weill-Marchesani and other syndromes.[9]

Table . Consensual surgical paradigms

Listing of the consensual surgical paradigms regarding AAD:
Operation with relative urgency, the application of the requirements of open heart surgery
Extracorporeal circulation (cardiopulmonary bypass): considering the total surgery, pump and circulatory arrest time; pre- intra- postoperative monitoring[16,37]
Avoidance of cerebral complications, minimizing risk factors[29,37,38]
Antibiotics, antithrombotics, neuroprotection (steroids), myocardium protection and the corresponding specific treatments [47,52]
Supportive, drug therapy, controlled haemodynamics

Surgical treatment

The successful surgical strategy in the treatment of AAD, besides solving the tactical and technical challenges are clearly the question of preventing the hypoxical brain damage. [1,2] Since DeBakey, Cooley and Creech first published about their successful aortic replacement in 1955, the solutions and methods with its pros and cons for the treatment of aortic dissection are still under debate. Nowadays it is a fact that the results improved regarding this questions whereas the mortality rates of the surgical treatment (20%) compared to the conservative therapy (50%) is 30% less in the first 30 days.[4] The goal of the operation is reconstructive, mostly carried out by the replacement of the dissected part of the ascending aorta or aortic arch. (Table I).[29,32,37,38,47,55]

Bypassing the circulation of the affected part of the aorta extracorporeal circulation is essential. The key moment of the cardiopulmonary bypass is the arterial cannulation and selective cerebral perfusion.[6,8,56] The cannulation of the right axillary artery has more advantages, it can be done directly or with an intermediary vascular graft and it provides isolated anterograde cerebral perfusion (through right subclavian artery – brachiocephalic trunk – right carotid artery and right vertebral artery) compared to the femoral cannulation (Figure 3).[2,52] The femoral artery cannulation in AAD is a make-do solution with its disadvantages concerning the retrograde flow in the aorta, threat of embolism, cerebral microemboli and potential perfusion of the fake lumen (malperfusion).[15,56] In case of femoral artery cannulation the mortality rate is 20-40% while in axillary artery cannulation it is 5-10%. In a certain study the two methods compared, the total and cerebral mortality rates decreased with 20% and 14% when axillary artery cannulation is used.[6,43,52] Alternatively the right brachial artery can be used, the caliber of the artery is usually suitable without applying a graft for cannulation.[12]

As perfusion through right axillary artery is unilaterally selective the circle of Willis has a definitive role in the blood supply of the left brain hemisphere. Anatomical records describe an imperfect hexagon of 14% in the normal population although functionality is practically sufficient according to evidence gained from Doppler and oximetric as well from angiographic verifications.[18,22,41,42] If supposed or proved that the anastomotic ring does not supply enough blood to the left hemisphere during unilateral axillary perfusion the cannulation of the left common carotid artery is indicated too.

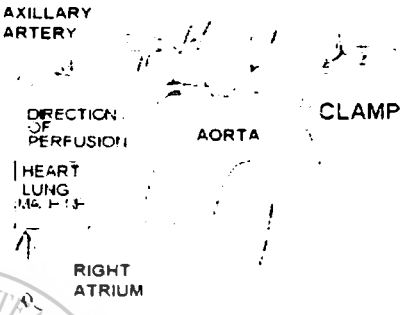

Figure 3. Axillary artery cannulation[52]

The circulatory arrest time in operations performed during deep hypothermia in case of femoral cannulation can be significantly prolonged with axillary cannulation under hypothermic protection. Hypothermia has protective effect on brain and myocardium cells and in case of axillary artery cannulation isolated cerebral blood flow is maintained which grants that the ischaemic time of the rest of the body can be even 2-3 hours. The cooling - according to the temperature of the perfusion solution - can be moderate (25-28°C) or deep (15-25°C). The perfusion solution's temperature cannot differ more than by 10°C from the core body temperature which can be measured oropharingeally, transesophageally or rectally.[9,16,26]

The surgical solution – depending on the indications – during the temporary suspension of the aortic circulation with clamps, is the resection of the dissected part and plastic vessel substitution with the use of Dacron vascular prosthesis. Exploration is outlined from median sternotomy or sternothoracotomy (in the left 3rd and 4th intercostal space).[25,54]

The surgical technique depends on the affected part of the aorta. If it occurs isolated and the valve and arch are in a good state a vascular interpositum is implanted.

At ascendent aorta reconstruction when the bulb and the valve are also involved, the resection is done by separation and perfusion the coronary arteries ostia with simultaneous aortic valve replacement. Between the vascular prostnesis (conduit) and resected ends of the aorta end-to-end anastomoses are carried out with the reimplantation of the coronary arteries into the conduit. The total aortic root exchange procedure can be performed according the methods of Bentall-DeBono or Cabrol with a separate vascular graft and a mechanical valve or with a so-called composite graft.[3,5] In case of untouched valves and intact ascending part valve preserving surgery can be done if no aortic-root enlargement is present (like in MFS, annuloaortic ectasia). In case of the involvement in the dissection of the aortic arch, partial or total arch replacement is advised with the use of modern tissue adhesive to reattach the split layers at the origin of the great arteries. (Figure 4)

Figure 4 Ascendent aorta replacement[5]

The aortic wall is strengthened with periaortic Teflon sheet, the sutures with the rarely used gelatin-resorcine-fuchsin (GFR) glue.[19,24,49]

The surgery of the dissection of the aortic arch is rather complicated. After the resection of the arch, the origins of the supra-aortic arterial trunks are removed and the stubs are cannulated, selective perfusion is performed (left common carotid and subclavian artery). The cannula in the right axillary artery provides the right side vessels with blood. The vascular graft according to its structure has either two or three branches (bifurcated, trifurcated). It is anastomosed with the remaining supra-aortic arterial stubs. The distal part of the graft which is elephant-trunk like is sewed to the thoracic aorta.[44,48] Periaortic echocardiographic control may be used during the reconstruction process of the aorta. Changes in surgical aspects of AAD are listed on the following table (Table II).

Another, less invasive solution for the reconstruction may be the endovascular flexible stent-graft used at real aortic aneurysms as well. It can be introduced in a transcatheter way percutaneously via the femoral artery or with a hybrid method approached by thoracotomy and aortotomy. Long term results of the endovascular stent graft methods are still under a debate.[1,45,58]

Prevention and prognosis

Identifying the condition and registering people with MFS in a national database, the regular check-ups, preventive operations are important. First of all health preservation, the change of lifestyle (quit smoking, avoiding straining movements, managing hypertension), certain medications (beta blockers, certain types of angiotensine receptor blocking anti-hypertensive drugs) can help to restrain the progression of the disease regarding the aorta.

In the strategy of primary surgical prevention of AD and aortic rupture (AR) in case of aortic root dilatation, certain diagnostic and therapeutic algorithms are followed. For screening purposes the different sizes of the aortic root are obtained through 2-dimensional echocardiography. Aortic root dilatation in case going with complaints for the patient is an aggressive surgical indication. Surgery is indicated if the dilatation goes without complaints and the aortic root size is over 55

Table II. Changes in surgical methods of AAD

Method	Cannulation	Workout of operation	Surgical technique	Mortality	Main cause	Problems/advantages
1.	Femoral artery and right atrium	Clamping of the ascendent aorta befor the origin of anonymous artery	Interpositum	20-40%	Bleeding, cerebral complication	Retrograde perfusion in fake and real lumen
2. From the begin of the '90s	Femoral artery and right atrium	No clamping of aorta, deep hypothermia, circulatory arrest	Bentall-DeBono, Cabrol	15-30%	Bleeding, cerebral complication	Retrograde perfusion in fake and real lumen, time limit (45 min max.), long reper-fusion time
3 Sabik et al[43]	Right axillary artery and right atnum	Branches of the aortic arch are clamped, 25-28C hypothermia, continuous cerebral blood flow	Bentall-DeBono, Cabrol	5-10%	Bleeding	Anterograde perfusion in real lumen, effective cerebral protection- there is practically no time limit (2-3h)

mm or between 50 and 55 mm and any of the following occurs: dissection in family history, mitral insufficiency of 3rd, 4th grade or when patient is before pregnancy. In other recommendations the aortic root size, diameter of annulus and Valsalvae sinus, sinotubular junction and their ratios play a role in setting up the indication for surgery.

Secondary prevention is vital for patients that underwent cardiac surgery with MFS. The purpose of the follow-up is the detection of the late complications (aneurysm, expansion, redissection, anastomosis leak, rupture). The severe late prognosis of acute AAD requires monitoring of the patients, long term handling of the disease. After the acute event the control with medical imaging methods is recommended the 1st, 3rd, 6th, 12th months then on a regular annual basis or more frequently if necessary or complications are suspected.[53] At preventive operations echocardiographic control is recommended every 3 months in the first year then yearly.

The prognosis of acute AAD after the successful treatment is influenced by the still underlying disease (MFS) and in consequence of the surgery chronic type B AD can remain. The operation itself can have late complications as well like anastomosis leak, pseudo-aneurysm formation or the benign thrombotic obstruction in the fake lumen of the distal AD.

Redissection, aortic aneurysm or rupture develops over 5 years at one-third of patients.[46] With appropriate setup and managed blood-pressure values, the incidence of redissections reduced from 45% to 17%. Reoperation is needed in 15% of the patients. The survival of successful acute proximal AD surgery patients over 5 years is 65-80%, and over 10 years is 40-50%.[7,22]

Prognostically in a certain study, the acutely and the earlier type B AD operated aorto-annulectatic group was the most sensitive, while the most protected was the aorto-annulectatic and preventively operated group of patients. Consequently the operated patients with MFS should be continuously monitored and carrying out preventive operations has importance as well.[53]

The effective prevention of acquired AD, carrying out the prophylactic surgery together with the continuous check-up of the predisposed and operated patients depends on a professional nationwide register for people with MFS, and a national register for patients with aortic dissection should be established as well.

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Immunohistochemical expression and in situ hybridization for Epstein-Barr virus in nasopharyngeal carcinoma – a study on 30 cases

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Epstein-Barr virus (EBV) is strongly associated with many epithelial and lymphoid tumors, including nasopharyngeal carcinomas (NPCs). The objectives of the present study were to determine the occurrence of EBV infected tumoral cells in NPCs, and to investigate the possibility of EBV infection as an early, initiating event in the development of NPC. EBV was detected by a bistadial indirect immunohistochemical (IHC) technique, using antibodies against EBNA2, and by in situ hybridization using the EBV probe ISH (EBER1) kit according to the manufacturer's instructions. We analyzed retrospectively archived samples of 30 primary NPCs. A number of 17 cases were EBNA2 positive (56.7%). EBER1 positivity was noticed in 19 cases (63.3%) of undifferentiated and nonkeratinizing types. EBV identification by ISH for EBER1 is more effective than IHC. The positivity for EBER1 may suggest a causal association and a sign of latent infection with EBV. EBV identification by IHC or ISH may be useful considering EBV as a potential biomarker for early detection and a possible target for therapy, like immunotherapeutic vaccines.

Key words: *Nasopharyngeal carcinoma, Epstein-Barr virus, EBNA2, in situ hybridization*

Viral infection plays an important role in malignant cells transformation, being estimated that approximately 15% of all human tumors have viral etiology.[2] The Epstein-Barr virus (EBV), a gamma-herpes virus, infects over 90% of the world's adult population by the age of 25.[1,3,6,7,12] Healthy virus carriers present a higher risk to develop tumors, especially those who have hereditary or acquired immune deficiencies. EBV, originally recognized for its ability to infect and transform lymphocytes, is now clearly recognised to infect epithelial cells as a part of its normal cycle of persistence in the human host.[5] Despite a relatively benign course in most carriers, it has been demonstrated that, etiologically, EBV is strongly linked to nasopharyngeal carcinoma (NPC), Burkitt's lymphoma, post-transplant lymphomas, Hodgkin's disease, malignant lymphomas, etc.[2,6,14]

NPC is a malignant tumor arising from the epithelial lining of the nasopharynx, consistently associated with the EBV. NPC is the third most frequent virus-associated malignancy in humans.[4]

Viruses that establish persistent infections must avoid detection and recognition by the immune system, that otherwise would eliminate the infection. Different EBV evasion strategies have been identified, such as: the restricted expression of viral genes and proteins that makes the infected cells nearly invisible to the host (e.g., EBV in B cells); the inhibition of antigen processing; and the MHC class I-restricted presentation.[2]

Life-long infection with EBV may be explained in part by EBNA1's function of inhibiting antigen processing, which allows infected cells to escape cytotoxic T lymphocyte surveillance.[9]

NPC-associated EBV could be a potential target for immunotherapeutic vaccines, through cell response stimulation against viral antigens expressed in tumor cells.[13,16]

If immunocompetent hosts fight against virus-infected and neoplastic transformed cells efficiently, ultimately eliminating them and preventing tumor outgrowth, immunosuppressed hosts (e.g. HIV-infected individuals, immunosuppressed organ transplant

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recipients) are at increased risk of virus-associated cancer (e.g. EBV-associated lymphomas), because neoplastic cells may escape the host's immune control.[2] Even though very few cells in the host may be infected at any given time, the chronic infection presents a long term opportunity for a rare event to occur, allowing the survival of cells with virus-modified growth control mechanisms.[2]

In early stages, NPC is a radiosensitive tumor and local control rates of more than 80% can be obtained; combinated chemotherapy usually can achieve a brief remission in metastatic disease.[10,15] However, a significant number of patients relapse, particularly when disease is advanced at diagnosis - the most common presentation due to a lack of early symptoms.[12,11] Moreover, radiation and chemotherapy are accompanied by severe short- and long-term side effects, including secondary malignancies.[18] After disease recurs or progresses, no additional effective therapy is available and patients die a few years later.[8,17] Hence, there is a need for therapies that will improve disease-free survival and that may be associated with reduced toxicity.[15]

To prove whether EBV infection is an early, initiating event in the development of NPC, we determined retrospectively the presence of EBV nuclear antigen (EBNA) and viral transcripts of EBV encoded RNA (EBER1) in patients with primary nasopharyngeal carcinomas.

MATERIAL AND METHODS

Our retrospective study was carried out on 30 patients diagnosed with primary NPC. Tissue samples were formalin-fixed, paraffin-embedded, and were selected from the archive of "Victor Babes" National Institute of Pathology.

An indirect bistadial technique performed with a polymer based detection system (EnVision* Dual Link System-HRP, Dako, Carpinteria, CA) using monoclonal antibody to EBNA2 (clone C51-4) (Dako, Glostrup, Denmark, dilution 1:100) was carried out.

In situ hybridization (ISH) was performed using the EBV probe ISH kit (Novocastra Labs, Newcastle upon Tyne, UK) for EBV RNA transcript EBER1, according to the manufacturer's instructions.

Data were analyzed using the Analysis Tool Pak of Microsoft-Excel 2003, running under Windows XP Professional. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSIONS

The study group was composed of 30 patients (age range 12 to 83 years; M=44.87; mode= 20; SD= 18.6; M:F=5:1) diagnosed with primary NPC. Ten cases presented lymph node metastasis (pN1) and 20 cases had no lymph node metastasis (N0).

Histopathological examination was performed by standard H&E staining. WHO classification was used to differentiate tumors in three subtypes: keratinizing squamous cell carcinoma (type I); nonkeratinizing carcinoma, including differentiated (type II) and undifferentiated carcinoma (type III).[3,11,12] Accordingly, we detected 2 cases of keratinizing squamous cell carcinomas (WHO type I) (6.66%) (Figure 1), 21 nonkeratinizing differentiated carcinomas (WHO type II) (70%), and 7 undifferentiated NPCs (WHO type III) (23.33%) (Figure 2).

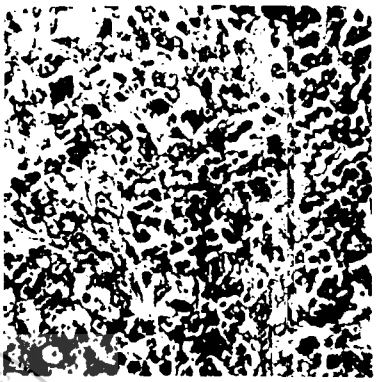

Figure 1. Undifferentiated NPC, H&E stain, Ob. 10x

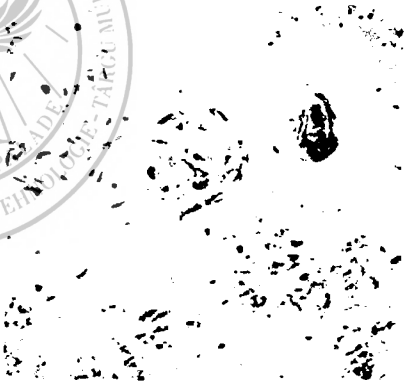

Figure 2. Keratinising squamous cell carcinoma , H&E stain, Ob. 4x

A number of 17 cases were EBNA2 positive (17/30) (56.7%) (Figure 3, Figure 4) with a strong nuclear expression.

EBNA2 was expressed in 6/7 undifferentiated carcinomas, and only in 11/21 nonkeratinizing carcinomas. All squamous cell carcinomas were EBNA2 negative.

EBER1 nuclear positivity was noticed in 19/30 cases (63.3%). All seven undifferentiated carcinomas were positive for EBER1, compared to only 12/21 nonkeratinizing carcinomas. All squamous keratinizing carcinomas were EBER1 negative.

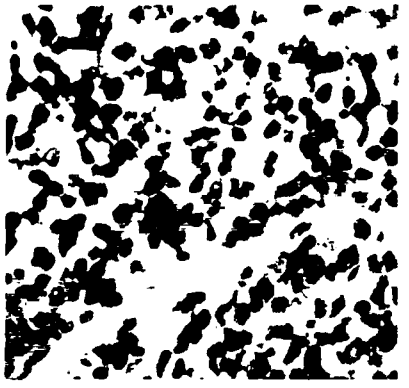

Figure 3. EBNA2 positive nuclear in tumor cells, Ob. 10x

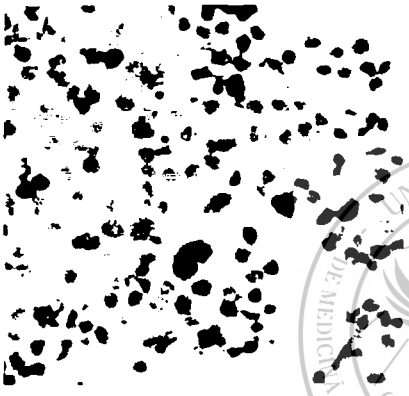

Figure 4. EBNA2 positive in rare nuclei of tumoral cells, Ob. 10x

EBER1 was identified more frequently in undifferentiated NPCs compared to nonkeratinizing NPC.

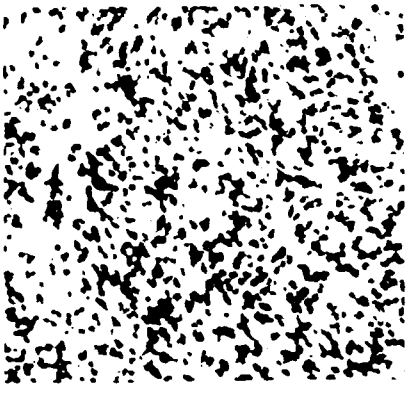

Figure 5 EBER1 positive in numerous nuclei of tumoral cells, Ob. 10x

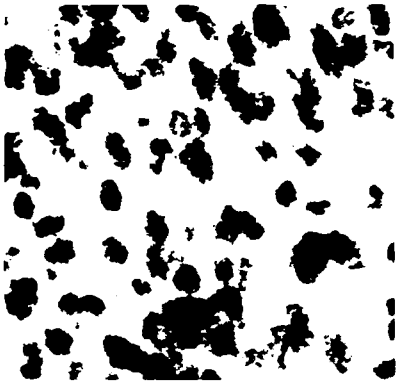

Figure 6. EBER1 positive in numerous nuclei of tumoral cells, Ob. 40x

All EBER1 positive cases had lymphocyte rich stroma, including cases without a classical appearance of lymphoepithelioma. This observation can suggest the importance of lymphocytes presence in NPC in triggering the carcinogenic events in epithelial cells, as recently mentioned.[10,18]

Our results are similar to those already reported, which showed that EBV is frequently detected in patients with NPCs.[2,4,6,14] In this context, EBV identification by IHC or ISH may be useful, considering EBV as a biomarker for early detection and a possible target for therapy in NPC.

In our study, EBV identification by ISH for EBER1 was more effective than IHC for EBNA2. The immunohistochemical identification of EBV has to be completed by molecular techniques such as ISH for EBV RNA transcripts.

The positivity for EBER1 is highly suggestive for a causal association and a sign of latent infection with EBV. Statistical analysis revealed no significant correlation (p=0.32) between EBNA2 and EBER1 expression in tumor cells.

The frequent presence of EBV in NPCs, especially in undifferentiated and nonkeratinizing types, recommends EBV as a potential target for novel therapies such as immunotherapeutic vaccines against viral antigens expressed in tumor cells.[13,16]

CONCLUSIONS

EBV infection is closely associated with the development of nasopharyngeal carcinoma.

EBV identification may be used as a biomarker for the early detection of nasopharyngeal carcinoma and also as a possible target for therapy (e.g. immunotherapeutic vaccines).

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Papillary microcarcinoma of the thyroid: incidence and prognostic factors

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The objectiv of this study is to define the factors of prognostic of the papillary microcarcinoma of the thyroid and to analyse their epidemiological and histological characteristics. The group of study included 57 patients which were diagnosed postoperator with papillary microcarcinoma of the thyroid, after the final histological examen. The factors of prognostic were elaborated from the results of the histological examination. The epidemiological and histological characteristics were also evaluated The histological characteristics represented by the vascular extension of the tumour, the invasion in the adjacent parenchyma or in the thyroid capsule are factors of unfavourable prognostic.Two different versions of the papillary microcarcinoma of the thyroid, with and without risk of metastasis, were deduced after analysing the factors of tumoural prognostic.
Key words: thyroid cancer, papillary microcarcinoma of the thyroid, factors of prognostic

The papillary microcarcinoma of the thyroid is relatively frequent, representing 25-30% from the papillary carcinomas.

Defined through its dimensions, which is smaller than 1 cm (< 0, 5cm in 80% of the cases), the papillary microcarcinoma of the thyroid is an uncapsuled or circumscribed sclerosant tumour. Clinically, it can appear as a lateral-cervical metastatic adenopathy (10%) or, as an accidental detection (90%), on a piece of partial or total thyroidectomy, performed for another pathology: adenoma, multinodular goiter, hyperthyroidism. These tumors can also be detected through the agency of systematical studies of the pieces taken from the necropsies, where their frequency is very changeable: 7-36%.

The aim of this study consists in analysing the epidemical and morphopathological characteristics of the papillary microcarcinoma of the thyroid and defining the factors of tumoural prognostic in accordance with this information.

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MATERIAL AND METHOD

This study is based on a longitudinal retroactive study of the information from the observational charts, of the operating proceedings and of the histopathological analysis reports for a group of 430 patients who have had a total, undertotal and quasitotal thyroidectomy, between 1998-2008. The group of study has been divided into two groups: patients with cervical adenopathy and/or metastasis on distance and patients without cervical adenopathy and metastasis on distance. The following histopathological characteristics of the papillary microcarcinomas of the thyroid have been evaluated: dimension, architecture, multifocality, the presence of the capsule and the invasion in the adjacent parenchyma, the vascular extension and at the level of the thyroidal capsule. The unfavourable factors of prognostic have been defined by correlating these characteristics with the presence of metastasis.

RESULTS

74 patients have been diagnosed with papillary carcinoma of the thyroid, from them, 57 presented the morphologic version of the papillary microcarcinoma.

Table I. Epidemiological characteristics of the papillary microcarcinoma of the thyroid

	All cases	N+/M+	No/Mo
Number	57	17	40
Sex males	9 (15,8%)	6 (35,5%)	3 (7,5%)
Age average	48 yers	47 years	48 years
≥45 years old	35 (61,4%)	10 (58,8%)	25 (62,5%)
≥60 years old	11 (19,3%)	3 (17,6%)	8 (20%)

N+/M+ - patients with cervical adenopathy and/or metastasis on distance
No/M0 - patients without cervical adenopathy and metastasis on distance

Table II. Histopathological characteristics of the papillary microcarcinoma of the thyroid according to the sex

	All cases	Males	Females
Number	57	9	48
Multifocality	14(24,6%)	2(22,2%)	12(25,0%)
Infiltration into the adjacent parenchyma	26(45,6%)	6(66,6%)	20(41,7%)
Extension into the thyroid capsula	17(29,8%)	3(33,3%)	14(29,2%)
Vascular extension	6(10,5%)	2(22,2%)	4(8,3%)

Table III. Histopathological characteristics of the papillary microcarcinoma of the thyroid according to the metastatic spread

	All cases	N+/M+	N0/M0
Number	57	17	40
Multifocality	14(24,6%)	7(41,2%)	7(17,5%)
Infiltration into the adjacent parencyma	26(45,6%)	13(76,5%)	13(32,5%)
Extension into the thyroid capsula	17(29,8%)	10(58,8%)	7(17,5%)
Vascular extension	6(10,5)	6(35,5)	0

N+/M+ - patients with cervical adenopathy and/or metastasis on distance
No/M0 - patients without cervical adenopathy and metastasis on distance

In 4 cases, the papillary microcarcinoma of the thyroid is connected with metastasis at the level of cervical ipsilateral ganglions, and two cases are associated with determinations on distance (osseous – 1 case, pulmonary and osseous – 2 cases).

In the studied population, the epidemical characteristics of the papillary microcarcinoma of the thyroid, are shown in table I. The medium age of the population was 48, with a ratio of sexes M/F of 1 to 5,3, which corresponds with the data from the specialty literature. Besides, the age of 48 is unanimously accepted as an important element of favourable prognostic in the differential thyroid cancer.[2] In 16 cases we found capsuled papillary microcarcinoma of the thyroid, without lateral-cervical adenopathy, and in 27 cases, they presented partial capsule tumours (without adenopathy in 17 cases, with adenopathy and metasthasis on distance in 11 cases). The uncapsuled form, diffused or sclerotic, had been found in 13 cases (7 patients without lateral-cervical adenopathy, 6 patients with lateral-cervical adenopathy).

The histopathological characteristics of those 57 cases of the papillary microcarcinoma of the thyroid are revealed in table II and III, in accordance with age and with the presence or absence of the lateral-cervical tumoural determinations and on distance.

In the studied group prevailed the smaller lesions, of 5 mm (75% of the cases), correlated with the young age of the patients.

From a histological point of view, the papillary form of the infracentimetric thyroid carcinoma had been found in 14% of the cases, the follicular shape in 35% of the cases, and the mixed form in 49% of the studied population, without significant statistical differences between sexes.

DISCUSSIONS

The papillary microcarcinoma of the thyroid represents 6% from the total of the cases that were included in the present study. An increased incidence of the papillary microcarcinoma of the thyroid had been found during the necropsies that were done deceased patients for other affections, which suggests the benign evolution of a raised percentage (2,5 – 30%) from these tumours.[3,6]

The clinical significance of the papillary microcarcinoma of the thyroid is a disputed subject, the prognostic of this affection, generally being excellent, from the surviving point of view, but also from the one of recurring.[1] The most frequent secondary tumoural determinations are in the lateral-cervical ganglions, lungs and osteo-articular system.[2,5]

The presence of metastasis in the lateral-cervical ganglions at the patients having papillary microcarcinoma of the thyroid raises the risk of local recurring, metastasis on distance and mortality. In the group of study, two patients have metastasis on distance (osseous and pulmonary).

A classification of the papillary microcarcinoma of the thyroid, in accordance with their dimension had been suggested by Kasai et al.[12] They noticed that the papillary microcarcinoma of the thyroid, between 5-10 mm in diameter, had a significant raised prevalence of the extrathyroidal extensions and of the ganglion metastasis. The grown aggressiveness of tumours larger than 5 mm had been reported by Noguchi et al as well, which registered a rate of recurrence at 35 years of 14%, confronted by 3% for the lesions smaller than 5 mm. The same authors noticed the significant prognostic impact of the age, at the patients older than 55, the affection recurred in 40% of the cases but only in 10% at the younger ones.[7,8] The age doesn't seem to be an unfavourable prognostic factor in the mentioned group, despite the studies of specialty.[6,5] Also, in the group of study, the dimension of the papillary microcarcinoma of the thyroid had been equal or smaller than 5 mm (75% of the cases), not being an unfavourable prognostic factor.

The follicular variant of the papillary microcarcinoma of the thyroid is considered an aggressive form. In the present study, the follicular or papillar architecture of the papillary microcarcinoma of the thyroid doesn't raise the risk of local-regional metastasis and on distance.

Choosing the surgery technique doesn't rely on the dimension of the tumoral lesion. The majority of the endocrinological surgery centers recommend a total thyroidectomy, as a treatment of election of the papillary microcarcinoma of the thyroid, taking into consideration the aggressiveness of some histological variants of the tumour, also considering its multifocusing and the risk of recurrence.[5,12]

CONCLUSIONS

Yearly, the incidence of papillary microcarcinoma of the thyroid is growing. The histopathological characteristics, as the vascular tumoural extension, the invasion of the adjacent parenchyma or of the thyroidal capsule and the localization in its closeness, are factors of unfavourable prognostic.

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Second trimester prenatal screening: comparison between biochemical and cytogenetic analysis

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In a retrospective study, we evaluated the first three years of prenatal screening within the Maternal and Fetal medicine department belonging to the Filantropia Hospital in Bucharest, Romania. The risk of trisomy 18/21 was calculated for 970 pregnant women in the second trimester of pregnancy, by combining the personal data, the ultrasound screening and the maternal serum markers with the help of Priska 4.0 software. Only 173 high-risk women accepted the amniocentesis for the detection of chromosomal anomalies. Thus, the fetal karyotyping from the "in situ" culture, using GTG banding techniques was performed. By comparing the "triple test" results and the cytogenetic methods, we reached some conclusions concerning the importance of the two methods for the prenatal diagnosis of malformations

Key words: triple test, Priska 4 0

Several studies showed that for the first pregnancy trimester there is a significant difference of the serum values of AFP, hCG and free-estriol as regards the pregnancies affected by a trisomy 21 compared to the normal pregnancies. After the arbitrary introduction of a cut-off value of 1/250 corresponding to a 37 old pregnant woman risk, bearer of a normal embryo –for all the patients over this value we recommended more prenatal investigations, including the amniocentesis.[1,2,3,10] Taking into account the fetal cells karyotyping (for the women who accepted the amniocentesis) and following the evolution of pregnancies for all women investigated by the triple test, we reached a conclusion concerning the predictability of the biochemical investigations and implicitly the "false positive" rate of these non-invasive methods.

MATERIAL AND METHOD

There are two categories of investigations for the congenital malformation screening: non-invasive (biochemical and ultrasonographic) and invasive, considered to be risky for the pregnancy evolution, like: amniocentesis, cordocentesis, and chorionic villus sampling.

A. Non-invasive methods of prenatal diagnosis for the second trimester: the biochemical "triple test ". In order to evaluate the trisomy 18/21 risk we took the following steps of triple test :

1. collecting 5-10 ml of maternal peripheral blood

2. introducing the patient data in a special form containing: maternal age, maternal weight, pregnancy age, the date of the last menstruation, some maternal specific conditions like: smoking, diabetes, lost pregnancies, if the pregnancy was spontaneous or as a result of in-vitro fertilization.

3. by using chemiluminescent DPC Immulyte assay, we analyzed the following maternal serum markers:

a) AFP – alpha –fetoprotein, a hormone produced by the embryo in development, by the gastrointestinal and liver fetal cells and which passed in maternal circulation in small quantities.

b) In significant quantities by the placenta during the pregnancy. An abnormally low value can suggest a chromosomal anomaly, like Down or Edwards syndrome. It can present variations in more pathological conditions like preeclampsy , anemia, kidney disorders, that is way this marker can be considered to have the most reduced predictive value for the triple test [4,5,6]

c) Chorionic gonadotropin hormone - a peptide hormone produced by the embryo right after the conception and subsequently by the syncytiotrophoblast.

The correlated values of the 3 hormones represent the "triple test" and it is associated with the following conditions (Table I):

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3 - University "Carol Davila", Bucharest

Tablel. The variations of the 3 maternal serum markers in case of certain fetal malformations

Detected values	Fetal conditions associated with detected values
low values for: AFP and unconjugated estriol high values for hCG	Down syndrome
low values for all three markers	trisomy 18/Edwards syndrome
high values for AFP	neural tube defects, omphalocel, gastroschisis

Table II Biochemical and cytogenetic investigations in women divided into age groups

Age group	Number of biochemically tested cases	No of cases with risk>1/250	No. of "risk" pregnant women who accepted amniocentesis	Number of cytogenetic cases confirmed	The pregnancy evolution (normal new borns/abortions)	Fpr (false positive" rate)
15-19 years	9	-	-	-	-	-
20 -24 years	105	10	4	1	104 normal new borns	0,086 (8,6%)
25 -29 years	334	90	50	3	330 normal new borns/1 iatrogenic abortion	0,26 (26%)
30 -34 years	350	110	61	5	344 normal new borns/ 1 spontaneous abortion	0,30 (30%)
>35 years	172	70	56	1	170 normal new borns/ 1 iatrogenic abortion	0,41 (41%)

Together with the personal and ultrasound data, the biochemical results are introduced in the trisomy 18/21 risk evaluation software and finally a specific diagram is obtained (Figure 1)

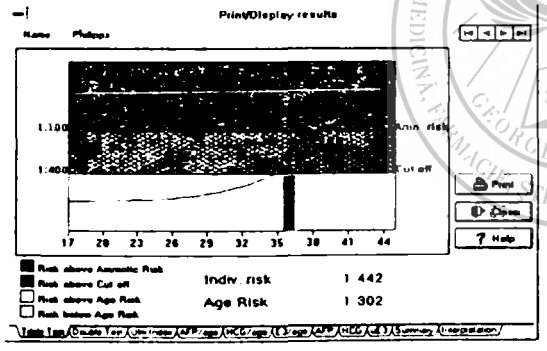

Figure 1. Diagram of trisomy 21 evaluation risk (Priska 4.0 software display)

B. Invasive methods for the prenatal diagnosis: cytogenetic investigations.

Out of 230 trisomy high-risk patients, 173 accepted amniocentesis. "In situ" cell cultures were performed, with a 7-10 days average incubation. 15-20 metaphases were analyzed for each case, by conventional coloration and GTG banding. Out of all cases investigated by cytogenetic methods, only 10 showed an abnormal fetal karyotyping; four pseudomosaicisms, a trisomy 18, three trisomy 21 and two trisomy 21 in mosaic.

RESULTS AND DISCUSSIONS

We carried out a retrospective study following the evolution of pregnancy for all cases (970) analyzed by the "triple test" and who gave birth in our hospital. Thanks to this study, we managed to compare both methods of prenatal screening. Finally, the comparison between the biochemical screening and the amniocentesis results were added in the following table (Table II), that reflects the false positivity of the triple test on age groups.

The false positive rate (fpr) is defined as the probability for a normal subject to present a "false positive result" and is calculated according to the formula[5,9]:

$$fpr = a/a+b = 1-s$$

where:

fpr - represents the false positive rate,
a-number of normal cases with positive results
b- number of normal cases with negative results
s- the specificity of the method

We noticed an increased tendency of pregnant women who came to our Maternal and Fetal medicine service to refuse the amniocentesis based on their fear of fetal injury. The average acceptability percent was at 61%, with an increase in amniocentesis acceptance in the age group over 35 years. A better counselling of these women is important after the age of 35, because they are more informed on the fact that the risk of pregnancy following the invasive procedure is smaller than the risk to give birth to a child with chromosomal trisomy[4].

Moreover, within the department of maternal-facial medicine from Filantropia Hospital a reduced number of fetal losses following amniocentesis were noticed: only 2 iatrogenic abortions for 171 amniocentesis, the results being comparable to the ones at the national level.

In the present study we calculated the false positive rate of the biochemical screening in pregnancy and we noticed an increased growth of this value with the maternal age. Also, we remarked the tendency of pregnant women to refuse amniocentesis as a confirmation method of an increased risk of chromosomal anomaly obtained through triple test. This reduced acceptability (of approximately 61%) is due to information from different sources that women have on the risk of iatrogen abortion following the invasive procedures and upon the increased false positive of the triple test[8,10]. We must underline the fact that for the women aged over 35, the risk of iatrogenic abortion is

lower than the risk to have a child with Down or Edwards syndrome[1]. Moreover, in our hospital the percentage of fetal affection following amniocentesis was below 1%. As a consequence, we will do a better counselling of pregnant women, taking into account that after the age of 35, once with the decrease in the accuracy and relevance of biochemical methods with the maternal method it is necessary to use invasive methods of prenatal diagnosis.

To reduce the anxiety of pregnant women with a biochemical risk >1/250, until the getting of the final diagnosis through amniocentesis, these must be informed on the fact that the aneuplody risk calculated through the "triple test" represent only the probabilistic result of a statistical elaboration.[2]

CONCLUSIONS

A. The placental secretion and hormonal transport characteristics can increase the "false-positive" rate with maternal ageing.

B. Sometimes, even the cytogenetic investigations can lead to doubts, determining mosaicisms, pseudomosaicisms and sometimes to artefacts in culture.

C. The discrepancy between the very high positive rate for the screening tests compared to the cytogenetic one makes the difference of accuracy in the 2 methods.

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Cytogenetic aberrations in chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes. It is the most common form of leukemia found in adults.
An abnormal karyotype is observed in the majority of patients with chronic lymphocytic leukemia (CLL). The most common abnormality are trisomy 12 and deletion of 13q, which occurs in more than 50% of patients.
Our aim is to identify and to evaluate the frequency of chromosomal abnormalities in patients with CLL in the Hematology Clinic in Tg. Mures.
Between January 2006 and January 2009 we received for chromosomal analysis 34 samples of peripheral blood from CLL patients from Hematology Clinic from Tg. Mures. We carried out peripheral blood cell culture according to standard methods.
We successfully analyzed the leukemic karyotype of 30 patients (88,3%) and identified 12 (40%) cases with chromosomal abnormalities. In our study the most frequent abnormality was aneuploidy. These findings are similar to the results obtained in other studies using a similar approach.
***Key words:** chronic lymphocytic leukemia, cytogenetic, trisomy 12*

Chronic lymphocytic leukemia (chronic lymphoid leukemia, CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes. It is the most common form of leukemia found in adults. Clinical features may iclude generalized lymphadenopathy, hepathomegaly and splenomegaly. Clinical staging correlate with median survival.[2,12]

An abnormal karyotype is observed in almost 30-50% of patients with chronic lymphocytic leukemia (CLL) by standard cytogenetic analyses. Chromosomal aberrations can be detected in almost 80% of cases of CLL by use of interphase fluorescence in situ hybridization.[16]

Chromosomal aberrations in CLL

There is no single specific cytogenetic abnormality in CLL.[11] The development of new techniques, such as fluorescent in situ hybridization (FISH), has increased the detection of numerical and structural chromosome abnormalities. The most common cytogenetic alteration is deletion 13q14 (51%), followed by deletion 11q22-q23(17%-20%), trisomy 12(15%), and deletion 17p13. Complex abnormalities may be present; for example, CLL patients with trisomy 12 may have simultaneous 13q14 deletions.[6,15]

Interestingly, the general picture is dominated by unbalanced changes, mainly with deletions of well-definable chromosomal regions (1; 5). Translocations usually accompany the progressive phase of the disease and seem to be more sporadic.[9]

The most common clonal cytogenetic abnormalities in CLL are trisomy 12 and deletions involving the long arm of chromosome 13.[11] The most frequent and relevant chromosomal aberrations found in chronic lymphocytic leukemia are described below (Table I).

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Table I. Chromosomal Aberrations in Chronic Lymphocytic Leukemia

Chromosome	Frequency	Genes Involved	Clinical Features
Del 13q14.3	>50%	*RB1*	Low CD38, good prognosis
Del 11q22-q23	19%	*ATM*	Extensive lymphadenopathy, unfavorable prognosis
Trisomy 12	15%	*MDM-2*	High CD38, unmutated V_H genes, intermediate prognosis
Del 17p13.3	15%	*p53*	Richter's transformation, short survival, poor response to purine analogs

Trisomy 12

Trisomy 12 was the first abnormality found in CLL. The incidence varies from series to series. The reasons for this are largely technical. By standard cytogenetic analyses, approximately 10% of patients have trisomy 12, but by interphase FISH analysis the prevalence rises to about 15%. Other factors include patient selection, the most important of which is selection for patients with leukemia cells that have typical morphology. Leukemia cells with trisomy 12 have "atypical morphology" more commonly than leukemia cells that lack this cytogenetic abnormality.[6] Cells with atypical morphology have a larger diameter, less rounded shape, and lacier chromatin than the 'typical' well-differentiated lymphocytes that commonly are seen in CLL. Such atypical morphology more commonly is associated with cases that have trisomy 12 and other cytogenetic abnormalities.[7,8]

Long-term clinical studies point to an unfavorable nature of this aberration, although not all data could confirm a difference in the survival from patients with normal karyotype.[5] Trisomy 12 is also frequently combined with other karyotype abnormalities.[9]

Del (13q)

Deletion 13q is the most common genetic abnormality in CLL, being detectable in at least half of all cases. These deletions generally occur in the absence of chromosome translocation. Nevertheless, the rare CLL cells with translocations often are noted to have ones involving 13q with any one of several different chromosomes. Individuals showing 13q14 abnormalities have a relatively benign disease that usually manifests as stable or slowly progressive isolated lymphocytosis.[9]

Del (11q22-23)

Deletion of the q22-23 locus on the long arm of chromosome 11 is another aberration with unfavorable prognosis. This change was reported in 12-21% of all CLL patients. Within the given locus ATM (ataxia telangiectasia mutated) is deleted. This gene is known to be responsible for ataxia telangiectasia, a hereditary syndrome with neurological disorders and increased risk for tumorigenesis.[3] ATM is known as an important synergist in the apoptotic pathway, the failure of which has been demonstrated in many malignancies including lymphoproliferative disorders other than CLL. The normal ATM acts predominantly through the activation of the p53-dependent apoptotic pathway, and its loss results in reduced cell death similar to p53 deficiency.[7,9] Patients with CLL cells that have 11q deletions tend to have more aggressive disease. Patients with 11q deletions had a tendency towards younger age, lower hemoglobin levels, and more advanced Rai stages.[4]

Del(17p)

According to recent observations, the loss of the short arm of chromosome 17 (17p13) is associated with the worst outcome ever, predicting a disease progression within 1-2 years and a relative resistance to chemotherapy. The deletion of 17p has been found to be the strongest independent marker for disease- related death.[3] This change can be demonstrated both by karyotyping and FISH . The aberration can be found already at diagnosis in 5% of the CLL cases, the overall frequency was 7-12%. Later occurrence of del(17p) was usually reported together with accompanying additional aberrations.[9]

Normal karyotype in CLL

In 20-40% of CLL cases no genetic disorders can be demonstrated despite the use of refined cytogenetic technology. This fact is a strong argument for the secondary nature of all previously mentioned chromosomal changes. The negative cytogenetic finding – by exclusion of all presently known unfavorable changes – equals to a favorable clinical prognosis.[9]

Major prognostic factors in chronic lymphocytic leukemia (CLL) include chromosomal abnormalities that can be identified by conventional cytogenetic analysis or fluorescence in situ hybridization; 17p– and 11q– are associated with a poor prognosis; 6q- and 12q trisomy are associated with an intermediate prognosis; and 13q– (the most frequent cytogenetic abnormality in CLL) is a favorable marker. Other prognostic markers include presence (favorable) or absence of IgV_H somatic mutations, detected by polymerase chain reaction, and high levels of expression of CD38 and ZAP70 (both unfavorable) measured by flow cytometry.[14]

Our aim is to identify and to evaluate the frequency of chromosomal abnormalities in patients with CLL in the Hematology Clinic in Tg. Mures.

MATERIALS AND METHODS

Patients. Between January 2006 to January 2009, 34 samples of peripheral blood from patients with chronic lymphocytic leukemia were sent to the Genetic Laboratory of The University of Medicine and

Pharmacy in Tg. Mures, Romania for cytogenetic evaluation. The study included patients with CLL from Hematology Clinics from Tg. Mures. The median age was 68 years; 24 patients (70%) were males and 10 (30%) females. All the cases were classified according to the Rai and Binet staging systems, which are the most commonly used staging systems in CLL.

Cytogenetic analysis

Heparinized peripheral blood obtained at the time of diagnosis or during drug therapy were cultured for 3 days in RPMI 1640 medium supplemented with 20% fetal calf serum, 1% L-glutamine, 50 ng/ml penicillin/ streptomycin with mitogens. After incubation, the cells were exposed to Colchicine (10 ěg/ml), followed by hypotonic treatment (0.075M KCl), and were fixed with a mixture of methanol and glacial acetic acid (3:1). Chromosomes were spread on cold, wet slides. We used a special staining technique (Giemsa staining or GTG staining). Karyotype was interpreted according to International System for Human Cytogenetic Nomenclature (ISCN 1995) recommendation.[12] A minimum of 16 metaphases were analyzed. Analysis was carried out using a BX51 Olympus microscope and images captured with an automated image analysis system (Cytovision, Applied Imaging). Cell culture failure was defined as cases where fewer than 10 analyzable metaphases were found or with poor-quality metaphases.

RESULTS

Among the 34 samples received, 4 (11,7%) were unsuitable for cytogenetic analysis because of a very low mitotic index or poor-quality metaphases obtained from the cell culture. We successfully analyzed the leukemic karyotype of 30 (88,3%) patients, and identified 12 cases with chromosomal abnormalities. The results were obtained from cultures with added growth factors, as described in the methods.

Cytogenetic Findings

Clonal cytogenetic abnormalities were detected in 12 (40%) patients, while 18 cases with CLL had a normal karyotype (60%). The chromosome aberration was considered as clonal, if there were found two or more cells with the same rearrangement or at least three cells with the same aneuploidity.

An abnormal clone carrying t(7q;14q) was detected in 1 patient with CLL (Figure 1). In this patient the translocation between chromosomes 7 and 14 t(7q;14q) was part of a complex karyotype and was associated with trisomy X and an extra chromosome marker (mar) (Figure 1).

Trisomy of chromosome 12 was found in two cases: 46,XY/47,XY+12. Trisomy 12 (see figure 2) is considered one of the most common clonal cytogenetic abnormalities in CLL (Figure 2).

Other cytogenetic findings were: tetraploidy 46,XX/92,XXXX or 46,XY/92,XXYY in two of our CLL patients and trisomy 21 karyotype:46,XY/ 47,XY,+21 in three cases.

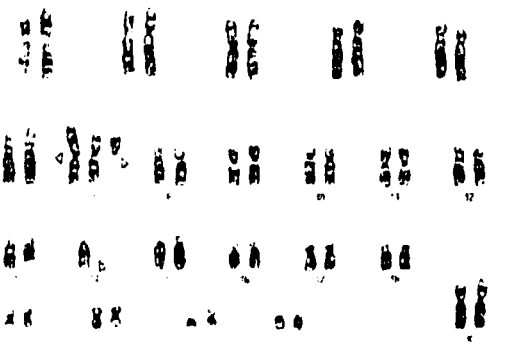

Figure 1. Karyotype 46,XX,t(7q;14q)

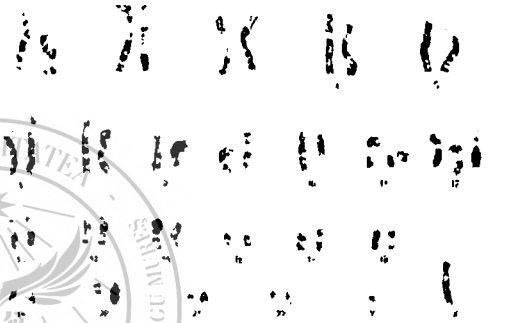

Figure 2. Karyotype 47,XX,+12

The structural rearrangements were found in 4 patients with CLL. The most important of them were: del(6p); del(11q) and t(7q;14q). The structural anomalies were associated with numerical chromosomal abnormalities in 2 cases.

Cytogenetic Risk Groups

According to Tefferi A. et al the major prognostic markers include: chromosomal abnormalities, presence (favorable) or absence of IgV_H somatic mutations, detected by polymerase chain reaction, and high levels of expression of CD38 and ZAP70.

We used the chromosomal abnormalities identified by conventional cytogenetic analysis for including our patients in different cytogenetic risk groups (Table II).

Table II. Cytogenetic Risk Groups

Risk Group	Cytogenetic abnormality	No. of cases
Favorable	Normal karyotype	18
	del(13q)	0
Intermediate	trisomy12	2
	del(6q)	0
Unfavorable	del(17p)	0
(adverse)	del(11q)	1
	translocation	1
unknown	Trisomy 21	3
	Trisomy 22	1
	hiperdiploidy	2
	del(6p)	1
	chromosomal heteromorphism	1

On the whole, our results demonstrate that the most frequent clonal karyotype alteration in CLL was aneuploidy, detected in 10 cases, while metaphases with structural anomalies were found in 4 cases.

DISCUSSION

Karyotyping or classical cytogenetic analysis is still the standard method for demonstrating chromosomal aberrations. As CLL lymphocytes show only very limited spontaneous growth, special B-cell mitogenic stimulation is necessary to drive the cells in the mitotic phase. For this purpose short-term cultivation of leukemic cells in the presence of B-cell mitogens from the peripheral blood is recommended.

Cultivation of bone marrow samples for this purpose promise only limited success, moreover, the many different cell types with spontaneous mitotic activity may significantly influence the results.

Karyotyping offers an overview of all existing aberrations within the same cells and also a clear comparison of the frequency of the changes, if the number of metaphases is satisfactory. With good practice a success rate of more than 70% can be achieved.

We successfully analyzed the leukemic karyotype of 30 (88,3%) patients, and identified 12 (40%) cases with chromosomal abnormalities. These findings are similar to the results obtained in other studies using a similar approach.

The structural rearrangements were found in 4 (13,3%) patients with CLL. The most important of them were: del(6p); del(11q) and t(7q;14q). The structural anomalies were associated with numerical chromosomal abnormalities in 2 cases. According to Méhes G. translocations usually accompany the progressive phase of the disease and seem to be more sporadic.[9] In our study translocation t(7q;14q) was part of a complex karyotype and was associated with trisomy X and an extra chromosome marker (mar).

We didn't find deletions at 13q even it is the most common genetic abnormality in CLL, being detectable in at least half of all cases.

Döhner H et al. reports that patients with 11q deletions had a tendency towards younger age, lower hemoglobin levels, and more advanced Rai stages.[4] We found in our work deletion of the long arm of chromosome 11 del(11q) in one male patient with CLL stage Binet C. According to Tefferi et al. 11q- is associated with a poor prognosis, so we include our patient in the unfavorable cytogenetic risk groups.

In our study the most frequently observed chromosome abnormalities are numerical aberrations, mainly +21, +12 and +X. Structural changes are observed less frequently, out of which deletions chromosomes 6 or 11 are more commonly seen.

An abnormal karyotype is observed in almost 40% of patients with chronic lymphocytic leukemia (CLL) by standard cytogenetic analyses. Chromosomal aberrations can be detected in almost 80% of cases of CLL by use of interphase fluorescence in situ hybridization (FISH). The application of fluorescence in situ hybridization with chromosome-specific DNA probes helps to further define molecular subclasses and cytogenetic risk categories for patients with this disorder. Moreover, FISH permits analysis of proliferating (metaphase cells) and non-proliferating (interphase nuclei) cells. This probe panel only detects prognostically important imbalances (gains or loss of DNA) in the chromosomes of interest. Chromosome alterations outside the regions complementary to these FISH probes will not be detected.

Classical and molecular cytogenetic analysis became an important tool for individual risk estimation. Unlike any other approaches, cytogenetic monitoring reflects the genetic heterogeneity and clonal growth dynamics during the course of the disease.

CONCLUSIONS

Our findings demonstrate that cytogenetic study may be of value in the clinical and prognostic evaluation of patients with chronic lymphocytic leukemia.

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The first descriptions of atherosclerosis in the records of the Institute of Pathological Anatomy from Cluj at the end of the 19th century

Szilágyi Marietta

Modern pathology took birth in the second half of the 19th century, and had representatives like Cruvielhier, Rokitansky, Virchow, Cohnheim and others. The new concept, the cellular pathology, was immediately accepted and developed in all famous European schools of pathology.

In this work we present in parallel the scientific concepts of the school of pathological anatomy from Cluj and the anatomo-pathologic diagnoses regarding the cardiovascular diseases at that time. There were studied the records of necropsy protocols from the Library of the Institute of Pathological Anatomy at Cluj from the period 1871-1895, scientific publications from outstanding contemporary medical journals and books of pathological anatomy published at the end of the 19th century

Our results show that cardiovascular diseases represented an important cause of death, which gained attention after the years 1870 when it's scientific investigation commenced

Key words atherosclerosis history of medicine, history of pathology, Cluj

Modern pathology took birth in the second half of the 19th century, and had representatives like Cruvielhier, Rokitansky, Virchow, Cohnheim and others [4]. The new concept, the cellular pathology, was immediately accepted and developed in all famous European schools of pathology. Founders of the Romanian schools of pathological anatomy were students of reputed teachers from Western Europe. In Cluj the Institute of Pathological Anatomy was founded by Genersich Antal.

In a previous study we performed a general investigation of this start-up period and the first 25 years of activity of the Institute (1871-1895). The analysis of the main illnesses causing death showed that at the first place was tuberculosis, followed by acute respiratory diseases and malignant tumors. On the fourth place were cardiovascular diseases.

In the present work we undertook a detailed analysis of this latter category of diseases by studying records of protocols kept in the Library of the Institute of Pathological Anatomy at Cluj, and the scientific publications of doctors of pathological anatomy of those times.

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Cardiovascular diseases represented 6.0 percent of total causes of death. In Table I are presented the cardiovascular diseases which have meant cause of death.

Table I. Cardiovascular diseases causing death in the studied anatomo-pathological records

Cause of death	Nr	%
Endocarditis	59	29.2
Sudden death	58	28.7
Heart failure	48	23.7
Sudden death of neonates and infants	19	9.4
Endarteritis (atherosclerosis)	9	4.5
Pericarditis	5	2.5
Myocarditis	4	2.0

The sudden death of infants I have classified in a separate category. But often nothing pathological was found at the necropsy. In such cases we referred to the clinical diagnosis, which in the modern terminology would be cardiorespiratory failure or respiratory distress syndrome of newborn.

Excluding this category of age, we found that majority of cases were subacute and chronic illnesses.

Most often were found cases of endocarditis. Most frequently we could find the anatomo-pathological description of endocarditis characteristic for rheumatic

fever. These cases were described as verrucous endocarditis, with fibrin deposits on the surface of valves and valvular lesions. Many times appears the diagnosis of acute endocarditis, with typical signs of infectious endocarditis, with large fibrin deposits, ulcerous injuries of the endocardium and valvular rupture. Heart failure could not always be strictly separated from chronic endocaditis with valvular injuries, the latter being the cause of the heart failure.

In the sudden death category I included: thrombembolia of large vessels (14 cases), pulmonary embolism (13 cases), thrombosis of the coronary arteries (11 cases), rupture of aortic aneurysm (9 cases), cases under the name of "cardiac paralysis " (7 cases), rupture of the myocardium or pericardium (4 cases).

I did not find the diagnosis of acute myocardial infarction in protocols studied. Occlusion (thrombosis) of coronary arteries, which causes heart attack, was found and described, but only in very rare cases. The reason could be, that very few patients have reached the hospital with symptoms of heart attack or angina pectoris, due to the lack of emergency services, and because of the lack of clinical experience to diagnose and treat this disease. Therefore, patients had not died in hospitals and did not get to be studied in anatomo-pathologic terms. The second cause might be that the disease was not so frequent. The way how myocardial damage was formulated as a consequence of thrombosis of coronary arteries has changed even during the studied period. In two protocols from 1871 we found the following diagnosis: "degeneratio adiposa cordis in sequelam obstructio arteriae coronariae dextra" and "degeneratio adiposa cordis in sequelam obturatoriam arteriae coronariae cordis posterior".

In 1883 are registered two cases, then in 1887 an other case of: "endocarditis chronica deformans, obliteratio arteriae coronariae cordis dextri et trombosis arteriae sinistrae rami superioris, paralysis cordis". The description of altered coronary arteries, „endarteritis", as a cause of coronary stenosis which could lead to sudden death ("cardiac paralysis") first appears in 1889, described as: "paralysis cordis, endarteritis arteriae coronariae cum stenosi ramuri sinistri".

Atherosclerosis as determining factor in the pathology of cardiovascular diseases, although very little was known about it, was already investigated, and explained by various theories, which in the years 1880–1890 started to reach the center of attention. In a series of articles published in 1896 in the journal Orvosi Hetilap (Weekly Medical Bulletin), dr. Ritoók Zsigmond is summarizing the theories known in that era about atherosclerosis.

"Atherosclerosis, which is one of the most frequent ailments, but is also physiological after a certain age, is not yet known and studied enough today, neither regarding it's etiology and pathological-anatomy, nor concerning its consequences. There are, however, several theories explaining them." From this source we can find that Marchand considered that the process of atherosclerosis begins with swelling, growing and hardening of the intima, which can lead to necrosis and partial calcification or continuous and extended sclerosis. This theory, which excludes the inflammatory processes from the etiology was accepted by most French pathologists. At that time it was uncertain whether atherosclerosis is an inflammatory condition or a degenerative process. Atheromatosis has been generally accepted as a part of the entire process.3 Martin argued that this is an obliterative inflammation of vasa vasorum, Virchow and Marchand denied the possibility of acute endarteritis, and Landouzy, on the contrary, proved it, presenting clinical cases. It was accepted Fraenkel's theory too, according to which arterial wall damage is preceded by illnesses that increase the work of the heart, or arterial resistance, consequently lead to increased systemic or local blood pressure[3]. Fraenkel, Bollinger, Marchand and Houchard agreed unanimously that the primary factor is the increased blood pressure and atherosclerosis is the consequence of this and not vice versa.[2]

It was accepted by all the classification of Houchard regarding the etiology of atherosclerosis.[5] According to this, there are:

I. Diathetic factors: 1. inherited; 2. gout, diabetes; 3. chronic rheumatism

II. Mechanistic factors

1. Toxic: alcohol, tobacco, chronic lead intoxication (saturnism), chronic dietary disorders, overweight, physical exhaustion, ageing, infections (acute infections and syphilis, malaria, tuberculosis)

2. Neurological factors without toxic effects: neuralgia, intellectual extenuation.

In Ritoók's review is ascertained that contemporary clinical statistics shows that atherosclerosis was found more often at men (70-71%), at women being far more rare. This was explained by increased exposure of men to the harmful effects of toxic substances.

"Beside atherosclerosis we find the illness of more internal organs, what is natural in view of diffuse nature of the disease, as well as the function of arteries to supply blood to the organs. I have found very rarely atherosclerosis alone, without organic illnesses.

There is of particular importance Martin's article, published in 1881 and of Duplaix from 1885, which emphasizes the pathological changes of heart, liver, spleen, kidneys and central nervous system, appearing together with arteritis. Both authors have found hypertrophy of connective tissue in the affected organs, which has developed due to an arteritis, which can be considered as starting point. This phenomenon has been presented with anatomo-pathological and histopathological preparations by Duplaix. Hypertrophy reaches parenchyma only if sclerosis of the connective tissue is accentuated. Hypertrophy is not an inflammatory process ... "

It was considered that heart diseases could be divided into three classes: valvular, vascular and arterial.

The valvular ones were of rheumatic etiology, the vascular disorders of diathetic origin, and the arterials referred to diseases of the myocardium or the valves of the left ventricle. In this category was included the formation of aortic aneurysms too, considering that this is of endarteritic etiology.

"Vascular type heart diseases, especially the myocardic subtype, known under the denomination of cardiosclerosis, were analyzed in a study of Huchard. He considers, together with other authors such as Martin and Duplaix, that the process of cardiosclerosis starts around an artery with irregular wall and narrowed lumen due to endarteritis obliterans, which is often associated with periarteritis. This is in the center of islands of sclerotic connective tissue, with irregular edges, diffuse, and between fasciculi of connective tissue are caught the fibers (cells) of the myocardium which suffered a granular and fatty degeneration."

Ritoók's article enters into details regarding the organs affected by arteriosclerosis, studying in turn the central nervous system, kidney, spleen and others.

From this series of articles we can find out what was known at that time about atherosclerosis and what kind of consequences were assigned to it.

If we try to track this topic in the necropsy protocols, even if we have relatively few cases, we can rebuild a contemporary conception about this disease, which in our times has become so frequent and important.

As pathological anatomic diagnosis, and causing death, endarteritis appears already starting in 1876, most of the times referring to the big arteries. After 1880 we find more frequently the diagnosis of endarteritis accompanied by sclerosis. For example, it appears in a very precise description of coronary sclerosis, in 1882, at man died at age 66. "Endarteritis chronica , sclerosis et ulcerationis atheromatosus cum stenosi arteriarum coronaria cordis".

In 1890 there were described the coronary sclerosis, hypertrophy and trophic disorders of the myocardium at a man who died at age 51: "Endarteritis deformans cronica aortae et arteriae coronariae cordis, degeneratio adiposa cordis, hipertofia cordis part sinistra, inclusio totalis arteria coronaria dextra", which had led to myocardial infarction.

Signs suggesting heart failure appear described in a protocol of 1891, at a man aged 66: "Hipertrofia cordis, ventriculi sinistri, degeneratio adiposa cordis, hydrops universalis, anasarca oedema, ascites".

In 1893, at a man died at the age of 52 years we find beside endarteritis, the diagnosis of atherosclerosis, under the denomination of atheromatous process: "Endarteritis chronica deformans (processus atheromatosus) majoris gradis, myocarditis chronica ventriculi cordis, hypertrofia cordis sinistri"

In conclusion, we can state that cardiovascular diseases represented a substantial category among causes of death in the 19th century too. Out of all etiologic factors of cardiopathies, on the first place were those infectious, causing illnesses that nowadays, due to antibiotherapy, appear very rarely.

But noninfectious pathologic modifications have been studied also in the past, as were other risk factors. But the underlying pathopysiological processes being largely unknown, it was granted no special attention to it, especially if the patient was clinically asymptomatic.

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The effect of the homeopathic treatment with Silicea CH 7 upon the evacuation of foreign bodies in rats

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The aim of the study was to asses the effect of the homeopathic treatment with Silicea CH 7 versus placebo upon the evacuation of foreign bodies. We studied 2 groups of male rats in which implanted by surgery a glass ball under the cervical skin. One group was treated with placebo and the other got homeopathic Silicea CH 7, by oral means daily. Some of the rats treated with Silicea CH 7 showed a delimitation of the ball under the skin, then a fistula appeared at that level, by which the glass ball was spontaneously evacuated. None of the rats from the placebo group presented the delimitation or expulsion of the glass ball. The statistics show the significant difference of reaction under Silicea CH 7 treatment versus placebo.

Key words: homeopathy, Silicea, foreign bodies, rats

Established since over 200 years, presented for the first time by the german physician Samuel Hahnemann, homeopathy is an entire medical system, based on the laws of similarity, dilution and dinamization of the substance.

It was experimentally observed, even at the time of Hipocrates, that a substance that can produce a symptom can cure that symptom if given in a diluted manner. Hahnemann perfected the system and introduced the centesimal dilution scale (CH - centesimal, hahnemannian), which means that the medicines are diluted successively in steps of 1/100, each step being followed by a vigorous shaking of the solution, called dinamization.

It is believed that the dinamization produces molecular collision, which leads to the transfer of the information from the substance to the solvent, by means of quantum physic processes incompletely elucidated at the moment. It was demonstrated that in the shaken solutions, certain forms of water aggregates appear, and the three-dimensional form of this aggregates, called clathrates, seem to be specific to the dissolved substance.

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The exact mechanism of action of the homeopathic dillutions is not clear yet. It is possible that the clathrates are somehow recognized by the living organisms, maybe at the level of membrane receptors, and they may be able to produce cellular reactions.[1,2,4,7]

Homeopathic anecdotic experience reports cases of fistulizaton of glass foreign bodies under treatment with the homeopathic medicine Silicea.[3,6] The medicine is prepared from flint through successive triturations in lactose, then centesimal dilutions, the resulting solution being impregnated in sucrose pellets. The process is standardised at industrial level and the medicine is officially registered and in current homeopathic use.

We found no scientific and statistic evidence of the mentioned effect of homeopathic Silicea, and from this fact aroused the idea of the present study.

The aim of the study was to asses the effect of the homeopathic treatment with Silicea CH 7 versus placebo upon the evacuation of foreign bodies in rats.

MATERIAL AND METHOD

We studied 18 male Wistar rats from the Târgu-Mureș University of Medicine and Pharmacy experimental station, with weights between 250-388g and age over 6 month.

The rats were randomized in two groups of 9 animals, group 1 - control-placebo, group 2 - treated with homeopathic Silicea CH 7.

The rats were put to sleep with ether and then the depilation and asepsis of the cervical zone, selected for this study, was performed.

After the incision we inserted a 5 mm glass ball under the skin and the suture was made using non-resorbable wire (Figure 1).

Figure 1. Glass ball inserted under the skin through surgical incision, suture with non-resorbable wire

The medication was given orally immediately after the animals were awake and then each morning, by means of a plastic syringe.

The preparation was made by dissolving a 4 mm homeopathic pellet in 1 ml water. For the placebo group we dissolved placebo pellets (sucrose) and for group 2 we used homeopathic Sillicea CH 7 pellets, factory produced, taken from pharmacy.

The dose for each rat was 0,5 ml from the solution each day.

RESULTS

The sutures healed on both groups. In group 1 (placebo) the glass balls remained mobile under the skin, with no local manifestations (Figure 2).

Figure 2. Glass ball is not delimited in placebo group

In group 2 (Silicea), 7 of 9 rats (77,7%) presented after day 7, the delimitation and the loss of mobility of the glass ball under the skin (Figure 3), followed in 5 of 9 rats (55,5%) by a necrosis of the skin over the glass ball (Figure 4) and the appearance of a fistula (Figure 5), by which the balls were spontaneously evacuated (Figure 6, Figure 7). None of the rats presented suppuration or other complications.

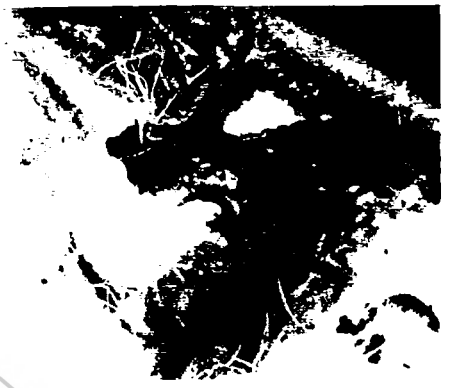

Figure 3. Glass ball is well delimited in Silicea CH 7 group

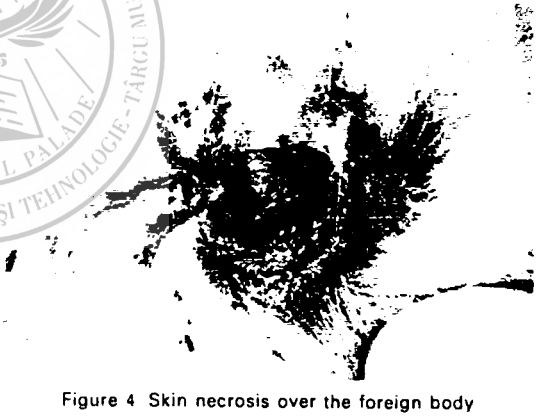

Figure 4. Skin necrosis over the foreign body

Figure 5. Glass ball is visible through the skin fissure

Figure 6. Glass ball was spontaneously evacuated and thus is not visible anymore

Figure 7. Suture wires were also evacuated and the skin is healing

All procedures were performed with respect to the European Convention for the Protection of Vertebrate Animals.[5] Before starting any procedure we solicited and obtained the written agreement of the Ethical Committee of the University of Medicine and Pharmacy of Târgu-Mureș.

Statistics were performed using GraphPad InStat and Chi square test, which showed that the difference between the two groups concerning the fistulization of the glass spheres was statistically significant, $p < 0,05$ (Figure 8).

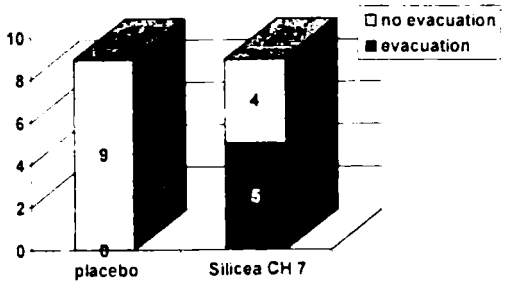

Figure 8. Number of rats with and without spontaneous evacuation of the glass ball

DISCUSSIONS

The homeopathic medicine Silicea CH 7 proved to be more efficient than placebo in producing the spontaneous evacuation of glass foreign bodies inserted experimentally under the skin in Wistar rats.

As the cases of fistulization under homeopathic Silicea treatment reported in the literature are not statistically evaluated, we could not compare our results with previous results.[6]

Other research done on laboratory animals sustain the statement that homeopathy is acting through certain mechanisms, which are different from placebo.[7]

CONCLUSIONS

Further research is needed on this subject; the potential benefit can be a clinical one. The medicine is actually in the current homeopathic use for multiple illnesses like abscesses, sinusitis, tonsillitis, chronic fatigue syndrome and many others, even for the expulsion of foreign bodies, either alone or in different commercial homeopathic combinations. But as this latter effect is statistically proven then in the presence of electronic pacemakers, hearing devices or different other surgical implants that must remain in place the contraindication of this medicine could be mandatory.

From other point of view, then medicine could be safely used for the intended expulsion of accidental foreign bodies under the skin.

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Evaluation of the risk factors in infective endocarditis by the experimental disease

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Infectious endocarditis (IE) is a microbial infection of the endocardium surface, in which despite the improvements in diagnostic methods, medical and surgical therapy, mortality remains high. Experimental models can simulate the human disease and provides data that permit statistical analysis, which can assess the degree of pathogenicity of the etiological agents: the number of bacteria/gram of tissue, the blood culture positivation rate, death rate after inoculation, etc. Although by experimental disease, the endocarditis is artificially induced, this system is the best in vivo model to determine the degree of colonization and efficacy of antibacterial agents in the treatment of these problematic infections. Experimental, mechanical lesion of the heart valves, required for the vegetation development, was achieved by placing a polyurethane catheter in the heart through the carotid artery. The inoculation of catheterized animal induced the IE; the most massive vegetation colonization was caused by Staphylococcus aureus. The inoculated but uncatheterized animals have not developed any endocardial injuries, the blood cultures being negative seven days after inoculation. Bacterial isolates from patients with IE are more pathogenic than the reference strains or those isolated from the community.

Key words: endocarditis, experimental disease, vegetation, pathogenicity, colonization

Infectious endocarditis (IE) is defined as a microbial infection of the endocardium. Despite medical progress, the incidence of infectious endocarditis has not changed in the last two decades. This can be explained by the gradual evolution of risk factors (such as intravenous drug use, prosthetic valves, or nosocomial infections with multi-resistant germs). In addition, many etiological agents are uncharacteristic ones, difficult to identify.[1]

The development of IE is closely related to the existence of concomitant valvular lesions and bacteremia. The disease pathogenesis involves 3 steps:

-mechanical or inflammatory heart valve injuries

-adhesion of bacteria to damaged valves (accomplished in a few minutes during a transitional bacteremia)

-bacterial multiplication with local or remote spreading, finally leading to tissue destruction[2,5]

The microbial etiology of the IE depends in its location (on native or prosthetic valve), but also in its nosocomial context. Staphylococcus aureus, Streptococcus spp. and Enterococcus spp. are responsible for over 80% of IE.[4] These microorganisms present surface adhesins that mediates their attachment to the vegetation. Since the first report of infectious endocarditis, there has been conducted various experimental models on various animal species (dogs, horses, pigs, opossum, rabbits and mice[3]). From these models, the most suitable are rabbits and mice; the induced disease is extremely similar to that in humans.[6]

The advantage of the experimental model is that beside the fact that it simulates the human disease, it provides data allowing statistical analysis: the number of bacteria / gram of tissue, the frequency of emergence of antibiotics resistance, the blood culture positivation rate, death rate after inoculation and therapy, etc. Although the disease is experimental endocarditis induced artificially, this system is the best in vivo model to determine the degree of colonization and efficacy of antibacterial agents to treat such problematic infections.

Working assumptions. Heart valves irritation (mechanical or due to marked pathogenicity of infectious agents) is a trigger factor in the pathogenesis of infectious endocarditis; bacterial strains isolated from nosocomial background present increased virulence.

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Purpose. The evaluation of the pathogenicity of the main bacterial species isolated from nosocomial and community environment, as well as the mechanical and inflammatory risk factors in the pathogenesis of IE, by using the experimental disease.

MATERIALS AND METHODS

Experimental model chosen for this study was the New Zealand rabbit. In the study we used two working groups: a first group of rabbits with induced mechanical valvular lesions, and a second one without valvular lesions. Each group was divided into two subgroups in which bacteremia was simulated by intravenous inoculation of different bacterial strains; first subgroup of rabbits were inoculated with bacterial strains isolated from patients with infectious endocarditis, or with strains with increased virulence that can induce lesions without a substrate by mechanical injury; the rabbits in the second subgroup were inoculated with bacterial strains isolated from the community, or with reference strains with known potential for causing infective endocarditis.

Induction of cardiac lesions in rabbits required surgical intervention, consisting in placing a polyurethane catheter in the left side of the heart, through the right carotid artery. Thus we used the following materials:

-Male New Zealand rabbits, 2000 - 4000 grams weight

-20G arterial catheter (0.6 mm internal diameter, 0.9 mm external diameter), 80 mm length

-Anesthetic, antiseptic, syringes and needles, scalpel blades, electric clippers, surgical and anatomical pliers, dissection pliers, scissors, suture material, 3/0 silk thread, sterile compresses.

Anesthesia and preparation of operating field. General anesthesia was achieved by intramuscular injection of Xylazin (5 mg/kg) and ketamine hydrochloride (35 mg/kg). Anaesthetized rabbit is maintained in dorsal position with the help of cords fixing the animal's paws to the surgical table. The operating field is prepared through a rigorous washing, the most difficult step being the removal of the fur. This has been initially done with the help of scissors and sterile blades, but the small skin lesions created in this process could have interfered with the study's results; to avoid this, we used depilatory cream for complete removal of the fur, which also has an antiseptic and astringent effect. Tegument was subsequently disinfected with iodine solution (Betadine). To reduce intra- and postoperative trauma, we also used an additional local anesthesia, achieved by subcutaneous injection of lidocaine.

Preparation of the artery and catheter insertion. A 3—5 cm longitudinal incision was made along the right side of the trachea. The right carotid artery was exposed through the muscle groups of the neck. The artery was properly prepared, requiring the complete removal of perivascular connective tissue, without affecting the vagus nerve and the adjacent nerve threads. At the distal end of the prepared artery, a 3/0 silk thread was mounted and tightened in order to prevent blood ebbing from the brain. Another thread was introduced at the proximal end of the artery, but not yet tightened. Between the two threads, the arterial wall was punctured with a needle, and the polyurethane catheter was inserted into arterial lumen with the help of its guide. Catheter was then carefully inserted further into the heart until resistance was met; characteristic pulsations of the catheter are detected when the catheter is in the heart. From this point, the catheter must be withdrawn about 3-5 mm, so as its peak to position next to the semilunar cusps of the aortic valves. The catheter was then tied upon itself with the previously mounted thread. The catheter insertion and its ligature must be made quickly in order to minimize blood loss. During the artery preparation, catheter insertion and ligature mounting, a constant damping with sterile saline solution is required in order to prevent tissue destruction. After checking all ligatures, the loose end of the catheter was sectioned with a fine scissors as close as possible to the place of insertion. The operating field was checked for any bleeding, and the skin incision was closed with "U" mounted threads. The catheter was left in place about a week to produce endocardial lesions.

Animal inoculation. The bacterial suspensions used for the rabbits' inoculation during the study were prepared from 6 different bacterial species:

-Staphylococcus aureus – reference strain ATTC 29213

-Staphylococcus aureus – clinical isolate from a patient with IE

-Staphylococcus aureus – Panton-Valentine leukocidin producing strain (PVL +)

-Enterococcus faecalis – reference strain ATCC 29212

-Enterococcus faecium – clinical isolate from a patient with IE

-Granulicatella adiacens (nutritionally deficient streptococcus) – clinical isolate from a patient with IE

Bacterial suspension was made in saline solution and adjusted to 0.5 McFarland. After shaving the external part of the ear, both vasodilatation and disinfection was produced by applying ethyl alcohol. Using a syringe with fine needle, the animals were inoculated with 0.05 ml/kg of bacterial suspension into the main ear vein.

Animal sacrifice and the evaluation of the inoculated germs pathogenicity. Animals which didn't die spontaneously after infection were sacrificed by diethyl ether inhalation. The thoracic chest was opened following the parasternal line, exposing the heart. The arteries and veins adjacent to the heart were ligatured and then sectioned. The collected heart was opened by incision along the aorta, looking for the presence of endocardial vegetations. If present, these were excised using a fine sterile pliers and scalpel. The excised vegetations were weighed using an analytical

Table Numbers of CFU/g of vegetation

Bacterial species	Incubated days	Status	Rabbit nr.	Vegetation weight	Dilution factor	Colony nr.	CFU/g
Staphylococcus aureus ATCC 29213	3	deceased	3	5,6	-	8000	142.857
Staphylococcus aureus from IE	-	deceased	-	-	256	306	14.140.072
Staphylococcus aureus PVL+	3	deceased	5	39,8	8192	1142	1.175.284.422
Enterococcus faecalis ATCC 29212	9	sacrificed	3	35	8	400	457.143
Granulicatella adiacens from IE	7	sacrificed	0	-	-	-	-

balance and homogenized by vortexing in 1 ml sterile saline solution in the presence of sterile glass balls. After homogenized, it was serially diluted. From each dilution, 200 µl was cultured on appropriate agar plates. The plates were incubated for 24 hours, after which the bacterial colonies were counted using the "IUL Flash & Go" automatic colony counter within Microbiology Department. Then the dilution, weight and volume adjustments were calculated, thus achieving the colonization degree of vegetation (CFU/g - the number of colony-forming units/gram of cardiac vegetation).

RESULTS

The rabbits with endocardial lesions induced by catheterization developed cardiac vegetations.

Numbers of CFU/g of vegetation are presented in table I.

Nutritionally deficient streptococci caused minimal endocardial damage, without vegetation development; in this case, the animal's blood culture was negative at the sacrifice time.

Rabbits inoculated with bacterial suspension but not being previously catheterized, did not develop any symptoms of IE and their blood cultures were negative. In these cases, the autopsy didn't reveal any endocardial vegetation.

DISCUSSIONS AND CONCLUSIONS

The endocardial lesion is the main risk factor for developing infectious endocarditis. Preexisting endocardial damage leads to accumulationm of platelets and fibrin, producing non-bacterial thrombotic endocarditis; this sterile lesion serves as an ideal site to trap bacteria as they pass through the blood stream.[6]

In the study of Garrison et al., they concluded that the polyethylene catheters with their tips at the entrance to or within the right side of the heart produce sterile endocarditis and tricuspid vavulitis; introducing as few as 10^2 microorganisms within the catheter predictably produces staphylococcal endocarditis. This model is suitable for the study of the bacteriologic, pathologic and imunologic aspectes of bacterial endocarditis and reproduces some of the complications indwelling venous catheterization that have been observed in human.[4]

Our study concluded that *Staphylococcus aureus* is one of the most pathogenic bacterial species, producing massive colonization of endocardial vegetations, in relatively short time leading to septic shock and death.

Among the strains of tested staphylococci, those coming from hospitalized patients with IE and those with present pathogenic factors, caused a more intense colonization compared with reference strains. Such a case is that of Panton-Valentine Leukocidin-producing *Styaphylococcus aureus* (PVL+); the PVL gene is frequently detected in community acquired MRSA (CA-MRSA), through this strain is rarely found in non-cutaneous infections (including IE).

In the study of Huang et al., PVL genes were detected in 50% of their CA-MRSA isolates. The detection of PVL gene in the CA-MRSA isolate with IE may be due to a clonal spread of PVL genes between community and hospital. The clinical significance of PVL gene in IE needs further study. They concluded that the PVL gene-positive CA-MRSA should be considered as a potential pathogen in IE.[7]

It is still not certain why the staphylococci isolated from patients with IE are able to produce a greater colonization compared with the reference strains; this requires further investigations such as the identification of virulence factors or the growth rate of this bacteria.

E. faecalis is responsible for most cases of enterococcal IE. Although *E. faecium* is isolated in some cases, including patients with hospital acquired IE, an association between bacteremia with this species and its involvement in IE could not be made. This is important because the incidence of *E. faecium* bacteraemic syndromes is increased, and in some centers they became the main isolated species of enterococci.[1]

On undamaged endocardium, the enterococci have a lower virulence than other germs, such as *S. aureus*, mortality of these patients being much reduced.[8] Our study has identified a lower colonization of vegetations with *E. faecalis* than with *S. aureus*; also, the rabbit survived for 9 days of inoculation.

During the experiments, we also managed to establish a working experimental model which can be successfully used in further studies regarding the pathogenicity of bacterial strains, pathogenetic mechanisms of IE, risk factors in IE, and antibiotic

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The importance of stress test in assessing patients with new onset angina

Voichita Sîrbu, C. Macarie, Adriana Mitre, I Vița, Mihaela Opriș

As first appearance of myocardial ischemia, new onset angina has major implications in patient's outcome and life expectancy Aim. Establishing the importance of stress test in patients with new onset angina, based on correlations between stress test results and coronarographic findings. Method and material. Retrospective and prospective study including 299 patients admitted between 01.01.2007 and 31.12.2008 for new onset angina, with performed angiocoronarography. Sensitivity of stress test results in detecting coronary artery disease was followed. Results. The stress test was more frequently positive in patients over 51 years of old and in those with more than one risk factor. Although sensitivity of stress test in patients with coronary atherosclerosis was comparable to that found in metaanalyses, we weren't able to establish a statistically significant correlation between stress test and coronary atherosclerosis, even in patients with significant coronary stenosis. Discussions, conclusions. In an important number of patients with significant coronary stenosis (17-25%), the stress test was false negative. More precise noninvasive diagnostic methods to exclude coronary artery disease are required.
Key words new onset angina, stress test, coronaro-graphy

Recent onset chest pain (under one month) suspected to be angina, requires thorough investigations in order to establish optimal therapeutic approach. In some cases, present EKG changes facilitate the diagnosis, but in most cases, especially in patients with suspected class I and II angina according to the Guidelines of Canadian Society of Cardiology, stress test are necessary to confirm the diagnosis.[1,5,8]

EKG stress test was initially used to highlight ST segment changes induced by myocardial ischemia. Although, in the last decade, new diagnostic methods that theoretically enhance accuracy have been developed, data provided by stress tests, by using individualized protocols, prediction equations, blood gas exchange analysis and carefully assessment of recovery period, significantly increased the value of stress tests.[9]

The stress test provides especially clinical data (occurrence of angina, timing of angina, symptoms limiting the effort, intensity of angina being assessed on a 4 grade scale, KATTUS scale), haemodynamic data (heart rate, blood pressure, double product = heart rate x systolic blood pressure, representing oxygen myocardial consumption) and electrocardiographic data (induced ST segment displacement, elevation or depression, ST slope type, number of leads in which they occur, persistence of changes during the recovery time, ventricular arrhythmias).

Sensitivity of stress tests is defined by the probability of a positive test occurring in patients having coronary artery disease - truly positive test.[2,3,4] In a metaanalysis including 147 studies and 24047 patients, sensitivity was estimated at 68% and specificity at 77%, with a predictive accuracy of 73%, growing at 92% by using prediction scores (compared to CT exam which had a prediction accuracy of 61% and stress echo exam of 80%).

Aim. Establishing the importance of stress test in patients with new onset angina, based on correlations between stress test results and coronarographic findings.

MATERIALS AND METHODS

This is a retrospective and prospective study performed between 01.01.2007 and 31.12.2008 in the Cardiology Clinic of The Institute of Cardiovascular Diseases and Heart Transplant in Tg.Mures, Romania. 299 patients admitted with new onset angina and coronarography done were included. Patients with known previous coronary events, those with valvular disease, cardiomyopathy or heart failure were excluded.

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Outcome and sensitivity of stress test in detecting coronary artery disease were followed up. Clinical symptoms, the presence of cardiovascular risk factors, stress test outcome and coronarographic findings were used as references. For statistical analysis we used CHI test, Student test and Fisher's Exact test.

RESULTS

Out of the 299 patients with new onset angina, 139 patients performed an exercise test. In 100 cases the stress test was positive, representing 71,94%. Most of the patients with positive stress test were over 51 years of age, and all patients over 70 had a positive stress test (Fig.1).

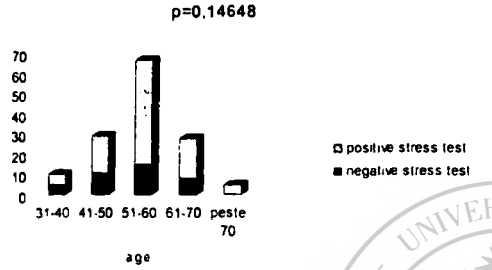

Figure 1. Relationship between age and positive stress test

No statistically significant correlation between patient's sex and positive stress test was found.

The stess test was also positive in patients with more than one cardiovascular risk factor, though we were not able to establish a statistical correlation between the number of risk factors and positive stress test (Fig.2).

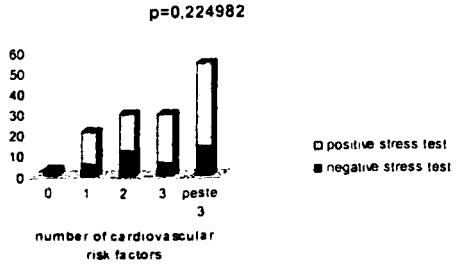

Figure 2. Relationship between cardiovascular risk factors and positive stress test

Coronarographic exam has been performed in all patients, afterwards two groups being formed:

A: patients with atherosclerotic coronary lesions, with significant coronary stenosis (161 patients) and nonsignificant stenosis (41 patients).

B: patients with nonatherosclerotic lesions: muscular bridge (39 patients), micovascular disease (53 patients), and normal coronaries (5 patients).

Stress test sensitivity was estimated (Table I).

Table I. Sensitivity of stress test in patients with new onset angina

Type of coronary lesions	Stress test sensitivity
Coronary atherosclerosis	75%
Significant coronary stenosis	80,70%
Left main stenosis	75%
LAD stenosis	83,33%
RCA stenosis	81,81%
LCx stenosis	76,47%
Insignificant coronary stenosis	65%
Nonatherosclerotic lesions	67,21%
Muscular bridge	55,55%
Microvascular disease	78,12%

Although stress test sensitivity is higher in patients with atherosclerotic lesions than in those with nonatherosclerotic disease, we could not find a statistically significant correlation between stress test outcome and coronary atherosclerosis. (p= 0, 322612) (Fig. 3).

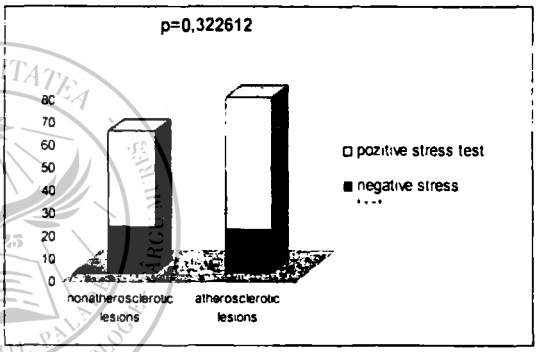

Figure 3. Relationship between stress test and coronary atherosclerosis

There is a positive significant relation between important left anterior descending artery stenosis and positive stress test, this one having the highest sensitivity (83,33%, p=0,066766).

The lowest sensitivity was observed in patients with muscular bridge on left anterior descending artery, which correlates with a negative stress test. (55,55%, p= 0, 034743).

CONCLUSIONS AND DISCUSSIONS

Exercise test is a widely used investigation method, that can be performed in an office (72% of tests in USA); it is cheaper compared to other methods, (echo stress is 2,1 times, SPECT is 5,7 times and coronarography is 21,7 times more expansive in the USA), and safe (mortality less than 1: 50000).[10]

In American Heart Journal were recently published the results of a clinical study made in England by Sekhri et al.[6] who assessed the prognostic value of EKG exercise test in patients with suspected new onset angina. 8176 patients were included, in 4848 of them a stress test was performed, all patients being followed for 6 years, the primary end point being

mortality caused by coronary artery disease and acute coronary syndrome. They found out that clinical evaluation (the presence of risk factors and typical chest pain) correlates with primary end point, with an additional minimal increase of prognostic value through resting and exercise EKG. They also ascertained that 47% of the patients with negative stress test, have had later coronary events. These results require the development of new techniques capable of higher diagnostic precision.

-Stress tests are a useful and available method in assessing patients with new onset angina.

-The sensitivity of stress tests in patients with coronary atherosclerosis is comparable with that expressed by recent metaanalysis.

-False negative stress test was observed in an important number of patients with coronary atherosclerosis and significant stenotic lesions (between 17 and 25%).

-New noninvasive diagnostic methods to rule out coronary artery disease are required.

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The role of noninvasive tests of hepatic lesions in chronic hepatitis B

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The chronic hepatitis with HBV represents an important issue for public health, as the chronic infection with VHB affects approximately 400 million people in the world and more than 1 million people in Romania. The hepatic fibrosis is a progressive constituent of the disease, so that the hepatic lesions obtain a dynamic perspective.
FibroScan and FibroMax are two hepatic non-invasive tests, easy to be reproduced, which distinguish hepatic injuries (fibrosis).
Our study included a group of 380 patients with chronic infection with HBV, AgHBs+,and it was studied the role of the two corelating tests in staging disease, establishing the moment when the treatment is initiated, the imagistic and injury monitory, the precocious intervention on hepatic nodules. The easier diagnose of fibrosis brings better knowledge of the liver disease and a better monitory of the evolution and therapeutic conduct.
Key words hepatic fibrosis, chronic hepatitis with B virus, serical markers, liver biopsy

Chronic infection with hepatitis B virus (HBV) represents an important issue for public health, the chronic infection with HBV affects over 400 million people in the world and more than 1 million people in Romania.[4]

The hepatic fibrosis is a progressive constituent of the disease, so that the liver lesions obtain a dynamic perspective. Since the liver biopsy distinguishes a moment in the evolution of hepatic injuries and because it can be hardly reproduced, the introduction of non-invasive hepatic tests was necessary in order to estimate the injuries (liver fibrosis), such tests being represented by elastometry (FibroScan) and FibroTest/ FibroMAX.[3,4]

FibroScan - measures the rigidity of the liver, analyzing the propagation speed of a wave produced by a mechanical impulse. The scissoring wave produces an echo in each tissue that is captured and gives back the progression speed in that particular fragment of tissue. The standard analyzed fragment is 2,5cm. Validation of the measurement is done automatically.[3]

Chronic hepatitis is histologically defined, but it has a serical resonance through several markers that can be modified in biochemical specific ways (ALT, AST, cholesterol, triglycerides, glycemia, GGT, total bilirubine) and serical proteins (the apolipoprotein, the haptoglobin, the alpha 2 macroglobulin). FibroTest, respectively FibroMAX processes these serical markers into an equivalent of the respective disease.[1]

The characteristics and the limits of the 2 non-invasive hepatic tests are distinguished in Table I.[2]

Aims: focus on the role of correlating the non-invasive hepatic tests in the staging of chronic hepatitis with HBV, in establishing the moment of initiating the treatment, in imagistic and injury monitory, in the precocious intervention on the hepatic nodules.

MATERIALS AND METHODS

Patients with VHB chronic infection directed to the the Clinic of Infectious Diseases II, "Matei Bals" Infectious Diseases Institute of Bucharest , were evaluated during the period June 2007 – December 2008. We selected for the study those patients who had at the firsts evaluation, the results correlated of the tests FibroScan and FibroTest/FibroMax, VHB-DNA level, VHD ARN level and ALT<1,5N.

RESULTS AND DISCUSSIONS

From 380 selected patients, 60 had double infection with VHB-VHD, and at least F3 fibrosis level. From 45

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Table I. characteristics and the limits of the 2 non-invasive hepatic tests

	FibroTest	FibroScan
Simplicity	Serical markers	Rapid results
Aplicability	95%	80% succes rate, IQR<30%, 10 measures and acute necrosis
Risk category	Gilbert diseas, hemolyze, acute inflamation	Fat tissue , necroinflamatory activity, thoracic fold>1,5cm, little intercostal space
Variability	(CV<10%) valid kituri and analisors	The best spot was not defin;dates intra si inter observators very variabil
Acuracy	without fals pozitiv results on 1000 blood donors, Sensibility for distingush F0,F1,F2 Very specific for F4	Very specific for F4 Standard cutoffs Need more studies on blood donors, can not distinguish F0,F1, F2
Prognostic value	Better then biopsy and APRI to 5 years	undeterminate

patients with double infection, over the age of 45 years old, 33 had a level of fibrosis F4, from which 24 presented dysplasia nodules. From those who presented dysplasia nodules, (evaluation CT/RMN), 4 patients were chemoembolized and 1 patient had surgical resection.

Within the patients with HBV infection only, 90 of them presented a fibrosis level >F2 after the first evaluation, and almost half of them, 40, had serum HBV DNA >10.000ui/ml, and antiviral therapy was applied on 30 of them; 12 had ALT in normal limits.

The correlation of non-invasive hepatic tests allows the facil distinguishing of fibrosis; it determines better Knowledge of the degree of hepatic disease and has an important role in determining the moment when the antiviral treatment should be applied.[5] If the advanced fibrosis is precociously discovered, then more efficient and therapeutic action can be taken – search of dysplasia nodules at F4-issues confirmed by the studies from literature.[3]

CONCLUSION

Distinguishing of hepatic fibrosis with non-invasive tests is a good predictor of severityof fibrosis to pacients with cronic hepatitis VHB.

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Utility of the Framingham Risk Score to predict the presence of ischemic heart disease in patients with rheumatoid arthritis

Mirela Parvu[1], Simona Coman[2] Lia Georgescu[3]

The prevalence of ischemic heart disease and atherosclerosis is increased in patients with rheumatoid arthritis. In the general population, the Framingham risk score identifies patients at increased cardiovascular risk and helps determine the need for preventive interventions. We examined the hypothesis that the Framingham score is increased in patients with rheumatoid arthritis and associated with ischemic heart disease. The Framingham score were compared among 53 patients with rheumatoid arthritis (33 with early disease, 20 with long-standing disease) and 41 control subjects. The ischemic heart disease was determined by clinical interview, ECG. The patients with long-standing disease had a higher Framingham score (18,65[13-25]) (median[interquartile range]) compared to patients with early rheumatoid arthritis(12,57[3-23]) or control subjects (11,02[2-16]).

In conclusion, a higher Framingham risk score is associated with the presence of ischemic heart disease in patients with rheumatoid arthritis.

Key words: Framingham score, rheumatoid arthritis

Patients with rheumatoid arthritis (RA) have increased mortality, largely attributable to cardiovascular disease.[4,5] The contribution of traditional and novel cardiovascular risk factors has been examined, but the mechanism for this increased cardiovascular risk in RA remain unclear. Hypertension, smoking and increased concentrations of C-reactive protein are more frequent in patients with RA than in control subjects. Furthermore, patients with RA and ischemic heart disease are older and have a higher cumulative exposure to cigarettes and a higher erythrocyte sedimentation rate than patients without atherosclerosis, suggesting that traditional risk factors and inflammation may both play a role in the process. The Framingham risk score is an extensively studied index to predict cardiovascular risk in the general population.[1,6] It includes age, gender, smoking, blood pressure, and cholesterol concentrations and estimates the risk of coronary events by stratifying individuals into three risk categories: low (<10% risk of an event in 10 years), intermediate (10% to 20%), and high (>20%).

Although the Framingham risk score is widely used in the general population to determine prognosis and the need for intervention, the value of this risk score is less clear in younger patients, women, and patients with inflammatory diseases. For example, in patients with systemic lupus erythematosus (SLE), another rheumatic disease associated with increased coronary calcification, there were no differences in the Framingham risk score in patients and controls, and the majority of the SLE patients with ischemic heart disease had a low 10-year risk according to the Framingham calculations.[2] In contrast to the general population, there is no information about the relationship between the Framingham cardiovascular risk score and ischemic heart disease in patients with RA. Therefore, we tested the hypothesis that the Framingham cardiovascular risk score would be associated with ischemic heart disease in patients with RA.

MATERIALS AND METHODS

Thirty-three patients with early RA who had a median (interquartile range) length of disease 2,06 (1 to 3) years and mean age of 53,33 years (43-62) (mean ± standard deviation) years. Twenty patients with long-standing RA (median disease duration 12,8 [8 to 17] years), aged 60,3 years (51-67), who met the classification criteria for RA,[1] and forty-one control subjects aged 52 ± 10 years were studied. Control subjects did not meet the classification criteria for RA or any other autoimmune disease and were frequency-matched for age, gender, and race with patients with RA (early and long-standing disease combined).

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Clinical Assessment

Patients and control subjects were assessed according to a standardized clinical interview, physical examination, laboratory tests, and (in patients) chart review. Family history of coronary disease was defined as a first-degree relative who had had a myocardial infarction or stroke before the age of 55 in males or 65 in females. Height and weight were measured, and body mass index was calculated by dividing the weight in kilograms by the square of the height in meters. Blood pressure was recorded as the mean of two measurements obtained 5 minutes apart after subjects had rested in a supine position for 10 minutes.

Framingham Score

The composite simplified coronary prediction model built on the blood pressure and cholesterol categories proposed by the Joint National Committee on Blood Pressure and the National Cholesterol Education Program was used. This model includes age, total and HDL cholesterol, blood pressure, and smoking and was designed in the setting of a community-based cohort (Framingham) of more than 5,000 people followed for 12 years.

Ischemic heart disease

All subjects underwent clinical interview, family history of coronary disease, ECG and classified in: level 0-absent-without ECG change, without clinical interview of angina, without myocardial infarction before, level 1-minimal- with ischemic change on ECG, without angina or myocardial infarction, level 2-moderate- with ischemic change on ECG, with angina on clinical interview, without myocardial infarction, level 3-severe-with myocardial infarction before.

Laboratory Tests

After an overnight fast, blood was collected for measurement of cholesterol, high-density lipoprotein (HDL) – using colorimetric method with AEROSET device, and for anticardiolipin antibody-IgG- we used Elisa method on TECAN device.

Blood pressure was classified using JNC indication:

Systolic BP	If Untreated	If treated
<120	0	0
120-129	1	3
130-139	2	4
140-159	3	5
160+	4	6

Statistic Analysis

Clinical characteristics and the cardiovascular risk scores were compared in patients with early RA, patients with long-standing RA, and control subjects using Fisher test for categorical unpaired variables . The correlation between Framingham score and continuous clinical characteristics was performed using the Spearman correlation coefficient (ρ). A multiple linear regression model with adjustment for age and gender was used to assess the difference in the Framingham score among control subjects and patients with early and long-standing RA.

Ischemic heart disease were classified as absent (0), minimal (1), moderate (2), and severe - level 3, and the association between these categories and the risk score was examined by proportional logistic regression in patients with RA and control subjects.

RESULTS

Correlation between Framingham score and duration of disease (figure 1) is 0,60, that was very significant. 36% from Framingham score variation is because of duration of disease .

Correlation between Framingham score and level of ischemic heart disease (figure 2) was 0,89 , that means a very strong correlation between risk score and ischemic heart disease. 79% from heart disease in RA is because of risk factors from Framinghan score.

The Framingham risk score was compared on patients with early RA(table I) and patients with long standing RA (table II) , and in control subjects (table III). Framingham scores were significantly higher on patients with RA compared with control subjects ($p<0,001$). Framingham score were significantly higher in patients with long –standing of disease RA- mean 18,65 (13-25) compared with patients with early RA – mean 12,57 (3-23)

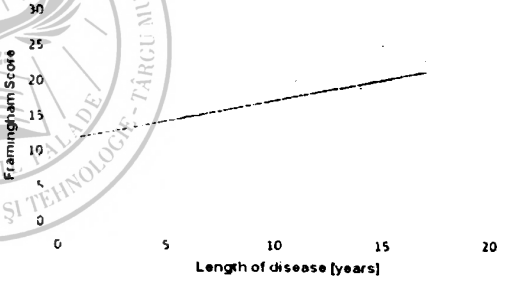

Figure 1. Correlation between Framingham risk score and length of disease

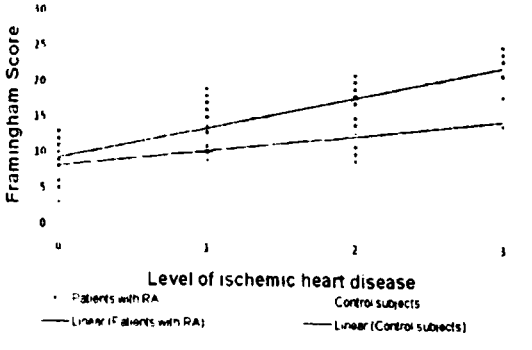

Figure 2. Correlation between Framingham Risk Score and level of ischemic heart disease on patients with RA and control subjects

Table I. Patients with RA

Nr	Length of RA (years)	Age (years)	Ischemic heart disease	ACL	Cholesterol (mg %)	HDLc	BP	Smoking	Framingham Score
1	2	51	2	+	190	56	2	-	10
2	11	56	2	-	267	38	4	-	19
3	1	58	0	-	189	60	-	-	9
4	3	51	1	-	245	45	3	-	15
5	13	61	2	-	267	36	-	-	17
6	2	53	0	-	156	57	-	-	5
7	2	52	0	-	180	56	-	-	8
8	15	64	3	-	256	36	6	+	25
9	1	52	0	-	158	60	-	-	5
10	1	51	0	-	128	57	-	-	6
11	3	50	2	-	245	49	3	+	19
12	14	64	3	-	305	45	4	+	21
13	16	60	2	-	335	42	3	-	18
14	17	63	2	-	289	44	4	+	20
15	1	49	2	-	223	32	-	+	18
16	3	54	0	-	227	49	-	-	11
17	2	58	0	-	190	54	3	-	13
18	2	53	0	-	245	53	-	-	11
19	3	52	1	-	235	44	2	-	13
20	3	58	1	-	289	45	-	-	16
21	3	62	0	-	145	45	-	-	11
22	17	67	3	-	289	38	5	-	23
23	15	64	2	-	245	42	4	-	18
24	13	58	2	-	134	45	-	+	15
25	3	56	1	-	145	55	3	-	11
26	2	47	2	+	203	33	-	+	11
27	2	62	3	-	345	35	6	+	24
28	1	48	1	-	210	34	-	+	18
29	1	55	1	-	223	43	-	+	17
30	1	58	0	-	234	56	-	-	12
31	3	54	2	-	222	33	6	-	18
32	11	59	2	-	306	47	4	-	20
33	3	59	1	-	226	56	5	-	17
34	3	58	1	-	256	56	4	-	17
35	2	47	0	-	156	55	-	-	3
36	2	55	0	-	123	58	-	-	8
37	2	51	0	-	145	67	-	-	6
38	9	58	1	-	305	48	3	-	19
39	1	43	2	-	256	35	-	+	17
40	1	45	2	-	201	52	-	-	9
41	2	54	0	-	189	56	-	-	8
42	17	61	2	-	277	47	5	-	19
43	8	61	1	-	198	56	2	-	13
44	14	57	2	-	278	38	4	-	19
45	15	59	2	-	256	37	6	-	21
46	11	62	3	-	305	34	5	+	23
47	2	56	0	-	178	56	-	-	10
48	3	57	0	-	190	46	-	-	11
49	8	59	1	-	189	45	4	-	15
50	9	60	2	-	267	47	-	-	14
51	10	62	2	-	204	45	6	-	19
52	13	51	3	+	256	34	5	-	18
53	2	51	2	-	289	35	1	+	20

Table II. Patients with early RA

Nr	Length of RA (years)	Age (years)	Level of ischemic heart disease	ACL	Cholesterol (mg %)	HDLc	BP	Smoking	Framingham Score
-	2	5*	2	+	190	50	2	-	10
2	1	58	0	-	189	60	-	-	9
3	3	51	1	-	245	45	3	-	15
4	2	53	0	-	156	57	-	-	5
5	2	52	0	-	180	56	-	-	8
6	1	52	0	-	158	60	-	-	5
7	1	51	0	-	128	50	-	-	6
8	3	50	2	-	245	49	3	+	19
9	1	49	2	-	223	39	-	+	18
10	3	54	0	-	227	40	-	-	11
11	2	58	0	-	190	54	3	-	13
12	2	53	0	-	245	53	-	-	11
13	3	52	1	-	235	40	2	-	13
14	3	58	1	-	289	45	-	-	16
15	3	62	0	-	145	45	-	-	11
16	3	56	1	-	145	55	3	-	11
17	2	47	2	+	203	33	-	-	11
18	2	62	3	-	345	44	6	+	23
19	1	48	1	-	210	34	-	+	18
20	1	55	1	-	223	43	-	+	17
21	1	58	0	-	234	56	-	-	12
22	3	54	2	-	222	43	6	-	17
23	3	59	1	-	226	56	5	-	17
24	3	58	1	-	256	50	4	-	17
25	2	47	0	-	156	55	-	-	3
26	2	55	0	-	123	48	-	-	9
27	2	51	0	-	145	53	-	-	7
28	1	43	2	-	256	35	-	+	17
29	1	45	2	+	201	52	-	-	9
30	2	54	0	-	189	46	-	-	9
31	2	56	0	-	178	56	-	-	10
32	3	57	0	-	190	46	-	-	11
33	2	51	2	-	289	35	1	+	20

Tabel III. Patients with long-standing RA

Nr	Length of RA (years)	Age (years)	Level of schemic heart disease	ACL	Cholesterol (mg %)	HDLc	BP	Smoking	Framingham Score
1	11	56	2	-	267	38	4	-	19
2	13	61	2	-	267	36	-	-	17
3	15	64	3	-	256	36	6	+	25
4	14	64	3	-	289	45	4	+	21
5	16	60	2	-	280	42	3	-	18
6	17	63	2	-	289	44	4	+	20
7	17	67	3	-	281	38	5	-	23
8	15	64	2	-	245	42	4	-	18
9	13	58	2	-	134	45	-	+	15
10	11	59	2	-	240	47	4	-	18
11	9	58	1	-	305	48	3	-	19
12	17	61	2	-	241	47	5	-	19
13	8	61	1	-	198	56	2	-	13
14	14	57	2	-	200	38	4	-	18
15	15	59	2	-	256	37	6	-	21
16	11	62	3	-	305	34	5	+	23
17	8	59	1	-	189	45	4	-	15
18	9	60	2	-	267	47	-	-	14
19	10	62	2	-	204	45	6	-	19
20	13	51	3	+	256	34	5	-	18

Tabel IV Control subjects

Nr	Lenght of disease(years)	Age (years)	Level of ischemic disease	Cholesterol (mg %)	HDLc	BP	Smoking	Framingham Score
1		53	1	234	56	2	+	14
2		42	1	200	56	-	+	15
3		63	2	267	50	1	-	14
4		56	0	156	55	-	-	7
5		53	1	200	54	1	-	12
6		49	1	189	50	-	+	13
7		67	2	202	52	2	-	15
8	-	54	2	155	51	2	-	9
9	-	63	1	189	52	1	-	12
10	-	47	0	158	60	-	-	2
11	-	67	2	167	50	1	-	14
12	-	63	2	190	47	1	-	11
13	-	53	1	107	59	2	-	9
14	-	55	2	156	46	2	-	11
15		59	1	156	50	1	-	9
16		60	1	267	57	-	-	14
17		57	2	216	54	2	-	15
18		56	2	238	47	-	-	13
19		44	2	158	54	2	+	9
20	-	58	2	235	51	1	-	11
21	-	65	2	278	51	1	-	14
22	-	55	1	190	49	2	-	13
23	-	51	0	187	52	-	-	8
24	-	50	0	160	56	-	-	8
25	-	67	1	189	49	2	-	16
26	-	53	1	201	52	1	-	9
27	-	60	2	149	55	1	-	11
28	-	50	2	234	49	2	-	10
29	-	57	1	267	58	0	-	13
30	-	54	2	205	51	2	-	12
31	-	47	1	178	55	1	-	8
32	-	55	2	198	53	0	-	10
33	-	52	0	109	59	1	-	8
34	-	58	3	170	55	2	-	14
35	-	44	0	167	62	-	-	3
36	-	63	3	205	48	1	-	14
37	-	59	2	230	54	0	-	10
38	-	56	1	179	53		-	10
39	-	45	0	140	50	-	+	10
40	-	48	2	201	49	2	-	14
41	-	58	1	190	52	-	-	8

DISCUSSION

Patients with rheumatoid arthritis (RA) have increased mortality, largely attributable to cardiovascular disease.[4,5]

The findings in this report are that the patients with long-standing RA had higher Framingham risk scores compared with patients with early disease and control subjects. On the other hand, the patients with long-standing RA' witch had severe ischemic heart disease – level 3 - had a Framingham score higher than the patients with early RA witch had a lower Framingham risk score .

The Framingham risk score includes age, gender, total and HDL cholesterol, blood pressure, diabetes, and smoking to derive an estimated risk of developing coronary heart disease within 10 years.[3,6] However, despite widespread use, the guidelines underestimate the presence of coronary calcification in certain populations, particularly women.[2] Because RA affects predominantly women, the utility of the Framingham risk score in this patient population is of interest, particularly considering that in patients with SLE, the Framingham risk score does not differ among patients and control subjects, and is inadequate for cardiovascular risk stratification.

Anticardiolipin antibody-IgG-(ACL) was found present only in 4 patients with RA, and that patients had a very high level for ischemic heart disease despite a Framingham risk score with lower or moderately values. In order to establish if this has both scientific

and statistical significance, we further need to extend our research on an large prospective studies.

CONCLUSION

Patients with RA and ischemic heart disease have higher Framingham cardiovascular risk scores even after adjustment for age and gender. Further prospective studies are needed to examine the utility of the Framingham and the modified Framingham scores to predict hard events in patients with RA.

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Arterial hypertension as a risk factor for the progression of cardiovascular disease in a subgroup of Romanian patients

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Arterial hypertension represents an important health problem and has been identified as the most frequent reason for cardiovascular morbidity and mortality according to the Framingham study. As high blood pressure (BP) is one of the most common cardiovascular diseases in our country (40% in general population as the SEPHAR study showed), the purpose of our research was to assess the prevalence of arterial hypertension in the population from Campulung County as well as other cardiovascular risk factors, associated to arterial hypertension. 650 hypertensives were enrolled in a prospective epidemiological study with a follow up period of 2 years. 50,37% of the patients had hypertension for more than 10 years, 75,08% of the population had at least 1 cardiovascular risk factors associated and 41,23% had metabolic syndrome. The clustering of other risk factors associated to hypertension impaires the prognosis of these patients.

Key words: arterial hypertension, risk factors, metabolic syndrome.

High blood pressure is an important risk factor for cardiovascular disease and mortality, both in western and also in eastern countries.[1,2] The number of patients with hypertension is likely to grow as the population ages, since either isolated systolic hypertension or combined systolic and diastolic hypertension occurs in over one-half of persons older than 65 years.[2] The rising incidence of obesity will also increase the number of hypertensive individuals. Although the prevalence of some cardiovascular risk factors has decreased in economically developed countries[14], the corresponding prevalence has increased in economically developing countries.[8] Despite the prevalence of hypertension and its associated complications, control of the disease is far from adequate.

Data from NHANES show that only 34 percent of persons with hypertension have their blood pressure under control, defined as a level below 140/90 mmHg.[14] Slightly higher rates of control were reported in a regional population study (approximately 45 and 55 percent of men and women, respectively, have controlled hypertension). There are numerous potential reasons for low rates of blood pressure control, including poor access to health care and medications, and lack of adherence with long-term therapy for a condition that is usually asymptomatic.[3] The latter may be particularly true when the therapy may interfere with the patient's quality of life and when its immediate benefits may not be obvious to the patient.[9] Thus, hypertension will likely remain the most common risk factor for heart attack and stroke.[5,9] In hypertensive adults, multiple randomized trials have shown that reduction of BP by antihypertensive therapy reduces cardiovascular morbidity and mortality. The magnitude of the benefit increases with the severity of the HTN.[3]

The correlation between the risk of adverse outcomes (including death) and blood pressure is a continuous variable, rather than an increased risk that is only observed above certain critical blood pressure values.[4] Furthermore, at any blood pressure, the cardiovascular risk is importantly affected by the presence or absence of other risk factors.[6,7,11] In providing a context for policy planners and health education programs in economically developing countries, it is important to assess the proportion of the population at high risk for cardiovascular disease.[10,13] Such data provide an understanding of the size of the population in need of targeted interventions to lower

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the population burden of illness due to high blood pressure.[3,4] The goal of this study was to assess the proportion of hypertensive patients who had 1 or more of the following major modifiable cardiovascular risk factors: dyslipidemia, metabolic syndrome, diabetes, current smoking and overweight.

MATERIAL AND METHODS

Study Population

A total of 650 individuals, aged between 20-80 years with a positive diagnosis of arterial hypertension were enrolled in our prospective epidemiological study. The follow up period lasted for 2 years.

Data collection was conducted in local county hospital in the participant's residential area. During the study visit, we administered a standardized questionnaire. Of relevance to the current analysis, the questionnaire assessed age; sex; education; cigarette smoking; a self-reported history of stroke, myocardial infarction, and congestive heart failure; and the previous diagnosis and treatment of hypertension, high cholesterol, and diabetes. Current smoking was defined as having smoked at least 100 cigarettes in the subject's entire life, having smoked cigarettes regularly, and smoking currently.

Blood Pressure Measurement

For each participant, 3 blood pressure measurements were obtained by a standardized protocol adapted from the procedures recommended by the European Society of Cardiology (ESC). Blood pressure was measured by trained physicians or nurses with participants in the seated position after they had rested for at least 5 minutes. Participants were advised to avoid alcohol, cigarette smoking, coffee, tea and excessive exercise for at least 30 minutes before their blood pressure measurement. A calibrated mercury OMRON M5-I sphygmomanometer was used, and 1 of 3 cuff sizes (short, regular adult or extralarge) was chosen on the basis of the participant's arm circumference. Hypertension was defined as an average systolic blood pressure (SBP) =140 mm Hg and/or an average diastolic blood pressure (DBP) =90 mm Hg and/or self-reported current treatment for hypertension with antihypertensive medication.

Height and Weight Measurement

Body weight and height were measured by trained observers following a standard protocol. Body mass index (BMI) was calculated as body weight (in kilograms) divided by height (in meters) squared. Overweight was defined as having a BMI =25 kg/m^2.

Definition of the Metabolic Syndrome

The metabolic syndrome was defined according to the guidelines of the National Cholesterol Education Program Third Adult Treatment Panel (NCEP-ATP III). On the basis of the baseline examination, the metabolic syndrome was diagnosed in our hypertensive patients when at least 2 of the following criteria were met. The first criterion was elevated body mass index (BMI =25 kg/m^2). The second criterion was elevated triglycerides (=150 mg/dL); the third, low high-density lipoprotein (HDL) cholesterol (<40 mg/dL in men, <50 mg/dL in women); the fourth, impaired glucose tolerance (=110 mg/dL by the NCEP-ATP III definition).

Laboratory Measurements

After subjects had fasted for 12 hours overnight, blood was obtained for measurement of serum total cholesterol (TC), HDL cholesterol (HDL-C), triglyceride (TG), serum creatinine and glucose levels in the Campulung county hospital's chemistry laboratory. LDL cholesterol (LDL-C) levels were estimated with the use of the Friedewald equation.

Statistical methods

Descriptive statistics for categorical variables are presented for the study cohort.

RESULTS

Overall, 328 women and 322 men were available for analysis. The distribution of patients according to their age is shown in Figure 1.

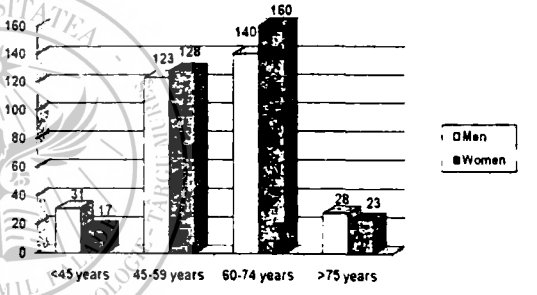

Figure 1 Age distribution of study population

50,37% of patients had been diagnosed with high blood pressure for more than 10 years, 21,84% had hypertension between 5 to 10 years and 19,53% had a positive diagnosis of HT between 2 to 5 years (Figure 2).

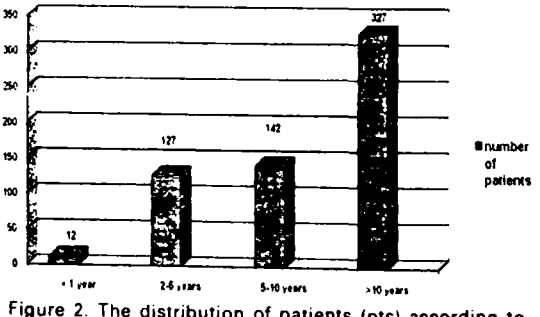

Figure 2. The distribution of patients (pts) according to the onset of hypertension

The prevalence of the risk factors in study population was very high. Overall, 7,23% and 7,69% of hypertensive men and women, respectively, did not

have any of the risk factors investigated (age, dyslipidemia, impaired fasting glucose, family history of high blood pressure, current smoking, positive CRP or overweight). In contrast, 14,30%, 14,3%, and 13,53% of men and 13,38%, 14%, and 16,46% of women had 1, 2, and =3 of these risk factors, respectively. Overall, 14,92%, 27,69%, 27,38%, and 30% of hypertensive patients had 0, 1, 2, and 3 of these risk factors, respectively. In total, 75,08% of the population had 1 or more cardiovascular risk factors (Figure 3).

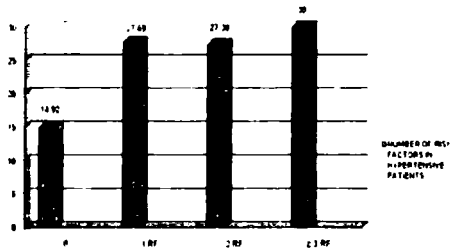

Figure 3 The number of risk factors (RF) in hypertensive patients

The blood pressure control (according to target values from the ESC/ESH 2007 Guidelines for the Management of Arterial Hypertension) was obtained in only 18% of hypertesives after 6 months of treatment (Figure 4).

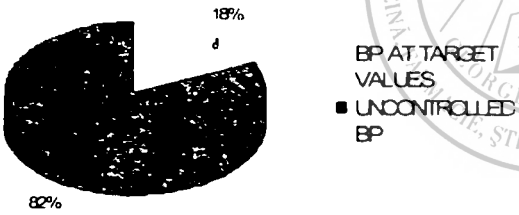

Figure 4 Blood pressure (BP) control according to target values after 6 months of treatment

We also assessed the prevalence of individual metabolic syndrome components and number of fulfilled components. Our study found that the metabolic syndrome was present in 268 subjects (41,23%) according to the NCEP-ATP III definition. The prevalence of the metabolic syndrome was higher in women (21,23%) compared with men (20%). The distribution of the prevalences of individual metabolic syndrome components in hypertensive patients are shown in Table I.

Table I. The prevalence of individual metabolic syndrome components

Characteristics	Number of pts	%
Metabolic syndrome components		
Overweight	239	36,76
Triglycerides	250	38,46
Low HDL cholesterol	223	34,30
Impaired glucose tolerance	101	15,53

DISCUSSIONS

In our study, 54% of the population had more than 60 years, data that are consistent with the prevalence of hypertension in men and women according to age and race/ethnicity in the United States from the NHAHES survey,[1] The number of patients with hypertension is likely to grow as the population ages, since either isolated systolic hypertension or combined systolic and diastolic hypertension occurs in over one-half of persons older than 65 years.[3]

Although 50,3% of the patients were diagnosed with hypertension for more than 10 years, the blood pressure control to target values after 6 months of treatment had been achieved only in a small number of subjects. These data contrast with the findings from different authors from US or Western Europe who reported higher levels of blood pressure control in their patients.[8,9]

In this community-based study, we have found that 85,08% of hypertensives had at least 1 identifiable cardiovascular risk factor for hypertension, data that are consistent with the findings from Dongfeng and from Yusuf and his coworkers.[4,15]

CONCLUSIONS

Arterial hypertension is a major cause of cardiovascular morbidity and mortality in our area. Overall, 72,31% of hypertensive patients have at least 1 cardiovascular risk factor from the 7 cardiovascular risk factors assessed in the current study. Effective population-based interventions such as smoking cessation, improved diet, and increased physical activity can safely and effectively lower the risk of cardiovascular morbidity' and mortality. Programs to enhance efforts aimed at prevention, detection, and treatment of dyslipidemia, hypertension, diabetes, smoking, and overweight may greatly reduce the future burden of major cardiovascular events in the general population.

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Pharmacological implications of certain predictive factors in arterial hypertension – target the renin-angiotensin system

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Introduction: Quality assured therapy is a further, more specific request of the evidence based therapy. In case of cardiovascular diseases this implies the screening of several predictive factors. Material and methods: This prospective observational study was performed in purpose to evaluate the correlation between certain predictive markers, the used pharmacotherapy and the progression of the cardiovascular disease. Results and discussion: The patients included in this study were between 40-80 year old and all of them were diagnosed with hypertension. The main parameters that we followed are: the ankle-brachial index, the serum creatinine-level, C-reactive protein and creatinine clearance The applied treatment was also recorded. We observed a positive correlation between the decrease of the ankle-brachial index and the increase of serum creatinine level, and also a benefic effect of most renin-angiotensin-system blockers on these markers. Conclusions: Important studies have revealed that major cardiovascular outcomes are influenced by certain risk factors. Using these markers to set up a pharmacological therapy can be helpful in assurance of a better control of the disease

***Key words**: quality assurance, pharmacotherapy, hypertension*

The prevention and management of hypertension are major public health challenges for the WHO and also for physicians all around the world.[1,6,27] If the rise in BP with age could be prevented or diminished, much of hypertension, cardiovascular and renal disease and stroke might be also prevented.[23,26] Whereas the short-term absolute risk for hypertension is conveyed effectively by incidence rates, the long-term risk is best summarized by the lifetime risk statistic[1,7,9,22], which is the probability of developing hypertension during the remaining years of life. Guideline committees recommend targets of treatment based on trial data on efficacy and effectiveness.[24,28] Quality-assurance initiatives apply these parameters in the general practice setting.[8,16,30] The goals for hypertension management that have been suggested by guideline committees and quality-assurance organizations are reasonable and achievable.[4,5,25] The establishment of these guidelines represents the compilation of contemporary experimental data, and adherence to these guidelines[2,12,13,19] would reap enormous benefit should control rates be improved by this type of surveillance and by using goal-oriented management.[18,19]

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MATERIAL AND METHOD

This prospective observational study was performed in purpose to evaluate the correlation between certain predictive markers, the used pharmacotherapy and the progression of the cardiovascular disease. An important issue to consider when trying implementing goal-oriented management is the global cardiovascular risk control. Quality-assurance methods give the possibility for recording the most important parameters regarding the medical condition in purpose to analyze, evaluate and document it.[11,14,15,17] The recruitment of the subjects was performed based on certain inclusion and exclusion criteria. Basically the main criteria for inclusion were as follows: diagnosed hypertension, age between 40-80 years; and we excluded all patients with diabetes mellitus and/or bilateral renal artery stenosis. During the study several risk factors were documented and evaluated: age, gender, smoking habit, laboratory parameters and the ankle-brachial index. Regarding the ankle-brachial index we considered pathological dose below 0.9. Statistical analysis was performed to determine significance levels, using tStudent test.

RESULTS AND DISCUSSIONS

This initial part of the study was performed on 96 hypertensive patients. At this point we recorded several cardiovascular markers (Table I) in aim to evaluate them and establish possible correlations with the used pharmacological therapy.

Table I. Mean values of predictive markers

Age	BMI	BP	ABI	CRP
72.33	27.32	160/86	0.69	3.23
UA	Cr	Gly	Chol	TG
6.06	0.96	104.33	207.66	161.66

*BMI – body mass index, BP – blood pressure, ABI – ankle brachial index, CRP – C-reactive protein, UA – uric acid, Cr – creatinine, Gly – glycemia, Chol – cholesterolemia, TG – serum triglycerides

We studied the incidence of decreased ABI in correlation with increased serum levels of creatinine. Our findings are represented considering the different groups of age (Figure 1).

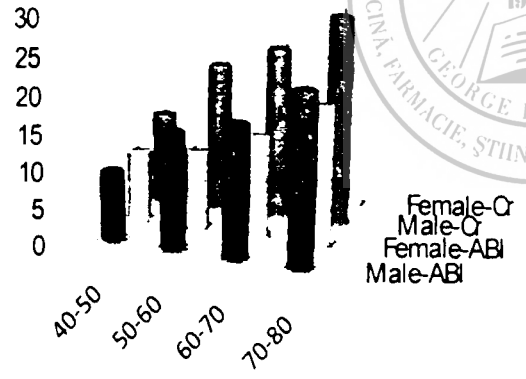

Figure 1. Incidence of decreased ABI in correlation with increased Cr

We observed a positive correlation between the decrease of the ankle-brachial index and the increase of serum creatinine level, and also a benefic effect of most renin-angiotensin-system blockers on these markers. According to some very recent findings[15] the renin/angiotensin system is more complexly implicated is blood pressure regulation as it was believed. Our results show that using either an angiotensin-converting inhibitor or an angiotensin-receptor blocker will disrupt the above mentioned correlation. That's the cause wherefore we will continue our study in aim to evaluate in a more specific matter the effects of the renin-angiotensin system.

The purpose of the pharmacological therapy is to reduce the incidence of major outcomes, to decrease overall mortality and to improve the quality of life. The quality assured therapy requires an adequate use of medicines, considering also the predictive markers and the global cardiovascular risk of the patient. In aim to assess the global risk we collected data – meaning risk factors – as mentioned above and using the "Assessment of cardiovascular risk in primary and secondary prevention" (Figure 2) software[10], developed under coordination of Guido Grassi and Giuseppe Mancia at the University of Milan, which allows a direct quantification of the cardiovascular risk profile.

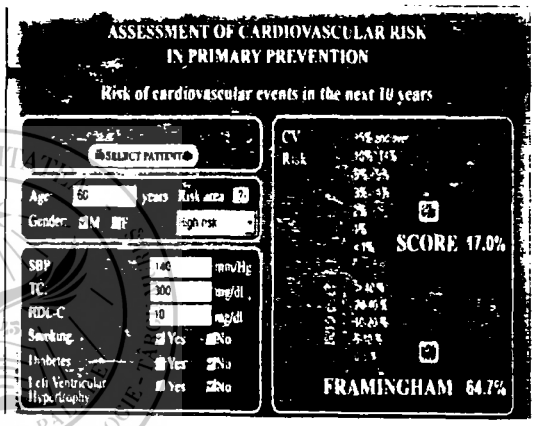

Figure 2. Simulation of risk quantification

Randomized controlled trials are needed to assess different blood pressure lowering strategies and different first-line drug classes in patients with hypertension[20,21] to help us ensure efficient pharmacotherapy.

CONCLUSIONS

Important studies have revealed that major cardiovascular outcomes are influenced by certain risk factors. Using these markers to set up a pharmacological therapy can be helpful in assurance of a better control of the disease. The observation that inhibiting the renin-angiotensin system improves in a very short time the global risk may be helpful for implementing a quality assured therapy. But this requires a wider study, which is our objective for near future.

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Complications of protein C and protein S deficiency in an idiopathic thrombocytopenic purpura case

Liliana M. Isacianu, Ingrid Miron, Silvia Dumitras, I. Tansanu

Introduction: protein C and S are plasmatic proteins which inactivate factors V-a and VIII-a (preservative effect), neutralize the third inhibitor of plasminogen activator and mediate the protein C activate. The deficiency of these proteins can lead to deep venous thrombosis, lung embolism and arterial thrombosis.
Objectives: the interest of study is to present the clinical particular, biological aspects of this rare case. Material and method: we report a case of a girl, 17 years with obesity, diagnosed with idiopathic thrombocytopenic purpura three years before, with schizophrenia previously to actual admission; she was hospitalized in our clinic for an important edema at the left foot and thrombocytopenia. Bone marrow showed partial ineffective megacariopoesis. The deep venous thrombosis was assessed by Doppler echocardiography.
Results: evolution: thrombocytopenia was remitted, while hereditary thrombophilia will need the administration with anticoagulant continuous treatment.
Conclusions: deficiency of protein C and protein S induced the deep left iliofemural venous thrombosis, lung ischemia and right hemiparesis following rolandic Central Nervous System (CNS) ischemic event.
Key words: protein C and S, idiopathic thrombocytopenic purpura, thrombosis.

Protein C and S are plasmatic proteins which inactivate factors 5 and 8 (preservative effect), neutralize the third inhibitor of plasminogen activator and mediate the protein C activate. The deficiency of these proteins can lead to deep venous thrombosis, lung embolism and arterial thrombosis. Objectives: the interest of study is to present the clinical particular, biological aspects of this rare case.

CASE REPORT

We report a case of a girl, 17 years with obesity, diagnosed with idiopathic thrombocytopenic purpura three years before, with schizophrenia previously to actual admission; she was hospitalized in our clinic for an important oedema at the left foot and thrombocytopenia. Bone marrow showed partial ineffective megacariopoesis. The deep venous thrombosis was assessed by Doppler echocardiography.

Case presentation

The teen-ager girl, D.M., 17 years (in 2005), who was diagnosed in 2002 with Idiopathic Thrombocytopenic Purpura (represented from recurrent epistaxis and purpura), with low values of platelets (75.000 – 125.000/ mmc), the presented in august 2005 a new episode of thrombocytopenia for who initiated treatment with corticotherapy (Prednison) in Emergency Clinical Hospital for Children "St. Mary" Iasi, Second Clinic of Pediatrics (figure 1).

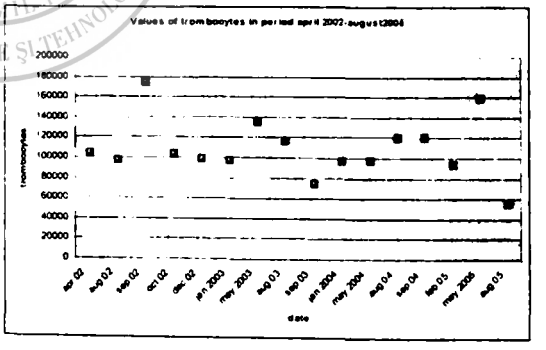

Figure 1. Graphic of values of trombocytes during April 2002 – August 2005

After two weeks, the patient, install oneself a right hemipareza, and CT exam showed thrombosis of superior longitudinal sinus and rolandic Central Nervous System ischemic event left, (this vascular accident was remitted with Plavix (future heart attack or stroke) = 1capsule/day, Pentoxifilin (intermittent claudication) = 1 dragee/day, Aspirin (as anti-inflammatory medication) = 1/2 tablet/day). After the event, the patient was hospitalize in Emergency Clinical Hospital for Children "St. Mary" Iasi, Fourth Clinic of Pediatrics, Oncohematology Department with

Emergency Clinical Hospital for Children "St Mary" Iasi, Fourth Clinic of Pediatrics, Oncohematology Department

68.000 number of platelets, while bone marrow showed partial ineffective megacariopoesis with moderate plasmocitosis.

It was suspicion the diagnostic of LES (this been disproved by absent lupic cells, ANA anti-bodies – negative, anti-DNA-dc anti-bodies – negative) and the existence of anti-phospholipid anti-bodies syndrome cancelled by absence of anti-cardio-lipidic anti-bodies. The patient received treatment with Celestene (anti-inflammatory properties) = 100drops/day (Trombocytes = 103.000/mmc), Dicarbocalm (antacids) = 3 tablets/day, Topamax (anticonvulsant) = 2 capsule/day, Plavix = 1 capsule/day.

In the October 2005 started a deep venous thrombosis left member[4] (violet oedema, pain at this level, extensive volume at the inferior left member) for who she was hospitalized Emergency Clinical Hospital for Children "St. Mary" Iasi, whose diagnostic was confirmed by Doppler exam effectuate in Emergency Clinical Hospital "St. Spiridon" Iasi, First Clinic "C.I. Negoita". The patient received treatment with Fluxum (antithrombotic properties) (Parnoparina or ENOXAPARINA SODICA) 0,6 millilitre x 2/day for four days and antibiotics, with a good evolution by reduction oedema (- 2 centimetre) and pain calming[1,3].

In context of history and pathology presentation we can emphasise two problems for discussion:

1. Idiopathic Thrombocytopenic Purpura like hematological symptom at the LES disease, or
2. Deficiency of anti-thrombosis factors disease (protein C, protein S or factor V Leyden)[1,2].

For continuation[3] of investigation and proper treatment the patient was transferred in Emergency Clinical Hospital "St. Spiridon" Iasi, First Clinic "C.I. Negoita". As well as complication of venous thrombosis mention right lung infarct, clinic support by twinge at right hemi-thorax support, tachycardia, radiology exam: - triangular opacity in inferior lobe of right lung, and echocardiography exam showed easy overdemand a right heart and easy hypertension.

It was performed measure of plasmatic protein C and protein S in this way:

- the IL TestTM protein C kit is a protein C assay based on a chromogenic substrate. synthetic[1,2]. Normal value = 69,1 – 134,1 %.

- the protein S kit determines the functional activity of protein S (PS) by measuring the degree of prolongation of a pro-thrombin time in the presence of bovine thromboplastin calcium ions and activated Protein C[1,2]. Normal value = 61,9 – 145,3%.

In our case these values had been Protein C = 51,19% and Protein S = 56,8%.

These new values of plasmatic protein going at the new diagnosis hereditary thrombophilia through deficiency Protein C and S[3,4].

The followed treatment was with anticoagulant Trombostop(anticoagulant and antithrombotic properties) = 3 milligrame/day (with lunar control of pro-thrombin time (Quick) and INR (*international normalised ratio*), (figure 2 and figure 3).

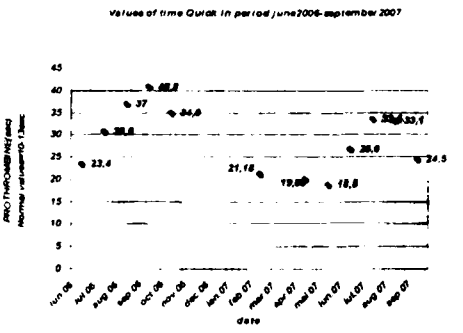

Figure 2 Graphic of values of time Quick during June 2006 – September 2007

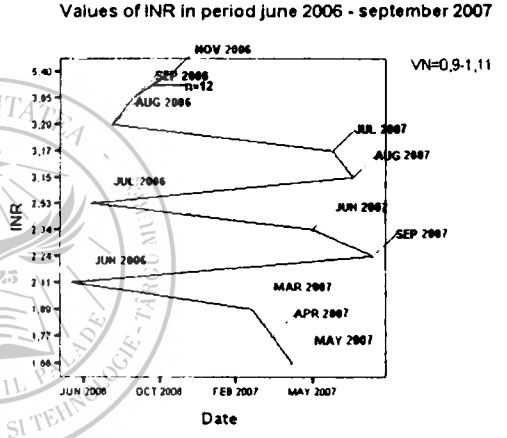

Figure 3 Graphic of values of *international normalised ratio* (INR) during June 2006 – September 2007

CONCLUSION

Deficiency of protein C and protein S induced the deep left iliofemural venous thrombosis, lung ischemia and right hemiparesis following rolandic CNS ischemic event.

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Prognosis factors in the treatment of hepatocellular carcinoma with arterial chemoembolisation

Camelia Colțescu[1], F.Habersetzer[1], G.Oltean[2], Simona Bățagă[2], D.Georgescu[2]

Chemoembolization is a palliative treatment in the case of hepatocellular carcinoma whose efficacity is controversial. Our study compares overall survival according to the alcoholic or viral etiology of the cirrhosis. The study comprises a retrospective analysis of patients with cirrhosis treated through chemoembolisation between 2000-2007. They analyzed survival according to the etiology of the disease, the Child-Pugh score, the CLIP score, the tumoral extension, the number of treatments. Average survival in case of hepatocellular carcinoma occurring on a viral cirrhosis was of 29 months as compared to the survival average in the hepatocellular occurring on the alcoholic cirrhosis of 15 months

The results of our analysis show that survival after the chemoembolization of the hepatocellular carcinomas occurring on alcoholic cirrhosis was less frequent as compared to the hepatocellular carcinomas occurring on viral cirrhoses.

***Key words**: hepatocellular carcinoma, chemoemboli-zation, survival*

Hepatocellular carcinoma (HCC) represents 5% of the cases of cancer in the world, the worldwide incidence is from 560,000 to 1,000,000 new cases per year.[1]

Recent epidemiological data show a major increase in its incidence in Western countries and the USA.[2] This can be partly explained by a more effective and early diagnosis due to new imaging techniques and could enroll mainly within important epidemiological changes importance such as: increasing incidence of viral hepatopathies, particularly HCV. A French study provides an increase in mortality in the CHC C viral cirrhosis of 150% in men and 200% in women in 2020.[3,22]

The prognosis is bad and variable depending on the period when it is found (average survival of 5.6 months in the symptomatic stage and up to 40 months in the absence of symptoms, metastases and vascular invasion).[14]

Curative treatment for CHC includes surgical resection, transplantation and more recently radiofrequency has demonstrated its efficacy in the small lesions, few in number and nonresectable.[12] Hepatocarcinomas are resectable in 15-20% of cases.[1]

Among patients with CHC 70% currently receive palliatives treatment of which chemoembolization (CEL) occupies a major and controversial role.[13]

If nonresetcable HCC we can have either an arterial embolization with a resorbable material, or a chemoembolization combining embolization with liver intra-arterial chemotherapy liver, associated or not with lipiodol. These palliative treatments are the most frequently used (10-15%) in HCC. Two studies conducted in Spain and Asia have shown the superiority of palliative treatment by CEL compared with symptomatic treatment[16,17] and other French studies are negative.[19]

CEL superiority to symptomatic treatment has been underlined in other 2 recent meta-analyses.[5,15]

The main purpose of our study was to compare overall survival according to the viral or alcoholic etiology of the cirrhosis. In parallel some prognostic factors for survival have been studied: age, gender, Child-Pugh score, CLIP score, tumor extension, the presence of segmentary portal thrombosis, the number of cures.

MATERIAL AND METHODS

The study is retrospective and included all patients who developed HCC cirrhosis having a viral B or C or alcoholic cirrhosis and treated by CEL during 2000-2007.

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[2]Departament of Internal Medicine, 1st Medical Clinic, Faculty of Medecine UMPh Targu Mures

Viral markers for hepatitis B and C were performed in all patients. In the case of mixed alcoholic and viral cirrhoses we retained only the viral etiology as the cause of cirrhosis study.

Diagnosis of HCC was established on the basis of ecoguided hepatic biopsy from tumour lesions or through the intersection of biological criteria(AFP ≥ 400 ng/ml) with the morphological ones (typical aspect in CT and MRI).

Diagnosis of cirrhosis was confirmed by PBF of liver adjacent to tumor or on criteria clinical-morphological and biological criteria. The retrospective study of patients with HCC fulfilled the diagnostic criteria of the European Conference in Barcelona from 2000.[12]

Among the exclusion criteria of the study were: HCC appeared on healthy liver on the cirrhoses from non-alcoholic steatohepatitis, the presence of extrahepatic metastases, of refractory ascites, hepatic encephalopathy patients treated only surgically.

CEL technique involves the supraselective intraarterial injection of an antimitotic agent in the form of emulsions associating lipiodol and an embolization with fragments of resorbable gelatin (spongel).

Patients were hospitalized in the previous day for a biological assessment and clinical examination and to start an intravenous hydration. A medical protocol including antibiotics, antiemetics, diuretics, antalgics, antipyretics was started in the day of the procedure and continued in the following days. A regular monitoring of biological hemogram, transaminases,the prothrombin time(PT), ionogram, creatinine, and plasma bilirubine was made.

Tumoral response was evaluated at 1 month after each treatment by morphologic examination (CT or MRI) and a clinical and biological balance is also made. Treatments were repeated in 2-3 months depending on clinical and biological tolerance of the tumoral response assessed by morphological examination and by the AFP.

Survival analysis in statistics is defined as time in months from the date of the first CEL received to the date of death or loss from supervision.

RESULTS

The study included 36 patients who were treated with CEL in the Hepatogastroenterology Service in Strasbourg in January 2000-November 2007. The average age was 61 years. 83% of patients were men and alcoholic cirrhosis dominated (61.5%). In 57% of cases HCC was multinodular. Most patients were Child A (68%) with a CLIP score of 0-1 in 49% of cases. Segmentary portal thrombosis was present in 17% of cases. Of the population with viral cirrhosis 77% had a chronic hepatitis C and a 23% a chronic hepatitis B. Mixed origin B and C was 9.5% in this group. The average number of cure was 3.5 ± 2.32. From the comparison of patients in the group with alcoholic cirrhosis to the group with viral cirrhosis there was a significant difference in the group of women with viral cirrhosis (8% vs 29%, p <0.00001).

Median survival in group with viral cirrhosis was 29 months versus 15 months in the group with alcoholic cirrhosis (p <0.00001) (Figure 1).

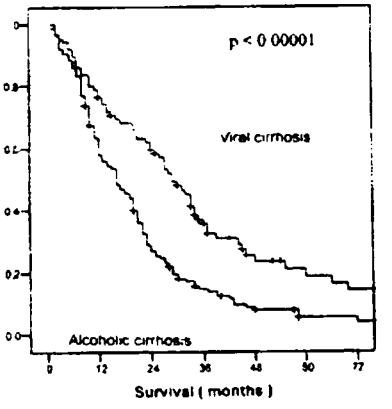

Figure 1. The probability of survival in function of etiology of the cirrhosis calculated by Kaplan-Meier method

The probability of survival at 1, 2 and 3 years calculated by Kaplan Meier method was 56%, 25% and 12% in the group with alcoholic cirrhosis and 74%, 57% and 33% in the group with viral cirrhosis (p <0 , 00001).

Average survival according to the Child score was 22 months in patients with Child A and 10 months in patients with Child B (p <0.00001) (Figure 2).

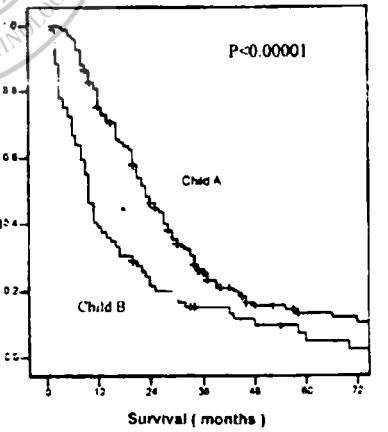

Figure 2. The probability of survival in function of Child's score calculated by Kaplan-Meier method

The average survival was 28 months in patients with CLIP score 0-1 and 5 months in those with CLIP≥4 (p <0.00001).

Depending on the segmentary portal thrombosis average survival was 11 months in patients with thrombosis and 23 months in those without thrombosis (Figure 3).

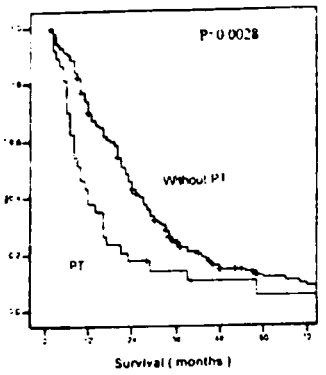

Figure 3. The probability of survival in function of the presence of segmentary portal thrombosis calculated by Kaplan-Meier method

Median survival was 24 months for unifocal HCC and 15 months in multifocal HCC.

DISCUSSIONS

Among HCC affected patients 70% currently have a palliative treatment, including CEL that plays an important and still controversial role.[15]

In 2002, two randomized prospective studies compared this technique to symptomatic treatment or with a suboptimal treatment with tamoxifen and demonstrated the superiority of CEL in HCC developed mainly on viral cirrhosis B and C.[16,17]

A study on an Asian population with mainly HCC related to hepatitis B virus suggested that the presence of AgHBs is an independent negative factor for survival after CEL.[16]

Some Western studies have shown a longer survival after CEL in CHC patients with viral cirrhosis B or C compared to the ones with alcoholic cirrhosis.

Survival of patients with CHC on cirrhotic liver is conditioned by the absence of aggravation of hepatopathy due to ischemic phenomena given by the embolization or the hepatotoxicity of the chemotherapy.

Our study examined other predictive factors: Child-Pugh score and CLIP score, the number of treatments. Many studies have found the Child-Pugh score[10,11], the CLIP score[9], the number of treatments[7,8] as factors predictive for survival.

These independent predictive factors must be known to improve selection of patients for CEL. Objective tumoral response (20-53%) is not always correlated with survival due to the risk of the aggravation of the existing hepatopathy.[4,7,10,18,21]

CONCLUSIONS

The results of our retrospective study suggests that survival after CEL in patients with hepatocellular carcinoma on alcoholic cirrhosis was much lower compared with those with hepatocellular carcinoma on viral cirrhosis B or C. Also they might explain the difference in results between different studies that assessed the CEL.

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Clinico-paraclinical correlations in child gastritis

Cristina Oana Marginean[1], Simona Stolnicu[2], Ana-Maria Pitea[1]

The diagnosis of the gastritis in child is based on the Sydney endoscopico-histological criteria , the recovery being evaluated after two months, when there is clinical and histological recovery.
The aim: analysis of gastritis cases in child, regarding the diagnosis phases (clinical, endoscopical, histopathological, serological), and the estimation of treatments' efficacy.
Material and methods: there had been evaluated 978 children between 2-18 years, hospitalized in Pediatry Clinic I Tg.-Mures between 01.03.2001–01.07.2008. We made a retrospective study and correlations between clinical aspects and histological changes, evaluating the efficacy of the treatment according to clinical status and endoscopical aspects at revaluation.
Results: the endoscopic examination detected antral damages in 63,4% of the cases, the remaining are pangastric changes or located at the gastric body. It has been found a clinical-endoscopical correlation in over 90% of the cases. Only 658 children came for an examination. The treatment led to a clinical and histological recovery in 655 cases (CI: 63,98–69,87, with "p"< 0,0001).
Conclusions: far of being rare in the paediatric pathology, the real incidence of gastritis could be better estimated through new methods of diagnosis. In our lot it has been found a clinical-histological correlation in 92,8% from the patients, and the treatment caused the recovery in 66,97% of the cases. Thus emerges the importance of an accurate diagnosis of this disease and of the gastritis treatment in child.
Key words: gastritis, child, endoscopy, treatment

Gastritis are inflammatory processes of the gastric mucosa, with histopatological arguments, having sudden onset, with nausea and vomiting, or slow onset as a dispeptic syndrome with regular alimentation and seasonal periodicity.[4,5]

The following can be a result of gastritis in childhood: epigastric pain, with regular alimentation and seasonal periodicity, nauseas, vomiting (food, bilious or bloody vomiting), anorexia, diarrhea, constipation.[4,8,11,18]

Gastritis classification uses The Sydney (endoscopic, histologic, etiologic) criteria. Using endoscopic criteria there are: exsudative gastritis, erosive gastritis, atrophic gastritis, hemorrhagic gastritis, and reflux gastritis with hypertrophy of gastric mucosa folds. Using histological criteria there are: acute, chronic, special gastritis (eosinophilic, lymphocytic, granulomatosa).[5,8,15]

The recovery of gastritis is usually evaluated after two months of treatment, when there is clinical and histological recovery of the treated cases.[11,18,19]

The aim of this paper is the retrospective analysis of gastritis cases in children regarding the phases of diagnosis (clinical manifestations, endoscopical aspects, histopathological examinations and serological tests), as well as elements related to treatment – the evaluation of treatment's efficacy correlated with clinical evolution of the disease.

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MATERIAL AND METHODS

There were 978 children assessed, having between 2 and 18 years, hospitalized in Pediatry Clinic I Tg.-Mures between 01.03.2001–01.07.2008. We made a retrospective study based on medical observation sheet, with corelations between clinical aspects and superiour digestive endoscopical findings and histological changes, assessing the efficacy of the treatment according to the clinical status and the endoscopical aspects at the revaluation.

The cases allotment was studied after age and sex groups, based on the most important simptoms at the first examination, as well as related to endoscopical findings, Urease Rapid Test, histopathological changes and evolution under treatment from a clinic, endoscopic and histologic point of view.

Accordind to CDC (Centers for Disease Control and Prevention) data, the incidence of gastritis in children is 1,3 per 100.000 inhabitants.[5,8,15,18] In this paper, we have studied more cases (for almost eight years) because we wanted to select a significant number of children presented for control evaluation, being known that our country the sanitary education is scanty and if the patient feels well, it often does not appear to revaluation.

It is approved by present knowledge stage in digestive pathology that Helicobacter pylori brings the gastritis and the peptic ulcer disease, and that the long-term infection associates a high risk for gastric adenocarcinoma or limphoma maligna in adult.[17]

In chronic active gastritis, the prevalence of infection exceeds 80%. In children aged between 8 – 10

Table I. Clinical manifestations on the study lot

Clinical manifestations	Abdominal pain	Vomiting and nausea	Anorexia	Deacrease of weight	Other cases
Number of cases	515 cases (52,65%)	182 cases (18,60%)	62 cases (6,33%)	11 cases (1,12%)	208 cases (21,26%)

years with recurrent abdominal pains, the prevalence of H. pylori infection is 25%, whereas in non-simptomatic children it is only 7,5%.[5,15]

These are criteria for patients' acceptance in our study:

-recurrent abdominal pains with occasional regular alimentation
-epigastric pains at first examination
-periumbilical pains
-low appetite
-weight loss
-nausea
-alimentary vomiting

The following are criteria for patients' exclusion from this study:

-abdominal functional pains
-abdominal epilepsy
-urinary tract infections
-intestinal proved parasitosis
-bilious vomiting

RESULTS AND DISCUTIONS

The allotment after sex is described below (Fig. 1). Our lot included 978 children aged between 2-18 years, 59,10 % of them (578) being girls and 40,89% (400 cases) - boys. The average of patients' age is 11,51 years.

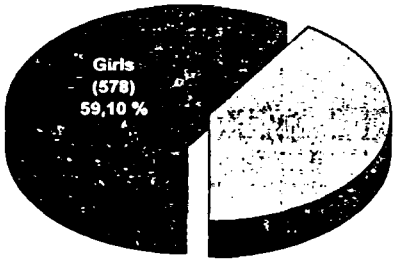

Figure 1. Allotment after sex

Allotment after age groups shows (Fig. 2):

- 2 – 6 years old: 86 cases (8,79 %)
- 7 – 12 years old: 354 cases (36,19 %)
- 13 – 18 years old: 538 cases (55,01 %)

Thus, it results that a high incidence is found at the group aged between13–18 years, probably because of the high stress at this age, as well as on account of disorganized alimentation, alcohol and coffee intake, or smoking; the data are similar to those found in speciality literature.[6,12,13,14]

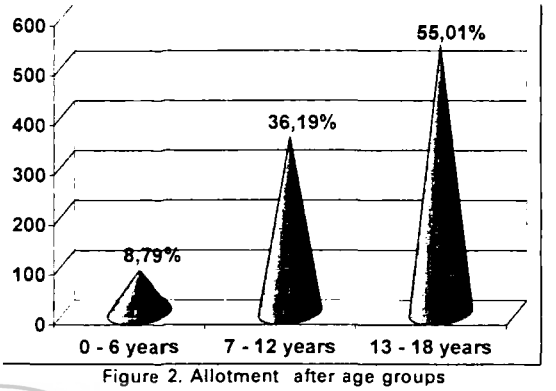

Figure 2. Allotment after age groups

We saw the following symptoms to our lot (Table I):

- abdominal pain - 515 cases (52,65 %),
-vomiting and nausea - 182 cases (18,60 %),
-anorexia - 62 de cases (6,33%),
-loss of weight - 11 cases (1,12%),
-other cases (bitter taste, heartburn, constipation, skin lesions) at 208 cases (21,26%).

The localisation of abdominal pains was different, as shown below (Table II):

-epigastric pains in 433 children (84,24% from those having abdominal simptoms)
-periumbilical pains in 35 children (6,80 % from those with abdominal simptoms)
-diffuse pains in 46 children (8,94% from abdominal pains)

It was performed a superiour digestive endoscopy with Olympus endoscop; it detected important modifications in 908 cases (92,84% from the patients).

The endoscopical findings were (Table II):

-gastric changes in 763 cases (78,01%)
-gastric and duodenal changes in 145 cases (14,82%).

Table II. Localisation of endoscopic modifications (gastric and duodenal)

Localisation of endoscopic modifications	Gastric modifications			Duodenal modifications
	Gastric Antrum	Gastric body	The whole mucosa	
Nr. of cases	620 cases (63,39%)	44 cases (4,49%)	99 cases (10,12%)	145 cases (14,82%)

The endoscopic aspect of gastric mucosa detected the following aspects, the percent of the changes being reported to the entire no. of cases (Fig. 3):

-eritemato - exsudative mucosa in 256 cases (26,17%)
-follicular, granulosum mucosa in 249 cases (25,46%)
-paving-stone appearance in 317 cases (32,41%)
-hemorrhagic mucosa in 64 de cases (6,54%)
-pale, atrophic mucosa in 23 cases (2,35%)
-other changes in 55 cases (5,62% from the lot of children)

Other associated anomalies of gastric mucosa were found. Thus, in 99 cases, different degrees of esophagitis were detected (10,12%), in 146 children were found type duodenitis changes (14,92%), and 160 patients presented gastitis caused by bilious reflux (16,36%).

The Helicobacter pylori infection was detected by Urease Rapid Test (only episodic available), associated with histopathologic examination of bioptic fragments. The Urease Rapid Test could be done in 513 cases (52,45%) and it was positive for 415 children (42,43% from the entire lot, representing 80,89% from those who could be tested).

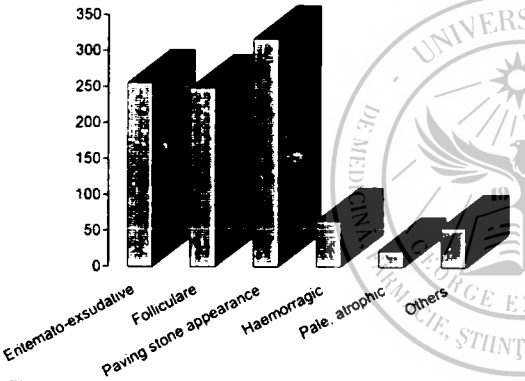

Figure 3. Distribution of different endoscopic modifications of gastric mucosa

"The gold standad" for diagnostic and evaluation of the recovery is the histopathologic examination.[1,5,7,8,18,19]

At the histopathologic examination, the following aspects were found (Fig. 4):

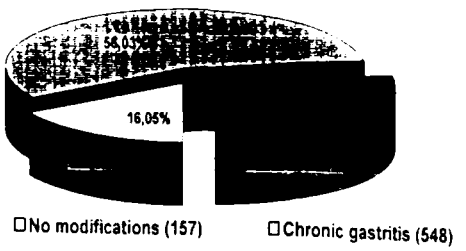

Figure 4. The histopathologic aspects of gastric mucosa

In 157 cases (16,05%) there was no change of gastric mucosa;

-chronic gastritis - lymfo-monocyte infiltrated in 548 de cases (56,03%)

-accute gastritis – neutrophils infiltrated in 273 cases (27,91%)

These data do not entirely correspond to those in literature[2,3,5,8,18] situation accountable by the fact that there could be some deficiences related to working technique.

For treatment there have been used proton pump inhibitors for 4-6 weeks.[3,8,10]

For Helicobacter pylori infection, those 415 children who have been positively detected were treated; the antiinfectious therapy included Amoxicillin and Metronidazole, or Amoxicillin with Claritromicin for 7 – 14 days.[6,12,13,16]

A number of 658 children were submitted to medical review (67,28%). The following represent their status after treatment: 497 children (75,53% from those presented to revaluation) presented no simptoms at the revaluation, and 161 children (24,46%) evinced abdominal epigastic or periumbilical pains, nausea, vomiting, decreased appetite, etc.

There were disease relapses; in 68 cases (10,33%), the Rapid Urease Test was positive again, and then the second diagram of antiinfectious treatment was started (Amoxicillin + Claritromicin) associated with proton pump inhibitors.

The clinic and histologic recovery after treatment was found in 655 cases (66,97%, with CI 63,98 – 69,87) and "p"< 0,001 (p significantly at 0,01); our data are similar to those in speciality literature.[6,9,12,15]

CONCLUSIONS

1. The new methods of diagnosis allow a better estimation of the real incidence of gastritis in pediatric pathology.
2. In our lot of 978 cases, the incidence of gastritis was greater in feminine gender (59,10%), comparative with the masculine gender.
3. By age group, higher incidence of gastritis was in the group of 13 – 18 years old (55,01%).
4. The abdominal pain was found in 514 cases (52,56 %), succeeded by nausea, vomiting in 189 cases (19,33 %), decreased appetite in 60 cases (6,13%), other simptoms having much less implication.
5. The localisation of abdominal pain was especially in epigastric region, in 433 children (84,24%) and only in 81 patients (8,28%) in other parts.
6. The endoscopic findings were especially at the gastric antrum, in 763 cases, the remaining being localised at the gastric body or pangastritis.
7. Acute gastritis were found in 27,91% cases according to histopathologic aspects and chronic gastritis in 56,03% cases.
8. The Helicobacter pylori infection can be diagnosed using Urease Rapid Test and the examination of bioptic fragments, and it was present in 415 cases (42,43%, meaning 80,89% from those who could be tested).

9. A number of 658 children came to revaluation (67,28%), the other children having probably a favourable evolution; only 658 cases from those presented to revaluation had some symptoms (24,46%).

10. The clinic and histologic recovery after antiinfectious and treatment with proton pump inhibitors was found in 655 cases (66,97%), (with CI 63,98 – 69,87) and "p"< 0,001 (p significantly at 0,01).

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Cutaneomucosal viral infections at HIV infected patients

Cristina Gîrbovan[1], O. Gîrbovan[2], Brândușa Țilea[1], Anca Georgescu[1], Carmen Chiriac[1], Adriana Martin[3]

Skin disorders can be frequent, severe and difficult to treat for patients with HIV. The aim of the present study is the evaluation of viral cutaneomucosal manifestations in HIV-infected patients. We analyzed the type of cutaneomucosal manifestations, lesions' evolution correlated to the stage of disease, and antiretroviral therapy. The retrospective study was accomplished using a group of 215 HIV-infected patients with cutaneomucosal affections; the age of patients is between 4 and 42 years, 110 patients being males and 105 being females, monitorized at the Infectious Diseases Clinic Tg-Mureş, during 2004- 2008. 120 patients (55,81%) showed herpes viruses infections : genital herpes - 10 cases, herpes simplex type infection - 110 cases; herpes zoster - 31 cases, varicella - 5 cases; molluscum contagiosum - 24 cases, human papilloma virus associated warts - 46 patients, herpetic stomatitis -15 cases, lingual leukoplakia - 5 cases, and 3 patients were diagnosed with Kaposi' sarcoma. In 130 patients (60,46%) clinical - immunological stage was category C3 and 55,34% of patients were under HAART therapy. Herpetic infections were treated with Aciclovir. The incidence of herpetic infections is raised at immunocompromised patients and the recurrence is much more frequent. Data of reference material are corroborating with high incidence of viral cutaneomucosal manifestations

Key words: cutaneomucosal viral infections, HIV

The skin is the largest and most visible organ of the body. Approximately 90% of people living with HIV develop skin changes and symptoms at some stage during the course of their disease. Skin infections (bacteria, fungi, virus, or yeast), various rashes, skin cancer, drug rashes and other drug-induced skin changes are all seen, but cutaneous manifestations which may represent the first sign of immunodepression, are frequently associated with the clinical aspect in HIV/ AIDS infection, from the very early stages. The diagnosis and early treatment of cutaneomucosal affections at the HIV positive patients lowers morbidity and improves the quality of life.

The aim of this study is to evaluate the epidemiological, clinical-evolving and therapeutical data of the cutaneomucosal viral infections at the HIV positive patients monitored and the Mures Regional Center in the period January 2004 – December 2008.

Some of our patients, with advanced HIV infection and severe immunodeficiency, presented during hospitalization two or more lesions of viral etiology.

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MATERIAL AND METHOD

The studied group was made up of 215 patients who presented lesions of viral etiology. At them, the lesions of viral origin were monitored, respectively the infections with herpes viruses, molluscum contagiosum, papilloma viruses, Epstein Barr virus infection, and human herpesvirus-8 infection. The diagnosis was established after the clinical and dermatological examination. The patients were framed in conformity with the clinical – immunological classification of HIV infection (CDC 1993), and they also were in different stages of evolution of the illness.

RESULTS

The distributed on sexes showed a dominance of the males 51,62% in comparison with the females 48,83% (Figure 1).

The average age of the patients was 21 years.

The stadialization of the infection was the following: stage C3 -130 (60,46%) patients; stage C2 – 18 (8,37%) patients; stage C1 -3 (1,39%) patients; stage B3 - 34 (15,8%) patients; stage B2 -17 (7,90%) patients, stage B1 -9 (4,18%) cases, stage A1 - 4 (1,86%) patients (Figure 2).

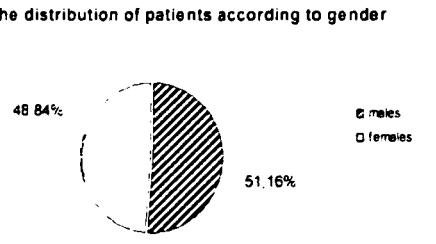

Figure 1 - The distribution of patients according to gender

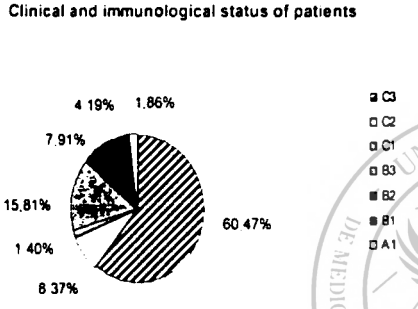

Figure 2 - Clinical and immunological status of patients

The cutaneomucosal manifestations of viral etiology were: 174 (80,93%) cases with herpetic infections, 24 (11,16%) patients with molluscum contagiosum, 46 (21,39%) patients with human papilloma viruses, 5 (2,32%) cases with oral hairy- leukoplakia, and 3 patients with Kaposi sarcoma.

A patient may present several types of cutaneomucosal manifestations during the hospitalizations. (Figure 3)

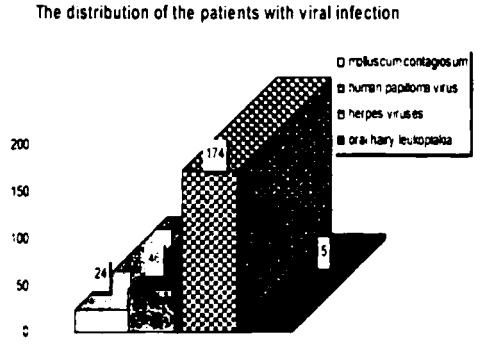

Figure 3 - The distribution of the patients with viral infection

In our group study herpes viral skin infections were: 110 (63,22%) patients presented herpes simplex located on labial and nasal areas, 10 (5,75%) cases with genital herpes, and 15 (8,62%) patients with herpetic stomatitis.

Herpes simplex virus (HSV) can produce recurrent, painful lesions on the genitals, anus, mouth, and face in people with HIV. Infections with the varicella-zoster virus appear more frequently at seropositive persons; in the studied group there were 31 (17,82%) patients with herpes zoster and 5 (2,87%) cases with varicella, predominant at females. (Figure 4)

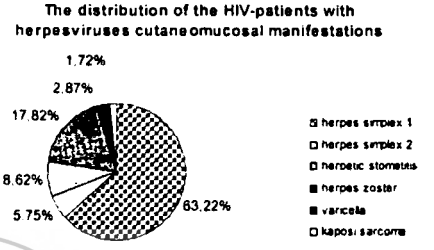

Figure 4 – The distribution of the HIV-patients with herpes viruses cutaneomucosal manifestations

The patients with viral affections were in 55,4% during antiretroviral treatment. (Figure 5)

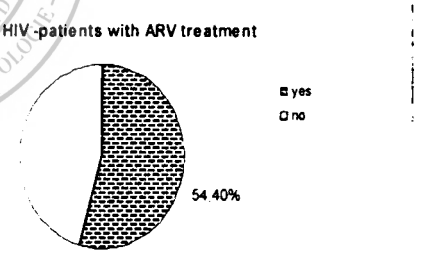

Figure 5 – HIV - patients with ARV treatment

From the studied group, 151 (70,23%) beneficiated of treatment with Acyclovir. (Figure 6)

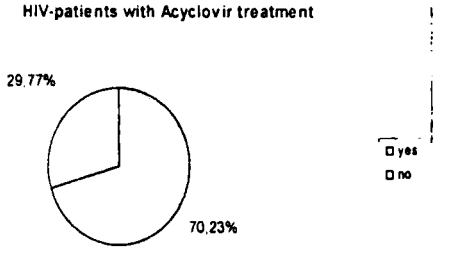

Figure 6 – HIV-patients with Acyclovir treatment

DISCUSSION

In HIV, herpes simplex may recur more frequently, take longer to heal, be more severe and widespread, and these infections have a fundamental connection to AIDS: chronic HSV lesions on the skin and mucosa are an AIDS-defining diagnosis and HSV genital lesions are believed to be a risk factor for subsequent or concurrent HIV infection.[3,4]

Shingles is another viral skin disorder that occurs in HIV-positive people. It is caused by the varicella zoster virus (VZV), the herpes virus responsible for childhood chicken pox. After a childhood episode of chicken pox, VZV is not eliminated from the body. It remains latent for years in the nervous system. Reactivation of latent VZV is more common in HIV-positive people.[9]

The patients with varicella present a greater percent of complications. We met with cases in which there were recurrences at intervals of time on different dermatomes or at the same dermatome. At a number of 15 patients, these manifestation led to the HIV infection diagnosis.[4,5]

Molluscum contagiosum is a benign viral infection, and the lesions are pimple-like which occur in both children and adults; the primary sites of involvement for adults are face, buttocks and genitals. In HIV-infected people, molluscum contagiosum lesions can number in hundreds or thousands, which is uncharacteristic of molluscum in those uninfected with HIV. In our study were not cases with an increased number of lesions. The rarity of the affection is also due to the antiretroviral treatment under which the majority of our patients were.[1]

The infections with papilloma viruses were present at 21,39% of the patients with the most frequent localizations at the level of hands and face; genitally it was located at 5 patients, 3 men and 2 women. Most often, the lesions were numerous, rebel at treatment and recurrent as long as the immunological status of the patient was deteriorated (on average the CD4 lymphocytes were 200 cells/ml at the moment of diagnosis). We also encountered cases with a spontaneous favorable evolution under the HAART treatment.[9]

OHL- oral hairy-leukoplakia, associated to Epstein Barr virus infection and considered a specific infection of the HIV disease was seen at 5 (2,32%) of the patients. The dramatic decrease of the OHL prevalence is due to the HAART therapy. Being asymptomatic it did not need treatment, it spontaneously remising at the improvement of the immune status of the patient.[8]

The Kaposi sarcoma (a rare cancer that can cause skin lesions) in whose etiology the human herpesvirus-8 is involved, was present at 3 male patients. In contrast to the classical Kaposi's sarcoma (KS) found in older men, in whom the tumors usually occur on the lower legs and feet, HIV –associated KS does not have a preferential pattern of localization. It can begin on any area of the skin, but may also appear on oral, genital, or ocular mucous membranes. Typical findings are initially solitary, or a few asymptomatic purple macules or nodules, which have a prediction for distribution along relaxed skin tension lines.[2,6]

CONCLUSIONS

HIV – infected patients with advanced disease often suffer from common skin disease, but they also may present rare lesions, unique to HIV infection.

The spectrum of skin changes in HIV infection is quite wide and with improvements in the antiretroviral treatment, the skin lesions associated with HIV infection have also changed.

Viral infections of the skin and mucous membranes are frequent opportunistic infections in HIV-infected patients.

Primary clinical infections are more frequent in the C3 stages of the illness, a fact also confirmed by our data.

The incidence of herpetic infections is raised at immunocompromised patients and the recurrence is much more frequent.

Viral infections with herpes simplex viruses were found in relatively equal proportions at both sexes, and the infection with varicella-zoster virus was predominant at females.

We met cases of patients that presented recurrences at variable time intervalson different dermatomes or on the same dermatome.

Patients infected with Molluscum contagiosum have a few number of skin lesions.

HIV-infected patients have a high prevalence of warts and they can appear anywhere on the body.

Oral hairy leukoplakia and the Kaposi sarcoma represent a revealing clinical manifestation and an unfavorable forecast factor of the HIV infection.

Despite the fact that HIV - associated opportunistic infections are less frequent in the HAART era, knowledge about these diseases and adequate treatment is still of high clinical relevance.

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Fever of unknown origin in human immunodeficiency virus-infected versus neutropenic patients

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We conducted a retrospective chart review of 49 HIV (human immunodeficiency virus) infected and 34 neutropenic patients with fever of unknown origin (FUO). Data on etiology, diagnosis and outcome were registered. Infections were the most common cause (66.63%) of FUO in the HIV infected group, followed by neoplasms (13.63%) and miscellaneous diseases (9.08%). Tuberculosis accounted for the majority of infections in this group. FUO was caused by infections such as bacterial or fungal sepsis in 42.86% of the neutropenic patient group. Empiric anti-tuberculous treatment served as the main diagnostic method in the HIV infected group, whereas the blood culture was the most useful method in the neutropenic group. The outcome of the neutropenic patients was less favorable with higher death rates in this group. The empirical treatment of the opportunistic infections and the antiretroviral therapy is necessary in the HIV infected with FUO, and the empirical antibacterial and antifungal treatment in the neutropenic patients with FUO.

***Key words**: FUO, HIV, neutropenic, diagnosis*

Fever of unknown origin (FUO) was first defined as fever higher than 38,3 degrees Celsius on several occasions, persisting without diagnosis for at least 3 weeks in spite of at least 1 week's investigation in hospital. Later a distinction was made between classical FUO and three other types, namely nosocomial FUO, neutropenic FUO and human immunodeficiency-virus (HIV)-associated FUO. The spectrum of underlying disease and the clinical approach is different in these entities. Neutropenic FUO is defined as recurrent fever above 38 degrees Celsius in a patient whose neutrophil count is 500 per mm3 or less and who has been assessed for three days without establishing an etiology for the fever. In most of these cases, the fever is caused by opportunistic bacterial infections. These patients are usually treated with broad-spectrum antibiotics to cover the most likely pathogens. Occult infections caused by fungi such as hepatosplenic candidiasis and aspergillosis must be considered. HIV-associated FUO is defined as recurrent fevers over a four-week period in an outpatient or for three days in a hospitalised patient with HIV infection. The differential diagnosis of FUO in patients who are HIV positive includes infectious etiologies such as Myocabacterium avium–intracellulare complex, Mycobacterium tuberculosis, Pneumocystis carinii pneumonia, and cytomegalovirus. Non-infectious causes are less common and include lymphomas, Kaposi's sarcoma and drug-induced fever.[4,8,10,11]

MATERIAL AND METHOD

We retrospectively reviewed the medical records of HIV-infected patients admitted to the 1st Infectious Diseases Clinic of Târgu Mureş during 1997-2006 and to the National Institute of Infectious Diseases of Bucureşti in 2005, and of neutropenic patients admitted to the 1st Medical Clinic of Târgu Mureş, Department of Haematology during 1999-2008. Charts of patients fulfilling the FUO definition were reviewed for demographic data, stage of the underlying disease, cause of fever, duration of fever and hospital stay, time until diagnosis was established, diagnostic methods and outcome. The CD4 cell count, determined by flowcytometry, the HIV viral load, determined by polymerase chain reaction, the presence of the antiretroviral treatment were noted in the HIV infected group, and the nutrophil count and leucocyte count in the neutropenic group. The statistical analysis was performed with the unpaired t-test.

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RESULTS AND DISCUSSIONS

49 HIV infected patients and 34 neutropenic patients met the criteria of FUO. The mean age was 18.87±12.51 SD years in the HIV infected group and 55.02±15.26 SD years in the neutropenic group. The lower age of the HIV infected reflected the epidemiologic characteristics of the HIV infection in Romania. There was a male predominance in both groups: 50.17% in the HIV infected group and 61% in the neutropenic group.

The underlying disease was in advanced stage in most of the cases: 89% of the HIV infected were in C3 stage, 7% in B3, 2% in B2, 2% in B1. The mean CD4 cell count was 126.48±171.99 SD / mm3. The mean viral load at the time of FUO diagnosis was 726755.28±836954 SD copies/ml. In only 7% of the patients was there an undetectable viral load. Only 27% of the patients were receiving antiretroviral therapy at the time of presentation.

The use of antiretroviral therapy seems to translate into a lower number of HIV-infected patients being admitted to the hospital with a diagnosis of FUO. When FUO does occur, it seems to preferentially affect patients with profound immunosuppression and virologic failure[6,12] (Figure 1).

In the neutropenic group the underlying disease was acute myeloid leukemia (41.17%), acute lymphocytic leukemia (11.76%), non-Hodgkin's lymphoma (11.76%), myelodysplasia (11.76%), chronic lymphoid leukemia (8.82%), chronic myeloid leukemia (2.94%), myeloma multiplex (2.94%), drug-induced agranulocytosis (2.94%), idiopathic myelofibrosis (2.94%), vasculitis associated pancytopenia (2.94%). Patients with acute leukemia undergoing intensive antileukemic treatment develop frequently FUO during the neutropenic phases[3] (Figure 2).

23.54% of the haematologic malignant diseases were in stage IV, 23.52% in stage III, 29.41% in stage II, and 23.52% in stage I (Figure 3). The mean neutrophil count was 282.65±211.01 SD/mm³, the mean leucocyte count was 1892.44±1942.6 SD /mm³ in the neutropenic group.

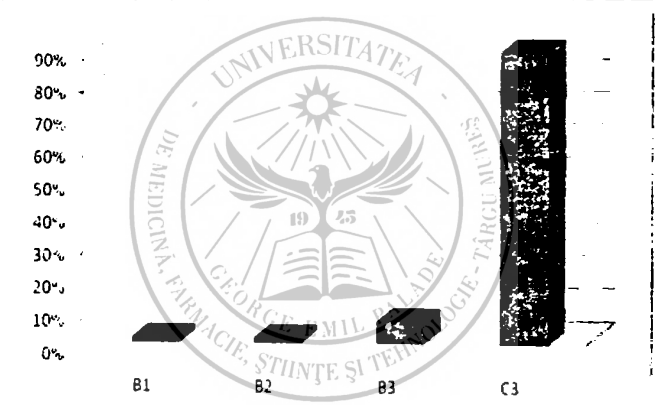

Figure 1. The stage of HIV infection in the HIV infected patients with FUO

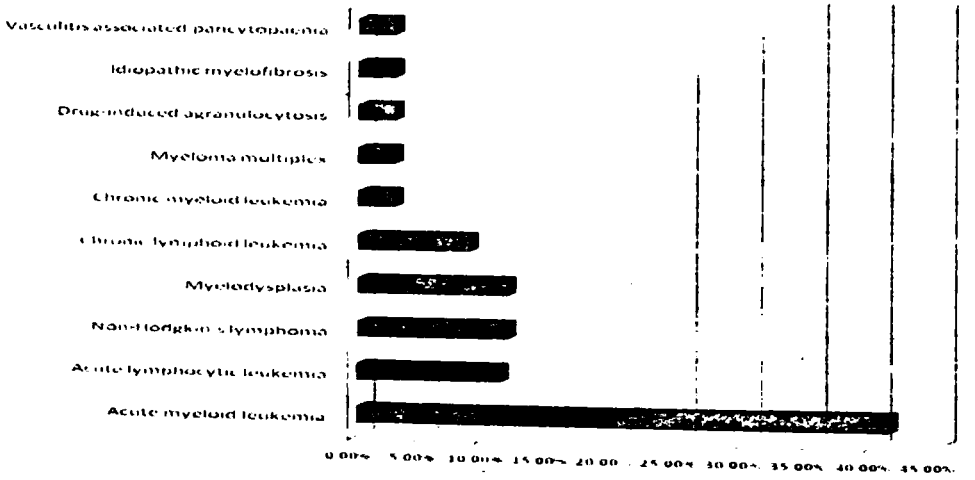

Figure 2. The underlying disease in neutropenic patients with FUO

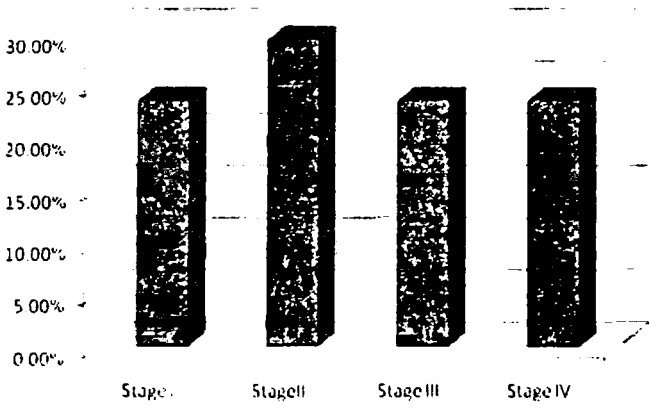

Figure 3 The stage of the haematologic malignant diseases in neutropenic patients with FUO

Table I. The causes of FUO in the HIV infected versus neutropenic patients

Causes of FUO in the HIV infected patients		Causes of FUO in the neutropenic patients	
Infections			
Tuberculosis	45.45%	Coagulase-negative Staphylococcal sepsis	14.28%
Mycobacterium avium complex	6.06%	Aspergillus pneumonia	8.57%
HIV	6.06%	Candida sepsis	2.85%
Lung abscess	1.51%	Escherichia coli sepsis	2.85%
Myocarditis	1.51%	Staphylococcal pneumonia	2.85%
Candida sepsis	1.51%	Candida pneumonia	2.85%
Cryptococcal meningitis	1.51%	Pneumonia with unknown etiology	2.85%
Urinary tract infection with Escherichia coli	1.51%	Urinary tract infection with Escherichia coli	2.85%
Klebsiella pneumonia	1.51%	Acute cholecystitis	2.85%
Neoplasms			
Lymphoma	12.12%		
Colon cancer	1.51%		
Miscellaneous			
Central fever (in the lesions of the central nervous system)	4.54%		
Immune reconstitution syndrome	3.03%		
Drug-induced fever	1.51%		
Undetermined			
	10.60%		57.14%

Infections were the most common cause (66.63%) of FUO in the HIV infected group, followed by the neoplasms (13.63%) and miscellaneous diseases (9.08%). In 10.6% of the cases the cause remained undetermined. Mycobacterial infection should be considered as a first-line diagnosis in HIV-patients with FUO. In about 10% of the cases the diagnosis cannot be determined.[1,2,6] FUO was caused by infections in 42.86% of the neutropenic patient group, in the rest (57.14%) the cause could not be established. The majority of the cases of FUO in neutropenic patients are caused by bacterial or fungal infections, but cause can be documented only in about 40-60% of the cases[4,5,12] (Table I).

The diagnostic methods that conducted to a diagnosis in the HIV infected group were the empirical anti-tuberculous therapy (22.58%), pneumological examination (18.27%), thoracic-abdominal computer-tomography (9.67%), serial chest X-rays (9.67%), lymph node biopsy (9.67%), introduction of the antiretroviral therapy (5.37%), examination of Ziehl-Nielsen smear (3.22%), cerebrospinal-fluid analysis (2.15%), haematological examination (2.15%), and empirical prednisone therapy, cytologic examination of the peritoneal fluid, cardiac ultrasound, blood culture, surgical examination, the bacteriological examination of the sputum, urine culture (1.07% each). The cause of FUO in the HIV infected is established mostly by examinations referring to the the mycobacterial infections.[2] The diagnostic methods conducting to a diagnosis in the neutropenic group were blood culture (35%), serial chest X-rays (20%), thoracic-abdominal computer-tomography (10%), pneumological examination (10%), the bacteriological examination of the sputum, urine culture, surgical examination, empirical Voriconazol-therapy, and abdominal ultrasound (5% each). Blood culture and serial chest X-rays are the most useful diagnostic methods and they must be used in cases of FUO in neutropenic patients[10] (Table II).

Table II. Diagnostic methods conducting to diagnosis in HIV infected and neutropenic patients with FUO.

Diagnostic methods conducting to a diagnosis of FUO n HIV infected patients		Diagnostic methods conducting to a diagnosis of FUO in neutropenic patients	
Methods	%	Methods	%
Empirical anti-tuberculous therapy	22.58%	Blood culture	35%
Pneumological examination	18.27%	Serial chest X-rays	20%
Thoracic-abdominal computer-tomography	9.67%	Thoracic-abdominal computer-tomography	10%
Serial chest X-rays	9.67%	Bacteriological examination of the sputum	5%
Lymph node biopsy	9.67%	Urine culture	5%
Introduction of the antiretroviral therapy	5.37%	Surgical examination	5%
Examination of Ziehl-Nielsen smear	3.22%	Empirical Voriconazol-therapy	5%
Cerebrospinal-fluid analysis	2.15%	Abdominal ultrasound	5%
Haematological examination	2.15%		
Empirical prednisone therapy	1.07%		
Cytologic examination of the peritoneal fluid	1.07%		
Cardiac ultrasound	1.07%		
Blood culture	1.07%		
Surgical examination	1.07%		
Bacteriological examination of the sputum	1.07%		
Urine culture	1.07%		

There was no significant difference between the time until the establishment of diagnosis in the two groups: 36.17±24.67 SD days in the HIV group versus 20.69±25.65 SD days in the neutropenic group (p=0.0571). The duration of fever was significantly longer in the HIV group: 44.22±37.93 SD days versus 19.68±21.40 SD days (p<0.0001). The mean hospital stay was also longer in the HIV infected group: 42.34±27.70 SD days versus 19.82±9.77 SD days (P<0.0001). 2.04% of the HIV group versus 17.23% of the neutropenic group had FUO repeatedly. The death rate in the first month following the FUO was 20% versus 34.48%, in the months 1-6 after the FUO 17.5% versus 13.79% for the HIV and the neutropenic group. The duration of fever, the mean hospital stay is longer in the HIV infected, however, the outcome is worse with a higher death rate in the neutropenic patients. Therefore the management of the neutropenics represents an emergency, empirical antibiotic treatment with beta-lactamic antibiotics associated with aminoglycosides, and possibly with antifungals is needed. The optimisation of the antiretroviral therapy and the empirical treatment of the opportunistic infections is needed in the HIV infected with FUO.[4,7,9,10]

CONCLUSIONS

FUO appears mostly in the advanced-staged HIV disease, with low CD4 lymphocyte level, high viral load, in the absence of the antiretroviral treatment. Neutropenic FUO appears frequently in patients with acute leukemia.

Infections are the most common cause of FUO in the HIV infected and neutropenics also, with the predominance of mycobacterial infections in the first, and bacterial and fungal infections in the latter. The cause cannot be determined in about 10% versus 40-60% of the cases in the HIV infected versus neutropenics.

According to the main etiology of FUO in the HIV infected, the most useful diagnostic methods are those investigating mycobacterial infections, whereas in neutropenics common methods such as blood cultures and chest X-rays are essential.

Although the duration of fever and the mean hospital stay are longer in the HIV-infected, the outcome is worse in the neutropenic group, with a higher death rate. Therefore these patients need urgent empirical antimicrobial treatment, whereas the HIV infected might need empirical treatment of the opportunistic infections, also, after proper investigations.

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Individualized, holistic therapies for nursing home residents - a study

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"House of Hope", is an Assistance and Nursing Center located in the city of Reghin, district of Mures. The facility offers, as part of the National Plan of Social Assistance, medical care and assisted living for the elderly with disabilities. At the time of admission each individual is evaluated by a multidisciplinary team consisting of a physician -medical doctor, a psychologist, a social worker, physical therapists and occupational therapists. Each discipline uses its own evaluation tool to reach an individualized program for the patient.

The goal of this individualized holistic program is the improvement of the medical status, independence and the overall well being of each individual. Every person is reassessed periodically, at least half-yearly , based on their responses to treatment and the set goals.

The combined treatment modalities consist of medication, physical therapy, psychotherapy and ergo-therapy.

Key words evaluation, reassessment individualized, holistic therapy

Healthcare for the disabled geriatric population in Assistance and Nursing Center involves a wide variety of sciences and multiple disciplines working together, adjusting their therapeutic approach based on the residents needs. The teams involved in the care of these individuals consist of an Internal Medicine specialist, a Psychologist, Registered Nurses, Social Workers, Physical Therapists, Occupational Therapists, Dieticians and Nursing Aids. Continuing education of all personnel involved in the care of this specific population extends and involves the patients themselves and their family members or paid caregivers.[1] The care involves not only preventive medicine in the geriatric population but also anti-aging medicine, early detection and treatment of old age ailments.

Modern medicine offers endless choices to maintain inner youth and to promote health above the chronologic age; the concept of biological clock that measures age is outdated. At the base of medical treatment the changes in the lifestyle should take precedent as a measure against aging. Daily physical exercise, a well balanced diet, an adequate caloric intake as well as a balanced psychological well being should precede treatment with medication. The healing and/or stabilization of chronic degenerative illnesses should follow with early cancer detection and treatment, all playing an important role in the anti-aging medicine.[4]

MATERIALS AND METHOD

In order to improve and correct outdated treatment modalities and to follow international standards of care, at "House of Hope", Assistance and Nursing Center, we applied a combined treatment plan developed by of a multidisciplinary team.[5]

The team consisted of a medical doctor, social workers, physical-therapists, psychologist and an ergo-therapist. At the time of admission, the medication was prescribed by the medical doctor, based of each individual's medical needs, and dosages were adjusted based on patient response and well being.

Physical therapy was devised based on each patient's need and treatment was received by the majority of the patient population. The occupational therapy was also construed based on each individual's need to correct or adapt to deficiencies and for the proper use of medical devices in order to increase

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independence. Ergo-therapy consists of different types of art programs geared to develop and improve independence in the activities of daily living (ADL). In our dietary approach, we tried several, different modalities throughout the study. Initially we instituted a "no added salt" diet (NAS), due to the high number of patients with hypertension and status post cerebro vascular accidents.[1] Later we modified the way food was prepared, eliminating unhealthy cooking techniques such as deep frying, toasting, or the use of animal fat altogether. To help swallowing, we mostly use soft foods, purees and jellos.[2]

RESULTS

There were 39 patients participating in our study. Of the 39, 37 were placed on different types of medication required by their medical conditions. These medication were periodically adjusted based on their particular diseases and responses to treatment. Of the 39 residents, 33 had physical therapy to maintain muscular strength, tone and elasticity using different techniques, all geared to improve balance and coordination in order to achieve the safe transfer from bed to wheelchair. Twenty-seven patients had electrotherapy, 11 hydrotherapy, 31 ergo-therapy and 23 psychotherapy (Figure 1).

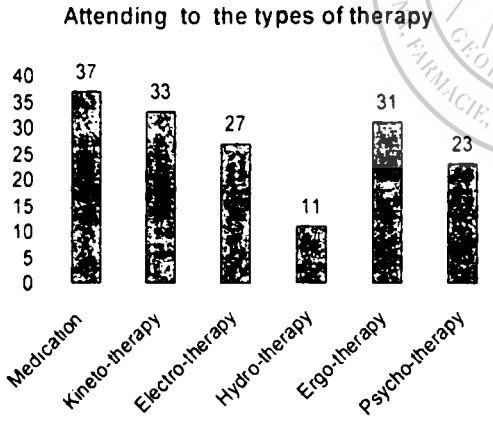

Figure 1 Attending to thye types of therapy

Some of our patients being bed-bound could not participate in ergo- therapy. These patients had extra physical therapy of passive mobilization and massage to improve muscle tone.The dietary approach was started with weighing each participant at the beginning of the study. As you can see in figure, the variant for weight loss is insignificant, except for the patients that were overweight and weight loss was one of our goals (Figure 2). To maintain and improve hydration we introduced a mandatory "10 o'clock tea".

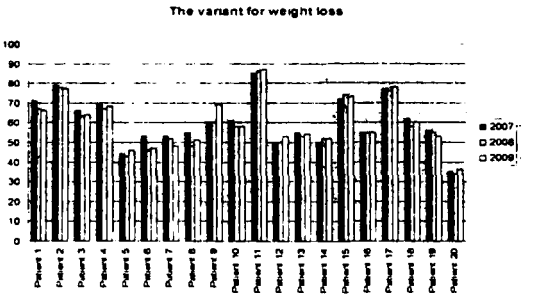

Figure 2

The results were significant, being noted by family members and caregivers alike. Twelve of our residents were status post cerebro-vascular accident with hemi paresis at the beginning of the study, regained independence, walking without the help of medical devices. Other seven of our residents gave up the use of wheelchairs, walkers, or canes and made us conclude that our individualized holistic treatment approach was working. Nine patients of the 31 participating in ergo-therapy regained their ability to eat independently. As a result of psychotherapy, the number of conflicts between residents diminished significantly and the rate of integration into the community of those newly admitted increased. During the 4th quarter of 2008 we continued our existing flu-vaccination program and, as a result, we had no influenza in our facility.

DISCUSSIONS AND CONCLUSIONS

Care provided in facilities of Assistance and Nursing Center is far superior to care at home by relatives and is a protective source of health care of the disabled elderly.[3] The therapeutic goals in these facilities take into consideration the patient's desire to get well, their co-morbidities, levels of independence in ADLs and IADLs, as well as their generalized well being. In the literature are described separate method of assessment based on daily activities or the assessment of mobilities.[2,6] In our study we have done and use one assessment file, which combine those tests and which we consider more suitable for the situation from our center to allow, initially, to stabilise individualised programme, or for reassessing to permit to monitorise the state of health and how the disease caused old age are progressing. The introduction on individualized holistic therapies based on the needs of each resident resulted in a healthier environment, giving a renewed meaning to the name of our facility " House of Hope". The introduction of the new treatment modalities were not always welcomed and easy to implement due to cultural differences, set habits, language barriers and communication difficulties with patients and their family members. With all difficulties along the way, the results were encouraging and as a result the study is ongoing. In May of 2009, we will participate in a comparative study with similar facilities in Germany and France.

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Factors influencing neonatal mortality and morbidity in premature infants with extremely low birth weight

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The aim of this study was to evaluate the demographic, obstetrical and neonatal factors that can influence mortality and morbidity of premature infants with extremely low birth weight (ELBW).
An observational retrospective study was performed in a group of 65 children with birth weight below 1000 g, born prematurelly, hospitalized during 1 January 2007 – 1 January 2009 at the Neonatal Intensive Care Regional Center, Targu Mures
There were only 39 infants out of the total 65 with low weight at birth who survived during the mentioned period. 12.3% had associated morbidity out of the total survival rate of infants at discharge from the hospital.
The frequency of handicaps among those who survived was in direct relationship with the place of birth, medical procedures at birth, number of days in Neonatal Intensive Care Unit, obstetric management, and the state of infant at birth.
Key words: premature birth, extremely low birth weight, morbidity, mortality

Approximately 12.5% of births in the United States are preterm (occurring before 37 weeks' gestation). Preterm infants with "very low" birth weight are those who weight 1500 g or less; ELBW neonates are defined as those who weight 1000 g or less.

Although they account for only 1.5% and 0.7% of live births, respectively, these infants contribute disproportionately to neonatal morbidity and to health care costs.[6]

The often complicated medical outcomes in extremely premature infants have generated discussion of the ethics of investing medical and financial resources in those infants who are on the border of viability.[6]

To facilitate more informed and better justified decisions, we assessed a small group of infants born with a birth-weight of 1000 g or less and treated at NICU Regional Center Targu-Mures, to relate gestational age and other risk factors assessable at or before birth to the likelihood of death or adverse outcomes.

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MATERIAL AND METHOD

This is a retrospective and observational study, including 65 extremely low birth weight premature infants, delivered at Obstetrics and Gynecology Clinic No. I Targu- Mures from January 2007 through December 2008, and addmited to the Neonatal Intensive Care Regional Center at the same hospital.

We also included in our study the extremely low birth weight premature infants who were delivered in non tertiary district hospitals and transferred to the Neonatal Intensive Care Regional Center after birth. We did not count the premature infant with Apgar score 0 (antenatal death), with congenital malformation, or infants who are small for gestational age (i.e. birth weight less than 1000 g but older than 30 gestational weeks).

The information was recorded and collected in a database using Microsoft Excel program and analyzed.

For the pregnant women we recorded: age, place of residence, school level, pregnancy- associated pathology (e.g. gestational hypertension, diabetus melitus, genito-urinary infections, placental pathology), antecedents of premature birth, antenatal transport to tertiary hospital, number of prenatal consultation, and obstetrical management.

For the premature infants we recorded: gestational age, Apgar score, pH from the umbilical cord artery, number of days with ventilatory suport, and number of days spent in intensive care unit until discharge or death.

We consider favorable outcome for the infant if: normal neurological exam at 40 weeks corected age, normal ophthalmologic exam, and „pass" the hearing exam.

For quantification of survivor infants' morbidity we consider the presence of at least one of following: respiratory distress syndrome with ventilatory intermitent positive pressure more than 7 days (a condition that predisposes to bronchopulmonary dysplasia), periventricular hemorrhage grades III-IV with ventricular dilatation, necrotizing enterocolitis and retinopathy of prematurity.

RESULTS

In our study 39 pacients out of 65 had survived, which means a 60% survival rate. Among the survivors, 25% had associated morbidity. 26 pacients out of 65 died after a period of 6 hours (minimum) – 57 days (maximum) after admission in the NICU. The average period of hospitalization until death for these infants was 10.6 days.

Concerning the place of birth, 21 out of the total 65 infants were admitted to the NICU after birth in the same maternity unit. Delivery was performed in a non tertiary hospital for 12 cases at a 1st level hospital, 7 cases at a 2nd level hospital and 2 cases were home deliveries. For each of the 21 cases who were admitted to the Intensive Care Unit after birth, the transportation to a tertiary level hospital was according to the standard procedure.

Concerning obstetrical management, antenatal steroid treatment was administrated in 33 cases. Out of these, 23 survived (69.7%) and 11 died (33.3%).

Regarding the route of delivery, 23 cases were delivered by cesarean section with a survival rate of 56.52% (13 infants) and a mortality rate of 43.4% (10 infants).

42 cases were deliverd vaginally with a survival rate of 62% (26 infants) and a mortality rate of 38% (16 infants).

General morbidity was 38% of all the newborns out of which 70,9 % were female babies.

A relationship between the neonatal outcome and an Apgar score of less than 7 or an umbilical cord artey pH below 7 was found in 28 cases (43%) out of which 19 (50%) had died.

Concerning multifetal pregnancy, 50% of the twins had associated morbidity and 19.2% of the twins died.

Other demographic parameters like parity, mother's age, place of residence, and marital status, had no influence on the neonatal outcome.

DISCUSSIONS

For multiple reasons, the effects of intensive care on extremely premature infants are unlikely to be determined in randomized trials. Observational studies like this one are more subject to bias, particularly at the lowest gestational ages, when intensive care is used most selectively. A population-based study is needed to verify the absence of an important effect of race or ethnic group on the outcome for extremely premature infants.

When are the burdens of intensive care justified by the likelihood of benefit? Traditional estimates of this likelihood are based on the proportion of births of infants in the highest-risk groups with a good outcome.

Because some infants die without receiving intensive care, this approach underestimates the likelihood of a benefit from intensive care.

To avoid this problem and provide an upper bound for the likelihood of such a benefit, we assessed the maximum potential benefit, assuming the same outcome among infants who died without receiving intensive care as among infants who received intensive care in the same risk category. In any risk category, the true likelihood of a benefit from intensive care is likely to be intermediate between the observed and maximum potential percentage of infants with a favorable outcome.[22]

Whether intensive care should be mandatory (i.e., given even if the parents object), optional, investigational, or unwarranted (i.e. not given even if requested by the parents) can be considered in terms of the likelihood of a benefit.[21]

In the literature, approximately 85% of infants with a very low birth weight survive to be discharged from the hospital. Within 2 years after discharge, 2 to 5% had died from medical complications related to their preterm birth.[1]

During the past decade, survival has improved, particularly in infants with extremely low birth weight, but the incidence of most short-term major medical complications associated with prematurity has remained relatively stable, despite this improvement in survival.[7,8] Because of improvements in neonatal care, the standard definition of a "neonatal death" – death within 28 days after birth — may be biased by the exclusion of continuously hospitalized infants who die after 28 days. Thus, several authors use the term "neonatal-related deaths" to refer to all neonatal deaths plus any deaths that occurred between 29 days and 1 year after delivery if the infant was continuously hospitalized.[6]

From our results, it is clear that among children who had morbidity, most were born in the center of lower level and were subsequently tranferred under appropriate conditions to a Regional Center neonatal intensive care after birth.

Extremely premature infants born in perinatal centers for high-risk infants, especially those with a high volume of such infants, have better short-term outcomes than infants transferred to such centers after birth.[2,15,18] The medical condition at birth for ELBW babies was affected when birth occurred in a lower level maternity unit, with later reffreral to NICU. Also, differences among hospitals in the level of obstetrical

care, especially the ability to perform very rapid cesarean deliveries, can result in the live birth of infants who would otherwise die in utero.[8]

Birth of ELBW children with a good Apgar score (more than 7) or an umbilical cord artery greater than 7, in which the mother had received antenatal corticosteroids was associated with a lower morbidity and mortality than in cases in which no antenatal steroid prophylaxis was performed. The better outcomes for infants who received antenatal steroids result at least in part from their use when obstetricians are committed to optimizing outcomes and were reported also in the literature.[19]

In 90% of cases we found at least two of the predictive factors for premature birth: precarious socioeconomic level, lack of proper antenatal care, history of preterm delivery, pregnancy- associated pathology, but these parameters were not significantly correlated with the prognosis of ELBW neonates.

Our study has some limitations: we quantify the morbidity indices only for IVH grade III and IV, necrotizing enterocolitis, bronchopulmmonary displasya (required additional oxygen at corrected age of 36 weeks) or RDS, which required more than 7 days support IPPV ventilator type.

Our study includes a small amount of cases and was developed over a short period of time, which does not allow us to formulate some conclusions about long-term prognosis of these children, but this will be the subject of a later study. Total days with ventilary support or total number of days of hospitalization before discharge per infant with a favorable outcome were computed as indexes of cost, resource use, parental distress, and infant suffering due to painful procedures, prolonged intubation, and such complications as intracranial hemorrhage, necrotizing enterocolitis, and recurrent episodes of hypoxia.[21]

Whatever minimum probability of a favorable outcome is judged to warrant intensive care, consideration of multiple factors is likely to promote treatment decisions that are less arbitrary, more individualized, more transparent, and better justified than decisions based solely on gestational- age thresholds.[22]

A simple Web-based tool (www.nichd.nih.gov/neonatalestimates) allows clinicians in estimating the likelihood that intensive care will benefit individual infants, after considering the extent to which outcomes in their center might differ from those we identified.[22]

In deciding whether to administer intensive care, Paris[27] contends that "The best one can do is to make a human judgment based on probabilities" but the decision whether to initiate or not intensive care for extremely premature infants is still highly controversial[25,01,26,13,14,19,21], and in some centers, intensive care is provided to all very premature infants. In our center, like in most centers, intensive care is provided selectively on the basis of specific gestational-age thresholds, with the limit at 25 week' gestational age.

CONCLUSIONS

Our study shows a strong correlation between place of birth, obstetrical management and ELBW mortality. ELBW mortality was lower in children born in level III centers, regardless of the fact that these women at risk of extreme premature birth, were submitted for admission directly to our NICU service or have been transferred to the prenatal center level III.

The delivery of such children by emergency Caesarean section for signs of fetal distress, and correct reanimation by a team of trained and experienced neonatologists is associated with better results for this ELBW babies.

Barring major therapeutic advances, our findings indicate that extending intensive care to all of the most immature infants would entail considerable suffering, resource use, and cost in order to benefit only a small proportion of infants.

Physicians should do their best to estimate and interpret these findings while counseling parents about immediate and long-term outcome.

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Invasive fungal infections in children with malignancies

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Invasive fungal infections (IFI) are becoming more frequent among patients with malignancies and represent a challenge for doctors regarding rapid diagnosis and effective treatment. The scope of our investigation was to study the etiology and favourable host factors in IFI during 2006-2008. Results: nine patients out of 105, aged 6 months-16 years, developed a total of 13 IFI episodes. The mean WBC was 1160/mm³, with ANC 330/mm³. Nine IFI episodes occurred under corticotherapy and 12 under additional chemotherapy. Candida parapsilosis *was identified from 6 blood cultures, all from central lines,* Candida krusei *from 1 blood culture,* Aspergillus spp. *from one skin lesion and disseminated aspergillosis was found in one patient who died in respiratory insufficiency. ESR was raised in 8 cases and CRP positive in 10 cases. No relationship was found between the fungi isolated from blood cultures or histological examinations and from skin or mucous lesions. The mean duration of antifungal treatment was 19 days. Conclusions: Overall incidence of IFI in our patients was 8,5%, being 17,30% in those under intensive chemotherapy. The favorable host factors for developing IFI were severe and prolonged neutropenia, indwelling central lines, corticotherapy, intensive chemotherapy. Overall mortality caused by IFI was 2,85%, from 0% in candidaemia to 66,66% in invasive aspergillosis.*

Key words: *malignancy, fungal infection*

Fungi and moulds are ubiquitos organisms living on the human skin, mucous membranes and gastrointestinal system but also in the environment on plants, trees, dry leaves, in food, thermal insulations, air conditioning systems, tap water, etc. Harmless to persons with healthy immune system, they are a threat to patients with declined immunity, causing high rates of morbidity and mortality. *Candida spp.*(85%) and *Aspergillus spp.*(1,3%) are the most common species found in human pathology. Up to 25% of infectious deaths in leukemic patients are caused by invasive fungal infections (IFI). The average mortality rate in IFI is 50%, ranging from 38% in candidaemia up to 95% in aspergillosis in transplanted patients.[16] IFI represent a challenge to doctors because of lack of fast laboratory tests with sufficient sensibility and sensivity, because of the emerging resistance to antifungal drugs and the omnipresence of serious comorbidities as severe neutropenia and malnutrition.

Aim of the paper: is to study IFI in patients treated with hematologic and oncologic malignancies at the Pediatric Clinic nr. I from Tg-Mures, in order to find relationships between invasive fungal infections and host factors like neutropenia, corticotherapy, chemotherapy, central venous catheters, as well as the major clinical signs and symptoms, laboratory characteristics, imagistic findings and outcome.

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MATERIAL AND METHODS

A retrospective analysis of the patients' clinical records and laboratory data between 2006-2008 was made.

RESULTS

During this period we diagnosed and treated a number of 105 children with malignancies, 65 with leukemia and 42 with solid tumors. Out of 63 leukemia patients 28 were newly diagnosed cases and 6 were relapses. Seven patients had acute myelo-blastic leukemia (AML) and 56 patients acute lymphoblastic leukemia (ALL). Two patients abandoned treatment and 11 patients died. In the solid tumor group, 20 were newly diagnosed cases and 2 were relapses. In this group 4 patients died. The most frequently found solid tumors were the lymphomas (n=22) followed by Wilms tumor (n=5), neuroblastoma (n=3), rhabdomyosarcoma (n=3), cerebral tumors (n=2), osteosarcoma (n=2) but there were also rare tumors like germ cell tumor and nasopharyngeal carcinoma. IFI was found in 9 pati-ents, corresponding to 8,5% of all patients and 17,8% of patients on intensive chemotherapy. Among the 9 patients with IFI, 8 suffered from malignancies (2 AML and 6 ALL) and one patient had chronic idiopathic thrombocytopenic purpura (ITP) recently treated with splenectomy. The 9 patients had a total of 13 episodes

Table I. The results of the fungigrams

Cultures	Type of fungus	Sensibility to Fluconazole	Voriconazol	Caspofungin
1 Blood	Candida parapsilosis	+	NT	NT
2 Blood	Candida parapsilosis	+	+	NT
3 Blood	Candida parapsilosis	+	NT	NT
4 Blood	Candida parapsilosis	+	NT	NT
5 Blood	Candida parapsilosis	NT	+	+
6 Blood	Candida parapsilosis	NT	NT	+
7 Skin lesion	Aspergillus	NT	+	+
8 Blood	Candida Krusei	NT	+	NT

Legend: + (sensible)
NT (not tested)

of fungal infections. All cases with IFI were in the group of hematologic malignancies, no patients from the solid tumor group experienced invasive fungal infections. The age of the patients was between 6 months and 16 years. Severe and prolonged neutropenia, splenectomy, cortisone treatment, chemotherapy, indwelling venous catheters were predisposing factors to severe fungal infections. The mean leucocyte count in leukemic patients was 1160/mm^3 (150-2800/mm^3) with absolute neutrophil count (ANC) of 330/mm^3. Much higher value was noted in the ITP patient with Candida non-albicans sepsis after splenectomy, with 20.000/mm^3 WBC (white blood cell) and 15.000/mm^3 ANC. The average hemoglobin value was 9,24 g%, whereas the platelets ranged between 7000- 647.000/mm^3 with a mean of 133.000/mm^3. Renal function tests (blood urea nitrogen, creatinin) and bilirubin were normal in all patients, transaminases showed a slight raise to a maximum of 300 U/l. Nine of the IFI episodes occurred during or immediately after cortisone treatment and 12 under additional chemotherapy. Central venous lines were present in 10 episodes of fungal infections, in 6 cases being infected with Candida parapsillosis. A raise in body temperature has been documented in 100% of IFI episodes. Fever over 39°C was noted in 2 patients, between 38-39°C in 4 patients and subfebril condition in 7 patients. Three patients experienced pulmonary symptoms such as dyspnoe, decrease in alveolar respiration, cough, respiratory insufficiency, low oxygen saturation in capillary blood. All three patients had proven or probable pulmonary aspergillosis.

Mycotic skin and mucosal lesions were found in 4 cases: *Aspergillus spp.* was isolated from a skin lesion and *Candida albicans* from throat swabs. No relationship was found between the types of fungi isolated from skin and mucous barriers and those identified from histological specimens and blood cultures. All patients with lung aspergillosis had negative blood cultures. Septic shock symptoms appeared in 4 cases.

The three patients with proven or probable pulmonary aspergillosis were investigated with lung X-ray analysis, which showed interstitial pneumonia, and in two cases computed tomography (CT) was done, which showed characteristic lesions for aspergillosis. Thirteen blood cultures were made from 9 patients and in 6 patients *Candida parapsilosis* was identified, associated in all cases with the presence of central venous catheters (Table I).

ESR (erythrocyte sedimentation rate) was increased in 8 episodes of IFI (61,5%) and reached extremely high values, namely 118 mm/h and 115 mm/h in two cases of interstitial pneumonia (probably lung aspergillosis), but remained relatively low (20 mm/h) in the patient who died from multiorgan aspergillosis. C-reactive protein (CRP) was qualitatively positive in 10 IFI episodes (76,92%). Identification of the causative fungi in the 13 episodes of IFI seen in 9 patients with haematological malignancies revealed *Candida parapsilosis* in 6 cases, all were associated with the presence of central lines; *Candida Krusei* in one blood culture and Aspergillus from a skin lesion. In one case, *Aspergillus spp.* was proved only postmortem from lung and liver histology (Figures 1 and 2). Antifungal treatment was conducted with caspofungin in 2 cases, voriconazol in 5 cases and fluconazol in 9 cases. Antifungal treatments lasted for an average of 19 days. The outcome of IFI was favorable in 6 cases and fatal in 3 cases. Significant statistical relationship has been found between the isolation of *Candida parapsilosis* in blood culture and the presence of central venous catheters (Odds ratio 10,11). We had 4 cases of *Aspergillus* infection, two proven by histological examinations and two probable lung aspergillosis based on imagistic aspects. One of the proven *Aspergillus* infection was a multiorgan aspergillosis (lung, liver) in a T cell acute lymphoblastic leukemia patient, who unusually presented severe neutropenia from the beginning of the disease (WBC 0,3 G/l and ANC 0,01 G/l) and died after 30 days of intensive chemotherapy in respiratory failure, diagnosis being made postmortem with autopsy. The other histologically proven aspergillosis was a skin infection in a relapsed high-risk ALL patient, who made a full recovery and is alive after 2 years of follow-up. Two probable lung aspergillosis were diagnosed in AML patients, one of them died in relapse, which probably was enhanced by temporary interruption of chemotherapy because of aspergillosis but the second patient recovered and is alive after 6

months of follow-up. The incidence of aspergillosis among leukemic patients was 6,15%. Our mortality rate in all aspergillus infections was 50%, and in systemic aspergillosis 66,6%.

Mortality caused by IFI was 2,85% among all patients cared for during 2006-2008, 4,61% among leukemic patients and 33,33% among patients with invasive fungal infections.

Figure 1. Lung aspergillosis, macroscopic view Lung abscesses which contain necrotic tissue and the causative pathogen, aspergillus in great quantities. Though macroscopically the abscess appears to be well delimitated, microscopic examination showed a less precise demarcation between the abscess and normal lung tissue

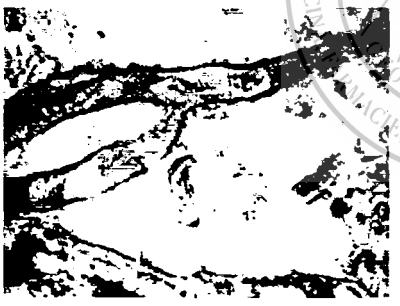

Figure 2 Microscopic view of pulmonary aspergillosis Silver impregnation has been used for optimal visualization of the pathogen. The aspergillus appears in the form of septate hyphae which ramifies in sharp angles. It is associated with tissue necrosis and acute inflammatory infiltrate.

DISCUSSIONS

Nosocomial mycotic infections in immunocompromised hosts are produced mainly by opportunistic yeasts like *Candida albicans* and *non-albicans* species (*Candida tropicalis, parapsilosis, glabrata, krusei, lusitaniae)* and filamentous fungi like *Aspergillus species (Aspergillus fumigatus, terra, flavus)*. *Candida krusei* produces endophtalmitis in leukemic patients, whereas candida glabrata appears more likely in patients with solid tumors and non-oncological diseases.[16]

Invasive candidiasis has been investigated in an intensive care unit and found that 81% of the patients were infected, 40% out of them with *Candida albicans* and 28% with *Candida parapsilosis.*[4] In our study, 66,6% of the patients were infected with *Candida parapsilosis*, all of them had central lines inserted. Mortality among patients with invasive candida infections in our paper was 0%, compared to the 45% mortality rate reported in a larger study on ICU patients.[4] This difference may be due to the excellent sensibility to fluconazol of the Candida parapsilosis species. *Candida parapsilosis* has been associated with the presence of intravascular devices and produces the lowest mortality rate (30%).[13]

Aspergillus infection is most likely located in lungs but in severe cases it may be multisystemic[6] and rarely, especially in children can be located on skin lesions.[3] Adhesion of *Aspergilus* to lung tissue is enhanced by the presence of preformed air cavities in the lung tissue, as seen in tuberculosis or sarcoidosis. The incidence of aspergillosis in our leukemic patients was 6,15%, whereas a 15-year review reports an incidence of 5,35% among AML patients,[3] another paper finds invasive aspergillosis in 14% out of the 215 patients enrolled in a study.[15] Our mortality rate in all aspergillus infections was 50%, and in systemic aspergillosis 66,6%, in a larger study (24 patients with proven or probable aspergillosis) Crassard found a mortality rate of 87%, Walmsley 74% and Abbasi 85%.[3] The prognosis of IA is very poor, uncontrollable pulmonary haemorrhage appears in 1/3 of the cases. Bronchoalveolar lavage, trans-thoracic lung biopsy, surgery are being carried out for the diagnosis of pulmonary invasive aspergillosis.[1,6] No blood cultures were positive for aspergillus in our IA patients, similar findings are reported in the literature.[3]

For diagnostic and research reasons, in invasive aspergillosis (IA) there were introduced three definitions: (1) proven IA, (2) probable IA and (3) possible IA.[1,3] Proven IA needs histological or cytopathological evidence of aspergillus hyphal invasion or positive culture from a radiologically suspect area. Probable IA is defined by positive microbiological results in a patient with favorable host factors for aspergillus infection, such as severe neutropenia, fever refractory to antibiotic treatment, corticosteroid treatment, previous fungal infections, chemotherapy, severe immunodeficiency. Microbiological criteria include sputum or bronchoalveolar lavage culture positive for *Aspergillus* or two blood samples positive for galactomannan aspergillus antigen. Possible IA is defined as the presence of favourable host factors but lacking the clinical and microbiological features.[3]

Fever of different degrees was present in 100% of our patients, close findings were found by Crassard, 90% of his patients had fever.[3]

C-reactive protein (CRP) was qualitatively positive in 76,92% of our IFI episodes, Crassard found positive results in 95,83%.[3]

The mean antifungal treatment in our patients was 19 days, in other studies varied between 11 days[4] and 26 days.[3]

Treatment of IFI can be prophylactic and curative. For prophylactic reasons it is recommended to avoid building areas, to use air filters with regular monitoring of the filters for spores, to avoid rooms where carpets are being cleaned with aspirator. It is adviced not to keep flowers in the patient's room and frequent hand washing. Judicious use of antibiotics, avoidance of long hospitalizations, exclusion of fresh vegetables, cheese with noble must, raw salamis from the alimentation of immunocompromised patients may help preventing IFI.[5,6] For curative reasons, we have antifungal drugs, but it is very important to maintain a proper nutrition and to reduce the neutropenic period. Antifungal drugs can be from the class of polyenes (amphotericin B), azoles (fluconazole, voriconazole, itraconazole, posaconazole, ravuconazole) and echinocandins (caspofungin, micafungin, anidulafungin).[15,16] Amphote-ricin B (AmB) is a fungicide drug which binds the ergosterol in the cellular membrane of the mould, disintegrating the cell. AmB is available in 4 pharmaceutical forms: AmB deoxycolate, lipid complex AmB, colloidal AmB and liposomal AmB.[15] Their therapeutic effects are the same but liposomal AmB is less toxic and also very expensive. Their oral biodisponibility is very poor (5%), so per oral suspensions can be used only for the prophylaxis and treatment of superficial mucosal mycoses. The intravenous AmB precipitates in normal saline solution, so it has to be dissolved in 5% glucose solution and perfused in a central vein to prevent phlebitis. In liver damage dose reduction is not necessary, but in renal impairment this is mandatory because of the renal toxic effect of the drug. It is used in the dose of 0,5-1 mg/kg in intravenous infusion for at least 6 hours. In a large study conducted over 15 years, AmB was the most frequently used drug in invasive aspergillosis (92%) with a duration of the treatment between 2-131 days (mean 26 days). In aerosoled form it can prevent pulmonary aspergillosis but not other localizations of aspergillus infection and does not improve survival.[3]

Fluconazole is fungistatic by decreasing ergosterol synthesis, essential for the integrity of cellular membrane of the mould. It is active against *Candida albicans* but ineffective for *Candida glabrata and krusei*, the latter showing intrinsic resistance to fluconazole, not to mention *Aspergillus*. It is very well absorbed from the gastrointestinal system, is excreted by the kidneys and penetrates in the cerebrospinal fluid.

Itraconazole has an erratic intestinal absorbtion, is effective against *Aspergillus*, is metabolized and excreted by the liver.

Voriconazole is well absorbed orally, is bound in 70% to serum proteins and metabolized in the liver. It is active against *Candida* and *Aspergillus species* but not against zygomycetes. Hepatotoxicity is its major side effect. The intravenous form contains cyclodextrine, which is toxic for the kidney and consequently it is not advised to be used for long periods of time or in case of renal impairment with creatinine clearance below 50 ml/min.

Posaconazole has proven its efficacy in the prevention of IFI, especially aspergillosis in AML and myelodysplasic patients.[2,8]

Echinocandins are fungicide drugs by inhibition of mould wall synthesis. Caspofungine is efficient against *Candida* and *Aspergillus species*.

Surgical intervention for resection of aspergilloma from the lung is a definite treatment choice.

The treatment of IFI is an emergency, microbiological results cannot be waited for.

The Clinical Practice Guidelines for the Management of Candidiasis updated in 2009 recommends for the treatment of invasive candidiasis in neutropenic patients an echinocandin (caspofungin, micafungin, anidulafungin) or lipid formulation of amphotericin B (LFAmB) started within 24 hours after a positive blood culture and continued two weeks after blood cultures become negative. Follow-up blood cultures should be obtained every other day until they no longer yield yeast. All patients with Candida sepsis should undergo ophtalmological evaluation for the detection of a possible candida endophtalmitis.[10]

Several studies have been made regarding prophylactic and curative treatment for invasive aspergillosis. A double-blind trial on prophylactic voriconazole versus placebo during induction chemotherapy for AML proved its efficacy though the study was stopped prematurely, when another trial demonstrated reduced mortality by antifungal prophylaxis with posaconazole, thus rendering randomization against placebo unethical.[14] Another study proved better outcome in IA with voriconazole treatment (52,8%) than with amphotericin B (31,6%).[11] Caspofungin has been approved by the FDA for treatment of patients with IA who have refractory disease or are intolerant to other therapies.[9] In a review of 4 clinical trials involving patients with proven or probable IA, 5 out of 12 patients (41,7%) treated with caspofungin as first line therapy had favourable responses. Another study of 32 patients with hematologic malignancy and neutropenia showed an overall response rate in 18 of 32 patients (56%) with proven or probable invasive pulmonary aspergillus infection.[12] Micafungin has also been evaluated in IA and appears to be an effective treatment.[7]

One of the major problems in IFI is the lack of rapid specific and sensitive diagnostic procedures. Blood cultures need several days to become positive, in some cases of IFI such as IA, blood cultures stay negative. Clinical suspicion is essential, lung imagistic methods (X-ray, CT) may show halo-sign or nodular infiltrates. Histopathologic examination confirms the diagnosis. In order to improve diagnosis, molecular biology PCR method (polymerase chain reaction) are being introduced. Detection of galactomannan antigen for invasive aspergillosis and 1,3 beta-D-glucan PCR-EIA (enzyme immunoelectrophoresis assay) for candidemia have a sensibility of 90% and specificity of 97%.[15]

CONCLUSIONS

1. During the period 2006-2008 out of a number of 105 patients with hematological and oncological diseases, 9 patients (8,5%) had IFI. This percentage was 17,30% among patients who underwent intensive chemotherapy.

2. IFI appeared only in haematological malignancies and after splenectomy for ITP and was not noted in solid tumor patients.

3. The favorable host factors for developing IFI were severe and prolonged neutropenia, indwelling central lines, corticotherapy, intensive chemotherapy.

4. The most frequently isolated yeast from blood cultures was Candida parapsilosis, which preserved its sensibility to fluconazole.

5. Aspergillosis appeared in 4 patients (16,15%), out of which in two cases the infection was proven by histological methods and in another two cases was probable based on pulmonary imagistic findings.

6. Aspergillus was demonstrated in a skin lesion in one patient with relapsed high-risc ALL.

7. Mortality caused by IFI was

a. 2,85% among all patients cared for during 2006-2008

b. 4,61% among leukemic patients

c. 33,33% among patients with invasive fungal infections.

8. We did not have any deaths among the patients infected with Candida parapsilosis but in aspergillosis the death rate was 66,66%.

9. The availability of rapid PCR tests for the assessment of IFI may have a positive effect upon the early diagnosis and more successful treatment of IFI in immunocompromised patients.

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Simultaneous tension-free mesh abdominal wall repair and extraanatomic ilio-femoral crossover bypass

O. Jimborean, Ş. Bancu, Gina Şuruianu

We performed an original technique in 6 high-risk patients who presented both peripheral critical ischemia of a limb and contralateral inguinal hernia. All these patients presented postmyocardial unstable angina and congestive cardiac failure, thus indication for extra-anatomic bypass. Associated disease: contralateral inguinal hernia. Technique: 1.Retroperitoneal approach through herniotomy; 2.Donour anastomosis between vascular graft and iliac artery; 3.Continous suture in tension-free manner of the polypropylene mesh; 4.Contralateral passage of the vascular graft through the mesh, 5 Distal anastomosis to the contralateral femoral artery. We use this technique in other 4 patients without inguinal hernia in order to prevent the graft strangulation, especially when an autogenous vein is used. Postoperatively: 2 late above-knee amputation:1 after 22 months for graft trombosis, 1 after 46 months for late prosthesis infection. Conclusion: the original technique intended to correct simultaneously 2 surgical diseases using the crossover ileo-femoral bypass through the tension-free mesh; the technique was succesfully exteded in patients without hernia. The preffered vascular grafts were the autogenous veins.
Key words: extra-anatomic bypass, critical limb ischemia, tension-free mesh

Based on the data of the literature, where there was demonstrated that 2 different surgical procedures are feasible, we performed a new approach which combines these different operations into one single: extraanatomic ilio-femoral crossover bypass and tension-free mesh abdominal wall repair.

MATERIAL AND METHOD

We applied an original surgical technique in 6 patients with the following features:

1. Comorbidity:

-unilateral critical limb ischemia with unilateral iliac artery occlusion

-left inguinal hernia:

-contralateral to the ischemic limb

-ipsilateral to the donour iliac artery

2.High-risk patients :

-postmyocardial unstable angina

-congestive heart failure

3. Indication for indirect arterial reconstruction (extra-anatomic bypass)

4. The need of a short, not shocking potential operation:

-spinal anesthesia

-contralateral iliac artery as donour source beyond the concomitant ipsilateral hernia (concomitant hernia repair in tension-free abdominal wall closure)

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In the period 2002 – 2006 we selected 6 patients, all males between 53 – 73 years old; all of them had critical limb ischemia due to unilateral iliac artery occlusion and femoro-popliteal occlusions. The only chance to avoid major amputation was to performe an arterial bypass to the profunda femoris of the affected leg in 5 patients and a prolonged femoral bypass for 1 patient.

We proceeded to an original technique which had the principle to transfer the arterial graft from the donor iliac artery through the tension-free mesh inserted for abdominal wall repair after the cure of hernia. The technical steps were the following: 1. retroperitonel approach through herniotomy + the cure of the hernial sac (Figure 1).

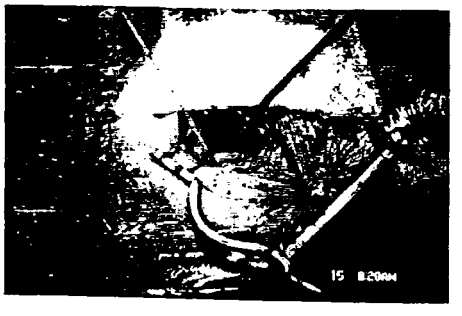

Figure 1. Retroperitoneal approach through herniotomy + the cure of the hernial sac

2. Donour anastomosis (termino-lateral) between vascular graft and iliac artery (Figure 2);

Figure 2 Donour anastomosis (termino-lateral) between vascular graft and iliac artery

3. Declamping donour iliac artery;

4. Abdominal wall repair with continous suture in tension-free manner of the polypropylene mesh (Figure 3);

Figure 3 Abdominal wall repair with continous suture in tension-free manner of the polypropylene mesh

5. Hole in the mesh for the passage of the arterial graft from retroperitoneum to the surface (Figure 4);

Figure 4 Hole in the mesh for the passage of the arterial graft from retroperitoneum to the surface

6. Retroperitoneal drainage + declamp → reclamp the arterial graft above the mesh before the completion of the mesh suture;

7. Subcutaneous suprapubian contralateral passage of the vascular graft to the contralateral femoral artery;

8. Distal anastomosis to the contralateral femoral artery +/- profundoplasty (Figure 5);

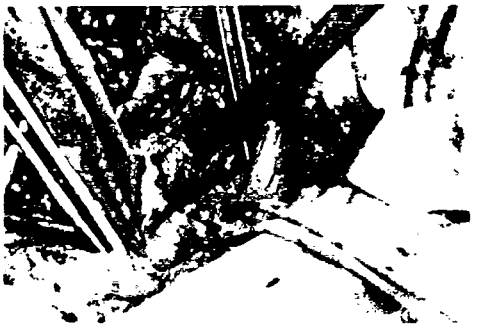

Figure 5 Distal anastomosis to the contralateral femoral artery +/- profundoplasty

9. Autogenous vein through the mesh.

We operated another 4 high-risk patients without hernia in the same manner in order to prevent the strangulation of the arterial graft in its passage from the retroperitoneal space toward contralateral subcutaneous tissues.

All the 10 patients had invalidant ischemia (2 patients in stage III Leriche, and 8 in stage IV Leriche)[5,7] The arterial graft was Dacron in 3 patients and pTFE (polytetrafluorethylene) in 1 patient. The other 6 grafts consisted of reversed autogenous veins. In one patient the donour artery was represented by a permeable branch in its iliac segment after an aorto-bifemoral bypass.

RESULTS AND DISCUSSIONS

We had late complications in 2 patients who lead to above knee amputations: 1 late thrombosis (22 month), 1 late prosthesis infection in the groin. All the other patients had good evolution without invalidant ischemia or hernial relapse after 2-6 years postoperatively.

The bypass had to be extra-anatomic because of the high-risk associated diseases (postmyocardial unstable angina and congestive heart failure).[1,2,3] We choosed the iliac artery as donour source in order to accomplish two goals: to avoid the contralateral Scarpa triangle (for a femoro-femoral crossover bypass) because of the high potential of infectious postoperative complications,.[9] The other goal was to correct simultaneously an inguinal hernia, ipsilateral to the donour iliac artery. In order to perform a surgical treatment in a single time for 2 concomitant diseases in these high-risk patients we used a benign spinal anesthesia.[4,6,8]

CONCLUSIONS

1. Our original technique is feasible in patients with comorbidities (critical limb ischemia and inguinal hernia ipsilateral to the iliac artery used as donour source for a crossover ilio-femoral extra-anatomic bypass;

2. This technique allows the simultaneous surgical treatment of 2 diseases in high-risk patients in a short operative time using a benign spinal anesthesia.

3. The technique is also feasible and may be routinely used for this type of extra-anatomic bypass even in patients without inguinal hernia. We consider that the use of the passage of the arterial graft through the mesh can avoid the strangulation of the arterial graft. This is more important when using the great saphenous vein as autogenous graft (our prefferate arterial graft in peripheral vascular surgery) (Figure 6);

4. We prefer the iliofemoral extra-anatomic bypass (crossover) instead of the classic femoro-femoral bypass: the groin is the site of choice for the early and late complications (lymphorheea, infections).

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Isoflurane pre- and postconditioning associated cardioprotection – interferences with prior anesthetic regimen in the rat heart in vivo

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Volatile anesthetics emerged as important cardioprotective agents in experimental models of ischemia/reperfusion injury through pharmacological pre-/postconditioning. Ketamine eliminates the protective effect of ischemic preconditioning. The present study was aimed at investigating the effects of two doses (high-H and low-L) of Ketamine, 80 mg/kg and 20 mg/kg, on Isoflurane induced cardioprotection. Anesthetized rats with H/L-Keta and subjected to regional ischemia followed by reperfusion were randomized to receive: no additional intervention; preconditioning with Isoflurane (prior to ischemia) and postconditioning with Isoflurane (at reperfusion). Myocardial infarct size was determined by the triphenyltetrazolium staining. Preconditioning with Isoflurane in presence of H-Keta significantly reduced myocardial injury, albeit to a lesser degree than in animals anesthetized with L-Keta, vs. the Ctrl group. Anesthetic postconditioning associated protection in L-Keta group was eliminated in H-Keta group. In the in vivo model of myocardial ischemia/reperfusion in rat hearts, administration of high- but not of low-dose Ketamine abolishes cardioprotection induced by Isoflurane postconditioning.
Key words: Isoflurane, preconditioning, post-conditioning, ischemia/reperfusion

Myocardial ischemic/reperfusion (I/R) injury remains a major public health burden and cardioprotection defined as the totality of both non-pharmacological and pharmacological interventions aimed at preventing the reperfusion injury, still represents a stringent necessity.

The two mechanical strategies widely investigated over the past years as major endogenous mechanisms of protection in the settings of experimental and clinical I/R of the heart are *ischemic preconditioning (IPreC)* and *postconditioning (IPostC)*. Discovered more than two decades ago[4], IPreC represents a powerful mechanism of endogenous adaptation to sub-lethal episodes of ischemia that results in constant anti-infarct protection against a subsequent prolonged lethal ischemia in every species tested. The more recently described phenomenon of myocardial IPostC is defined as a series of brief mechanical coronary artery occlusions and reperfusions that applied at the very onset of post-ischemic reperfusion elicits a protection similar to IPreC.[9]

Volatile anesthetics emerged as important cardioprotective agents in both experimental models of ischemia/reperfusion injury and humans with coronary artery disease through pharmacological pre- and postconditioning.[6,7] However, in experimental settings interferences with other anesthetics used in surgical preparation were reported. Racemic ketamine, was reported to block ischemic preconditioning associated cardioprotection in the in vivo rabbit hearts[2] and isolated rat hearts.[1] The present study was aimed at investigating the effects of ketamine on cardioprotection associated with Isoflurane pre-and postconditioning in the in vivo rat heart model of ischemia/reperfusion injury.

MATERIAL AND METHODS

All investigations were performed according to the Guide for the Care and Use of Laboratory Animals (US National Institute of Health no. 85-23, revised 1996) and were previously approved by the Institutional

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Committee of Ethics and Deontology of University of Medicine and Pharmacy in Timisoara. Data are presented as mean ± S.E.

Animal Preparation

Male Sprague-Dawley rats (250-350 g) anaesthetized with either low dose - 20 mg/kg or high dose - 80 mg/kg of Ketamine intraperitoneally were intubated and ventilated. The left carotid artery was dissected for continuously blood pressure monitoring and a standard electrocardiogram (DII) was recorded from subcutaneous limb leads. Rectal temperature was recorded and core temperature was maintained at 37-38°C. Left-sided lateral thoracotomy was performed in the 4th intercostal space, the pericardium was opened and a 5-0 suture was placed under the left coronary artery. The ends of the suture were threaded through a propylene tube to form a snare. The coronary artery was occluded by tightening the ends of the suture by a pair of clamps.

All animals underwent 30 minutes of ischemia and 120 minutes of reperfusion. Coronary occlusion was confirmed by the hemodynamic response (typical fall in blood pressure, the ECG aspect (ST elevation) and the appearance of ventricular arrhythmias after the first 8 - 10 minutes of occlusion. Reperfusion of the artery was initiated by loosening the snare and was confirmed by visualizing epicardial hyperaemia. At the end of the experiments, hearts were excised and the area at risk and the infarct size were determined by the means of triphenyltetrazolium staining.

Experimental protocol.

Animals (n = 6-8/group) were randomly assigned to one of the following groups: 1) Control group (Ctrl) - no other intervention; 2) Isoflurane preconditioning (Iso-PreC) - preconditioning by three episodes of 5 min isoflurane 2.1% interspersed with three 10 min washout episodes; 3) Isoflurane postconditioning (Iso-PostC) - 5 min postconditioning with isoflurane 2.1% started 3 min prior to and administered for 2 min within reperfusion (Figure 1). This protocol was used when either high- (H, 80 mg/kg) or low-dose (L, 20 mg/kg) ketamine was applied for background anesthesia.

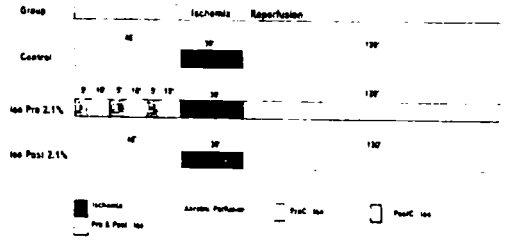

Figure 1 Outline of the experimental protocol. For explanation of abbreviations see the above text.

RESULTS AND DISCUSSIONS

Preconditioning with Isoflurane in presence of H-Keta significantly reduced myocardial injury (infarct size = 25+/-7%; mean +/-S.E), albeit to a lesser degree than in animals anesthetized with L-Keta (19+/-7%), vs. the Control group (51+/-8%; $p<0.05$). Anesthetic postconditioning associated protection in L-Keta group (28+/-8%; $p<0.05$ vs. Ctrl) was eliminated in M-Keta group (43+/-8%; p NS vs. Ctrl), (Figure 2).

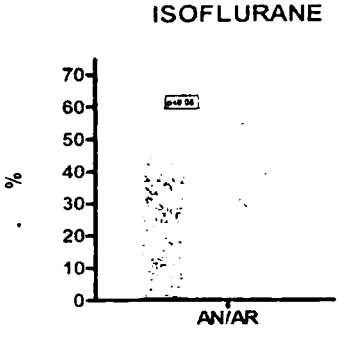

Figure 2. Infarct size - No anti-infarct protection of postconditioning with Isoflurane

In our hands the 5 minutes postconditioning protocol was not able to elicit cardioprotection in the presence of high-dose Ketamine. These results are in agreement with the findings described for the pre- and postconditioning protocols with Sevoflurane in the same experimental settings (Hentia C., unpublished results).

Racemic ketamine (in association with xylasine) is widely used as background anaesthesia in experimental models of acute in vivo ischaemia/reperfusion injury in rodents. The first papers reporting the observation that ketamine abolished cardioprotection elicited by ischemic preconditioning in rodents either in ex vivo[1] or in vivo[2,3] models can be traced back to 2001. These authors recommended the use of S(+) ketamine enantiomer (not available at us yet) in experimental settings in order to accurately assess the protective effects of conditioning strategies. We have shown that ketamine interferes not only with the ischemic preconditioning protocols but also with protection elicited by anesthetic postconditioning. This observation may be of clinical relevance since pharmacological postconditioning is nowadays considered as potential therapy in association with revascularization procedures.

The cardioprotective properties of volatile anaesthetics are known already for more than two decades[6,7], but their in vivo interferences with other anaesthetics are only partially described. Accordingly, further studies demonstrating in animal models in vivo all possible influences are required before speculating on possible associations in clinical settings.[8]

CONCLUSIONS

In the in vivo model of myocardial ischemia/reperfusion in rat hearts, administration of high- but not of low-dose Ketamine abolishes cardioprotection

induced by Isoflurane postconditioning. However, before transferring the results obtained in a peculiar animal model to the clinical more complicated situation, a cautious outlook is warranted. Further characterization of the in vivo interferences between currently used anesthetics and their dose dependency in other species will certainly contribute to our understanding of their use in targeted clinical situations.

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Clinical effects and the morphopathological aspect of the trans - scleral cyclo-photocoagulation with the laser diode in various forms of advanced painful glaucoma

Aurora Béres

The work proposes a retrospective evaluation of all the cases of advanced painful glaucoma hospitalized in the Emergency Hospital „Fogolyan Kristof" from Saint George, Covasna County during 2001-2009. I observed clinical effects such as painful syndrome, visual acuity and intraocular pressure on a short and medium period, respectively morphopathological changes of the trans-scleral cyclo- photocoagulation.
Key words glaucoma, trans-scleral cyclo- photocoagulation, intraocular pressure, visual acuity

Trans-scleral cyclo- photocoagulation is a therapeutic intervention which reduces the production of aqueous humor by the partial dysfunction of the ciliary body .It represents an alternative in anti-glaucoma therapy, generally used when the filtered anti-glaucoma therapies can not decrease the intraocular pressure and consecutively the pain. Trans-scleral cyclo-photocoagulation is a therapeutic method whose clinical effects appear relatively quickly, with the disappearance of the pain even from the first post operational day and the unexpected decreasing of the intraocular pressure in the first week, and the stabilization of the results one month after the intervention.

Indications

Using „test G" in trans-scleral cyclo-photocoagulation = TSCPC is indicated in secondary neo-vascular glaucoma, secondary post uveitis glaucoma, in glaucoma appeared during the childhood and secondary glaucoma appeared after vitreous retinal interventions. It is also used in uncompensated glaucoma after the filtered operations and in secondary glaucoma of the young adult, as well. Using „test G" makes possible the cure of the new cases of glaucoma in the underdeveloped countries where it proved to be a rapid practical procedure, well tolerated with good results in decreasing the intraocular pressure. The parameters of the cure have been changed since 1991. Currently many surveys are attesting the efficiency of the cure on 270 degrees. Some authors use big power and short duration (2,5W, 1s), others use small power and long duration (1,25W, 4s). It seems that it is needed 4-6 J for each application – the number of them being 20 -30.

Emergency Hospital Dr Fogolyan Kristof Sf-Gheorghe

MATERIAL AND METHOD

In January 2000 – April 2009, a retrospective survey was accomplished on all the cases of advanced painful glaucoma hospitalized at the Ophthalmology Department of the Emergency Hospital „Fogolyan Kristof" from Saint George. During this period 103 TSCPCs were made on 94 patients (the cyclo-photocoagulation was repeated at 2 patients, at 1 patient it was made on both eyes, and in 1 case it was made on both eyes, repeated then on one eye again). These 103 cases of glaucoma of different etiologies represent 1,08 % of the total of the hospitalized cases during this period. The maximum prevalence of the advanced glaucoma was registered in the 5th, 6th and the 7th decade. From the diagram it can be noticed the obvious repartition of the cases aged over 50 – 83% (Figure 1).

Figure 2 indicates us the prevalence of the secondary advanced painful glaucoma.

The patients were aged between 15-91 (the average 63 years old), 44,67 % coming from the urban area and 55,33 % from the rural area. The larger incidence of the cases coming from the rural area is bounded to the difficulties of diagnosing and hospitalizing the ocular affections and the metabolic and cardiovascular affections which lead to ocular affections. Men are more frequently affected - 61,7% (58 cases) – than women - 38,3% (36 cases). TSCPC was made by laser Diode/

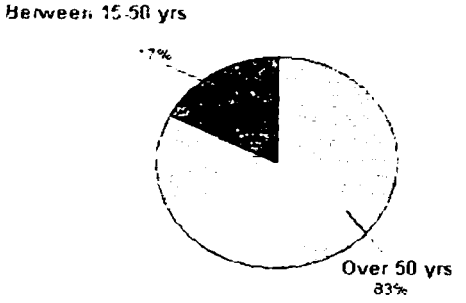

Figure 1 The percentage of the patients aged over 50

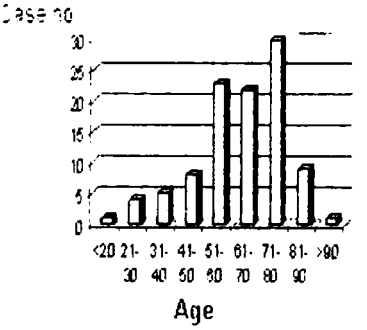

Figure 2 Prevalence of the advanced glaucoma

IRIS Medical OcuLight SLX 810 nm, equipped with a scleral applicator G-PROBE. After a previous retro bulbar anesthesia with xiline 4%, 4 ml were inoculated with 23-25 impacts on 180 degrees superior or inferior at 1,5 mm of the cornea scleral limb. The power varied between 1750-2500mW, the time between 1800-2000 ms and it was limited by the acoustic phenomenon „pop".

Visual acuity, intraocular pressure by applanation, bio-microscopic examination of the anterior pole and where it was possible of the posterior pole as well, ocular echography were made at all the patients. During the examination, the presence / absence of the ocular pain, the visual acuity and the intraocular pressure were under observation. Regarding the statistics, I expressed visual acuity in decimal fractions such as:

- pmm=0,006
- bright perception with good localization = 0,002
- bright perception without localization = 0,001
- without bright perception

Table I presents the percentage repartition and the numeric value of the cases with different values of visual acuity. Fig. 3 suggests the percentage proportion of visual acuity. This statistics indicates that 43,7% of the patients did not have bright perception, 3,9% perceived the light but the localization was confuse or some of them did not perceive it at all, 9,7% perceived the light making a good localization, 20,4 % perceived the movement of the hand, 21,4% had a view under 0,1 (could count fingers at a few meters far) and 0,9% could see over 0,1.

Table I. Percentage of cases with visual acuity problems

AV	No of cases	%
0=fpl	45	43,7
0,001=pl-	4	3,9
0,002=pl+	10	9,7
0,006=pmm	21	20,4
under 0,1	22	21,4
over 0,1	1	0,9
total	103	100

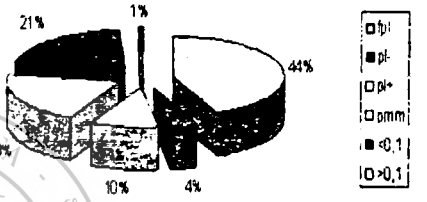

Figure 3 The percentage report of visual acuity

The measurement of the intraocular pressure ran according to the following scheme:

-before the intervention: P0
-after the intervention: P1 5 hours after the intervention
-P2 1 day after the intervention
-P3 1 month after the intervention
-P4 3 months after the intervention
-P5 6 months after the intervention
-P6 1 year after the intervention

The ocular examination was completed by a general examination, being more insistent on cardiovascular affections and making a metabolic and complete cardiovascular balance at all the patients. I could examine histopathology only at 2 eyes – at those patients who reached enucleation. It was used the technique of including in paraffin and of making serial sections of 6 microns, colored van Gieson and hematoxiline eosin.

RESULTS

Pain

The patients did not have any pain starting with the first post operator day, in 8 cases the pain relieved. I observed that in those cases the pain persisted but with very low intensity so I had to repeat the trans-scleral cyclo- photocoagulation (Table II).

Among those 8 cases where the pain persisted: 1 case reached the enucleation in 1 case the pain was remitted in 6 cases TSCPC were repeated

- in 5 cases it disappeared
- 1 case reached the enucleation

Table II. Occurence of pain

Pain	Lack of pain	Bearable pain	Pain appears again after the first CPC	Pain appears again after the second CPC
Number of TSCPC	95 cases	8 cases	6 cases	1 case
%	92,23%	7,76%	5,82%	0,97%

Unfortunately one week after TSCPC, 1 patient was hospitalized complaining unbearable pains even though IOP =30 mmHg and, at his request, the ocular globe was removed.

Repeating the cyclo- photocoagulation on 180 degrees, 5 patients from 7 were relieved of pain. 1 patient announced persistent unbearable ocular pains even after the second intervention although the intraocular pressure decreased to 36 mmHg, fact that determined the ocular globe removal.

Intraocular Pressure

IOP decreased significantly after the intervention (Figure 4).

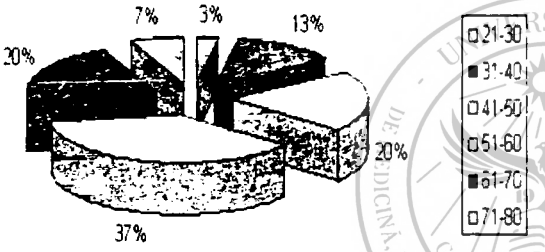

Fig.4 Percentage variation of the IOP before TSCPC

Visual acuity

One year after the intervention, in 87 cases (92,55%) visual acuity remained unchanged, in 7 cases (7,45%) it got worse : 2 of them reached enucleation of the ocular globe and at the other 5 it could be observed a decreasing of their eyesight due to the advance of the cataract.

Anti-glaucoma medication

Before hospitalization all the patients had got maximum therapy in other ophthalmology departments:

-topic: mixed therapy +

-general therapy: Ederen and transfusions with Manitol

Those who were hospitalize in our department got the following therapy scheme:

1. topic beta-blocking
2. Ederen 4 times 1 pill/day

After TSCPC we proceeded the following dosing:

1. atropine 1%, twice/day – 1 week
2. corticosteroid instillations, 5 times/day - 1 month
3. topic beta-blocking, twice/day
4. general analgesics, in case of necessity
5. Ederen 3 times 1 pill/day, only during hospitalization

At the first examination (after 1 month) I concluded to stop dosing the corticosteroid, the treatment consisting only in topic beta-blocking

At the second examination (after 3 months) the anti-glaucoma medication was reduced (only to 1 drop) or even suspended.

Complications

- conjunctive hyperemia was observed in 18 cases (17,5%) (Table III).

Table III. Complications

Complications	No. of patients	%
Conjunctive hyperemia	18	17,5
Keratic precipitates	7	6,8
Deformation of pupil	1	0,9
Uveal ectropion	10	9,7
Cataract	5	4,85
Hypheme	5	4,85
Atrophy of the ocular globe	0	0

- previous uveitis with Tyndallize phenomenon in the anterior chamber was observed at all the cured patients, and precipitate on endothelium was found in 7 cases (6,8%)

- uveal ectropion: 10 cases (9,7%). posterior synechiae

- in 4,85 % of the cases it could be observed the progress of the cataract, 1 year after the intervention: 5 cases

- deformation of pupil caused by: only 1 case (0,9%)

- hypheme of different degrees: 5 cases (4,85%)

- it was not registered any case of ocular globe atrophy, chronic hypotonic and retina displacement

- exacerbation of the pain - 7 cases (6,8%) – from which in 1 case it had been proceeded to enucleation at the patient's request before TSCPC was repeated, and in 6 cases the procedure was repeated on the other 180 degrees, after which 5 patients became asymptomatic (the pain ceased), and 1 patient reached enucleation.

DISCUSSIONS

As far as the statistics made by us shows, depending on etiopathogens, 56,4% of the cases (58 TSCPCs) were determined by the complications given by total or partial occlusions of the retina's central vein and of diabetic retina. Sometimes the secondary neo-vascular glaucoma was the consequence of the interaction of the mechanism of the two affections mentioned above generating severe ocular hypoxia responsible for its very severe evolution. Retina venous occlusions and diabetic retinopathies are the most important causes in

the apparition of neo-vascular glaucoma; that is because they determine a circulatory slow in large areas at the level of the previous pole enough to determine the production of important quantities of vessel forming factors.[4C] The hypothesis of synthetizing vessel forming factors at the ischemic posterior segment level nowadays is supported by most of the authors relying on clinical and experimental proofs. The identification of vessel forming and vessel protecting confirms the clinical data.[4C]

Arterial occlusions are less susceptible to be the cause of neo-vascular glaucoma, because in these situations there is a total absence of irrigation at the level of the respective segment, provoking cellular death, in which case the vessel forming substances can not be synthetized any longer. Nevertheless there are authors who support the idea that cellular death induced by ischemia occurs only at the level of internal layers of the retina, the hypoxic external retina being also susceptible of delivering vessel forming factors in the arterial obstructions.[4C]

The rest of the cases had old traumas as etiology, primary glaucoma absolute stage, pseudo-exfoliative glaucoma, post-inflammatory glaucoma etc (Table IV).

Table IV Complications

Etiology	No. of cases	%
Hyperglycemia	32	31,05
TVCR	26	25,25
Absolute primary glaucoma	21	20,38
Posttraumatic glaucoma	12	11,65
Post-inflammatory glaucoma	5	4,85
Pseudo-exfoliative glaucoma	2	1,95
OACR + DZ	2	1,95
Old retinal detachment	1	0,98
Sec gl. after kerato-plasty	1	0,98
Sec gl + cornea degen.	1	0,98

The risk of bleeding in the anterior chamber is higher in the case of neo-vascular glaucoma. Ophthalmoscope examination, when it is possible, highlights retina affection which is responsible of neo-vascular glaucoma occurrence.

According to Brown, in 208 cases of neo-vascular glaucoma, a retina ischemic pathology could be relieved on 97% of the patients.[11]

Not taking into account aetiopathogens, the clinic aspect is similar, with vessels of neo-formation which develop at the level of the big or small arterial circle of the iris developing towards the camerular angle.[2,4C]

According to Wand[50,51], 4 stages of iris neo vascularization can be described: incipient, moderate, advanced, secondary glaucoma:

- Incipient iris neovascularization is characterized by dilated capillaries at the edge of the pupil; the camerular angle is not affected.

- Moderate iris neovascularization, in which neo-formation vessels are disposed radial towards the periphery of the iris, at the level of the coleret, is characterized by a dilated vessel disposed circumferentially; the camerular angle is not affected.

- Advanced iris neovascularization – neo-formation vessels appears at the level of iridous cornea angle; neo vessels start from the big arterial circle of the iris, cross the scleral spur, branch at the level of the trabeculum; at the level of the angle goniosynechyae are formed.

- Neo vascular glaucoma - the camerular angle is completely obturated through ring shaped goniosynechyae; the pupil is deformed with ectropion of the iris pigmentary foil.

Taking into account the affection of the camerular angle, Weiss and Gold[52] propose another staging:

- Stage1 – characterized by discreet vascularization at the level of the pupil edge and angle, branching at its level in less than two quadrant.

- Stage 2 - characterized by the apparition of neo formation vessels in more than two quadrants of the camerular angle.

- Stage 3 – moreover, it can appear uveal ectropion, neovascularization of the iris root and goniosynechyae in 1 to 3 quadrates.

- Stage 4 – the signs mentioned in stage 3 are present in all the 4 quadrates.

Neo formation vessels have thin irregular walls and are located on the surface of the iris, fact that makes them easily visible.[18] A microscopic examination relieves the very different aspect of these vessels from the normal irian vessels which have a thicker wall presenting an adventice which is often duplicated at exterior by a conjunctive gland , both of the parts being separated by a lax tissue which permits the iris to move.[16,36] The presence of this tissue - absent at the level of the neo vessels - explains the frequent bleedings caused in its evolution.

Neo formation vessels are contained in a conjunctive neo formed membrane which causes uveal ectropion at the level of the pupil and the closing of the irido- corneal angle. At the level of the neo formation vessels it is registered a constant chronic inflammatory reaction.[1C,4C]

The positive diagnosis is given on the bio microscopic and gonioscopic examination which reveals the presence and the extent of the neo formation vessels at the level of the iris and the camerular angle. In more advanced stages at the level of the camerular angle it can be marked out more or less large gonyosinechyae. Visual acuity is much reduced - sometimes limited to the perception of light - and ocular pressure is up at the respective eye. In 1995 Leoni[32] studied AV after TSCPC on patients who had a good AV before the treatment and he realized that in 86,9% of the cases it remained stable. Our results and of other authors' do not show any considerable modification of the AV. Some other cases of chronic ocular hypotonic were also described: IOP<5 mmHg lasted more than 3 years. These cases appeared when the TSCPC procedure was repeated. Lima

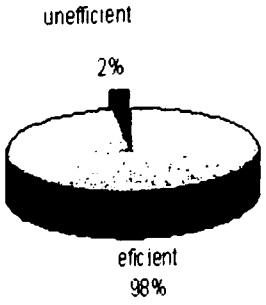

Figure 5 CFT-TS efficiency

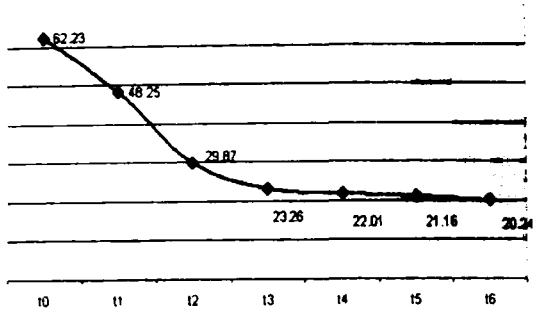

Figure 6. IOP variations timeline

(1996)[11] describes us a similar case at a patient suffering of neo vascular glaucoma. I did not find any case of chronic ocular hypotonic in my study. Ocular globe atrophy was described by

Lima 1,2% and Carassa 2,8%[13,14] after TSCPC was made on patients, 2 years after the intervention. I did not find any case of ocular atrophy in my study. According to Bandello[2], iris neovascularization can be detected much earlier by AGF than by bio- microscopic examination. The previous pole AGF can highlight the neo formation vessels at the level of the iris and of camerular angle even from the beginning when the bio-microscopic examination does not point out the neo vessels yet. On one hand, the irian vessels stop the colorant diffusion at the level of the aqueous humor; on the other hand, a very discreet colorant diffusion at the edge pupil can be frequently considered insignificant. Regarding the neo formation vessels, the colorant diffusion in the anterior chamber is fast and it begins early. The comparison with the congener eye can be useful in appreciating the incipient changes. Abnormal fluorescence of the iridous corneal angle can be revealed with a blue filter and a gonioscopy lens.[37] Histopathology was made on two enucleated eyes which previously supported TSCPC procedure but the pain did not cease fact that caused the removal of the ocular globe.

CONCLUSIONS

Transscleral cyclo- photocoagulation is an efficient method for decreasing intensity of the pain, intraocular pressure, anti-glaucoma medication. From 94 patients who were subjects for 103 TSCPCs, only 2 patients (2,12%) reached enucleation of the ocular globe (Figures 5 and 6).

In the simple cases TSCPC does not change AV.

Patients with secondary neo-vascular glaucoma present a high risk for complications especially bleeding after TSCPC. It is a method which can be also used in the secondary glaucoma at children and young persons.

It can be made even with minimum conditions of surgery,it does not need hospitalization. The procedure can be repeated after 1-2 months.

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A few aspects regarding children anesthesia outside the surgery room

Simona Marinescu

Anesthesia outside the surgery room for various invasive or non-invasive procedures under continuous extension is performed outside the „homelike environment" the anesthesiologist is familiar with, representing a real challenge. The societies of this category worldwide make efforts to standardize the conditions and formalities for anesthesia outside the surgery room with support from the Romanian Society of Anesthesia and Intensive Therapy (SRATI). Within the „regulations related to the activity of anesthesia and intensive therapy" there are mentioned the conditions of instrumentation necessary in „stations outside the surgery unit" or during the intra-hospital transport. The existent studies in this field contain few approaches regarding anesthesia outside the surgery room. Therefore I considered the presentation of my work referring to children anesthesia outside the surgery room to be helpful in this context.

Key words: *anesthesia outside the surgery room*

Along with the lately explosive development of diagnosis and therapy related works performed within various hospital departments or outside the hospital (day surgery center or private medical rooms); anesthesia also had to become extended to the level of these locations, representing "anesthesia outside the surgery room", meaning "anesthesia provided at alternative sites".

This phenomenon was possible due to the progress made in medical technology, in the development of monitoring devices or in the development of anesthetic drugs (LAURITO CE, quoted).[2]

Nowadays about 20% of the total amount of anesthesia is carried out outside the surgery unit[1] and surgery intercessions in USA may reach up to 70%.

The main advantages are medical (less medicines, faster recoveries) and economical, the costs being 25-75% lower than in hospitals.

Patients who reach to various hospital departments for such intercessions, may suffer of all kinds of chronic or acute diseases which cover all ASA risk degrees from 1 to 5; therefore the anesthetist has a big responsibility, being the main manager of these works.[3] He is responsible for the screening, evaluation, information and preparation of the surgery, therapeutic or exploring action.[4]

At the same time in case of anesthesia outside the surgery room, the rate of complications, morbidity and decease may increase due to the monitoring difficulties and/or lack of cooperation between the teams involved.[1]

Children anesthesia outside the surgery room therefore results as "one of the biggest challenges of clinical therapy" (R.S. LITMAN)

MATERIAL AND METHOD

In this work, among the children anesthesias carried out outside the surgery room, I selected the ones performed for computer tomography (CT) and nuclear magnetic resonance (RMN), which represent the majority of procedures (Figure 1) achieved outside the surgery room.

For a number of 179 children with ages between 2 weeks and 17 years (Figure 2), of different sexes (Figure 3), coming from various hospital units or from home (Figure 4), in whose case CT and RMN have been carried out, we performed various methods of anesthesia, monoanesthesia or combined anesthesias with Cloral Hydrate, Misazolam, Fenobarbital, Ketalar, Dormicum per os, intrarectal, intranasal, intramuscular or intravenous, in bolus, in adequate doses (Figure 5).

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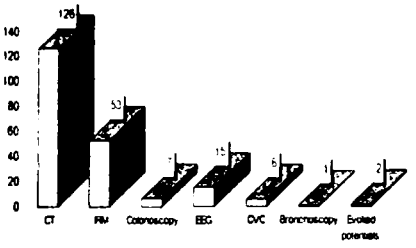

Figure 1. Represents the value weight of various procedures which needed anesthesia outside the surgery room from the total amount of the carried out procedures.

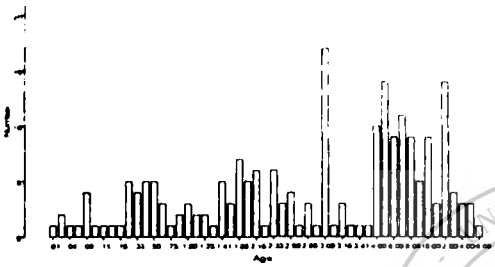

Figure 2. Distribution of submitted patients according to their age for anesthesia outside the surgery room.

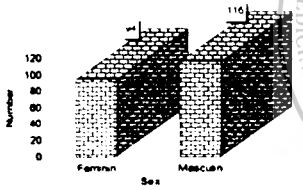

Figure 3. Distribution of studied patients according to their sex.

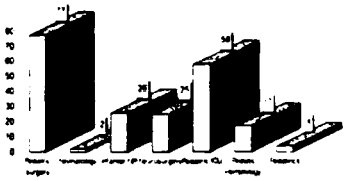

Figure 4. Origins of patients who had paraclinical investigations.

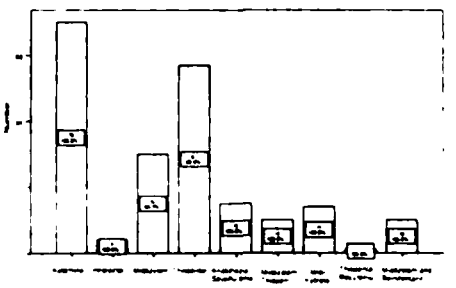

Figure 5. Weight value of various methods of anesthesia achieved outside the surgery room

RESULTS

Since the CT and RMN procedures for a good examination need a serious administration of sedatives, the favorite methods I used were the intravenous ones, in agreement with the international protocol.

1. For CT

* Midazolam 0,1 mg per kilo body weight + Fentanyl 2 mg per kilo body weight – 10 patients

* Propofol 1-2 mg per kilo body weight + Fentanyl 2 mg per kilo body weight – 12 patients

* Thiopental i.v. 3-4 mg per kilo body weight – 29 patients

* Ketamine 1 mg per kilo body weight + Midazolam 0,1 mg per kilo + Atropine 0,01 mg per kilo body weight – 51 patients

Ketamine may also be used intramuscularly.

*Propofol in bolus + continuous PEU – 7 patients

*Cloral hydrate for children under 1 year – 9 patients

*Metohexital 1-2 mg per kilo body weight – 0 patients

2. For RMN

* Propofol i.v. in bolus, continued with administration of shots – 13 patients

* Intrarectal cloral hydrate for children under 2 years – 10 patients

* Ketamine + Atropine + Midazolam intramuscular or intravenous in 10 minute repeated boluses if necessary – 42 patients

* Propofol + Ketamine, associated with various advantages – 5 patients

* Propofol + Fentanyl (with caution to apnea) – 5 patients

* Ketamine + Fentanyl also with caution to apnea – 0 patients

* Thiopental intravenous for brief procedures – 27 patients

3. The Main Complications:

* insuficient administration of drugs;
* laringospasm,
* bronchospasm,
* dolayed awarening

DISCUSSIONS

The administration of sedatives for anesthesia outside the surgery room to children may be conscious or profound. The conscious administration of sedatives supposes order responses, reflex preservation, without the necessity of a complex monitoring. The profound administration of sedatives to the patient when he has no response to orders or verbal stimuli, does not preserve reflexes of the breathing tract, needs a complex monitoring.[1,3-7,10,12,16,18-19,22,25,28]

Our medical team has been composed by a specialist physician, an Anesthesia – Intensive Therapy (ATI) resident physician, an anesthetist nurse and a sister.

Our instrumentation contained a stretcher with Oxygen bottle, equipments for IOT, emergency kit with drugs and materials for resuscitation, EKG monitor, transport air blower.

The team and equipment correspond to the minimal international standards.[4,6,13,15,28]

The pre-anesthetic examination was carried out inside the medical room or inside a room of the radiology section during the day of procedure performing, being a routine examination.

These kind of shortages are frequently reported in related literature.

The anesthesia methods corresponded to the procedure needs (CT, RMN) of profound administration of sedatives including limited monitoring procedures (in lack of nonferromagnetic monitors, therefore only the medical experience was important.

In our casuistic (similar to international readings) of all procedures, the most requested are CT and MRI, and the most frequently used drugs for anesthesia are Ketamine and Thiopental 70% and 57% (Figure 1).[16,20,22,23,28]

The complications arisen (Figure 6) were related to insufficient administration of sedatives, laryngospasm, bronchospasm and delayed awakening.[27,28,32]

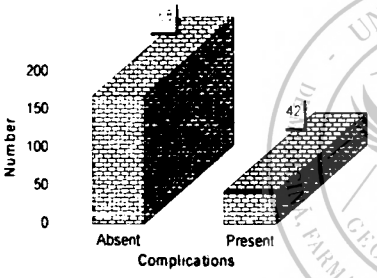

Figure 6 Rate of all type complications arisen in case of anesthesias performed outside the surgery room.

CONCLUSIONS

We will try to use our observations for the elaboration of some guide books dedicated to anesthesia outside the surgery room.

In romanian literature, the published studies are extremely limited, compared to the growing needs.

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Ethiology of urinary infection in patients with urolithiasis

Bianca Tudor [1], Felicia Toma[1], R. Boja[2], V. Osan[2], Teodora Cighir[2]

More than 600.000 patients are seen in the emergency department in the United States each year due to urolithiasis and the complication of this disease

To determine incidence of urinary tract infection in patients with urolithiasis, this study was conducted at Urology Unit of Mures county Emergency Hospital over a period of 4 months from December 2008 – March 2009. In this study fifty patients (50 patients) with urolithiasis were included, some exclusion criterias being taken into account.

Patients included were above 14 years old, with age range between 26-74 years. Out of 50 patients with urolithiasis , 37 patients were having urinary infection disease.

So the frequency of urinary infection in patients with renal stone disease was 74%.

The commonest organisms isolated according to culture report were E.Coli, Pseudomonas spp, Enterobacter spp, Klebsiella spp.

In conclusion urinary tract infection with certain bacteria place an important role in the synthesis of renal stone. Also there is a clear correlation between the bacteria that colonises the urinary stone and microorganisms which are responsible for urinary infection, which is proven in an undergoing study.

***Key words**: urinary stone, urinary tract infection, microorganism*

Intensive investigation of urinary tract infection has been carried out for past three decades in an attempt to define more accurately the epidemiology, pathogenesis, natural history, treatment and prevention of these infections.[2]

The evidence of urinary calculi has been found in a 7000 years old Egyptian mummy.[11]

With its multifactor etiology and high rate of recurrences, urinary tract stone disease provides a medical challenge. It is roughly estimated that the annual incidence of stones amounts to around 200 per million inhabitants in Europe and North America.[12]

Numerous risk factors responsible for or contributing to stone formation have been identified including environmental, metabolic, dietary, racial, gender, obstructive uropathology and infection of urinary tract.[8] In view of the high risk of recurrences in patients with stone disease, it is necessary to identify risk factors that might be of etiological importance and thus get some clues for predicting the further course of the disease.

The objective of this study was to investigate the correlation between urolithiasis and urinary tract infection, stone formation, chemical composition and urine culture.

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MATERIAL AND METHODS

This cross sectional study was carried out on consecutive 50 patients, both from outside of surgical unit and emergency department of Urology Unit - Hospital Targu-Mures, over a period of 4 months from December 2008 - March 2009. Both male and female patients with urinary stones were included, while patients with lower urinary tract stone disease, those with renal failure, renal tumour, ESWL extraction stone and previous history of renal stones were excluded.

A specially designed proforma, containing general information about the patient, urinary symptoms and signs, was filled out for every patient included in this study. After clinical examination every patient was investigated in this manner.

Urine Analysis

The morning urine's midstream was collected in sterilized containers, after a rigurous cleaning of the external genital organs. The urine analysis was performed using Uric 3.V strips, Uricon Biotech Korean.

The bacteriological analysis of the urine (uroculture, the isolation and identification of microorganisms, antibiogram) was performed using regular bacteriological methods.

Bacteriuria was not accompanied by leucocyturia, the presence of more than 2 bacterial species being the ground of asuming a urine contamination during emission.

Imaging

The plain abdominal film was the first imaging test in the evaluation of the patients with urinary symptoms. Ultrasonography was advised, with emphasis on urinary tract system, for the detection of hydronephrosis, site and size of radiopaque shadow, parenchymal thickness and structural abnormalities.

Blood Analysis

For the detection of renal functions blood urea, serum creatinine and serum electrolytes analysis were performed. Tests for serum calcium and serum uric acid were performed in order to identify hypocalcaemia and hyper-urecaemia.

Data Analysis

The data analysis was computer based and software SPSS v.10 was used. The different statistical tests used for data analysis were range, ratio, percentage, proportion, mean, and median.

RESULTS AND DISCUSSIONS

In this study over a period of four (4) months, consecutive 50 patients with urinary stones were studied.

The patients had ages between 26-74 years. Mean age of patients with urinary stone was 58,4 within median age of 58 years, while mean age of patients with urinary infection disease was 51,4 years and median age was 55,6 years respectively.

There was a slight female preponderence in patients with renal stone disease (29 women compared to 21 men, with a ratio of 1:1,4).

The incidence of positive urocultures in our study group was 74% (37 out of 50 cases).

The commonest microorganisms isolated according to culture report were : *Eschericia coli* (35%); Enterobacter spp (30%); *Pseudomonas aeruginosa* (20%); *Staphylococcus saprophyticus* (10%); Klebsiella spp(5%).

Alteration in urinary pH also plays an important role in the synthesis of renal stones, because in certain stones, the pH is specific.[1] Urinary pH was high (>7) in 21 cases, while in 29 cases the urine was acidic (4-5). Also, haematuria was present in 29(58%) cases, both macroscopic and microscopic haematuria. Associated bacterial pyurea was present 29(58%) of cases and crystallurea in 23(46%) of cases.

Chemical analysis of urinary stones revealed that 20% were calcium stones, 40% oxalate stones, 40% urate stones.

Out of 50 patients with urinary stone disease, 37 patients were having urinary infection.

In 94,4% of cases stones were unilateral.

In 67% of cases, the stones were on the left side, while 33% were on the right side, and bilateral were present only in 5,6%. Similar observations were made by Bucholz et al, which showed that most stones were in the left kidney.[5]

Urinary tract infection is one of the most common types of infectious diseases encountered in the practice of medicine today. It is one of an important cause of morbidity and mortality in the subjects, affecting all age groups across the life span. Intensive investigations of these infections have been carried out for the past three decades in an attempt to define more accurately the epidemiology, pathogenesis, natural history, treatment and prevention of these infections.

There are many intrinsic (such as urinary obstruction and pregnancy) and extrinsic risk factors (such as catheterization and other invasive procedures), which are leading causes of urinary tract infections.[8,9]

Urinary tract obstruction is one of the main causes of urinary tract infection, which produces the favorable environment for the growth of urinary tract pathogens. The presence of stone is one of the main causes of urinary tract obstruction.[2]

T.Ogata *et al* performed a study in which renal stones were mostly seen in the 3rd and 4th decades of life.[9] The results are similar to our study in which majority of patients with renal stones were between 30 - 40 years.

Naber revealed that the urinary tract infection was present in 74%of cases, while the observed frequency of renal stones in this study was 18,98% in patients with urinary tract infection. Huchereiter W showed a frequency of 59 % of infection associated with kidney stones.[6,7]

There is controversy regarding the role of urea splitting microorganisms in formation of renal stones.[4]

In this study 13,2% of cases had radiological evidence of Staghorn stones. P.Rieu has reported 2-3% of staghorn stones.[10]

Recent findings lead to more theories as to how infection leads to stone formation. Clinical suspicion should initiate studies directed at imaging the upper tracts as the source of recurrent as persistent urinary infections. Without removing the stones the infection will not clear. Further investigation is necessary to improve the outcomes and optimize prevention of patients with infections and urinary stones.[1]

CONCLUSIONS

Our study revealed the following aspects :

-urinary infection frequency was higher in patients with urolithiasis, and slightly higher in women compared to men.

-the preponderant ethiology of urinary infections in patients with urolithiasis was given by the following microorganisms: *Escherichia coli*, Enterobacter spp., *Pseudomonas aeruginosa*

-we consider important the need of a further study to demonstrate the importance of urinary infection as a factor in the formation of renal stones.

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Changes in uterine circulation in the first and second trimester of pregnancy

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Many studies demonstrated that the process of implantation plays an important role during the subsequent evolution of pregnancy. In this process, the trophoblasts has a key role by invading the spiral arteries of the miometrium, thus causing a decrease in vascular resistance in uterine arteries. The goal of this study is to investigate the utero-placental circulation for developing screening methods for early diagnosis of high-risk pregnancies. We followed up pregnancies in the first and second trimester. Biometry, morphology and utero-placental circulation were studied with ultrasound. Statistical analysis was performed with Student's t-Test. With the progress of pregnancy we found that the impedance to flow shows a significant decrease in the uterine arteries. This decrease in the impedance to flow is significant between the end of the first trimester of pregnancy (12th+/-1 week) and 17th+/-1 week; insignificant between 17th+/-1 week and 22th+/-2 week; and significant between 22th+/-2 week and the end of the second trimester. After 24 weeks of pregnancy the protodiastolic notch disappeared in both uterine arteries only in about 2/3 of subjects.
Key words: uterine arteries, Doppler ultrasound, notch, placenta

The blood supply to the uterus comes mainly from the uterine arteries (with a small contribution from the ovarian arteries). These vessels anastomose at the cornu of the uterus and give rise to arcuate arteries that run circumferentially round the uterus.

Many studies demonstrated that the process of implantation plays an important role during the subsequent evolution of pregnancy. Physiological modification of spiral arteries is required to permit the ten-fold increase in uterine blood flow which is necessary to meet the respiratory and nutritional requirements of the fetus and placenta. Trophoblastic cells invades spiral arteries and these are converted into uteroplacental arteries (which has a dilated and tortuous lumen, a complete absence of muscular and elastic tissue, no continuous endothelial lining, mural thrombi and fibrinoid deposition).

This conversion of the spiral arteries to uteroplacental arteries is termed 'physiological change'. It has been reported to occur in two stages: the first wave of trophoblastic invasion converts the decidual segments of the spiral arteries in the first trimester and the second wave converts the myometrial segments in the second trimester. As a result of this 'physiological change', the diameter of the spiral arteries increases from 15–20 to 300–500 mm, thus reducing impedance to flow and optimizing fetomaternal exchange in the intervillous space.

Impedance to flow in the uterine arteries decreases with gestation. The initial fall until 24–26 weeks is thought to be due to trophoblastic invasion of the spiral arteries. Increased impedance to flow in the uterine arteries is associated with increased risk for subsequent development of pre-eclampsia and intrauterine growth restriction. Women with normal impedance to flow in the uterine arteries constitute a group that have a low risk of developing obstetric complications related to uteroplacental insufficiency. The early diastolic notch disappears between 20 and 26 weeks. If it persists, the pregnant is at risk for developing pregnancy-induced hypertension.

MATERIAL AND METHOD

The goal of this study is to investigate the uteroplacental circulation for developing screening methods for early diagnosis of high-risk pregnancies. We used color Doppler ultrasound to assess flow in the uterine artery at the point where it crosses the external iliac artery, which is a more reproducible examination. The impedance to flow was calculated using the following:

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Table I Changes of resistance index (R.I.) and pulsativity index (P.I.) in the uterine artery at the first and second trimester of pregnancy

	11-13 week	16-18 week	20-24 week	>24 week
R.I	0.721+/-0.081 (V:11.33%)	0.594+/-0.076 (V:12.91%)	0.581+/-0.066 (V:11.43%)	0.484+/-0.061 (V:12.62%)
P.I.	1.146+/-0.197 (V:17.24%)	0.862+/-0.159 (V:18.53%)	0.829+/-0.131 (V:15.81%)	0.645+/-0.106 (V:16.55%)

Table II. Changes of resistance index (R.I.) and pulsativity index (P.I.) in the arcuate arteries at the first and second trimester of pregnancy.

	11-13 week	16-18 week	p
R.I	0.478+/-0.095 (V=19.9%)	0.421+/-0.109 (V=25.95%)	p<0.05
P.I	0.643+/-0.172 (V=26.83%)	0.548+/-0.194 (V=35.47%)	p>0.05 (p=0.055)

Table III The prevalence of the protodiastolic notch (P.N.) in the uterine arteries at the first and second trimester of pregnancy.

	11-13 week	16-18 week	20-24 week	>24 week
presence of P.N. in both uterine arteries (%)	100	75	14.29	11.11
presence of P.N. in only one uterine artery (%)	0	15	28.57	22.22
lack of P.N. in both uterine arteries (%)	0	10	57.14	66.67

RI=S-D/S

PI=2(S-D)/S+D

RI- resistance index,

PI- pulsativity index,

S- peak velocity in systole,

D- peak velocity in diastole.

We followed up pregnancies in the first and second trimester (n=52). Biometry, morphology and utero-placental circulation (uterine arteries, arcuate arteries) were studied with ultrasound.

We evaluated the pregnant women:

-at diagnosis of pregnancy (first trimester),

-at the end of the first trimester of pregnancy (11-13 week),

-between 16-18 week,

-between 20-24 week,

-after 24 week.

Statistical analysis was performed with Student's t-Test.

RESULTS

The pregnant women observed:

-were aged between: 28.82 + / - 3.68 years (V: 12.5%),

-BMI: 23.11 + / - 3.19 kg/m2 (V: 13.8%),

-51.06% had a history of pregnancy, 48.94% were at their first pregnancy,

-42.55% had birth in their history,

-17.02% had spontaneous abortions in anamnesis,

-none of the pregnants were diagnosed with hypertension.

These pregnancies were desired pregnancies with intrauterine live fetuses.

Biometry at the end of the first trimester of pregnancy was as follows:

-crown-rump length (CRL) was 59.9 + / -7.6 mm (V: 12.77%),

-biparietal diameter (BPD) was 20.36 + / -3.9 mm (V: 19.15%)

-average diameter of the gestational sack at the end of the first trimester of pregnancy was 73.2 + / -9.3 mm (V: 12.77%)

Changes of resistance index (R.I.) and pulsativity index (P.I.) in the uterine artery at the first and second trimester of pregnancy are presented in table I.

With the progress of pregnancy we found that the impedance to flow (resistance and pulsativity indexes) shows a significant decrease in the uterine arteries. This decrease in the impedance to flow is significant between the end of the first trimester of pregnancy (12^{th}+/-1 week) and 17^{th}+/-1 week ($p<0,05$); insignificant between 17^{th}+/-1 week and 22^{th}+/-2 week ($p>0,05$); and significant between 22^{th}+/-2 week and the end of the second trimester ($p<0,05$).

Changes of resistance index (R.I.) and pulsativity index (P.I.) in the arcuate arteries at the first and second trimester of pregnancy are presented in table II.

With the progress of pregnancy we found that the impedance to flow (resistance index) shows a significant decrease in the arcuate arteries ($p<0,05$).

The prevalence of the protodiastolic notch (P.N.) in the uterine arteries at the first and second trimester of pregnancy are presented in table III.

After 24 weeks of pregnancy the protodiastolic notch disappeared in both uterine arteries only in about 2/3 of subjects.

DISCUSSION

Impaired trophoblastic invasion of the maternal spiral arteries is associated with increased risk for subsequent development of intrauterine growth restriction, preeclampsia and placental abruption. Many screening studies have examined the potential value of Doppler in identifying pregnancies at risk of the

complications of impaired placentation. They studied the impedance to flow in the uterine arteries and in the arcuate arteries.

a. In the uterine arteries:

-Persona-Sliwińska *et al* found a mean resistance index of 0.866 +/- 0.066, and a mean pulsativity index of 2.469 +/- 0.618 between 5th and 12th weeks of pregnancy.[6]

-Valensise *et al* defined as an abnormal result a resistance index of more than 0.58 in the uterine arteries at 22 weeks of gestation.[7]

-North *et al* defined as an abnormal result a resistance index greater than 0.57 in the uterine artery, on the placental side at 19–24 weeks of gestation.[5]

-Irion *et al* defined as an abnormal result a resistance index of more than 0.57 in the uterine arteries at 26 weeks of gestation.[2]

-Zimmermann *et al* defined an abnormal result by a resistance index of more than 0.68 in the uterine arteries at 21–24 weeks of gestation in high risk pregnancies for hypertensive disorders.[8]

-Kurdi *et al* defined abnormal, when the uterine arteries presents P.N. in both side and the mean resistance index is greater than 0.55.[4]

We found in the uterine artery a mean resistance index of: 0.721+/-0.081 at 11-13 week, 0.594+/-0.076 at 16-18 week, 0.581+/-0.066 at 20-24 week and 0.484+/-0.061 at >24 week.

b. In the arcuate arteries:

-Persona-Sliwińska *et al* found a mean resistance index of 0.728 +/- 0.123, and a mean pulsativity index of 1.352 +/- 0.362 between 5th and 12th weeks of pregnancy.[6]

-Arduini *et al.* defined as an abnormal result a resistance index of more than 0.57 in the arcuate arteries at 18–20 weeks of gestation.[1]

-Jacobson *et al* defined as an abnormal result a resistance index of more than 0.57 in the arcuate arteries at 24 weeks of gestation.[3]

We found in the arcuate arteries a mean resistance index of: 0.478+/-0.095 at 11-13 week and 0.421+/-0.109 at 16-18 week.

CONCLUSIONS

-With the evolution of pregnancy in the first and second trimester, impedance to flow shows a decrease in the uterine arteries.

-This decrease in the impedance to flow is significant between the end of the first trimester of pregnancy (12^{th}+/-1 week) and 17^{th}+/-1 week; insignificant between 17^{th}+/-1 week and 22^{th}+/-2 week; and significant between 22^{th}+/-2 week and the end of the second trimester.

-With the evolution of pregnancy in the first and second trimester, impedance to flow (resistance index) shows a significant decrease at the arcuate arteries.

-After 24 weeks of pregnancy, the protodiastolic notch disappeared from both uterine arteries only in 2/3 of cases.

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The "Obstetric-Gynecology 1" Clinic's experience regarding the fibroid's surgical treatment

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Uterine leiomyoma plays an important role in women's pathology. According to the statistics, acquired in The Obstetrics-Gynecology 1 Clinic of Targu-Mures, during 01.01.2008-01.01.2009, from a total of 329 women diagnosed with uterine leiomyoma, more than 40% were under 40 years old, which represents an early manifestation of the disease, at a reproductive age. There were described a variety of treatments, from which the conservative methods, focused on treating the illness and maintaining the uterus, gained popularity among patients. In everyday practice, each woman needs to be carefully investigated in order to decide either to use a medical or a surgical treatment. It is well known that there are complex biochemical interactions involved in myoma's development and that ovarian steroids have a significant influence on this process. This is how these therapies manipulate the hormonal branch, causing tumor's regression From the surgical point of view, the "traditional" therapy (total hysterectomy) was replaced by alternative therapies, in order to maintain fertility. It is necessary to continue investigating these techniques, because it offers to every clinician great pieces of information.

Key words leiomyoma, surgical treatment, alternative therapies, postoperative complications.

Uterine leiomyomas, usually known as fibroids are the most common uterine pathology of women in the reproductive age.

Fibroid is one of the most common and most frequently solid pelvic tumors affecting 20-40% of females in fertile period.[16] Some studies showed that women with fibroids are on 5th place regarding hospitalization cases of women between 15-45 years old, not including pregnancy.[14]

It represents the main indication of hys terectomy.[15]

Most of uterine myomas are asymptomatic or poorly symptomatic and it is estimated that only 20-50% of women with fibroid have symptoms who can be directly associated with the tumor.[13] Approximately 62% of women with symptomatic fibroid have multiple symptoms associated with: number, size, location, or degenerative modification that occurs in fibroid's evolution.[6]

MATERIAL AND METHODS

This study emphasizes the surgical treatment and postoperative complications for uterine myomas applied to women hospitalized in the "Obstetric-Gynecology 1" Clinic of Targu-Mures between 1st of January 2008 to 1st of January 2009. Along this period in the Clinic were hospitalized, diagnosed and surgical treated 329 women for fibroid.

Most of uterine myomas are asymptomatic or poorly symptomatic and it is estimated that only 20-50% of women with fibroid have symptoms who can be directly associated with the tumor.[13] Approximately 62% of women with symptomatic fibroid have multiple symptoms associated with: number, size, location, or degenerative modification that occurs in fibroid's evolution.[6]

In this paper work we have studied the type and the method of the surgical abort correlated with the clinical symptoms and with the uterin fibroid's associated pathology in women diagnosed and surgically treated in the Clinic.

The traditional treatment of uterine fibroid is the surgical one. The hysterectomy is the most used procedure suggested to women who don't want future pregnancies.

Still, many women choose to avoid hysterectomy in order to maintain fertility, to avoid premature menopause or due to psychological reasons.

Therefore, those young women (under 40 years old) who still have home-planning to do and don't have an associated pathology of the uterine cervix or of the annexes, are recommended a conservative surgical method.

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I shall present the conservative surgical methods, without too many details concerning the operation technique that are used in the uterine myoma's therapy: The Myomectomy, Myolysis and the Occlusion of Uterine Artery.

Myomectomy is a surgical method of removing the uterine fibroid from the adjacent tissues (enucleere) being the first surgical procedure made for the uterus preservation.

1. Classical myomectomy or the myoma's removal from the uterus is the first option in the conservative surgical treatment. This procedure is handy for all gynecologists. That is the reason of its popularity despite of the subsequent impressive alternatives that appeared further on. This method is still the gynecologist's first choice for cases of big myomas (diameter > 10 cm) or in case of associated pathology which requires a major intervention. The abort depends on the topography, on the number and the volume of the fibroid nodules. After performing the myomectomy the recurrence risk is substantial: 50% of the women have transvaginal screening in the next 5 years.[1] The recurrence rate of the symptoms is between 15% to 30% in the next 10 years, 10% of this cases requering future surgical treatment.[1]The recurrence predictors include multiple fibroids and lack conception.[4]The rate of the pregnancy after myomectomy is variable depending on the myomas number, the size and their localization, being placed between 10% to 75%, with an average of 50% and the rate of uterine rupture to a future pregnancy is about 0.002% to 5%.[4]

2. Laparoscopic myomectomy has the advantage of a quicker postoperative recovery. In medical practice, in order to appreciate the laparoscopic myomectomy's importance, it must be compared with the classical myomectomy concerning: the execution time, the bleeding, the complications and postoperative fertility

The rate of operative bleeding is comparable postoperative fever is rare and the cost wasn't analyzed.[2] The reproduction after classical abdominal myomectomy in comparison with laparoscopic one has no significant difference.[12]

3. Hysteroscopic myomectomy performed for submucosal myomas has two big advantages: is less invasive and need no incision to external surface of uterus, reducing the risk of postoperative adhesion. The risk of uterine perforation, of bleeding, infection, adhesion and hydro-electrolytic disorders are good to know.[8] This method is valued because it increases the fertility rate.[11] One recent study suggests an increassing rate of fertility after resection of submucosal myoma.[9]

4. Myolysis consist in destruction in situ of fibroids nodules using laparoscopic electro-cauterize, laser energy or cold therapy. The method is based on tissue destruction or occlusion of the fibroid vessels .The advantage is that this procedure is less technically challenging, there is an absence of bleeding, and a rapidly recovery after surgery. Given the localized tissue destruction without repairing, there may be a theoretical increased risk for adhesion formation or uterine rupture during the pregnancy.[10]

5. Occlusion of Uterine Artery is a recent conservative method consisting in temporally laparoscopic stent the uterine artery under Doppler control, until the signs disappear.[6] Temporally occlusion of uterine artery decreases fibroid volume and abnormal bleeding but is to be avoided in women who want to keep their fertility.[7] This technique has been used for a long time for stopping the bleeding during or after delivery.

RESULTS AND DISCUSSIONS

Our study shows the clinic manifestation of fibroid, represented in Table I.

Symptoms found in the group of patients are in line whit literatures data in the sense that the phenomena of compression and abnormal bleeding are the main causes in the literature data too.[6,13,15,16]

In Obstetric-Gynecology 1 Clinic of Targu-Mures from a total of 329 fibroid cases surgically treated, 39,2% were performed total hysterectomy with bilateral anexectomy, 6,1% were performed total hysterectomy with unilateral anexectomy and in 20,7% were performed total hysterectomy and ovarian preservation. Conservative surgical treatment reported: abdominal myomectomy in 20,7% of cases, laparoscopic myomectomy in 9,7%, diagnostic hysteroscopy in 2,5%, and total vaginal hysterectomy in 1,1%.

We will note that the genital pathology associated with uterine fibroids is around 47% in the group of patients and it is represented in Table II.

Table I Symptoms found in the group of patients

Symptoms	Compression	Leucorrhoea	Urinary disorders	Pelvic pain	Abnormal bleeding	Vaginal bleeding	Menorrhagia
Percent %	16,4%	1,8%	1,7%	21,2%	18,7%	32,3%	7,8%

Table II. The genital pathology associated to uterine fibroids

Pathology	One ovarian disorder	Bilateral ovarian disorders	Tubing disorders	Secondary sterility	Primary sterility	Urinary disorders	Endometritis	Malignancy
Percent %	12,7%	4,5%	3,9%	2,5%	4,2%	2,3%	9,4%	7,5%

Table III. Early local postoperative complications

Abdominal plague complications	Evisceration0.3% (1 case)
	Suppuration 0.9% (3 cases)
Genital left organs complications	Near bont collection 0,3% (1 case)

Table IV. Early general postoperative complications

Complications	Frequency (%)
Febrile morbidity	Fever 0,6% (2 cases)
	Feverish 7,6% (25 cases)
Anaemia	Anaemia post haemorrhage 3,3% (11 cases)
Cardiovascular	HTA 0,6% (2 cases)
	Disorders of rhythm and conduction 0,6% (2 cases)
Urinary	Bladder injury 1,2% (4 cases)
	Uretheral injury 0,3% (1 case)
	Acute retention of urine 0,3% (1 case)

Table V. Complication's frequency after the type of intervention

Type of surgery	Frequency (%)
Laparoscopic myomectomy	0,9 % (3 cases)
Abdominal myomectomy	2,7% (9 cases)
Total hysterectomy with bilateral anexectomy	5,5% (18 cases)
Total hysterectomy with unilateral anexectomy	1,5% (5 cases)
Total hysterectomy and ovarian preservation	1,8% (6 cases)

Table VI. The average of postoperative hospitalization days on surgical intervention types

Type of intervention	The average of postoperative hospitalization days
Laparoscopic myomectomy	4 days
Hysteroscopy	3 days
Abdominal myomectomy	7 days
Total vaginal hysterectomy	7 days
Total hysterectomy and ovarian preservation	8 days
Total hysterectomy with unilateral anexectomy	9 days
Total hysterectomy with bilateral anexectomy	9 days
Total hysterectomy with bilateral anexectomy and organs ablations	15 days

Regarding genital pathology associated our dates are very interesting because the literature data do not establish a correlation between uterine myoma and genital pathology associated.

We have also studied early postoperative complications for all the cases (Tables III-VI).

General and local postoperative complication's type and frequency are similar with bibliographic dates.[1,14,15,16]

The average of postoperative hospitalization days obviously smaller in laparoscopic myomectomy, concords with bibliography[14] and apparent in our study.

We mention that all the patients received prophylactic or curative anticoagulant and antibiotic treatment.

It was necessary to correct post haemorrhage anaemia with: integral blood, erythrocytes mass (M.E.), fresh frozen plasma (P.P.C.).

CONCLUSIONS

1. Uterine fibroid plays an important role in women's genital pathology; one of three women with fibroid surgical treated is under 40 years old.

2. Regarding the size, location, number and associated disorders conservative therapies are to be preferred, because of the preserved fertility.

3. The incidence of fibroids is more increased in young females, but "not every fibroid must undertake surgery". Associated pathology (tubing disorders, endometriosis, ovarian disorders and sterility) was in many cases the main sign for surgical treatment.

4. The main surgical indications were: menstrual disorders, pelvic pain and pressure, sterility and abnormal bleeding.

5. Clinic and Doppler controls may be a method to indicate the moment and the type of therapy that we can use in treatment especially for young females.

6. Laparoscopic myomectomy was performed especially in cases of sterility.

7. The most frequent indication for surgical treatment on young females was represented by the fibroid volume and the fertility disorders (sterility).

8. Local and general early postoperative complications had been rare.

9. Increase number of complications after classics intervention in comparison with laparoscopic ones is explained by the complexity of cases.

10. The average days of postoperative hospitalization on surgical intervention types was twice higher for classics intervention.

11. Finally it is absolutely necessary to continue the investigations of surgical techniques, offering gynecologist new concrete and explicit information in order to be able to wisely advice their patients.

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Vaginal infection with Ureaplasma urealyticum and Mycoplasma hominis

Natalia Simona Chiujdea

Sexual experience is related to vaginal colonisation with the genital Ureaplasma urealyticum and Mycoplasma hominis. These microorganisms can be found commensal in lower genitourinary tract. Sometimes they cause many disorders as pathogens and can possibly cause many different pathologies like: non-gonococcal urethritis, bacterial vaginosis, cervicitis, endometritis or pelvic inflammatory disease. Sexually inexperienced woman have low rates of colonisation, similar to those see among preadolescent girls. At pregnant woman can cause chorioamniotitis and rupture of fetal membrane. Mycoplasma species have been implicated in the pathogenesis of prematurely, intrauterine growth retardation, low birth weight. The aim of present study was to evaluate the occurrence of Ureaplasma urealyticum and Mycoplasma hominis in vaginitis.
Key words: Ureaplasma urealyticum, Mycoplasma hominis, vaginitis

Ureaplasma urealyticum and Mycoplasma hominis are the most common pathogen cultured from the amniotic cavity of women with preterm labor and intact membranes, yet the significance of amniotic fluid infection exclusively caused by these microorganisms is unclear[5]. However, experience with antibiotic eradication of the pathogen in this setting is limited. This study goal was to determine the incidence of Ureaplasma urealyticum and Mycoplasma hominis in women experiencing chronic urinary symptoms and to determine whether antibiotic therapy targeting these organisms is effective. Drug resistant is observed and decision for proper antibiotic is proposed. The importance of study microorganism like Ureaplasma urealyticum and Mycoplasma hominis announced a guideline regarding the epidemiology, changing concept of diagnostic tools and treatment strategy for sexual disease[2].

MATERIAL AND METHOD

Female with discomfort and lower urinary tracts symptoms including dysuria, pollakyuria and nocturia and/or gynecological history were included. Patients had gynecological examination for infections and cystocele. Female with urinary tract infection and cystocele were excluded from the study. Urine analysis and urine culture were performed in all patients. The subject has no urinate for 3 hours. Vaginal samples were taken from the endocervical region after cervical mucus have been cleaned with a swab without using local antiseptics. Mycofast EvolutioN 3 was used for the isolation of Ureaplasma urealyticum and Mycoplasma hominis. For study were included patients with genital symptoms and no antimicrobial local or general treatment in the last 3 weeks. For Mycoplasma hominis the number of units formatting colonies CFU/ml was done. Colonies were evaluated after 24 and 48 hours.

RESULTS

This study was conducted to examine the presence of Ureaplasma urealyticum and/or Mycoplasma hominis at females. Of the 91 women included in this study were found 16 cases, 14 of this with Ureaplasma urealyticum and 4 of this with Mycoplasma hominis. 2 of them were with both microorganisms. 18% of female were positive and 82% negative (Fig.1)

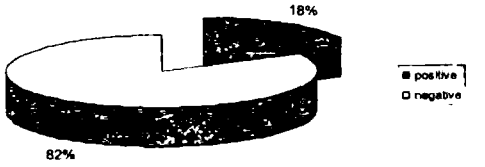

Figure 1. Cases tested for ureaplasma urealyticum and mycoplasma hominis

Laboratory of Medical Analysis, Gynecology Clinic II, Tirgu Mureş

Age of all women were between minimum 19 and maximum 52 years old, average was 31,37+/-stdev 8,46. Median or the middle of the set was 31 years and the most frequently was the same 31 years. 75% are under 35 years old and 25% are adults between 35 and 52 years old as was shown in figure 2.

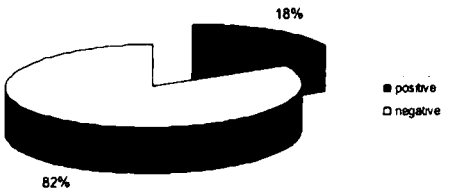

Figure 2. Age repartition of cases

15 women were detected with history of premature delivery, chorioamniotitis, intrauterine growth retardation, low birth weight or sterility.

For determine the statistics difference between two groups, one with gynecological history of disturbance followed in the study and the other one with no history. For this Pearson Test was done. (Table I)

Statistics include the Fisher and mid-p exact tests, chi squares, odds ratio, and relative risk P-value <0.05 showed that the difference is statistic significant with a Mantel chi test 5,047. Risk in exposure was 93,75% and for unexposed 65,33%, RR 1,435.

Table I. Pearson Test

Exposure	History present	History absent	Results
Ureaplasma/ Mycoplasma positive	15	1	X= 5,047, P=0,01
Ureaplasma / Mycoplasma negative	49	26	RR= 1,435
Total	65	27	Risk in exposed= 93,75%

DISCUSSIONS

Although often overlooked or improperly treated, Ureaplasma urealyticum and Mycoplasma hominis infections may account for a large proportion of unexplained chronic genitourinary symptoms[1]. Ureaplasma urealyticum is commonly isolated from the amniotic fluid of unselected individuals with intact membranes at the time of cesarean section even prior to onset of labor[4]. The risk of placental infection increases with onset of labor, rupture of membranes and number of vaginal examinations[1]. Microorganisms have no influence on spontaneous abortion. About 5% of febrile postpartum women have Ureaplasma urealyticum or Mycoplasma hominis isolated from the blood. These data suggest that both genital mycoplasmas can cause postpartum fever[1]. The degree of colonization with Ureaplasma urealyticum correlates with an adverse effect on pregnancy outcome[6].

CONCLUSIONS

History of complications associated in 15 of 16 patients with Ureaplasma urealyticum and/or Mycoplasma hominis suggest, but do not prove, that may have an adverse effect on outcome of pregnancy period.

Compared to the negative women increasing colonization with Ureaplasma urealyticum was associated with a significant history of decrease of birth weight, gestational age and with an increase of chorioamnionitis and preterm delivery ($p<0.05$).

Further studies are necessary to substantiate this implication and to determine the relationship between infection and a lot of female disturbing of reproductive pathology.

However, we consider a positive result to by treat with proper antibiotic after specific antibiogram. Culture and treatment should be considered before pursuing more costly and invasive tests.

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Genital infections with parasitic etiology

Camelia Guta[1], Adriana Turculeanu[2], Maria Balasoiu[2], Carmen Avramescu[2], Smarandita Cotarcea[3]

Infections of the male and female genital tract are a frequent cause for consulting the doctor. Genital infections may evolve as „specific" infections, that are exogenous infections determined by microorganisms with sexual transmision that can be: bacterias (Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum), viruses (Herpes or Pallinoma virous), parasitys (Trichomonas vaginalis) or fongus (Candida).In this paper we have tried to research the incidenceinfecion with Trichomonas vaginalis, at patients with symptoms specific to vaginitys, vulvovaginitys and acute uretritys, as well as associating altor etiologic agents towards producing such affections, on a number of 283 samples of vaninal secretions and 17 samples of uretral secretions. Regarding the mixing with other bacteria or fungi: Trichomonas urovaginalis is frequently encountered with Candida albicans, but also with other etiology agents.This is another proof of the great risk which is determinated by the lack of sexual education(the infestation in the same time with several illnesses sexually transmitted).

***Key words**: genital infections, Trichomonas vaginalis, Candida.*

The normal flora of the female genital tract varies depending on the pH and estrogens concentration of the mucus, in close correlation with age:

-prepuberty and postmenopause flora is dominated by staphylococci and bacteria (same flora that you find on the surface of the epithelium).

For sexually aëtive women microbial flora changes, being composed of Enterobacteriaceae, streptococci, staphylococci, anaerobic bacteria (lactobacili, anaerobic bacillus).

Lactic-bacilli represent a normal vaginal microbial flora for these women. Group B Haemolytic streptococci (Streptococcus agalactiae) is present in the vaginal flora and can be transmitted to the new - born (with the occurrence of neonatal complications).[3,4,5]

Female genital infections are a frequent cause for gynecological consultations. They may evolve as:

-endogenous infections, caused by microorganisms of the normal vaginal flora;

-exogenous infections, caused by microorganisms, mostly sexually transmitted ones

-Bacteria (Neisseria gonorrhoeae, Chlamydia trachomatis - serotypes DK, Treponema pallidum)

-viruses (Herpes simplex - type 2, Papillomavirus)

-protozoa (Trichomonas vaginalis)[1,4,5]

Infections of the male and female genital tract are a frequent cause for consulting the doctor. Genital infections may evolve as "specific" infections, that are exogenous infections determined by microorganisms with sexual transmission that can be: bacterias (Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum), viruses (Herpes or Papilloma virous), parasitys (Trichomonas vaginalis) or fungus (Candida albicans).[6]

Trichomonas vaginalis is a cavity parasite from Flagellata class frequently involved in genital pathology.

Protozoa are unicellular microorganisms that live in different conditions, parasite or free and present in their evolutionary cycle two forms:

-Vegetative form in which all vital functions take place active

-Cystic form with a resistant membrane and nutritional reserves, which has a latent life with metabolic processes reduced to a minimum.[1]

Morphological features:

It is an oval parasite, pear-shaped, easily deformable, which develops extensions.The citoplasma is granular, with many corpusculi, well-developed nucleus, located above.

In the front of nucleus there are blepharoplasts placed in a group, departing from which leaves 3-5 previous scourges, a recurent scourge forming a corrugated membrane, a rigid extension that surrounds the nucleus and has the role of an axial skeleton and a reinforcement fiber for the corrugated membrane (firming coast).[1]

Epidemiology

- how human is infected: by venereal way
- location: the vagina, urethra.[1]

Clinical aspects

a) incubation period: short, several days

b)The outset of the disease: suddenly, with pruritus, vulvo -vaginal or urethral burnings, (greenish-yellow secretion, fluid, odoriferous)

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c)The development: leukorrhea, painful intercourses

d)complications: infection extending to the neighboring organs- urethritis, family, bartolini, prostate (at men) or cause of sterility at women.[1,4,6]

Positive diagnosis

-the examination between leaf blade of a fresh sample of a vaginal and urethral secretion, which highlights the shape of the parasite Amoeba and extreme and featured mobility (in Zig-Zag)[1,3];

-Microscopic examination of a fixed and coloured smear, the most common blue methylene or May-Grunwald Giemsa (it shows the pear-shaped form, with blue citoplasma, eccentric nucleus, of a pink color).[1,3]

Objectives. Based on these data from literature, we study the incidence of this disease with parasitic etiology, in this case with Trichomonas urovaginalis, in persons with vaginal symptoms labeled as vulvovaginites or urethritis. We also study the association of this parasite with Candida albicans and etiology agents. All these cause vulvovaginites or urethritis classified as typical.

MATERIAL AND METHODS

The study was conducted on 300 samples collected from the genital area, namely: 233 samples of vaginal secretion investigated in order to detect these etiology agents, 50 samples of secretion from the female genital area collected for the purpose of Babes Papanicolau Test and 17 samples of urethral secretion. All these samples come from patients who attended the Philanthropy Hospital Craiova.

The technology of processing the samples collected was: - vaginal secretion samples were collected with a buffer of a pharyngeal exudates type. From these samples are performed fixed and colored smears of methylene blue 1% and May-Grunwald-Giemsa.

The samples of urethral secretions have been harvested with a buffer or with a bacteriological ansa, then we realized fixed and coloured smears, as we previously explained.

The samples we took for Babes Papanicolau Test required the harvest of three smears: F1-one sample from the bottom of posterior vagina (Douglas); F2-a sample from the external cervix;F3-a sample from the inner cervix. All these samples have been fixed and coloured May-Grunwald-Giemsa.

All these smears, after their execution, were examined on the optical microscope, using the morphological aspect of the parasite for its identification.

RESULTS

1. We had 300 samples and only 30 (10 %) of them were positive for Trichomonas urovaginalis, as it follows:

-27 positive vaginal secretions;

-3 samples harvested for Babes Papanicolau Test;

-3 samples for urethral secretion.

From all that we can that it is possible for the men to be only a link in the epidemiologic chain of this infection (they carry it and pass it over) and the women are the persons who develop the infection. They are the ones who have the clinical symptoms which makes them come to the physicians.

On the other side we have to say that at 3 persons we took samples for both vaginal secretion and Babes Papanicolau Test. In 2 of these cases we discovered Trichomonas urovaginalis at the Babes Papanicolau Test.

Thus it is proved that the act of harvesting the samples is essential for pointing the parasite, more precisely from the bottom of posterior vagina, even when we take a common sample of the vaginal secretiòn(a bacteriological, parasitic and mycologic test).

2. Among the 30 positive cases of infection with Trichomonas urovaginalis, 7 of them were double: Trichomonas urovaginalis and Candida albicans (Figure 1).

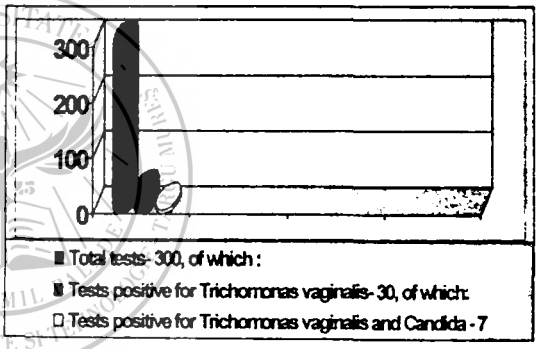

Figure 1. Cases of infection with Trichomonas urovaginalis

3. Regarding the infection with Trichomonas urovaginalis and Chlamydia trachomatis or gonococci we didn't discover any kind of association;only one case was reported at a man who was diagnosed and treated for a gonococical infection, post gonococical infection (the method we used for the Chlamydia diagnose was a chromatographic method which allows the emphasis of the chlamydial antigen in the urethral or vaginal secretion).

DISCUSSIONS

Trichomonas vaginalis is identified in the vaginal secretion of 20% of the whole feminine population and of 50% of the women who have whites. It was the main origin of 1/4 to 1/3 of the total of the vaginitis. It is found in the urethra of 70-80% of the partners of the women with trichomonazic vaginitis, most of them not presenting any clinical evidence. Trichomoniasic infection is more frequent in the urban area for the women with multiple sexual partners and with a critical

genital hygiene.His occurence doesn't seem too high on pregnant women.[2,4]

It is annually estimated at 2,5 millions the number of new cases in U.S.

In the specialized literature it is considered that the natural reservoir of the parasite is represented by women. Women infect men through sexual intercourse and this plays the part of transmitter.[2,4]

Extrasexual transmision is accepted but it is considered exceptional.It is admitted the possibility of infecting women by wet toilet objects, unwashed hands, swimming pool water, gynaecological unsterilized instruments. In the favour of extrasexual contamination come the rare cases vulvovaginal infections diagnosed on little girls.

The maximum frequency of vaginal trichomoniasis is in the same period with the highest period of sexual activity (third and fourth life decade). The receptivity is different at the beginning of the sexual life, virgin women taking sistematically the illness with the ocasion of the first sexual infecting intercourse.[2]

There are notable variations of the infection's intensity during the menstrual periods- the number of parasites is very high around periods and smaller between the 12-25th day of the menstrual periods.

The trichomoniasic infection is favourised by a number of elements (Table I). Using oral combined anticonceptionals (such as estro-progestatives) for some authors would favourise trichomoniasis and for others, on the contrary, would reduce its incidence.

Table I Elements that would favour the trichomoniasic infection[6]

Elements that would favourise the trichomoniasic infection:
1 hipoestrogenia (not the entire absence of estrogens too);
2.hiperestrogenia;
3 hipoviatminosis (especially A);
4.decreasing of the resistance to infections;
5.traumatising the vaginal mucous (local irrigation with concentrated antiseptics, sexual excesses ;

There are some authors who consider that Trichomonas vaginalis becomes agressive only in the conditions of disappearance of loctobacillus and of multiplication of associated flora, and that the presence of Candida gives an addition of aggressiveness to trichomoniasic infection.[2,3]

CONCLUSIONS

1.The Trichomonas urovaginalis infection is still frequent among people, especially the young ones, because they usually they have unprotected intercourses with various partners, since 10% in an important number. The existence of this infection proves a lack in health education among young people. They don't realize the risks that exist when they have unprotected intercourses with various partners.

2.To emphasis the parasite Trichomonas urovaginalis it is to harvest the sample from the bottom of posterior vagina (Douglas).

3.The test in optical microscope on fixed and coloured mix is an easy way to establish a fast diagnosis.

4.Regarding the mixing with other bacteria or fungi: Trichomonas urovaginalis is frequently encountered with Candida albicans, but also with other etiology agents. This is another proof of the great risk which is determinated by the lack of sexual education (the infestation in the same time with several illnesses sexually transmitted).

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Clinical results of prepatelar bursitis arthroscopic treatment - a five years clinical review

T. Bătaga, M. Denes, A. Ivănescu, A. Solyom, Bianca Ola

The prepatelar bursitis is the most frecvent extra-articular pathology of the knee.The goal of this study was to determine the clinical results of the prepatelar bursitis resection using arthroscopic debridment.We evaluated 85 consecutive patients who underwent arthroscopic debridment under the diagnosis of prepatelar bursitis.The size of the bursitis was assesed by measuring his diameter.The patellofemoral symptoms and function included anterior knee pain,erythema,swelling.No significant association was found between the size of prepatelar bursitis and anterior knee pain or any patellofemoral functional parameters.All the patients receive arthroscopic bursectomy.Good functional results was present in every cases.The diagnosis of the prepatelar bursitis must be suspected in early stage of the disease.Arthroscopic debridement should be the routine treatment.Bursectomy should be considered even as a primary procedure when significant patelar bursitis hypertrophy is present or when the conservative treatment did not lead to a fast improvment

Key words: patela, bursitis, treatment, artrhroscopy

Acute prepatellar bursitis is an inflammation of the largest knee bursa, located between the patella and the overlying skin. It is most commonly caused by trauma, as a result of a fall or the direct pressure and friction of repetitive kneeling ("housemaid's knee"). The prepatellar bursa is one of two bursa in the body (the other is the olecranon bursa) that can become infected, most commonly by Staphylococcus aureus[4,5]. The prepatellar bursa also may be inflamed by urate crystals[1].

Prepatellar bursitis becomes chronic in approximately 5 percent of patients. Chronic irritation and inflammation results in dilation of the normally paper thin bursal walls, which then thicken and become fibrotic.

A bursa is a small sac of fibrous tissue with a thin synovial lining that is filled with fluid. Numerous bursae are found in the body, around joints and in places where ligaments and tendons pass over bones. They can also form in other places as a response to unusual pressure or friction. Generally, bursae help to reduce friction and allow maximal range of motion around joints. Bursitis is inflammation within a bursa. The inflammation leads to an increase in synovial fluid production and causes the bursa to swell. There are 4 bursa located around the knee joint. They are all susceptible to bursitis but the prepatellar bursa is most commonly affected. Less frequently, the infrapatellar and deep patellar can become inflamed.[6]

The prepatellar bursa is located superficially on the anterior aspect of the knee between the skin and the patella. The bursa does not communicate with the knee joint and the knee joint itself is normal in prepatellar bursitis.

Prepatellar bursitis may occur due to:

-Acute trauma: fall/direct blow onto the knee.

-Recurrent minor injury: occurs after long periods of time spent kneeling forwards and putting pressure on the patella. Historically, this was typical of housemaids who spent long periods of time scrubbing floors on their knees, hence the term Housemaid's knee. Now more commonly seen in tradesmen, e.g. carpet fitters, concrete finishers, roofers.

-Infection: pyogenic prepatellar bursitis is common in children. May be mistaken for septic arthritis of the

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knee. Staphylococcus aureus is the usual causal agent[4]. There is usually a history of a break in the skin prior to its onset.

-A coexisting inflammatory disease: for example synovitis related to rheumatoid arthritis.

-A crystal depositing condition: prepatellar bursitis is more common in people with gout or pseudogout.

MATERIALS AND METHODES

Between 2004-2008, in our clinic,we have treated 85 cases of prepatellar bursitis in 56 patients.In 29 cases the bursitis was bilateral.32 patients were male and 24 were female.6 patiens have septic bursitis. All the patients have an average physical activity. The clinical symptoms and signs are :

-Knee pain, swelling and redness

-Difficulty kneeling and walking

-Tenderness and swelling superficial to the patella

-Erythema and localised warmth of the skin over the patella

-Reduced knee movement

All the patients fulfill a questionnaire wich involve this details :

-Occupation - does this involve excessive kneeling

-Physical activity level

-History of fall or acute knee injury

-History of any repetitive motion involving the knee

-Recent NSAID's or steroid treatment?

-is there a history of crystal arthropatny or inflammatory disease

-Use a knee orthesis or history of kinetotherapie or physiotherapy

-Impresions after arthroscopic bursectomy

In all cases we make arthroscopic bursectomy performed under local or spinal anaesthetic.

A sharp trochar was used to enter the inflated bursa 1 cm away from the edge. A pump was used to distend the space to visualize the resection with a motorized shave. The use of a curved tip on the shaver blade helps with corner access. The bursa was resected from the inside out technique.

A drainage for 24 hours is usually performed.The hospitalization duration was 2 days. A stick or cane may be needed to aid walking for the 10 days. Analgesic medication and local ice were used for 72 hours.

Figure 1 and 2 Preoperator images of the right knee prepatellar bursitis

Figure 3.4 Arthroscopic bursectomy procedure

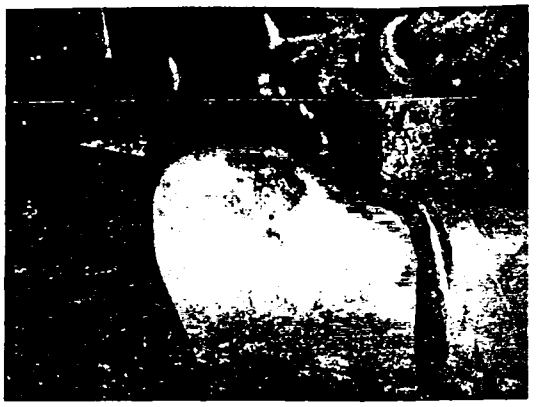

Figure 5. Posteoperative image of the right knee

RESULTS

All the patients fulfills the questionnaire at clinical review make at 1,3,6 and 12 months.76% had no pain whatsoever, but we did note some residual tenderness in 20% of the patients, and 4% had pain on kneeling. There were 2 recurrences; 1 patient had rheumatoid arthritis and 1 repetitive daily trauma to the knee. There were no significant complications. All the patients were satisfied after this procedure and his cosmetic aspect. Physiotherapy is indicate because exist a reduced range of movement in the knee joint. This treatment were made for 14 days and kinetotherapie for 6 weeks. The return to physical activity were made after 6 weeks.

DISCUSSION

We present this simple procedure for arthroscopic prepatellar bursectomy which may avoid the complications associated with an open procedure. Kerr and Carpenter reported a series of three patients who underwent arthroscopic prepatellar bursectomy using a three portal technique[4]. They described good results for two patients with traumatic bursitis, but a poor result for a third patient with CREST syndrome who had tethering of the skin to the quadriceps tendon and residual calcium deposition within the bursa. Ogilvie-Harris and Gilbart reported a series of 19 patients with prepatellar bursitis[5]. They describe a procedure for arthroscopic prepatellar bursectomy using medial and lateral portals positioned 1 centimetre distal to the bursa. 17 patients experienced complete symptomatic resolution and there was one recurrence in a patient who was a tile setter at nine months postoperatively.

Kaalund et al described endoscopic resection of the septic prepatellar bursa in four patients using two lateral portals.[3] They described resolution of the bursa and the infection in all cases with no complications. This procedure has, however been implicated in the progression of septic prepatellar bursitis to necrotising fasciitis in one case report.[1,2,7].

Our results are similare with this authors and we recommend to use this procedure in all the hospitals from Romania,because also reduce the hospitalization costs and became a very important economical factor.

Arthroscopic prepatellar bursectomy is a simple procedure which is effective and has a low complication and recurrence rate.

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Outcomes in the management of vascular prosthetic graft infections

Eliza Russu[1], C. Copotoiu[1], Bianca Grigorescu[2], D. Popa[1]

Despite routine antibiotic prophylaxis and refinements in implantation technique, microbial infection of the vascular prosthesis can occur. Infection involving a vascular graft is difficult to eradicate.
We retrospectively analysed the operative registers of our clinic as well as the regular archives, from 2000 until present, searching for reported graft infections which needed excisions and extraanatomical bypasses or for conservative therapy.
There were 50 patients in this interval admitted and treated in Surgical Clinic No 1, out of a total of 950 vascular interventions.
10 of them were early graft infections(< 4 months), 40 were late ones(>4 months).
Using Szilagyi's classification, 10 were grade I, 17 were grade II and 23 grade III.
We performed 20 graft excisions for infrainguinal graft infections, with the removal of the entire graft, radical debridement of infected perigraft tissues, closure of the arteriotomies with monofilament suture and the administration of systemic and topical antibiotics. We attempted graft preservation in 5 cases of infrainguinal prosthetic bypass graft infection(with serial surgical wound debridement, coupled with antibiotic therapy, early muscle flap coverage and repeated wound cultures to identify any development of bacterial resistance or change in the microbial flora.
Dissatisfaction with the morbidity and the mortality of treating vascular graft infections, regardless of location, by total graft excision and remote bypass has been the impulse for the expanded application of lately performed in-situ bypasses or even for the prophylactic use of antibiotic-bonded grafts, in carefully selected cases.
Key words: infection, vascular prosthesis, pathology, therapeutic solutions

Despite routine antibiotic prophylaxis and refinements in implantation technique, microbial infection of the vascular prosthesis can occur. Infection involving a vascular graft is difficult to eradicate. If not recognized or treated promptly, implant failure will occur by producing sepsis, hemorrhage or thrombosis. Surgical therapy is always required, often coupled with prosthesis excision, because antibiotics alone are insufficient to eradicate an established infectious process. Management involves graft excision alone, graft preservation within the implant wound, in/situ graft replacement, or graft excision in conjunction with extra-anatomic bypass grafting. Improved results have been reported folowing both graft excision coupled with extra-anatomic bypass and in-situ replacement procedures. Even when treatment is succesful, the morbidity associated with vascular graft infections is considerable, with outcomes worse than the natural history of the vascular condition that led to graft implantation.

The reported incidence of infection involving a vascular prosthesis varies from 0.2% to 5% of operations, being influenced by the implant site, indication for intervention, underlying disease and host defense mechanisms.

The presence of a foreign body potentiates the infectivity of bacteria. In 1957, Elek and Conen demonstrated that a single-braided silk suture significantly reduced the inoculum of staphylococci required to produce a local infection.[5]

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The risk of foreign body infection can be predicted by the formula: Risk of biomaterial infection = Dose of bacterial contamination x virulence/ Host resistance.

The initiating event is bacterial adherence to the biomaterial surfaces, followed by colonization and development of "bacterial-laden" biofilm that resists host defenses and antibiotic penetration. Both graft and bacterial characteristics influence the likelihood of colonization.

Bacterial adherence to polyester grafts is 10 to 100 times greater than to polytetrafluoroethylene (PTFE) grafts; gram-positive bacteria produce an extracellular glycocalyx, or "mucin" that promotes adherence to biomaterials in greater numbers than gram-negative bacteria.

The etiologic factors involved in graft colonization are:

1. perioperative contamination via the surgical wound
2. bacteremia seeding of the biomaterial
3. mechanical erosion into bowel or genitourinary tract or through the skin
4. involvement caused by a contiguous infectious process

MATHERIAL AND METHOD

We retrospectively analysed the operative registers of our clinic as well as the regular archives, from 2000 until present, searching for reported graft infections which needed excisions and extraanatomical bypasses or for conservative therapy.

There were 50 patients in this interval admitted and treated in Surgical Clinic No.1, out of a total of 950 vascular interventions.

10 of them were early graft infections (< 4 months), 40 were late ones (>4 months).

Using Szilagyi's[9] classification, 10 were grade I, 17 were grade II and 23 grade III.

In literature, the risk factors for graft infection are:

1. Bacterial contamination of the graft
-Faulty sterile technique
-Prolonged preoperative hospital stay
-Emergency surgery
-Extended operative time
-Reoperative vascular procedure
-Simultaneous gastrointestinal procedure remote infection
-Postoperative superficial wound infection/ skin necrosis/ seroma/ lymphocele

2. Altered host defenses
Local factors:
-Biomaterial foreign body reaction
-Bacterial slime production (producing a protective biofilm)
Systemic factors:
-Malnutrition
-Leucopenia/ lymphoproliferative disorder
-Malignancy
-Corticosteroid administration
-Chemotherapy
-Diabetes mellitus
-Chronic renal failure
-Autoimmune disease

We couldn't determine the impact of all this factors, but we noticed the most frequent one of each group: reoperative vascular procedure (72%) and diabetes mellitus (56%).

We followed antibiotic prophylaxis protocols in all of the cases, prior to first vascular intervention.

Present followed international protocols for antibiotic prophylaxis in adults undergoing prosthetic graft or patch implantation during clean surgical procedures are:

-Cefazolin 1-2 g IV slowly prior to induction of anesthesia and repeated (1-2 g) q 8 hours for 24-48 hours, or cefuroxime 1.5 g IV and q 12 hours for total of 6 g; a single dose of Cefazolin 1 g IV is recommended prior to endovascular stent deployment

-When methicillin-resistant Stapylococcus aureus is cultured on body surfaces or is a known important pathogen in hospitalized patients, add Vancomycin 1g IV infused over 1 hour

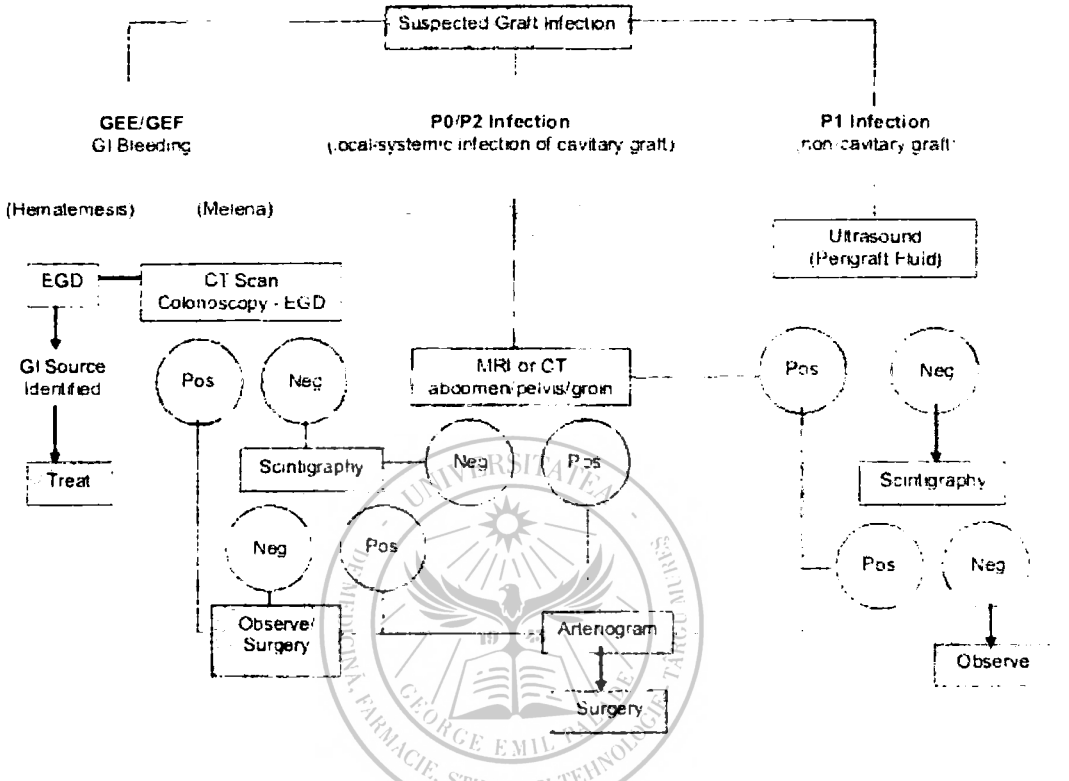
-If the patient has a cephalosporin allergy, give Aztreonam 1 g IV q 8 hours for 24 hours

-If the patient has a Vancomycin allergy, give Clindamycin 900 mg IV over 20-30 minutes followed by 450-900 mg IV q 8 hours for 24 hours

Staphylococcus aureus was the most prevalent pathogen (95%) found affecting our grafts, literature depictions are as well showing it accounting for one fourth to one half of infections depending on the implant site. Graft infections due to S. epidermidis or gram-negative bacteria have increased in frequency[3]. This change in the microbiology of graft infection is the result of reporting both early- and late-appearing graft infections, including aortic graft infections. Graft infections associated with negative culture results are caused by S. epidermidis or other coagulase-negative staphylococci, and on occasion by Candida species. Infections due to gram-negative bacteria such as E. coli, Pseudomonas, Klebsiella, Enterobacter and Proteus species are particularly virulent. Methicillin-resistant S. aureus (MRSA) now accounts for one fourth of early prosthetic graft infections. The recent increase in MRSA wound infections may justify use of specific antibiotic prophylaxis for all vascular device implant procedures.

Diagnosis was based on clinical examination, microbiologic methods and operative findings. Clinical evaluation includes: patient history, physical examination, diagnostic testing and vascular imaging (arteriography, contrast- enhanced CT, ultrasonography, MRI, endoscopy).

Table I Algorithm for evaluation of a suspected prosthetic graft infection taken from Rutherford Vascular Surgery, sixth edition, chapter 59:879 (written by Bandyk DF, Back MR[1]). GEE, graft-enteric erosion GEf, graft-enteric fistula, EGD, esophagogastroduodenoscopy, GI, gastrointestinal; Pos, positive; Neg, negative



RESULTS AND DISCUSSIONS

Management strategies. Treatment should be individualized. The surgeon should select a procedure that the patient can tolerate and that eradicates the clinical manifestations and potential complications of the infectious process. Available treatment options can include graft excision without revascularization[10], graft preservation[2], graft excision coupled with extra-anatomic by-pass[6,8] (conventional management) and graft replacement in situ[11].

The general principles of any chosen treatment are: determining the extent of graft infection, removing the graft, debridement of the arterial wall and perigraft tissues and drainage and antibiotic therapy. Regarding the revascularization of organs and limbs, several vascular groups have demonstrated decreased morbidity and mortality with staged or sequential treatment as compared with traditional treatment (total graft excision followed by immediate extra-anatomic bypass). If a monophasic Doppler arterial signal is present at the ankle after graft excision or if arterial systolic pressure is greater than 40 mm Hg at the ankle or forearm, delayed reconstruction is an option because sufficient collaterals are present to maintain limb viability. In the presence of critical limb ischemia, arterial revascularization should not be delayed because of the associated morbidity from ischemic compartment syndrome and nerve ischemia.

We performed 20 graft excisions for infrainguinal graft infections, with the removal of the entire graft, radical debridement of infected perigraft tissues, closure of the arteriotomies with monofilament suture and the administration of systemic and topical antibiotics. We attempted graft preservation in 5 cases of infrainguinal prosthetic bypass graft infection (with serial surgical wound debridement, coupled with antibiotic therapy, early muscle flap coverage and repeated wound cultures to identify any development of bacterial resistance or change in the microbial flora). We used the staged approach in 20 cases, beginning with drainage of the perigraft abscess, followed in 2 or 3 days by graft excision and autogenous vein grafting. We performed none in-situ replacements with Rifampin-bonded prosthesis, partly because they were not available until a few years ago, partly because we didin't consider it a proper option, given the case selection.

Calligaro[4] and coworkers recommended specific selection criteria and treatment adjuncts for selective graft preservation for early extracavitary infections, such as: (Table 2)

Table II. Selection criteria and treatment adjuncts for selective graft preservation for early extracavitary infections[5]

Selection criteria for graft preservation:
Patent graft that is not constructed of polyester (Dacron)
Anastomoses are intact and not involved with infection
Patient has no clinical signs of sepsis
Treatment adjuncts for graft preservation:
Repeated and aggressive wound debridement in the operating room
Daily wound dressing change at 8-hour interval using dilute povidone-iodine(1 ml of 1% povidone-iodine in 1L normal saline)
If wound is closed between serial wound debridement, antibiotic(vancomycin,tobramycin)-impregnated methylmethacrylate beads are implanted in the subcutaneous tissue
Administration of culture-specific antibiotics
Rotational muscle coverage of the exposed prosthetic graft segments

For the patients with infection localized to only a portion of an aortofemoral graft, we preferred, for the decreased morbidity, the excision of the infected portion of the graft (partial graft excision) and after solving the inguinal infection, a staged[7] extra-anatomical bypass.

As for the gold standard regarding the aortic graft-total graft excision and ex-situ bypass, we only performed 5 of them. 3 patients died and 2 required major amputation.

We performed none total graft excision and in-situ replacement.

We performed none obturator extra-anatomical bypasses, only axillobifemoral ones.

CONCLUSIONS

Dissatisfaction with the morbidity and the mortality of treating vascular graft infections, regardless of location, by total graft excision and remote bypass has been the impulse for the expanded application of lately performed in-situ bypasses, given also the revolutionary matherials (antibiotic-coated and bacterial colonization-proof grafts) available and for graft preservation procedures.

Clinical experience with this less aggressive treatment options is evolving and continued progress can be expected.

Prophylactic use of an antibiotic-bonded graft would be of most clinical benefit in patients judged to be at increased risk for infection.

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Plaque formation in young patients with diabetes mellitus

Adriana Monea, A. Monea

The present investigation was performed to evaluate the influence of gingival alterations that occur in patients with diabetes mellitus on the amount of de novo plaque that forms during a 24 hours period of no oral hygiene. Material and methods. 49 subjects participated in this sturdy, 20 healthy and 29 with diabetes mellitus type I. The condition of the gingiva and the presence of supragingival plarque were examined at 4 surfaces of each tooth, at a baseline examination Than all patients were subjected to a professional tooth cleaning and instructed to refrain from any measure of oral hygiene for the next 24 hours After this period the examination were repeated. Results. The clinical measurements revealed that during a 24 hours period of no tooth cleaning subjects with diabetes mellitus formed generally more plaque than healthy subjects. It was found more frequently on tooth surfaces adjacent to sites with gingivitis. Conclusions. The condition of the gingival plays an important role for de novo plaque formation. Patients with diabetes mellitus have more sites with inflamed gingival tissue, which favors plaque accumulation.
Key words: diabetes mellitus, plaque formation

Diabetes mellitus is a syndrome characterized by insufficient secretion of insulin, decreased glucose tolerance and a tendency for development of microangiopathy, neuropathy and arteriosclerosis.

Diabetically conditional periodontal changes have often been discussed in the literature, and it has been postulated that diabetes does not predispose to periodontal disease. Several studies in recent years have demonstrated, however, that there is a causal relationship.[4,9]

It has been demonstrated that child-diabetics with well-controlled disease had more gingivitis than age matched controls without diabetes; both groups had the same plaque score (Faulconbridge, 1981). In adults it had been shown, in a longitudinal study, that even patients with controlled diabetes have more gingivitis and loss of epithelial attachment than nondiabetics.[11]

Data indicate that altered neutrophil function may be responsible for accelerated periodontal tissue breakdown in poorly controlled diabetics. An impaired chemotactic and phagocyte activity of the polymorphonuclear leukocytes has been found in diabetics.

The aim of this study was to evaluate the influence of gingival status upon the rate of plaque formation in 24 hours in healthy and diabetes type 1 young patients.

MATERIAL AND METHODS

In this study 72 subjects were included, 34 healthy and 38 with diabetes type 1, with a mean age of 23 years old (range 18-27) (Figure 1).

They had no sites with pockets more than 3 mm deep and no open carious lesions. Gingival inflammation and the presence of plaque were recorded at 4 points of each tooth; a site was considered inflamed. When there was a marked change in color and texture or bleeding could be provoked by gentle probing in the orifice of the gingival sulcus. The % of inflamed sites per individual was calculated.

The tooth deposits were disclosed with Erytrocin (Diaplac, Astra Wallco AB, Sweden) and the presence of plaque was scored if a continuous band of organic material was found to be located in the marginal portion of a tooth surface.

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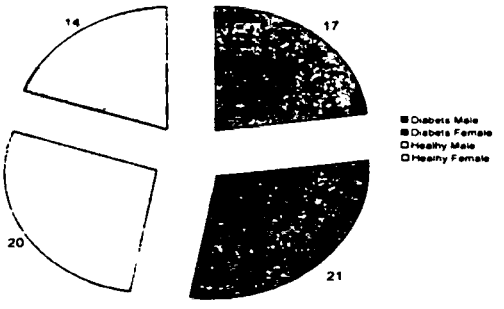

Figure 1. Representation of study groups

Table I. Mean individual percentage of sites showing gingivitis and plaque deposits on tooth surfaces and tooth groups in the study group

n = 38 subjects with diabetes type 1	gingivitis %	plaque deposits Baseline %	24 hours %
all surfaces	45	61	25
incisors and canines	39	58	25
premolars	40	55	19
molars	57	70	32
TOOTH SURFACES			
buccal	35	59	19
lingual	37	50	16
approximal	53	69	35

Table II. Mean individual percentage of sites showing gingivitis and plaque deposits on tooth surfaces and tooth groups in the control group

n = 34 healthy subjects	gingivitis %	plaque deposits Baseline %	24 hours %
all surfaces	38	49	16
incisors and canines	31	51	17
premolars	42	59	16
molars	47	67	23
TOOTH SURFACES			
buccal	28	41	15
lingual	31	52	16
approximal	42	60	23

Following this first examination, all participants were subjected to a comprehensive professional tooth cleaning and instructed to refrain from all oral hygiene measures for the next 24 hours. After this period, a re-examination regarding plaque was performed, using the plaque formation rate index [1]. All the measurements were made by the same examiner.

RESULTS

The findings from the baseline examination for the group of subjects with diabetes type 1 revealed that, on the average, 45% of all gingival units showed a marked change in colour, texture or bled on probing and 61% of all tooth surfaces had plaque deposits (Table I).

As a rule, the posterior tooth regions and approximal units exhibited more plaque and gingivitis than the anterior segments and buccal/lingual sites. Thus, inflamed gingival was found more frequently at molar sites (57%) than at incisors and canines (39%) and interproximal (53%) than at buccal (35%) sites. Plaque occurred more frequently on molar (70%) and approximal tooth surfaces (69%) than on incisors and canines (58%) and on buccal surfaces (59%).

The 24 hours reexamination disclosed that all subjects had formed plaque; on the average 25% of all tooth surfaces were found to harbor stainable material. De novo plaque appeared to be more frequent at molar surfaces and approximal surfaces (32% and 35%), compared to incisors, canines and buccal surfaces (25% and 19%).

The results for the group of healthy subjects are presented in Table II.

The data presented clearly demonstrated that in all segments of the dentition de novo plaque had formed more frequently at initially inflamed sites of subjects with diabetes type 1; these patients tend to have more destruction around the first molars and incisors, a condition that becomes generalized at older ages.

DISCUSSION

Endocrine disorders, such as diabetes and hormonal fluctuations that are associated with puberty and pregnancy are well known examples of systemic conditions that adversely affect the health of the periodontium. Endocrine disturbances and hormone fluctuations affect the periodontal tissue directly, modify the tissue response to local factors and produce anatomic changes into the gingiva that may favor plaque accumulation and disease progression.[2,8]

Diabetes mellitus is an extremely important disease from a periodontal standpoint; it is a complex metabolic condition characterized by chronic hyperglycemia whose long term complications are micro vascular disease (retinopathy, nephropathy, neuropathy), macro vascular alterations (cardiovascular and cerebrovascular), increased susceptibility to infections and poor wound healing.[3,11]

The influence of diabetes on the periodontium has been thoroughly investigated; although it is difficult to make definitive conclusions about the specific effects of diabetes on periodontium, a variety of changes have been described, including a tendency toward enlarged gingival, sessile or pedunculated gingival polyps, polypoid gingival proliferations, abscess formation, periodontitis and loosened teeth.[7,9,15]

Periodontitis in type 1 diabetes appears to start after age 12.44. The prevalence of periodontitis has been reported as being 9,8% in 13 to 18 years old, increasing to 39% in those 19 years and older. Severe gingival inflammation, deep periodontal pockets and abscesses often occur in diabetic patients with poor oral hygiene. Young subjects with type 1 diabetes tend to have more destruction around the first molars and incisors than elsewhere, but this destruction becomes more generalized at older ages.[10,13,14]

The results of the present investigation revealed that during a 24 hours period of no oral hygiene, subjects with diabetes type formed substantially more plaque than healthy individuals. In all parts of the dentition, dental deposits formed more frequently on tooth surfaces adjacent to sites with gingivitis.[5,8]

The observation that gingival inflammation can influence early plaque formation is not new, but the present findings have extended the previously published information[1] by using a large study population and including whole mouth examinations of plaque and gingivitis.

The findings of our study also corroborate data reported by Hillman and Hull who studied plaque formation on 6 selected teeth in 21 young patients during 2 periods of 7 days each; one starting when the participants had experimentally induced gingivitis and one when their gingivae were comparativelly healthy. The examination after 24 hours of no oral hygiene gave a plaque score of 1,12 in the gingivitis and 0,89 in the healthy dentition.

The findings of our study strongly imply that the enviroment at a gingivitis site promotes early colonization of plaque forming bacteria. These data seem to be in variance with those reported by Quirynen et al (2001)[12] who considered that the influence of gingival inflammation on the rate of plaque farmation could be first distinguished only after 36 hours of no tooth cleaning. This difference is most likely explained by the fact that in their study the scorings were confined to labial surfaces of selected teeth, gingival inflammation was experimentally induced during a 2/3 week period, while in our trial all surfaces of teeth in subjects with naturally occuring gingivitis were included in the examination.

CONCLUSIONS

·The conditions of the gingival tissues play an important role for de novo plaque formation.

·Patients with diabetes mellitus type 1 have more sites with inflamed gingival tissue, which favors plaque accumulation.

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Electronomicroscopic study of root surfaces debrided with different scaling methods

Blanka Petcu[1], S. Bocskay[2], L. Barbu[3]

Introduction: In the present study we comparatively assessed by scanning electronomicroscopy (SEM) different root surfaces debrided in vitro by ultrasonic, sonic and manual devices.
Material and method: In the study we used 8 extracted teeth, grouped into 4 categories: group A with teeth covered by calculus; group B consisting of teeth cleaned by sonic device, group C debrided manually and group D with teeth cleaned by ultrasonics. On the SEM images we analized smoothness, the presence of level changes or calculus residues.
Results: Group A offered images of extremely uneven, porous surfaces, scattered by craters and tunnels. Group B showed abundant calculus residues, significant changes of level, craters and grooves. Group C indicated smooth surfaces, with small amounts of mineralized structures and at group D we noticed the presence of significant residues of calculus, accompanied by reduced changes of level in cementum.
Conclusions: None of the tested scaling method offered a completely calculus removal, but manual scaling offered superior results, comparing to sonic and ultrasonic cleaning.
Key words: scaling, ultrasonic, sonic, manual

Tooth scaling and root planing is a non surgical routine dental procedure of scraping away the bacterial plaque and dental calculus from the teeth, especially from the root surfaces, below the gum line. The success of this intervention depends on the detailed thoroughness of the root surface debridement and the patient's standard of oral hygiene [5,6].

Since the 1960s studies regarding manual and power-drived instrumentation of root surfaces have consistently produced varied and conflicting results[1,2,3]. Ultrasonic devices seem to offer similar results to hand instruments for removing plaque, endotoxins and calculus[11,18,20]. In addition, several studies have shown that the debridement time spent per tooth may be reduced with sonics and ultrasonics as compared to manual scaling [4,19].

The major goal of initial periodontal therapy is to effectively remove plaque and calculus without causing root surface damage. Investigations evaluating differences with regard to the magnitude of root surface alterations produced by hand, sonic, and ultrasonic instruments are inconclusive[8,9,17]. When sonic is compared to ultrasonic, several studies find sonic to be similar[10] or inferior to ultrasonic with regard to root surface smoothness[7]. Several older studies show that curettes leave the root surface smoother than ultrasonics [12,16].

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Therefore, the present in vitro study was designed to evaluate with the use of scanning electronmicroscopic (SEM) examination the root surfaces debrided by sonic, ultrasonic and hand instruments. The images obtained by SEM indicated the morphological characteristics of the radicular cementum after calculus removal.

MATERIAL AND METHOD

Eight mandibular and maxillary teeth (4 incisors and 4 molars) extracted for periodontal reasons were selected for this study. All teeth had abundant subgingival calculus and they were randomly assigned to one of the four groups of the study: group A (control group) with two teeth covered by subgingival calculus and without instrumentation; group B consisting of two teeth cleaned by a sonic device (KaVo Sonicflex lux 2000, Perio tip, at medium power), group C formed by two teeth that were debrided manually (well shaped Gracey curettes 3-4, Hu-Friedy) and group D containing the last two teeth exposed to piezoelectric ultrasonic scaling (NSK, Varios 750, G6 tip, at medium power). All studied root surfaces received apical to coronal strokes until each surface got smooth and seemed clean. At the group treated with ultrasonics and sonics the active tips were used with a 10-15 degrees inclination between tip and root; at the hand instrumented group the curette was activated at an angle of 50-60 degrees. The 8 teeth were numbered to provide easy recognition.

All samples were prepared for the SEM examination: therefore they were rinsed two times in phosphate buffered solution at 37°C and fixed in 0.1 M cacodylate buffer containing 2.5% glutaraldehyde at room temperature for 45 minutes. This was followed by post-fixation in 1% osmium-tetroxide in 0.1 M cacodylate buffer for 15 minutes, rinsing in water and staining in 2% aqueous uranyl acetate for another 30 minutes. Afterwards specimens were dehydrated in ascending grades of ethanol. Ten-minute immersion in each alcoholic solution was performed at concentrations of 70%, 80% and 90% ethanol. Sixty-minute immersion in 100% ethanol was carried out. Specimens dried at room temperature during 10 hours. Teeth were fixed in aluminum stubs and then sputter-coated with gold-palladium during 2 minutes. All samples were examined by scanning electron microscopy (JEOL JSM 5510, Tokyo, Japan), operated at an acceleration voltage of 15 kV and with a tilt angle of 30° to 45°. The images aquired at relatively moderate magnification (of 25X, 100X, 500X, 1000X and 1500X) were used for the descriptive morphological analysis of the cementum, considering the smoothness, the presence of mineralized calculus residues or any level changes on de root surfaces

RESULTS AND DISCUSSIONS

Differences in surface roughness of the treated groups could be seen clearly on the SEM photomicrographs.

The cracks visible on the cementum surface are artifacts caused by the dehydration during specimen processing.

Group A, containing uncleaned teeth, offered images of extremely uneven, porous root surfaces, scattered by craters and tunnels (Figure 1). These characteristics are specific for the subgingival calculus.

Figure 1. Micrograph of not scaled root surface (control), magnification 100 X.

Specimens of group B, cleaned by the sonic device, showed abundant calculus residues (Figure 2), associated with significant changes of cementum level, several craters and irregular grooves on the root surface (Figure 3).

Figure 2. Micrograph of root surface cleaned by sonic instrument, magnification 100X. Note the rough surface with deep grooves and calculus residues.

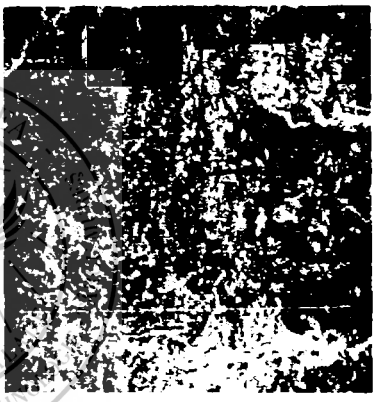

Figure 3. Higher resolution scanning electron micrograph (magnification of 1500X) of the root surface scaled by sonic device, showing large calculus deposits.

The photomicrographs of group C, cleaned by hand instrumentation, indicated the smoothest surfaces among the treated groups (Figure 4).

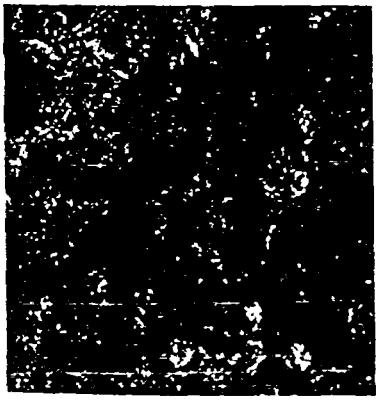

Figure 4. Micrograph of root surfaces of hand scaling, magnification 100X

this group, superficial grooves were observed following the same direction of the scaling movements and less roughness and better finishing surfaces were found when compared to ultrasonic and sonic groups (Figure 5).

Figure 5 Higher magnification (1000X) of the hand instrumented root surface. Note the parallel orientation of the grooves left by the curette

The teeth of group D (representing ultrasonic scaling) harbored significant residues of calculus, accompanied by the presence of deeper sulcus and rougher surfaces than the curette group (Figure 6).

Figure 6 Micrograph of the ultrasonic scaled root surface, magnification 100X, presenting mineralized deposits and depressions.

Although there are many advantages of using power-driven scalers instead of hand curettes[4,15], the present study showed that ultrasonic and especially sonic devices produced rougher root surfaces than curettes. The use of ultrasonic and sonic instruments created surface alterations, including scratches, nicks and gouges, more marked than in curette group.

Previous studies have evaluated differences regarding the roughness produces by sonic, ultrasonic and hand instruments[3]. However, the angulation and design of instrument tip, sharpness of working edge, the length of time the instrument is in contact with the root and the cumulative number of strokes has an impact on the degree of root damage[3] and this situation can be explained by the lack of standardization.

CONCLUSIONS

These findings show that manual instrumentation produced a smoother root surface than sonic and ultrasonic scaling. In addition, hand instrumentation with curettes produced lower roughness than sonic and ultrasonic debridement. The sonic group exhibited the highest surface roughness, as well as the deepest cementum defects caused by scaling.

Within the limits of the present study it can be concluded that none of the tested scaling methods offered a complete calculus removal, but manual scaling attained superior results, comparing to sonic and ultrasonic cleaning. Therefore, we recommend the use of manual scaling, either alone or in combination with the other two methods.

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Epidemiological and social implications on general health status of an aging population in three rural settlements of Mures county

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Changes in socio-economic status and various health problems adversely affect an individual's way of life during old age.

Objectives: to study the health and social problems of the target group and to identify the key-problems and factors that have the biggest impact on the quality of life of the elders population and more specific dental treatment.

Materials and methods: descriptive transversal randomized study carried out in the field with the help of medical personnel under contract with health services in three rural settlements in Mures County. A total of 213 elderly patients (60 years and above) were given to fill out a questionnaire on their visit to the medical clinic in their village. Findings were described in terms of proportions and percentages to study the socio-economic status of the sample and its correlations to social problems.

Results: around 73% of the patients belonged to the age group of 60-69 years old. Nearly half of the respondents had a low level of education. Around 48% were dissatisfied with their life. Majority of them had systemic diseases as hypertension followed by arthritis, diabetes, asthma and anemia. Almost 68% of the respondents felt that the main attitude of the society towards elders was the one of neglect.

Discussions: the need of dental treatment must be correlated with the need of general medical treatment and the modification of the general society towards this age category. It is imperative for the elders to understand the rights that derive form the medical insurance increasing the addressability and the impact of the primary and more specialized medical services, including here also the dental services.

Key words: elderly patients, medical insurance, dental services

There is no United Nations standard numerical criterion, but the UN agreed cutoff is 60+ years when referring to the elderly population in all the demographic documentation.[8] We also used this age limit in our study. After 1990, in Romania the aging process recorded a big loop by two mechanisms: one was the declining birth and the second was the migration of the young workforce to other areas of Europe. As a result, while in the 1992 census the elderly represented only 12% to a 37% young adult population and a 58% of working adult population, in the 2002 census we are talking about a percent of 19% elders to a young adult population of 17.6% and a working adult segment of 58.32% and a trend of continuously aging process and raising the retirement age.[5]

The physiological decline in elders refers to the physical changes an individual experiences because of the decline in the normal functioning of the body resulting in poor mobility, vision, hearing, inability to eat and digest food properly, a decline in memory, the inability to control certain physiological functions, and various chronic conditions.[7] Change in socio-economic status adversely affects the individual's way of life after retirement. The economic loss is due to a change from salary to pension or unemployment leading to economic dependency on family (children or relatives).[9]

The feeling of inutility given by the physical downfall is amplified by the economic deficiencies and the social alienation.

This article, based on a more complex study and with much greater implications tries to cover only two objectives as follow:

1. To study the background and socio-economic status of the elderly.
2. To study the social and health problems faced by the elderly and their attitude towards life.

MATERIALS AND METHODS

This study was carried out over a period of 11 months from February 2008 to January 2009, the subjects of the study were elders over 60 years old, home residents in three settlements in the vicinity of Tirgu Mures in which there was a medical facility and medical personnel working under contract with the National Health Insurance Company. The subjects were given a questionnaire when presenting to the

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doctor for different health problems, questionnaire based on an international QLRQ (Quality of Life-Related Questionnaire), which degree of understanding was pilot tested on 10 random subjects making the necessary changes. The 213 subjects were given the necessary help by the medical nurse who was also responsible for getting the signed approval for the use of personal data, answers were given individually. Data processing was made by a single individual.

RESULTS

Socio-demographic characteristics

Table I shows that a major fraction of the population was in the age group of 60-69 year old, while a small fraction (2.8%) were 80 years old or older. Males and females formed an almost equal proportion of the study sample. Majority of the subjects (89%) were of Romanian ethnicity and regarding the family system about 56.8% were traditional families with 2 or 3 different generations under the same roof, followed by nuclear families. 12.1% of males and 67.7% females were widows. 2.3% were singles, all males. The degree of education of the sample was a low one.

Table I. Demographic distribution of the respondents

	Males (92) (%)	Females (121) (%)	Total (n=213)
Age (years)			
60-69	57 (61,9%)	97(80,1%)	154(72,3%)
70-79	31(33,7%)	22(18,2%)	53(24,8%)
Over 80	4(4,3%)	2(1,7%)	6(2,8%)
Marrital status			
Married	70(76,1%)	31(25,6%)	101(47,4%)
Single	5(5,4%)	0(0%)	0(0%)
Divorced	6(6,5%)	8(6,6%)	14(6,6%)
Widow	11(12,1%)	82(67,7%)	93(79,8%)
Education			
Elementary unfinished	21(22,8%)	75(62%)	96(45,1%)
Elementary	1(1,1%)	1(0,8%)	2(0,9%)
Secundary unfinished	40(43,5%)	38(31,4%)	78(36,6%)
Secundary	14(15,2%)	5(4,1%)	19(8,9%)
Unfinished high-school	13(14,1%)	2(1,7%)	15(7,0%)
High-school	2(2,2%)	0(0%)	2(0,9%)
College	1(1,1%)	0(0%)	0(0,5%)

Health problems of the elderly

Table II shows that all respondents had multiple health problems, most common being hypertension, osteoarthritis, diabetes or bronchial asthma. Others included cataract, anemia or dermatological problems. Bone and joint problems were more common in females than males while other health problems were almost similar among both the genders.

Table II. Morbidity pattern of the respondents

Disease	Males(%)	Females (%)	Total(%)
Hypertension	53(57,6%)	73(60,3%)	126(59,1%)
Diabetes	11(11,9%)	11(9,0%)	22(10,3%)
Osteoarthritis	19(20,6%)	69(57,0%)	88(41,3%)
Lung diseases	13(14,1%)	10(8,2%)	23(10,7%)
Others	17(18,4%)	19(15,7%)	36(16,9%)

Attitudes towards old age

Table III shows that almost 98% of the respondents felt that old age had affected their day-to-day life. Among these, 86.4% felt that age had partially affected their daily activities. Half of the people interviewed felt neglected by their family members, while 47% felt unhappy in life and 36.2% felt they were a burden to the family. An unfavorable attitude was observed to be more among females than males.

Table III. Attitude towards old age

	Males(%)	Females(%)	Total (n=213)
Old age has affected day-to-day life	88(42,3%)	120(57,7%)	208(97,7%)
-partial	76(41,3%)	108(58,7%)	184(86,4%)
-completely	12(50%)	12(50%)	24(11,4%)
Feel neglected by family members			
-always	4(40%)	6(60%)	10(4,7%)
-sometimes	46(37,7%)	76(62,3%)	122(57,3%)
Feel a burden to family	34(44,2%)	43(55,8%)	77(36,2%)
Not happy in life	46(45%)	56(55%)	102(47,9%)
Not loved by family members	28(35,9%)	50(64,1%)	78(36,6%)

Table IV shows that females had poor perception regarding economic and social security as compared with males. Approximately 40% of the respondents interviewed had feelings of insecurity while around 56.3% were deprived of financial security. Other reasons of insecurity were diseases of family conflicts.

Table IV. Perceptions of elderly regarding economic and social security

Perception on security	Males(%)	Females(%)	Total(%)
Deprived of finances	47(39,2%)	73(60,8%)	120(56,3%)
Deprived of companions	4(28,6%)	10(71,4%)	14(6,6%)
Troubled with feelings of insecurity	35(41,1%)	50(58,9%)	85(39,9%)

Table V shows that 48% of the respondents considered the mainly negative factor that negatively influences the quality of life poverty followed by illness (41.3%). Other reasons for feeling sad were unwed children at home, alcoholic son/son-in-law, financial loss, illness of spouse, children staying away from them, recent death of children, or not owning a house.

Table V. Reasons for feeling sad

Reasons	No (n=213)
Poverty	102(47,9%)
Illness	88(41,3%)
Neglected	28(13,1%)
Loss of spouse	22(10,3%)
Loneliness	11(5,2%)
Others	54(25,4%)
Family failure of the children	14(6,6%)
Alcoholic member of family	11(5,2%)
No specific reason	4(1,9%)
Illness of the spouse/children	8(3,8%)
Children staying away	8(3,8%)
Financial loss	3(1,4%)
Death of children	3(1,4%)
Not owning a house	3(1,4%)

[illegible] of the respondents knew about the medical rights they got and only 14.6% ever benefited of those rights. Over 75% declined their trust in the medical insurance system and that they were obliged to pay for the medical services that in fact were free.

It was observed in our study that around 52% of the respondents felt that old age affected their role in the family. A total of 35% of the respondents felt they were not consulted by the family members for making decisions regarding themselves. They felt they were ignored by family members more because of their economic dependence than of the physical illness. In spite of being unhappy due to these problems, they all preferred their home to an old age home for their residence.

DISCUSSIONS

More than half of the participants were from traditional families (58,6%) and 33% were from nuclear families[1], confirmed also by other studies as the one of Smirnov I , Marghescu C. which concluded that the position in the traditional Romanian family is given by marital status. The percent of widows among females obtained was far more superior to the one recorded in similar social-demographic studies in Cluj county in 1995 and 2007.[1]

The data from the Romania 2002 census show that the population between 55 and 65 years in rural area has a low educational and professional training level. The results in our study follows the national data with a 46% of unfinished primary education of the age limit between 60-69 years as the same people form the 55-65 group during the period of the census. We could also conclude that the females had a lower level of training and education than males.[5]

Half of the respondents felt neglected by their families and almost half (49.7%) considered their lives unhappy lives. Another important discovery was that was a deep and consistent feeling of social alienation among the elderly from the group because of the lack of social contact (24.8%).[2] The reason presented for the lack of respect by family or community was the age and the fact that those individuals felt the have no contribution for the society.

Almost 50% identified as the major deficiency of the every day life the poverty by paradox with the claim of 78.8% as a part of the middle class and not the poor class. The next deficiency was the poor health condition (41.3%) or different family problems.[1] The most concerning fact was that the majority of people had absolutely no idea about their medical rights and also the lack of medical addressability[1] due to the fact that in 11 months and from 2600 patients that could have been qualified for the study by age and location only 213 subjects were recruited giving a result of 8.2% addressability of primal medical services in a year in a population with so many chronically developing diseases.

CONCLUSIONS

The results of this study proved the fact that most of the elders questioned are inactive dependent or partially depended on the family persons, suffering from many general chronic diseases and also the neglect from family and society. This study is only a fraction of a more in depth specific research that included also the addressability of the dental services in general and prosthetic treatments in particularly. This segment of the study wanted to represent a comparing point between the general health status, the state of mind among the third age population of the rural area and the oral health status and the need of dental treatment of this group. The main conclusion of the investigation was that there is a growing need for interventions to ensure the health of this vulnerable group and to create a policy to meet the care and needs of the disabled elderly. Interdisciplinary research, especially correlated with a specific comunitary medicine research is needed for the quality and quantity aspects of the problems.

We accept the limitation of the research reminding that this is only partial and incipient results of a larger study regarding the oral health status, need for treatment and frequency of edentulousness among the elders. Main purpose of this study was not to quantify by degrees but to probe and discover the everyday issues of this population.

Due to the randomization and the time length of the study the results and considerations could be extrapolated to the elderly population from the rural region of Mures county.

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Comparison of the obturation result of lateral condensation versus vertical compaction in a vitro experiment

R.M. Sireteanu Cucui

The purpose of this study was to evaluate the microleakage of two obturation methods: lateral condensation and vertical compaction 15 human teeth were prepared for in vitro experiment. All canals were instrumented and obturation performed for two groups of cases. Time for vertical compaction was longer then for lateral condensation. To quantify the amount of micro leakage on the teeth, a coloring method was performed for the evidence of methylene blue dye leakage After immersion in the dye, the teeth were sectioned longitudinally and dye penetration was evaluated using a stereomicroscope and micro leakage recorded in mm. Overfilling of obturation sealer was observed in all cases of canal obturation, but was just a little amount. No gutapercha overfill was made. This clinical investigation showed no statistically significant difference micro leakage after endodontic treatment using lateral or vertical condensation filling techniques. Although the dye penetration was 100%, indicated the presence of methylene blue in the canal, it is a small indicatory to say if technique or material will be successful.
Key words: lateral condensation, vertical compaction, microleakage

To achieve a successful endodontic therapy, the most important criterion is the well adaptation of the filling material to dentin walls and to prevent apical and coronal micro leakage.[1] After one of lateral condensation and vertical compaction as obturation techniques, to evaluate the apical seal of root canals, in a vitro experiment, methylene blue dye leakage was used to prove the sealing ability.

MATERIAL AND METHOD

The material for this study was 15 extracted human teeth with straight roots, included incisors, canines and premolars inferior and superior. Extraction was practice for complication of carious or paradontopathies and teeth kept in formol 10%.

Teeth were cleaned out of carious dentine, of remaining soft tissues, alveolar bone and tartar and straight acces was performed and the length was determine. The canals preparation was done by step back technique with file K and K-flex (Kerr). At the apex level the preparation length was 45. All the time of biomechanic treatment the canals were irrigate abundant with chloramine and hiperol, alternative. In time and after finnishing of preparation permeability of apex orifice was check up with file Kerr 15. Finally, the teeth were divide aleatory into 2 groupes of 7 for each obturation technique and one tooth positive control with no obturation. For lateral codensation was select a standardizate principal cone. For majority of the cases, after a few trial a 45 cone was used, for other teeth cone of 50. For adaptation no solven was used, only top section. A little amount of obturation seale Endomethasone was introduced with Lentulo file trying to be uniform and then principal cone. For each second cone a space was created with finger spreader (Kerr) and maintained in canal 15-30 sec, for uniform compaction. 6-10 accesory cones were introduced. For the others 7 teeth vertical compaction were performed with standardizate principal cone. 3 pluggers were used. After introduction of a little amount of Endomethasone the main cone was introduced and with a Black hot spoon, section, first cold compaction and after that a succession of warm and compaction. Finally, coronal obturation with zinc- eugenol was made. Teeth were covered with nail polish, excepted apical surface. Imersion of roots in color solution was done to compare the sealing ability, through the evidence of methylene blue dye leakage.

RESULTS AND DISCUSSIONS

Each series was divided like this: 1 tooth negative control and 6 teeth all obturated by lateral condensation/ vertical compaction, respectively. In the

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Table I Time distribution of lot for 7 teeth obturated by Lateral Condensation and other 7 by Vertical Compaction

Teeth	Position	Teeth	Position
D1 6 min	Incisor	D8 15 min	Premolar
D2 9 min	Premolar	D9 18 min	Premolar
D3 8 min	Premolar	D10 15 min	Canine
D4 10 min	Premolar	D11 20 min	Incisor
D5 8 min	Canine	D12 20 min	Incisor
D6 9 min	Canine	D13 17 min	Canine
D7 7 min	Premolar(control)	D14 18 min	Premolar(control)
average	8,12 min	average	17.57 min
stdev	1.345	stdev	2.07
median	8	median	18
mode	9	mode	15
min	6	min	15
max	10	max	20

Table II. Micro leakage in each group of obturation method

Lateral Condensation micro leakage		Vertical Compaction micro leakage	
Teeth	micro leakage	Teeth	micro leakage
D1 Incisor	0.9 mm	D8 Premolar	1.4
D2 Premolar	0,7 mm	D9 Premolar	2
D3 Premolar	1,3 mm	D10 Canine	0.6
D4 Premolar	1,2 mm	D11 Incisor	0.9
D5 Canine	1,7 mm	D12 Incisor	1.7
D6 Canine	2,0 mm	D13 Canine	1.9
D7 Premolar	0 mm (control)	D14 Premolar	0 mm (control)
average	1.3	average	1.42
stdev	0.487	stdev	0.564
median	1.25	median	1.55
min	0.7	min	0.6
max	2	max	2

situation of lateral condensation period of time for obturation was an average of 8 min ± st dev 1,345 minute, depending of number of accesory cones used. The median or the number in the middle of the set was 8, mode or the most frequently value was 9 minute. For vertical compaction, time of obturation was an average of 17,57 ± st dev 2,07 minute, median 18, mode 15, minimum 15 and maximum 20 minute (Table I).

The time for vertical compaction was longer than for lateral condensation, average difference of 11, 5 minute.

Overfilling of obturation sealer was observed in all cases of canal obturation, but was just a little amount. No gutapercha overfill was made.

For control teeth negatives, 1 with lateral condensation, one with vertical compaction, and for the remained one control positive with no obturation all surface were nail polish covered, included apical foramen. For the other 12 teeth, the apex surface was free and coloring with blue methylene was measured. After immersion in the dye, the teeth were sectioned longitudinally and dye penetration was evaluated using a stereomicroscope and micro leakage recorded in mm (Table II).

A deeper penetration suggests a higher micro leakage. The dye penetration for lateral condensation was between minimum 0,7 mm an maximum 2 mm, average 1,3 mm+/_ st dev 0,487, middle of the set or median 1,25 mm. Average 1,42 mm+/_ st dev 0,564 for vertical compaction, with a minim of 0,6 mm and maximum 2 mm, median 1,55 mm.

Lateral condensation presented the lesser average leakage, but a difference of 0,1 mm in the average results was not significant. In the situation of positive control, the dye was found on the entire canal length, and in the situation of negative control was no methylene blue penetration. The results are in concordance with literature, a One-Way Analysis of Variance indicated the two condensing methods were not statistically different ($p>0.05$). When micro leakage was compared within groups, there was also no statistical difference between coronal or apical micro leakage ($p>0.05$).[4]

Several clinical studies, representing the same two techniques, report the success rate of endodontic therapy ranges from 85.4% to 94.5%.[1]

CONCLUSIONS

On the slides made on each tooth the color penetration was observed on microscopic examination. This clinical investigation showed no statistically significant difference micro leakage after endodontic treatment using lateral or vertical condensation filling techniques. During endodontic surgery procedures are necessary to remove the pathological tissue and impermeabling the root-end in order to avoid leakage. It is obtained by the root-end preparation and retrograde obturation, this fact allows the healing. Both the lateral and vertical condensation had micro leakage on apical portions of the root indicating that regardless of the condensation method and the use of a pack able composite, there is still micro leakage that could potentially create further clinical problems. If bacteria

and irritants agents remain in the foramen, there is high possibility that the infection gain the external root surface. Although the dye penetration was 100%, indicated the presence of methylene blue in the canal, it is a small indicatory to say if technique or material will [illegible]success.[illegible]

More clinical comparison between vertical and lateral condensation techniques in the context of the treatment outcome is needed since such studies are scarce in the literature

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Hypodontia: a preliminary epidemiologic study

E. Bud

The purpose of the study was to determine (evaluate) the prelevance of developmental absence of second premolars and lateral incisors

We have chosen a study group of 400 children between 12 and 18 years as a first part of the study. At the end of the study, the group will comprise 1000 children. After a preliminary examination we used panoramic radiographs of the children who presented missing teeth. The prelevance of congenital absence was calculated by tooth type and sex. Results were compared to data from international literature.

Key words: hypodontia, prelevance, congenital absence

A missing tooth is considered a developmental tooth problem if it has not erupted in the oral cavity and is not radiographically visible. Developmental lack of tooth is a result of different factors during the early stages of tooth development.

Hypodontia is a relatively frequent anomaly of the permanent dentition, with a reported incidence between 3.5 and 8.8 percentage, when the third molar is excluded.[3,6,7]. This phenomenon can be observed more frequently in females, although gender distribution showed some local variations when studies were compared.[5,10,13,15]

Although there was some variation in the reported frequency of developmental absence, the majority of studies indicated that the most frequently absent teeth are: the lower second premolar, the upper second premolar, followed by the upper lateral incisor and the lower central incisor [6,11,12,14]. On the other hand, Muller et al.(1970) suggested that the most commonly absent teeth are: the upper lateral incisor, followed by the lower second premolar, the upper second premolar and the lower central incisor. Tavajohi-Kermani et al. (2002) stated that the order was: the lower second premolar, the upper lateral incisor, the upper second premolar and the lower central incisor.

Various aetiological factors are supposed to be the cause of failure in the development of permanent tooth germ, thus leading to its absence: physical obstruction, rupture of the dental lamina, limitation of space, functional anomalies of the tooth epithelium, initial error of the mesenchyme, or disturbances in the development of embryonic fusion of the maxilla and the medial nasal processes (a similar situation occurs in cleft lip and palate).

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Several genetic and syndromic conditions now are known to increase the risk of hypodontia, but congenitally missing teeth are commonly found in healthy, apparently normal people. Some genes, such as MSX1 and PAX9, increase the risk of hypodontia but with variable expressivity, so the extent of the missing teeth differs among people with one of these alleles.

MATERIAL AND METHODS

The research was performed on a study group of 400 children with their age ranking between 12 and 18 years, out of which 168 were boys and 232 girls.

In this age range, the subjects were old enough so that we could confirm the presence of each permanent tooth, notably the later-forming third molars, yet young enough to have lost few teeth, and anamnestic and dental histories were reliable for documenting extractions and avultions.

Patients with facial clefts and craniofacial syndromes were excluded.

The prelevance of congenital absence was calculated according to the type of tooth, and gender.

The data was analysed using the Chi-square test. The value of $P<0.05$ was considered significant.

RESULTS

The test we used to compare final results was the chi-square test. A chi-square test (also chi-squared or χ^2 test) is any statistical hypothesis test in which the sampling distribution of the test statistic is a chi-square distribution when the null hypothesis is true, or any in which this is *asymptotically* true, meaning that the sampling distribution (if the null hypothesis is true) can be made to approximate a chi-square distribution as closely as desired by making the sample size large enough.

Out of the total number of 400 patients (168 boys and 232 girls) 9 were identified as presenting missing superior lateral incisors, 5 girls (55,5%) and 4 boys (44,44%). Chi square was 0.022 which is smaller than the Chi square of 3.418 corresponding to a P=0.05. The results indicate that hypodontia strongly depends on the gender of the patient. The prevalence was 2.25 in 5 patients who had the second superior premolar missing corresponding to a prevalence of 1.25. The patients were 3 boys (60%) and 2 girls (40%). In the mandible there were 8 second premolars absent, a prevalence in 4 girls (50%) and in 4 boys.(50%).

Hypodontia can involve a single dental element but most of the time several teeth are affected. This revealed that a person's liability to hypodontia is a systemic rather than an isolated phenomenon.

DISCUSSION

Many authors have remarked that hypodontia is the most common human malformation, and it usually occurs without any other signs or symptoms of maldevelopment.

There are various causes for the congenital absence of a tooth, notably the absence of an epithelial signal to the ectomesenchyme, the failure of a tooth bud to reach a critical size or, more proximately, to receive the signal to continue development.

Developmental absence of the upper lateral incisor has proved to be more common in females[2,7,8].

CONCLUSION

Our results regarding this study agree with data published in the international speciality literature, missing teeth in females being more frequent. The reason is not clear, maybe sexual dimorphism in general skeletal growth and in tooth eruption.

Regarding the speciality literature prelevance of developmental absence of the permanent upper lateral incisors varies in different studies.

In our study we found a prelevance of 2.25 for the lateral incisors missing teeth. This is a little bit higher than the other values which were between 0.8% and 2%.

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Glass ionomer cements used in atraumatic restorative treatment technique

Gabriela Bereşescu[1], I. Bocskay[2]

The purpose of the study was to evaluate, in a clinical study, the deterioration of glass ionomer cements used with the atraumatic restorative treatment technique or approach. The restorations were placed in 25 adult patients, mainly in small occlusal preparations in molar teeth. Photographs, radiographs and replicas were obtained at baseline and subsequent recalls.The cements showed early high losses of sealant and restorative material. Restoration failures of 7% were from wear and fracture of the cements and reccurent caries.
Although the short-term clinical performance of the glass ionomer cements was resonable, the materials require further improvements in their mechanical properties.
Key words. atraumatic restorative treatment, glass ionomer cement

Glass ionomer cement (GIC) was first introduced to clinical dentistry by Wilson and Kent in 1972. GIC has undergone continuous development designed to take advantage of the material's unique characteristics.

GIC posses certain properties that make them useful as restorative filling materials[2]. These properties include a low coefficient of thermal expansion similar to that of tooth structure, physico-chemical bonding to both enamel and dentin, and the release of fluoride ions into adjacent tooth structures.

This study evaluated the deterioration of glass ionomer cements used with the atraumatic restorative treatment (ART) technique.

Atraumatic restorative treatment (ART) is an innovative, minimal intervention procedure for treating carious teeth[1,4]. The procedure involves removing carious tooth substance using hand intruments only and then restoring the cavity and any adjacent fissures, usually with a glass ionomer cement.

MATERIAL AND METHODS

The handling, deterioration and failure of four conventional glass ionomer cements used with the ART approach were investigated over three years in a clinical study. GIC restorations were placed randomly in 25 adults patients, mainly in small occlusal preparations in molar teeth. Photographs, radiographs and replicas were obtained at baseline and subsequent recalls.

Sharp hand instruments were used to gain access to the lesions and then to remove soft caries along the dentino-enamel junction. Hard, undetermined enamel and carious dentine close tp the pulp were left. No bases were placed. Although patients were treated in a clinic environment, rubber dam,local analgesia and the triple syringe were not used.

Further phptographs and impressions were taken at the subsequent 6-month, 12 month and 24-month recalls. Direct observations were made for bulk restoration fractures and restoration-associated caries demonstrated by cavitation and softened dentine. Indirect observations were made from colour transparencies, colour mismatch and sealant loss. The transparencies were compared against standard linear sets as has been described elsewhere.

The categories approximated the alpha, bravo and charlie categories of the USPHS-Ryge direct clinical observation system. Indirect observations were made from die-stone replicas for marginal discrepancies or fractures and occlusal surface wear or losses of material.

RESULTS

The patients was 8 male and 17 female. After three years, one male and one female patient had been lost from the original patients entering the study. Patients attented for recalls at mean times of 6, 12 and 24 months. After three years 84 restaurations were available for evaluation.

There were no significant differences present in the baseline distribution of the restorative materials by gender and patients age, or by molar tooth site and cavity opening width measured from the transparencies (Table I, Table II).

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Table I. Distribution of restorations by gender and patients age

Material	Gender		Patients age
	Male	Female	
GIC I	23	32	25 97±7 15
GIC II	16	29	27 40±

Table II Distribution of restorations by tooth site and cavity width

Material	Tooth site		Cavity opening width
	Maxilla	Mandible	
GIC I	24	31	2 00±1 21
GIC II	22	23	1 92±1 57

There were no patients with generalized tooth wear or heavy staining, and there were no immediate post-operative problems, although some soft caries was left in the deeper aspects of 11 preparations rather than risk possible vital pulp exposures.

Small surface voids were present in restorations at all time intervals, but ussually involved only four restorations at any one recall period. Relatively few restorations showed other than minor marginal discrepancies or fracture (Table III).

Table III. Number of restorations for marginal discrepancies or fractures

Rating	Material	Base	6 months	12 months	24 months
Good (alpha)	GIC I	51	54	54	24
	GIC II	44	44	43	30
Adequate	GIC I	4	1	1	23
(bravo)	GIC II	1	1	1	7

All glass ionomer cements showed relatively high cumulative occlusal wear rates from baseline values over the study period (Table IV).

Table IV Cumulative occlusal wear before adjustment

Material	Base	6 months	12 months	24 months
GIC I	18 5 ±38 2	64 7± 52 1	79±9 51 0	101 6 ±71 4
GIC II	25 7± 85 1	79 1±110 2	95 9±109 4	129 7±134 6

Relatively few restorations showed good initial colour matching with the adjacent tooth enamel at baseline usually being too light.

Some restorations, at subsequent recalls, became slightly darker than the restored teeth.

One restoration was partly lost from bulk fracture and three shallow restorations werecompletely lost, and not replaced. There were threee instances of recurrent marginal caries. These failed restoraions were replaced by other materials.

DISCUSSIONS

Two patients were lost to follow-up over the tree years study period and relatively few problems were encountered during restorations placement or subsequently. Although attemps were made to simulate field/placement of ART restorations, the main emphasis of the study was elatuate the deterioration behaviour of glass ionomer restorative materials[1]. Restoration deterioration was characterized by early occlusal wear of the glass ionomer cements, especially during the first 6 month.

The glass ionomer cements demonstrated a marked colour shift following their placement[2,5]. The subsequent darkening of the cements was possibly related to their later moisture uptake and maturation. The relevance of this colour shift to restoration deterioration remains to be determined[4].

CONCLUSIONS

From the findings of this tree year clinical study of glass ionomer cements used to restore the ART approach, it was comcluded :

1. The clinical procedures were unerventful and acceptable to patients.
2. The glass ionomer cements were easy to mix and place.
3. The glass ionomer cements evaluated appear suitable for restricted use only, when placed in the occlusal surface of posterior teeth[5].

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Clinical study regarding dental aesthetic restorations

Ileana Roman, M. Pop, Anca Torcătoru, Csilla Benedek, Imola Buka

The aim of the study is to examine clinically and to assess the quality of the esthetic coronal dental restorations.
Material and method: The study has been performed on 175 patients aged between 18 and 73, who attended consultation and treatment. Eight hundred and eight esthetic restorations were examined and data regarding their quality according to their age was recorded
Results: We found that most (40,23%) of the 808 composite restorations had been applied to individuals aged between 26 and 45. According to the type of the cavity in which these had been applied, most of the deficiencies were found in cavities of class 3 (31,67%)
Conclusions: The composite materials proved to be durable materials of good quality. The deficiencies found could be due to the non-compliance with the correct technique of the cavity preparation or in applying the restorations. Replacement of the composite restorations was made upon request or other reasons (secondary caries, color change, inflammation of the gum).
Key words: composite, marginal leakage

The advent of the composite diacrylic resins and their introduction into dentistry constituted a real progress. Initially recommended only for the restoration of front teeth, due to their physical and mechanical properties they have become the most often used materials in the restoration of front teeth and the lateral teeth as well.

The aim of the study was to evaluate the quality of the esthetic coronal restoration on a randomly chosen group of patients.

MATERIAL AND METHOD

The study was performed in 175 patients aged between 18 and 73, who got consultation and treatment.

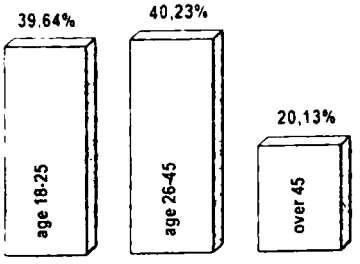

Figure 1. Number of restorations distributed by age groups

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Eight hundred and eight esthetic restorations were examined and the data regarding their quality according to their age were registered on a record elaborated for this reason. In this record, besides the personal data of the patients we also noted the age of each restoration according to the reports of the patients and the deficiencies found at the objective examination in each restoration: marginal leakage, marginal discoloration of the restoration or the wall of the cavity, and the loss of restoration.

The examination of the restoration was made by clinical, conventional means: dental mirror, dental explorer and air jet. We did not make any radiological examination.

An Excel table was used for the centralization and data analysis.

RESULTS AND DISCUSSIONS

Of 175 patients 53 (30,28%) were males, and 122 (69,72%) were females. Correlating the number of esthetic restoration with the age groups we found that the most esthetic restorations had been applied in patients aged between 26 and 45 (40,23%) respectively between 18 and 25 (39,64%). Approximately one fifth of the total number of restorations (20,13%) were found in patients aged over 45. Of 808 restorations, 252 (31,18%) had been applied to front teeth and the rest 556 (68,82%), more than double had been applied to lateral teeth. This fact is explained by the continuing improvement of the mechanical and esthetic parameters of the composites used in the last decades.[8,9]

Mair[4] shows that after 10 years the resistance of these materials applied to lateral teeth is similar to amalgam restoration.

Regarding the distribution of the restorations by Black type cavity, most of them had been applied in class II cavities (346 – 41,83%) and the least had been applied in class V cavities (14 – 1,73%). Of the total restorations applied in class II cavities 107 (30,92%) had insufficient marginal seal and of those applied in class V cavities only 1 (7,14%).

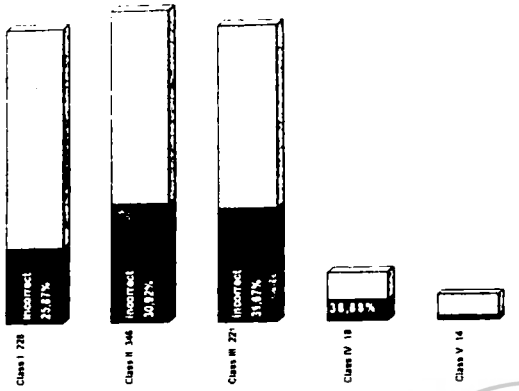

Figure 2 Restorations of incorrect marginal seal distributed by cavity types

Probably the most important feature that must be taken into consideration in appreciating the quality of any restorations is the marginal seal. In case of composites the sealing ability of the material may be damaged because the setting is achieved through a polymerization reaction due to which the material shrinks (Morwafi,).[7] The hybrid composites – the most often used ones – do shrink in a proportion of 2,5 – 3 % (Ferrari).[3] The reduction of the negative effect of the polymerization shrinkage over the marginal seal of the restoration can be obtained among others through the use of a high quality adhesive system.

Versluis and co. quoted by,[1] in recent in vivo and in vitro studies show that the stratified application of the photopolymerized composites reduces the linear shrinkage and assures a better marginal seal.

Kuijis and co. quoted by,[1] confirm the fact that the type of the composite material and the duration of the polymerization are key factors in reducing the tensions and the appearance of the marginal leakage.

In our study, 552 (68,31%) of the total 808 restorations had a good marginal adaptation.

In the evaluation of the marginal seal made by Disken and Horstedt,[5] a good adaptation of 96% was found in the restorations which had had more than 1 year, and Morwafi[7] studying the long time behavior of the composite materials found a 90% rate of success in restorations older than 5 years.

Regarding the physical integrity of the restorations, in our study 94,5% of the restorations were appropriate, they did not present any fissures or fractures.

Fracture of the cavity wall was found only in 9 cases (1,11%) and the fracture of the restoration in 14 cases (1,73%).

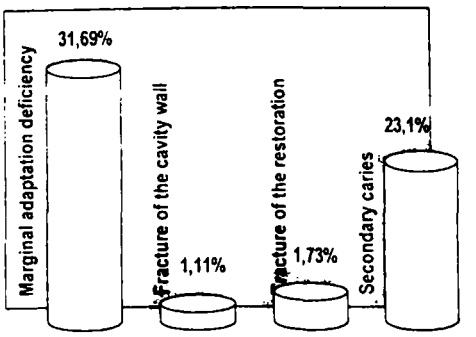

Figure 3. Deficiencies occurred in composite restorations

Disken and Horstedt[5] found a reduced frequency of the enamel fractures or composite restoration which are more than 1 year old.

Fonseca[4] through in vitro studies showed that the design of the different cavities prepared for restoration does not have any effects on teeth filled with composite materials but the less aggressive preparation is more adequate than an extended cavity in order to prevent the fracture.

Secondary caries were found in 186 (23,01%) restorations.

This high percentage is probably due to the incorrect preparation of the cavity or the incorrect restoration technique (etching dissolution, application of the material).

Perry,[6] in a clinical study performed on patients aged between 21 and 45 having restorations in the molar teeth 1 and 2 , at the evaluation performed after 3, 6, 9 months and 1, 2 years, found that on neither restoration did appear secondary caries.

As far as longevity of the composite restoration is concerned, Haikel quoted by[2] found that their durability ranges between 15 and 18 years. Of the restorations examined by us, the majority (527, namely 65,22%) had less than five years, and those having more than ten years were less (54, namely 6,68%).

In our study we found a direct relationship between the age of the restorations and deterioration of the marginal adaptation. Namely, from 18,8% which is being the incidence of the more than 5 years' marginal seal, this increases to 39,8% in the case of the restorations which are 6 to 10 years old, and to 41,6% in case of the restorations which are more than 10 years old.

After examining the patients we found that a part of the restorations had been replaced or they were to be replaced (325, namely 40,22%). 40,92% of the total had been replaced upon request, while 59,08% due to the dentist's recommendation.

CONCLUSIONS

Restoration composite materials turned to be durable materials of good quality.

The deficient marginal seal occurs in restorations of less than 5 years but their incidence increases to 40% in restorations that are more than 10 years old.

Our observations (94,9%) regarding the integrity of the restorations (lack of fractures and fissures) are similar to those found in the literature (90%).

The replacement of the restoration composite materials was made upon request in order to reduce the esthetic aspect or due to some deficiencies like secondary caries, inflammation of the gum.

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Interaction of some beta-blockers with diphenylpicrylhydrazyl free radical

Alina Balint [1], B. Tőkés[2]

The use of diphenylpicrylhydrazyl (DPPH) free radical for estimating antioxidant capacity of substances is specified in the literature. The aim of the study is to identify the interaction of beta adrenergic blockers with N-centered free radicals such diphenylpicrylhydrazyl. We chose to study beta-blockers such: atenolol, carvedilol and pindolol. Measurements are carried out using a spectrophotometer; the stable DPPH free radical has a deep violet color characterized by an absorption band in ethanol solution centered about in 517-520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color, although there would be expected to be a residual pale yellow from the picryl group still present. Therefore the absorbance values decrease because the capture of the free radical. Following after mixing the beta-blocker with free radical solution, can be observed in the spectra analysis a decrease of absorbance at the characteristic wavelength of free radical, especially in interaction with atenolol and pindolol, carvedilol presents no effect on N-centered radicals.
Key words: beta-blocker, diphenylpicrylhydrazyl, free radical, spectrophotometer

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body.[1] There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants. One such method is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH).[11] The molecule of 1,1diphenyl-2 picrylhydrazyl is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color characterized by an absorption band in ethanol solution centered about in 517-520 nm.[6]

As a donor molecule we can use a beta-blocker, to check the interaction with N-centered radicals, because the reaction with O-centered radicals such $OH^{\bullet}$ one was studied with electronic spin resonance method in previous researches.[5]

Beta blockers are a class of drugs used for various indications, but particularly for the management of cardiac arrhythmias, cardio protection after myocardial infarction (heart attack), and hypertension.[14] For carvedilol is already proved a antioxidant activity, therefore we try to extend the research over other beta blockers that can act as scavengers for free radicals, as additional benefit in therapy.[7,12]

MATERIAL AND METHOD

Measurements are carried out using a UV-VIS AnalitikJena Spectrophotometer, with standard 1cm-pathlength spectrophotometer cuvettes, with a maximum working volume of 2 ml.

The substances: atenolol, pindolol, carvedilol and diphenylpicrylhydrazyl where provide from LGC Standards, Germany.

All the solutions were prepared in ethanol, the stock solution of this "stable free radical" do slowly deteriorate, therefore it was fresh prepared and keep in dark place before measurement. The concentration for the stock solution of diphenylpicrylhydrazyl and the beta-blockers solution is the same, 100μm.

The working wavelength was between 190-1100nm, with change of UV lamp at 400nm.

After the analysis of the diphenylpicrylhydrazyl spectrum, with a maximum absorbance at 520 nm, the measurements where taken for atenolol, pindolol and carvedilol in UV, all the spectrum where registered in the absence of DPPH. Then we add together the beta blocker solution and free radical solution in stoichiometric

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proportions (1:1, reaction time 5 minutes because after that there is no change in the value of absorbance. The reaction is very rapid and after 30 minutes, for example, the spectrum is the same.

RESULTS AND DISCUSSIONS

The spectrum of diphenylpicrylhydrazyl is characteristic, with maximum absorbance 1,2 at 520 nm, after adding the beta blockers solution there is a visible decrease of the absorbance under 0,5 in case of pinolol (Figure 1), under 1,0 for atenolol (Figure 2) and for carvedilol is only a slight reduction of the absorbance (Figure 3), not significant, because even in the literature a reaction with N-centered radicals is not certified[11].

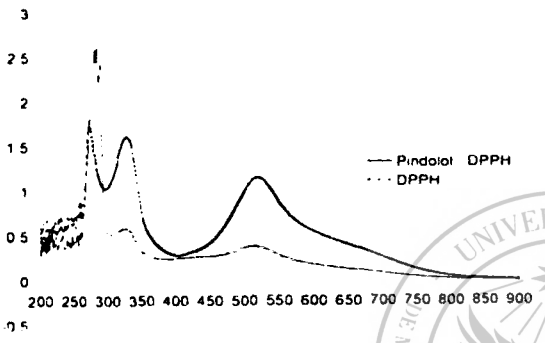

Figure 1. Pindolol + DPPH (1:1, 100μm)

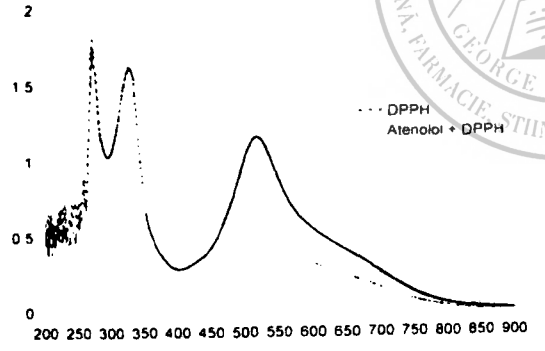

Figure 2. Atenolol + DPPH (1:1, 100μm)

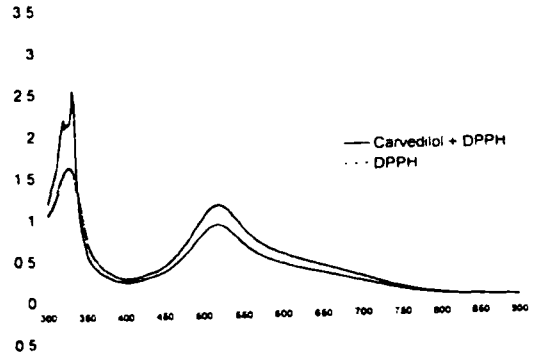

Figure 3 Carvedilol + DPPH (1:1, 100μm)

Pindolol is more reactive, in his case we can observe the larges difference between the spectrum of DPPH alone and after the mixture with beta blocker, pindolol, 2-hydroxy-3-(1*H*-indol-4-yloxy)propyl](propan-2-yl)amine, possible because of the indol NH and also is present in the molecule NH group from the side chain.

Atenolol, 2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy] phenylacetamide, has also a NH from the side chain, but the acetamide group is also capable to donate a H atom to free radical diphenylpicrylhydrazyl.

Carvedilol, 3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl] [2-(2-methoxyphenoxy)ethyl]amine, showed no effect at all on the reaction with DPPH radicals in ethanolic solution, but he is a scavenger for OH free radicals and the antioxidant activity of carvedilol was attributed to the carbazole moiety of the molecule.

We used the same concentration of the sampies, 100μm, and same proportion of beta blocker and diphenylpicrylhydrazyl solution, also we tried to eliminate the decomposition of free radical solution, in this way we can confirm that absorbance decrease is not because of the free radical degradation is the result of interaction with beta-blockers molecule.

For further investigations is necessary that the chemical mechanism of the reaction to be elucidate, this can be possible with aid of molecular modelling program and complete this results with other practical measurements such electronic spin resonance, nuclear magnetic resonance and chemiluminescence's.[8,9]

In case of pindolol and atenolol the reaction occurs, but is only a qualitative measurement following that the quantitative aspect of the reaction to be elucidate.

CONCLUSIONS

After the spectrophotometer analysis of diphenylpicrylhydrazyl free radical reaction with beta-blockers such atenolol and pindolol, we can conclude that reaction really occurs, this can be observed by absorbance decrease of free radical solution after reaction.

The most reactive is pindolol followed by atenolol and carvedilol has no effect on N-centred free radicals.

The chemical structure of pindolol offers many possible interactions with DPPH free radical, especially NH groups to donate an electron to the unpaired molecule of free radical.

Diphenylpicrylhydrazyl solution is stable, even after one week the absorbance is not very different from the fresh prepared solution.

Diphenylpicrylhydrazyl free radical is not complete reduced by beta-blocker, a higher concentration of beta-blocker is required, for example if we add a powerful antioxidant like ascorbic acid to the DPPH solution, the violet solution of free radical turns immediately yellow, the reduction is complete and rapid.

For further information a molecular and kinetic investigation is required, also other specific instruments such electronic spin resonance and nuclear magnetic resonance.

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Microequilibria in inclusion complexes IV. Information obtained from repartition of aliphatic alcohols

Csilla Kun[1], B. Tőkés[2], Gabriella Donáth-Nagy[2]

The aim of this work is to expand the rules applicable to the partition processes on the formation of inclusion complexes of cyclodextrins, considering these interactions as partition microequilibria of the host molecule between the nonpolar cyclodextrin cavity and the polar external environment. We obtained relations between the stability constant of inclusion complexes of aliphatic alcohols with linear chain, their thermodynamic parameters, and partition constants calculated from the additivity. These results confirmed and documented from several directions our initial assumption concerning microequilibria.
Key-words: inclusion complexes, cyclodextrins, aliphatic alcohols, partition constants

Studies on the linear correlations between lgK_{ow} and $1/\varepsilon$ in case of linear alcohols complete our research concerning inclusion complexes' microequilibria. In this series also includes the lgK_{ow} (or HLB) value of 1-octanol. This value can be considered as the partition constant of 1-octanol between 1-octanol and water. The lgK_{ow} value of lower alcohols is smaller – the denominator is bigger, meaning that they are more water-soluble – than the highest one, these being less soluble in water. The octanol/water partition constant is not identical with the solubility ratio of the given compound in the two solvents, because the two solvent-phases are not pure octanol and pure water. It is known from the experimental data given in literature[1-4], that in the state of equilibrium the organic phase contains 2,3 mol/L water and the aqueous phase contains $4{,}2.10^{-1}$ mol/L octanol (figure 1.).

n-octanol

(+27.5 mol% water)

water

(+7.5 10^{-3}mol% n-octanol)

Figure 1. Data of phases

In addition, the Kow is often also dependent on the concentration of dissolved substances. As we can see, the definition requirements of the partition constants are only partially fulfilled, but the results obtained are suitable for orientative purposes. The substance is added to an octanol/water solvent mixture, which volume is adjusted to the expected K_{ow} value. The lowest member of the studied alcohol-series is the methanol, i.e. its lgK_{ow} value. Let's suppose an extrapolation (based on the regression equation) of the data series to the n = 0 value of the $H(CH_2)_nOH$ general formula, namely to water. In this case we are talking about the partition of water between 1-octanol and water, i.e. – considering our supposition – the ratio of the water concentration in 1-octanol and in octanol-containing water. Therefore, the lgK_{ow} of water and 1-octanol describe the same phenomenon (the system itself is the same) and reflects the mutual solubility of the components.

MATERIAL AND METHOD

The experimental data were obtained partially from our measurements and partially from the literature.[1, 3, 4] The data were processed according to our supposition, and the results obtained lead to original conclusions. The experiments were made using reagents of p.a. quality. The system had been carefully shaken till the equilibrium established (between 15 minutes and 1 hour). The phases were separated by centrifuge. An adequate analytical

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method was chosen for the determination of the concentrations. The quickest way to determine the K_{ow} is the HPLC method, because there is a linear correlation between lgt and lgK values. This method was used in several cases to control the obtained results. The experimental data were processed by mathematical [illegible]

RESULTS

The idea was to determine the lgK_{OW} value of water, by extrapolating the octanol-containing (lgK_{OW}, $1/\varepsilon$) regression line of aliphatic alcohols to n = 0 value. This value can also be calculated using the mutual solubility of water and 1-octanol. To clarify this idea we used the relationship between the experimental lgK_{OW} values and the dielectric constants (Table I). These data refer to 20°C.

Table I Relationship between dielectric constants and partition constants in case of alcanols

Alcohol	Formula	n	ε	1/ε	log K_{OW}
water	HOH	0	81	0.012346	-
metanol	$H(CH_2)OH$	1	34.05	0.029	-0.66
etanol	$H(CH_2)_2OH$	2	25.2	0.039683	-0.16
propanol	$H(CH_2)_3OH$	3	19.7	0.050761	0.34
butanol	$H(CH_2)_4OH$	4	17.7	0.056497	0.88
pentanol	$H(CH_2)_5OH$	5	14.4	0.069444	1.4
hexanol	$H(CH_2)_6OH$	6	12.5	0.08	2.03
heptanol	$H(CH_2)_7OH$	7	11.1	0.09009	2.53
octanol	$H(CH_2)_8OH$	8	9.8	0.101523	3.03
decanol	$H(CH_2)_{10}OH$	10	8.1	0.123457	4.03

DISCUSSIONS

Considering the data above, we obtained a good linear correlation between lgK_{OW} and $1/\varepsilon$:

$lgK_{OW} = 52,76\ 1/\varepsilon - 2,14$ N = 12 R = 0,997

Introducing the ε=81 value for water in this equation, results a $lgK_{OW,water} = -1,51$ value for water. It means that in 1-octanol/water system $K_{OW,H2O} = 0.031$. In the same system the $lgK_{OW,octanol}$ value for 1-octanol is 3,03, meaning that $K_{OW,n\text{-}octanol} = 1071,52 = 1,07.10^3$. The ratio of the two partition constants is $K_{OW,octanol}/K_{OW,water} \approx 35.10^3$. For check, we can calculate these distribution constants and their ratio using the cross solubilities in case of the same system:

1. Composition of the upper (octanolic) phase:

- water: mole-fraction: 0,275 (27,5 mol%), or molarity: 2,77 mol/1000 g
- n-octanol: mole-fraction: 0,725 (72,5 mol%), or molarity: 7,31 mol/1000 g

These concentration values reflect that each 100 molecules of n-octanol correspond to 38 molecules of water. In such system both types of molecules can form H-bonds in any version. Water molecules can form twice as many H-bonds as n-octanol ones.

2. Composition of the lower (aqueous) phase:

- water: mole-fraction: 1- 7,5.10^{-3} ≈ 1(100 mol%), or molarity: 55,56 mol/1000 g
- n-octanol: mole-fraction: 7,5.10^{-3}(7,5.10^{-1} mol%), or molarity: 4,17.10^{-1} mol/1000g

The $lgK_{OW,H2O}$ value of water in the n-octanol/water system is: $K_{OW,H2O} = 2,77/55,56 = 0,050$, and $lgK_{OW,H2O} = -1,30$.

The $lgK_{OW,octanol}$ value of 1-octanol in the same system is: $K_{OW,octanol} = 1,78.10^3$, and $lgK_{OW,octanol} = 3,24$

These values excellently correspond to the partition data calculated from the above regression equation, especially given that the solubility was measured at 25°C, and the two methods were completely independent of each other. The ratio of distribution constants in this case is also about 35000. The match will also demonstrate that the inclusion of water in the alcohol-series is justified.

Besides the linearity of the (lgK_{OW}, $1/\varepsilon$) correlation – according to the additivity condition – there has to be a linear regression in case of (lgK_{OW}, n) and ($1/\varepsilon$, n) relationships. Indeed, although the distribution of (ε, n) value pairs is non-linear (Table I, Figure 2), the expected two correlations were excellently linear. The corresponding regression equations:

$lgK_{OW} = 0,5284n - 1,210$

N = 10 R = 0,9997

and

$1/\varepsilon = 0,0107n + 0,0162$

N = 10 R = 0,9981

These linear relationships, in addition to proof of the above-mentioned relations, can be used to predict the dielectric constants of alcohols which have not been studied yet, or for clarify questions of principle.

It is interesting, that the $1/\varepsilon$ values are additive within a certain family of compounds, therefore they can be used to explain structural questions. This character is not described in the literature (Figure 2).

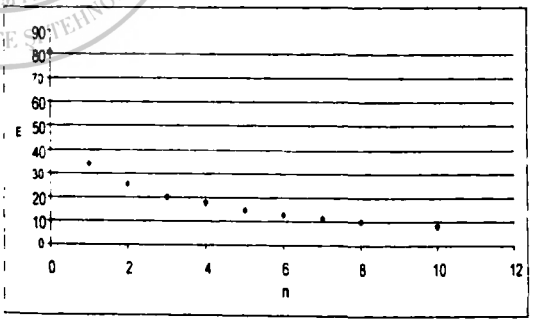

Figure 2. The graphic image of (ε, n) value pairs

The distribution of (ε, n) value pairs corresponds to an equal-side hyperbola, which is shifted from the ε-axis with 0,0162 units. In their synthetic work, Rekharsky and Inoue[2] presented the thermodynamic data of several inclusion complexes formed between aliphatic alcohols and α- or β-cyclodextrins (Table II). This makes possible the comparison of partition constants with stability constants. As one can see, in case of α-CD, with increase of n the lgK values increase, and ΔG, ΔH and ΔS decrease. However, in case of β-CD, with increase of n the lgK values increase, ΔG and ΔH decrease, but ΔS increase. So, the entropy changes in opposite direction (Table II).

Table II. Thermodynamic parameters of inclusion complexes formed between aliphatic alcohols and cyclodextrins

Alcohol	Log Kst	ΔG^0	ΔH^0	$T\Delta S^0$	lg $K_{ow\ exp}$
		α-cyclodextrin			
Ethanol	0,99	-0,57	-2,4	3,3	0,595
1-propanol	1,46	0,84	-6,4	2,2	1,04
1-butanol	1,91	-10,9	-10,2	0,7	1,485
1-pentanol	2,51	-14,4	-14,8	-0,4	1,93
1-hexanol	2,90	-16,6	-17,2	-0,6	2,375
1-heptanol		-17,5	-22,8	-5,3	2,82
		β-cyclodextrin			
1-propanol	0,65	-3,7	-6,0	9,8	1,04
1-butanol	1,20	-6,9	-4,3	11,2	1,485
1-pentanol	1,80	-10,3	-4,6	14,9	1,93
1-hexanol	2,34	-13,3	-0,4	13,7	2,375

The lgK_{ow} values can also be calculated by the additivity, and the resulting values can be compared with the lgK_{st} and the experimental lgK_{ow} values. Using the regression equations, the missing $lg\ K_{st}$ value for 1-heptanol can be determined. We obtained good correlations between the lgK_{ow} values calculated by the additivity, and the lgK_{st} values both in case of α-CD:

$$lgK_{ow} = 0,9105\ lgK_{st} - 0,2948$$
$$N = 5 \qquad R = 0,9968$$

or β-CD:

$$lgK_{ow} = 0,7845\ lgK_{st} + 0,5327$$
$$N = 4 \qquad R = 0,9996$$

We also found good correlation between the experimental lgK_{ow} values and the lgK_{st} values:

$$lgK_{ow} = 0,9849\ lgK_{st} - 0,3124$$
$$N = 5 \qquad R = 0,9966$$

CONCLUSIONS

Comparing the experimental and calculated data, we obtained linear correlations between the following parameters: lgK_{ow} and $1/\varepsilon$, lgK_{ow} and n, $1/\varepsilon$ and n, calculated lgK_{ow} and lgK_{st}, experimental lgK_{ow} and lgK_{st} in case of aliphatic alcohols. We found correlations between the thermodynamic data and calculated partition coefficients of inclusion complexes formed between linear alcohols and cyclodextrins. These results proved our initial supposition.

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Determination of macro and trace elements in *Physalis alkekengi* L. fruits and extracts by inductively coupled plasma atomic-emission spectrometry

Eszter Laczkó-Zöld[1], Klára Szentmihályi[2], C. Csedő[1], Sigrid Eşianu[1]

Physalis alkekengi L. (Winter cherry), a species of the plant family of Solanaceae is distributed in many regions of Europe, Asia and America. Its fruits are used in traditional folk medicine especially as diuretic, anti-rheumatic, analgesic and as a vitamin source. European winter cherry fruits have been extensively studied with regard to their carotene content. However, no specific studies on mineral content have been carried out. The aim of the present study was to determine the mineral element content of this fruits.

Inductively coupled plasma atomic-emission spectrometry (ICP-AES) was used to determine mineral element content of Alkekengi fructus and of different aqueous (infusion and decoction) and hydro-alcoholic extracts (20%, 40%, 70% and 96% alcohol), overall 13 elements (macro elements: Ca, K, Mg, Na, P, S; trace elements: Al, B, Ba, Cu, Fe, Mn, Zn) were considered. Generally, aqueous extract of fruits of Physalis alkekengi *L. contain macro and trace elements in higher amount as the hydro-alcoholic extract. Comparing the aqueous extracts, we observed that minerals extractability, excepting Ca and Na, is better with decoction than with infusion. The minerals extractability decreases with the increase in alcohol concentration of solvents used for the extraction.*

Therefore, when a mineral supply is required we suggest consumption of fruits or aqueous extracts of Winter cherry fruits

Key words: Physalis alkekengi *L.; mineral elements; fruits; extracts*

Physalis alkekengi L. (Winter cherry), a species of the family of Solanaceae is distributed abundantly in many regions of Europe (var. *alkekengi* L.) and Asia (var. *franchetii* Masters). The fruits are used in traditional folk medicine especially as a diuretic, anti-rheumatic, analgesic and also as a vitamin source.[2,3,6] European winter cherry has been extensively studied with regard to its carotene content[7,10,11], while the Asian var. *franchetii* to its physalin content.[4] However, no specific studies on mineral content have been carried out.

It is well known that the pharmacologic effect of herbal medicinal products or their extracts cannot be attributed only to their bioactive components but to mineral elements as well, since the preparation of extracts results in the presence of both organic and inorganic compounds.[5,8]

The aim of the present study was to determine the mineral element content of Alkekengi fructus and of different aqueous and hydro-alcoholic extracts. Inductively coupled plasma atomic-emission spectrometry was used for this purpose.

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MATERIAL AND METHOD

Plant material. The mature fruits of *Physalis alkekengi* L. were collected in Mureş district (Romania) in September 2003.

Extracts. A known amount of plant drug (5 g) taken for 100 ml solvent (deionised water; alcohol of 20%, 40%, 70% and 96%) was infused according to the description of the Romanian Pharmacopoeia[14], decocted for 5 minutes or in case of hydro-alcoholic extracts macerated in ultrasonic bath for 30 minutes.

Sample preparation for element measurement. Plant material (0.5 g) and the dried extracts (100 ml of solution) were digested with 5 ml HNO_3 (65%) and 2 ml H_2O_2 (30%). After digestion, the samples were transferred into a 25 ml calibrated flask, diluted to volume with deionised water. Elements were determined in three parallel measurements.

Table I. Macro and trace element content of Alkekengi fructus

Macro elements	µg/g d.f.	Trace elements	µg/g d.f
Ca	614 ± 5	Al	5 ± 0.4
K	14061 ±195	B	11 ± 0.3
Mg	1625 ± 11	Ba	0.39 ± 0.01
Na	42 ± 1	Cu	8.3 ± 0.08
P	3299 ± 34	Fe	30 ± 0.3
S	1866 ± 26	Mn	9.3 ± 0.1
		Zn	19 ± 0.6

d.f. = dry fruit

Table II Macro element content of Alkekengi fructus extracts

Extracts	Macro element content of extracts (µg/g d.f.)					
	Ca	K	Mg	Na	P	S
Infusion	580 ± 4	1877 ±177	1228 ± 7	42 ± 6	2225 ± 11	1292 ± 23
Decoction	481 ± 2	1940 ± 1005	1270 ± 6	31.6 ± 10	2289 ± 24	1372 ± 23
Hydro-alcoholic extract 20%	300 ± 0,5	1571 ± 221	764 ± 4	25.4 ± 3	1269 ± 19	754 ± 13
Hydro-alcoholic extract 40%	343 ± 5	1789 ± 208	834 ± 5	21.7 ± 2	1288 ± 12	951 ± 14
Hydro-alcoholic extract 70%	213 ± 2	1309 ± 432	630 ± 5	7.82 ± 4	923 ± 12	815 ± 10
Alcohol 96%	120 ± 2	837 ± 44	264 ± 3	5.72 ± 2	255 ± 8	206 ± 8

d.f. = dry fruit

Table III. Trace element content of Alkekengi fructus extracts

Extracts	Trace element content of extracts (µg/g d.f.)						
	Al	B	Ba	Cu	Fe	Mn	Zn
Infusion	4.4 ± 0.07	6.5 ± 0.1	0.091 ± 0.01	4 ± 0.06	16 ± 0.2	6.1 ± 0.03	17 ± 0.2
Decoction	4.8 ± 0.1	9.3 ± 0.1	0.145 ± 0.01	4.7 ± 0.06	15 ± 0.1	6.2 ± 0.05	18 ± 0.2
Hydro-alcoholic extract 20%	4.6 ± 0.06	5 ± 0.08	0.085 ± 0.01	2.8 ± 0.04	8.9 ± 0.05	3.6 ± 0.004	10.6 ± 0.1
Hydro-alcoholic extract 40%	4.7 ± 0.03	5.7 ± 0.1	0.032 ± 0.01	3.7 ± 0.06	12.9 ± 0.1	3.7 ± 0.02	11.1 ± 0.05
Hydro-alcoholic extract 70%	2.8 ± 0.02	4.1 ± 0.06	0.0079 ± 0.00	2.9 ± 0.03	5.4 ± 0.04	1.4 ± 0.02	6.5 ± 0.2
Alcohol 96%	-	3.7 ± 0.16	0.019 ± 0.01	2.1 ± 0.06	1.7 ± 0.07	0.05 ± 0.01	1.24 ± 0.1

d.f. = dry fruit

Investigation of element content. For the determination of macro and trace element content of dried drug and extracts inductively coupled plasma atomic-emission spectrometry (ICP-AES) was used[9], 13 elements were examined (Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn). Standardization of the apparatus was carried out with Merck ICP standards of similar composition and matrix as sample solutions.

Spectrometer: Thermo Jarrell Ash, type Atom Scan 25 ICP with generator (2 kW; 27.12 MHz) exciting argon plasma to 8000–10000 °K. The optical system is composed of a Czerny-Turner vacuum monochromator and two fotoelectron multipliers.

All results were expressed in microgram/gram dry fruit (mean ± SD) and extractability of minerals in solvents used for extracts preparation was calculated.

RESULTS AND DISCUSSIONS

In the present work content of 13 elements was determined in fruits of *Physalis alkekengi* L. and in extracts prepared by different procedures. Table I shows the macro and trace element content of fruits determined by ICP-AES.

The mineral element content in extracts prepared by various procedures shows significant differences (Table II and III).

According to the WHO recommendations[1], Recommended Dietary Allowances (RDA)[12] and Dietary Reference Intake (DRI)[13] and taking into consideration consumption of 100 g fruits, winter cherries are good sources for B (DRI: 0.87-1.35 mg/day), Cu (WHO: 1.2-1.3 mg/day), Mg (WHO: 200-250 mg/day), Mn (RDA and DRI; 2 mg/day; WHO: 2-3 mg/day) and Fe (WHO: 6-8 mg/day).

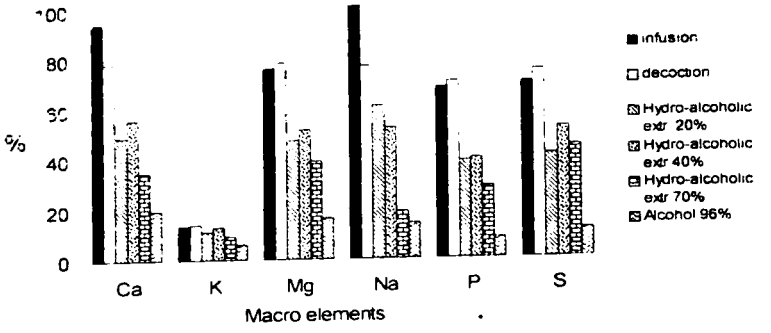

Figure 1. Extractability of macro elements in different solvents

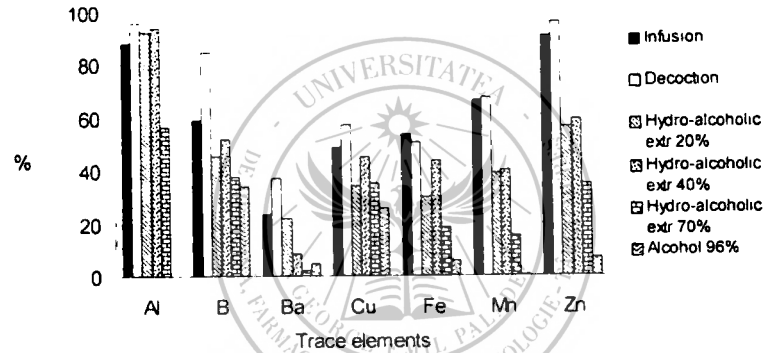

Figure 2 Extractability of trace elements in different solvents

Figure 1 and 2 shows extractability of minerals expressed in %.

Excepting Ca and Na, aqueous extracts (infusion and decoction) of fruits of *Physalis alkekengi* L. contain macro and trace elements in higher amounts as hydro-alcoholic extracts. The minerals extractability decreases with the increase in alcohol concentration of solvents used for the extraction. Therefore, when a mineral supply is required we suggest consumption of fruits or aqueous extracts of Winter cherry.

CONCLUSIONS

Inductively coupled plasma atomic-emission spectrometry (ICP-AES) was used to determine mineral element content of Alkekengi fructus and of different aqueous (infusion and decoction) and hydro-alcoholic extracts (20%, 40%, 70% and 96% alcohol), overall 13 elements (macro elements: Ca, K, Mg, Na, P, S; trace elements: Al, B, Ba, Cu, Fe, Mn, Zn) were considered. Extractability of minerals in solvents used for extracts preparation was calculated. The mineral element content in extracts prepared by different procedures shows significant differences.

Comparing aqueous extracts, we ascertained that minerals' extractability, excepting Ca and Na, is better with decoction than with infusion. The minerals extractability decreases with the increase in alcohol concentration of solvents used for the extraction.

Therefore, when a mineral supply is required we suggest consumption of fruits or aqueous extracts of Winter cherry.

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Contributions to the study of the native species *Portulaca oleracea* L.T. Preliminary research concerning its chemical composition

Adriana Anghel[1], Viorica Istudor[2], Mihaela Dinu[1], Ligia Duțu[2], Ioana Tudor[2], O. Olaru[1]

The present study has in view the practical application of the native species Portulaca oleracea L (purslane) traditionally used as an anti-inflammatory, diuretic, antidiabetic and antiulcerogenic remedy in different countries. The species is used in a tincture or extracts form, as an ingredient in various cosmetic products.
The objectives of the following consist in researching the phenolic and sterolic compounds.
For revealing the chemical compounds we made the chemical analysis of the solutions obtained by successive extractions with ether (A), ethanol (B) and water (C) through specific chemical reactions and through thin layer chromatography. The flavonoids were quantitatively determined by a spectrophotometrically method after complexing with aluminium chloride and the sterols by a colorimetric method using chloramine T and sulphuric acid. There were identified: sterols and carotenoids in solution A, coumarinic derivatives and phenolic acids in solution B, polyholosides and reductive compounds in solution C. The presence of beta sitosterol , coumarin, umbelliferone and caffeic and chlorogenic acids, kaempferol, quercetin was confirmed through thin layer chromatography. The total flavonoids content varies between 0.030 and 0.066g%, that of sterols is 1.125g% (in 100g of dried plant material).
Key-words Portulaca oleracea, chemical screening, phenolic, sterolic compounds

Portulaca oleracea L. (*Portulacaceae*) is a succulent plant that grows spontaneously on sandy soil, cropped and uncroped land, in areas with temperate climate[3]. This plant grows wildly in all the parts of our country and is used as a leaf vegetable[11]. It is also used as a traditional remedy in digestive inflammatory disease (gastritis, duodenitis, colitis) and urinary inflammatory disease (cystitis). According to literature *Portulaca oleracea* contains numerous active principles: omega-3-fatty acids (eicosapentaenoic acid, docosahexaenoic acid, alpha linolenic acid) and omega-6-fatty acids (linoleic acid), beta-sitosterol, aminoacids (tryptophan, valine, threonine, methionine, lysine, hystidine, alanine, phenylalanine, tyrosine and aspartic acid), phenolic acids (caffeic acid)[5], alkaloids (robustin, oleraceins A - E), allantoin, diciclohexyl urea, isoflavones (genistein), coumarins (scopoletin, bergapten, isopimpinellin, lonchocarpic acid)[2], flavonoids (kaempferol, apigenin, myricetin, quercetin, luteolin)[15], carotenoide (beta-carotene, lutein)[11], monoterpene glucoside (portuloside B)[17].

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Different plant extracts were investigated for the diuretic, antimicrobian, analgesic, antiulcerogenic and hypoglycemic effect[4,7,8,9,10,12]. Purslane is used in the USA in tincture form (Portulaca oleracea tincture from Amazon Herbs) as a remedy for skin inflammations (burns, cuts, dermatitis, psoriasis), urinary tract, chronic cough, diarrhea etc.[14]. In China it is used as a dietary supplement, recommended for stimulating the immunitary system, digestion and detoxifying.

Since the chemical composition of plant species depends on the climate and soil, we have proposed to evaluate the indigenous species for its content in active principles and therapeutic potential. In this preliminary research we have performed a chemical screening and study of phenolic and sterolic compounds.

MATERIAL AND METHODS

The raw material used for pharmacognostic study consists of the aerial parts of the plant (herba), harvested in Bucharest, in October 2007. Samples of are currently in the custody of Faculty of Pharmacy, Botany Laboratory, Bucharest, "exicatta" collection.

To quantify the quality of the arial parts we have proceeded on a chemical analysis of solutions obtained from successive extractions with ether (A), ethanol (B)

and water (C). Half a part (as volume) of solutions (B) and (C) had been hydrolyzed for 2-3 hours with HCl 1N. After cooling, aglycones were extracted using ether. The obtained solutions (BA and CA) were used to identify aglycones by specific chemical reactions [6], and by thin layer chromatography (TLC).

Conditions for TLC:

- Stationary phase: silica gel GF_{254} on already prepared Merck plates (20X10 cm) for all compounds because they are fluorescent.
- Mobile phase: toluene - ethyl acetate - formic acid (5:3:1) (solvent 1) for flavone aglycones; ethyl acetate - distilled water - formic acid - acetic acid (72:14:7:7) (solvent 2) for phenolic acids; acetic acid - ether - toluene (10:50:50) (solvent 3) for coumarins; chloroform - acetone (80:20) for sterols.
- Reference substances: quercetin, kaempferol, caffeic acid, chlorogenic acid, rutoside, coumarin, *ortho*-coumaric acid, 7-hydroxy-coumarin, beta-sitosterol (alcoholic solutions).

Reagents: diphenyl-boryl-oxy-ethyl-amine (reagent 1) - for flavones and phenolic acids; potassium hydroxide 10% in methanol (reagent 2) - for coumarins, acetic anhydride, sulphuric acid: ethanol (1:1) (reagent 3) for sterols.

The plates were examined in visible and ultraviolet (366 nm) light.

The quantitative determination of the flavonoids was performed with a spectrophotometric method – officinal in FRX,specified in the monography *Cynarae folium* –The calibration curve we created using rutoside (Sigma) as standard. The measurements was done at 427 nm, using a UV/VIS Jasco V 530 spectrophotometer[16]. The quantitative determination of sterols was made spectrophotometrically. The calibration curve we created using beta-sitosterol (Sigma) as standard. The measurements was done at absorption maxima at 400 nm using a UV/VIS Jasco V 530 spectrophotometer[1].

RESULTS AND DISCUSSIONS

The phytochemical screening revealed: sterols, carotenoids, coumarins, polyphenolic acids, polyholosides, reductive compounds (table I).

The values of the R_f of the thin layer chromatography separated spots are described in table II.

Table I - Phytochemical screening of herba *Portulaca oleracea* L

Nr crt.	Active principles	Reaction	Etheric Extract	Alcoholic Extract	Aqueous Extract
1	sterols	Liebermann-Burchard	++	+	-
2	flavones	Shibata	-	+	±
3	polyphenolicarboxilic acids	Arnow	-	+	+
4	polysaccharides	acetone methanol precipitation Molisch	-	-	++
5	coumarins	fluorescence in UV 366 nm	-	+	±
6	tannins	ferric chloride	-	+	+
7	carotenoids	Carr-Price	+	++	+

Legend ++ intense positive reaction, + positive reaction, , ± low positive reaction, - negative reaction

Table II - The results of cromatographic analyses

Nr. crt.	Extract-Solvent	Rf-	Spot color		Observations
1	etheric	0.45	Green	+	coumarin
		0,11	Blue	+	umbelliferone
2	hydrolysed alcoholic	0.45	Green	+	cumarin
		0,11	Blue	+	umbelliferone
		0,31	Green	+	*o* coumaric acid
3	hydrolyzed aqueous	0.45	Green	+	coumarin
		0,11	Blue	+	umbelliferone
		0,31	Green	+	*o* coumaric acid
		0.26	Blue-green		kaempferol
		0.31	Yellow-green		quercetol
4	unhydrolysed alcoholic	0,56	Blue	+	chlorogenic acid
		0,88	Blue	+	caffeic acid
		0,58	Orange	+	rutin
5	unhydrolysed aqueous	0,88	Blue	+	caffeic acid
		0,58	Orange	+	rutin
6	alcoholic	0.73	Pink-violet	+	beta-sitosterol

Legend: + identified

The chromatographic analysis of extracts of *Portulacae oleraceae herba* revealed following aspects:

- from the etheric extract two spots with coumarinic properties (one green spot with R_f 0.45, corresponding to coumarin and one blue spot with R_f 0.11, corresponding to umbelliferone) have been separated;
- from the etheric solutions containing the aglycons resulted after hydrolysis of the alcoholic and aqueous extract three spots with coumarinic properties (one green spot with R_f 0.45 corresponding to coumarin, one blue spot with R_f 0.11 corresponding to umbelliferone, one green spot with R_f 0.31, corresponding to *ortho*-coumaric acid) have been separated;
- from the etheric solution containing the aglycons resulted after hydrolysis of the aqueous extract spots with flavonoids properties (one blue-green spot with R_f 0.26 corresponding to kaempferol , one yellow-green spot with R_f 0.13 corresponding to quercetol) have been separated;
- from the alcoholic extract three spots with phenolic compounds properties (one blue spot with R_f 0.56, corresponding to chlorogenic acid, one blue spot with R_f 0.88, corresponding to caffeic acid, one orange spot with R_f 0.58 corresponding to rutoside) have been separated, the last two spots being encountered in both extracts (alcoholic and aqueous);
- from the methanolic extract a pink-violet spot (R_f 0.73) corresponding to beta-sitosterol have been separated.

The results of the present study are in conformity with the reports that the plant possesses sterols, coumarins, polyphenolic acids, flavonoids [5,15].

The total amont of flavonoids is situated between 0.030 and 0.066g% (rutoside in 100g of dried plant material).

The quantitative determination of sterols revealed the presence of 1,125g%, the result being expressed in g beta sitosterol in 100g of dried plant material.

CONCLUSIONS

Preliminary studies regarding chemical compounds of *Portulaca oleracea* L., the native species in our country, lead to the identification of polyholosides, beta-sitosterol, coumarin, umbelliferone and *ortho*-coumaric, chlorogenic and caffeic acids.

The quantitative determinations suggest that the native species, *Portulaca oleracea* L, collected in October doesn't contain a significant amount of flavonoids, probably as an effect of the impact of climate and soil on their biosynthesis.

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Identification and dosing of Ceftazidime by spectroscopy methods

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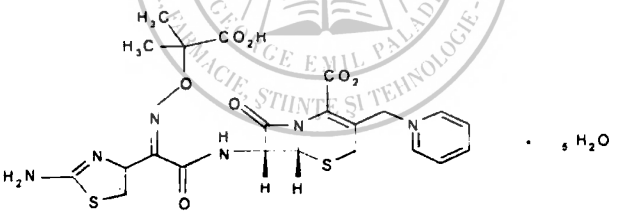
Ceftazidime is identified by means of infrared (IR) spectroscopy and dosed by ultraviolet (UV) spectroscopy. IR spectra for both the reference substance (ceftazidime) and the commercial product containing this substance (Fortum® 1g - powder for injection) were obtained under identical conditions. The technique involving potassium bromide tablets was used for preparation of samples in IR. Assignment of the main absorption bands is achieved, characteristic of the studied molecule. We deployed the IR method for identification of ceftazidime in the commercial product, i.e. the UV dosing spectrometric method.

Key words: Ceftazidime, Fortum, IR spectra, UV spectra

Our objective in this paper is to obtain the infrared spectra for Ceftazidime (Figure 1), with the assignment of the main absorption bands and the specification of the most useful ones for analytical purposes, with the aim of using these spectra to identify the relevant substance.[1,2,3] We have also set the goal to develop a method for the dosing of ceftazidime in Fortum® 1g – powder for injection, by using the UV spectrometric method.[4,5,6,7]



$C_{22}H_{22}N_6O_7S_2 . 5H_2O$

Figure 1. The structure of Ceftazidime,; (6R,7R)-7-[[(Z)-2-(2-aminotiazol-4-il)-2-[(1-carboxi-1-metiletoxi)acetyl]amino]-8-oxo-3-[(1-piridin)methyl]-5-tia-1-azabiciclo[4.2.0]oct-2-ene-2-carboxilat pentahydrate

Working methods for UV spectrometric determination: we prepared solutions (in sulphuric acid 0.1 M) at a concentration of 24.00 μ g/mL for both the reference substance ceftazidime and the product Fortum® 1g – powder for injection.

MATERIALS AND METHODS

Substances: KBr, Sigma; Ceftazidime x $5H_2O$ ref. s., Sigma; Fortum® 1g – reconstitute powder for injection, GlaxoSmithKline; Methanol Merck, Sulphuric acid 0,1 M, distilled water.

Apparatus: spectrometer FT-IR from BIO-RAD model FTS 115; hydraulic press Grasey Specac 15.011; vibrating agitator Narva; analytical scale Mettler Toledo AT261. Spectrophotometer UV-VIS Jasco V-550, quartz cells 1 cm.

Procedure for identification: the samples were prepared as tablets in potassium bromide. The spectra were recorded in the same conditions for the investigated substances and for the reference substance, in the domain 4000 – 400 cm^{-1}. In order to increase the certainty of the identification of the substances by this method, we have assigned the absorption bands both for the investigated substances and for the reference substances. For the intensity of the absorption bands we have used the following expressions: v. i. = very intense, i. = intense, a. = average, w. = weak, v. w. = very weak.

Procedure for dosing: we prepared solutions (in sulphuric acid 0.1M) at concentration of 24.00 μ g/mL for the reference substance ceftazidime and the product Fortum® 1g – powder for injection.

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RESULTS AND DISCUSSIONS

THE IR SPECTER OF CEPHTAZADIME. The IR spectra of the Ceftazidime ((6R,7R)-7-[[(Z)-2-(2-aminotiazol-4-il)-2-[(1-carboxi-1-metiletoxi)acetyl]amino]-8-oxo-3-[(1-piridin)methyl]-5-tia-1-azabiciclo[4.2.0]oct-2-ene-2-carboxilat pentahydrate) is shown in Figure 2. The substance was extracted from reconstituted powder for injection and the spectra was recorded in the 4000 – 400 cm^{-1} domain. The absorption bands are grouped in two spectral regions, 4000 – 2000 cm^{-1} and 2000 – 400 cm^{-1}. The assignment of the absorption bands was made for both spectral regions. In the first region (4000 – 2000 cm^{-1}) there are two bands, one at 3400 cm^{-1} (w.) and one at 3206 cm^{-1} (w.), corresponding to the valence vibrations (ί) of the NH_2 group and a band at 3103 cm^{-1} (v. w.), corresponding to the valence vibrations (ί) of the NH group. Also in this region there are three bands, the first one at 3058 cm^{-1} (v. w.), corresponding to the valence vibrations (ί) of the CH_3 group, a second one at 2536 cm^{-1} (w.) corresponding to the valence vibrations (ί) of the CH group and a third one at 2347 cm^{-1} (v. w.) corresponding to the in-plane deformation vibrations of the CH group (δ_{H}). In the second spectral region (2000 – 400 cm^{-1}) there are the bands for the CO, COOH, CN, CH, CC, CN etc. groups. At 1756 cm^{-1} (v. i.) and 1706 cm^{-1} (i.) there are the two bands corresponding to the valence vibrations (ί) of the CO group (i_{CO}) and at 1618 cm^{-1} (v. i.) another very intense band, corresponding to the in-plane deformation vibrations of the NH_2 group (δ_{NH2}) (Figure 2).

At 1578 cm^{-1} (i.), 1536 cm^{-1} (m.) and 1395 cm^{-1} (w.) there are the bands corresponding to the valence vibrations of the COOH (ν_{COOH}), CC (ν_{CC}) and CN (ν_{CN}) groups and at 1435 cm^{-1} (w.) and 1470 cm^{-1} (w.) two bands corresponding to the in-plane deformation vibrations (δ) of the CH_3 group (δ_{CH3}). At 1268 cm^{-1} (w.) and 1187 cm^{-1} (w.) there are two absorption bands corresponding to the in-plane deformation vibrations of the CH functional group (δ_{CH}). At 1155 cm^{-1} (m.) there is a band corresponding to the out-of-plane deformation vibrations of the CH_3 group (γ_{CH3}) and at 1064 cm^{-1} (w.), 1093 cm^{-1} (w.) and 1017 cm^{-1} (w.) there are three bands corresponding to the out-of-plane deformation vibrations of the CH group (γ_{CH}). At 957 cm^{-1} (m.) and 918 cm^{-1} (m.) there are two bands corresponding to the out-of-plane deformation vibrations of the NH (γ_{NH}) and at 726 cm^{-1} (w.) a band corresponding to the out-of-plane deformation vibrations of the NH_2 group (γ_{NH2}). At 686 cm^{-1} (w.) and 500 cm^{-1} (w.) there are two bands corresponding to the out-of plane deformation vibrations of the CC (γ_{CC}).[1,3,4,5,6]

DOSING OF CEFTASIDIME IN THE PRODUCT FORTUM® 1g – POWDER FOR INJECTION BY UV SPECTROSCOPY. We evidenced the spectra for the reference solution and for the tested solution. We obtained a spectra with the maximum at λ 259.4 nm (absorbance 0.8624) for the reference solution, (Figure 3) and a spectra with the maximum at λ 259.8 nm (absorbance 0.9252) for the analysed solution (Figure 4), respectively.

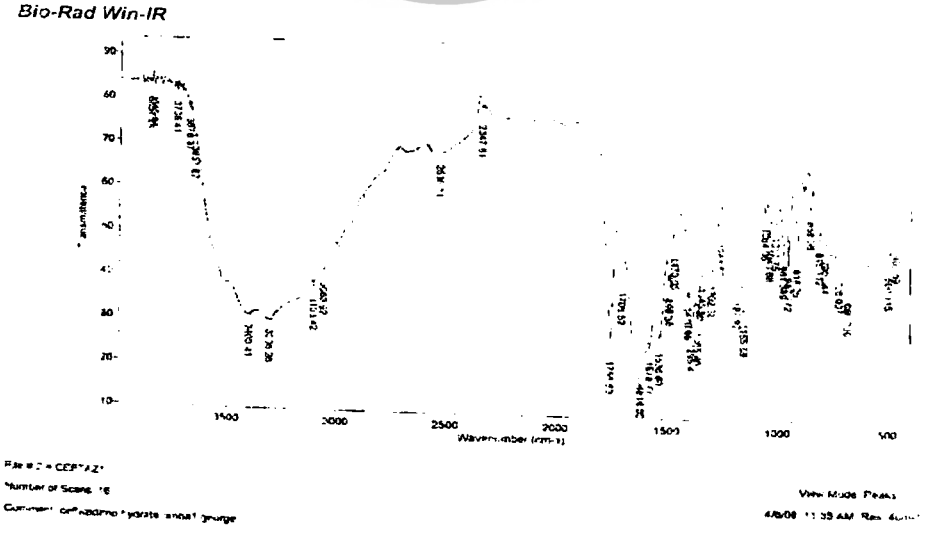

Figure 2. The IR spectra of Ceftazidime in the product Fortum® 1g – powder for injection

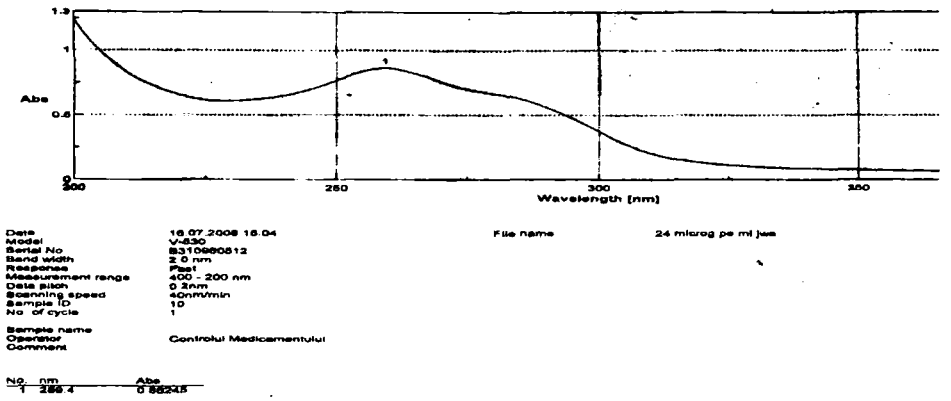

Figure 3. The UV spectra of Ceftazidime (s. r.) 24 µg / mL

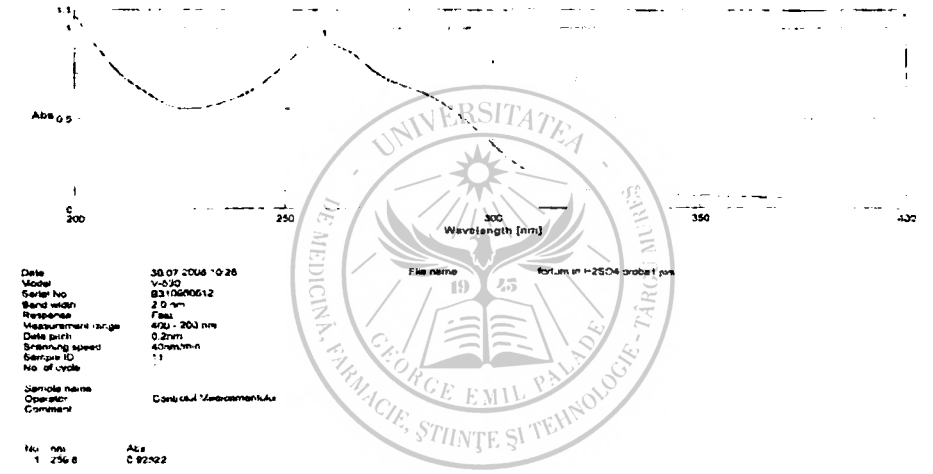

Figure 4. The UV spectra of product Fortum® 1g – powder for injection

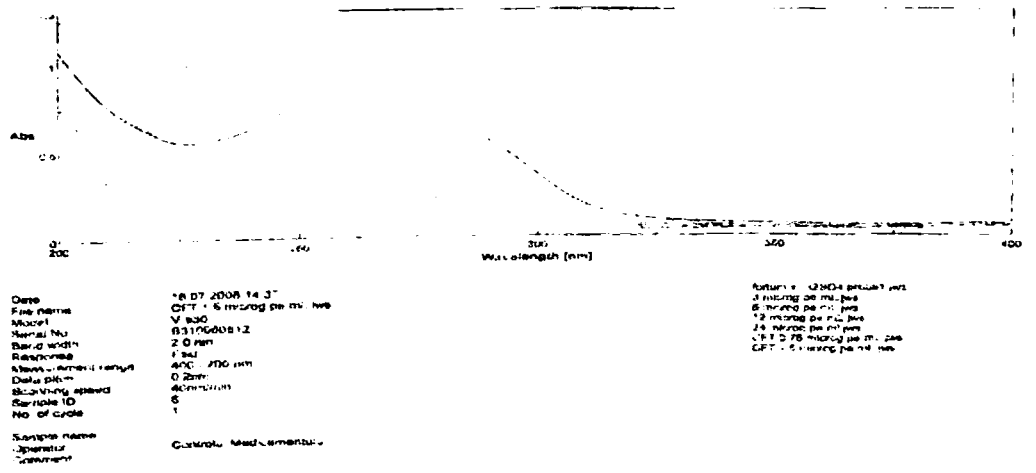

Figure 5. The over posed UV spectra of Ceftazidime s. r. (0.75 – 24.00 µg / mL) and of Ceftazidime in the product Fortum® 1g – powder for injection 24 µg / mL

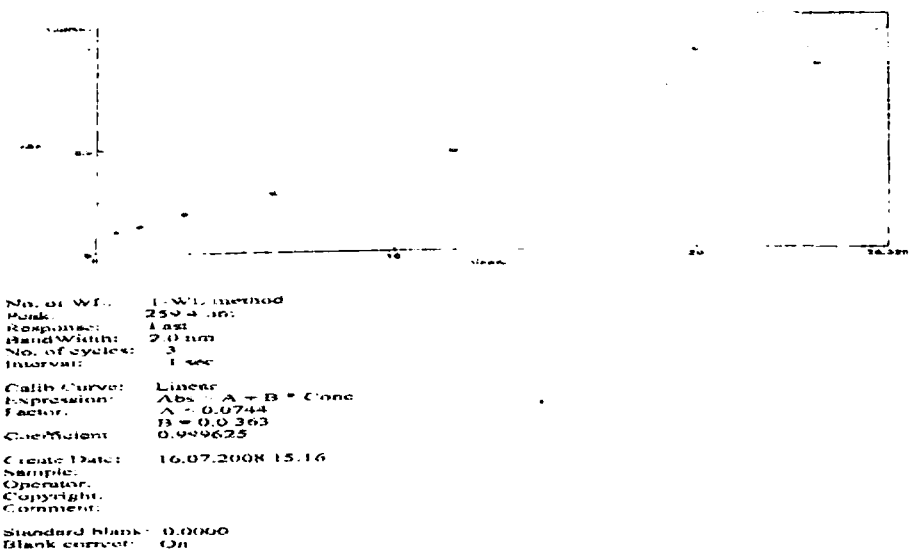

Figure 6. Calibration curve for solutions with Ceftazidime in the product Fortum® 1g – powder for injection

Linear dependence of absorbance in relation to concentration was determined in the concentration interval 0.75 – 24.00 µg/mL ceftazidime reference substance. Over the spectra of the six reference solutions we superposed the spectra of ceftazidime in the pharmaceutical product Fortum® 1g – powder for injection (Figure 5).

The study of the absorbance values function of concentration by linear regression analysis has resulted in the calibration line with the equation:

$A_{hi_{259,4}}$=0.0744*0,0363*[ceftazidime] with the correlation coefficient R^2 = 0.999625 (Figure 6).[1,2,7]

CONCLUSIONS AND DISCUSSIONS

We have identified Ceftazidime by the method of comparing the IR spectra obtained under identical conditions for both the reference substance and the analysed samples; the method may be used for the analysis of the studied cephalosporin. The specification of the main absorption bands allows for a firm identification of Ceftazidime by infrared spectroscopy.

We have dosed ceftazidime in Fortum® 1g – powder for injection through UV spectroscopy and we have determined the linearity and scope of linearity in the concentration interval 0.75 – 24.00 µg/mL with a correlation coefficient higher than 0.99. The developed method is to be further validated.

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The rheological study of valproic acid suppositories

Alexandrina Tăurean[1], R. Cazacincu[2]

For this work were studied the rheological parameters (penetration capacity and stretching capacity) of the obtained suppositories with valproic acid with lipophile and hydrophile bases by classical metod in accordance with current good manufacturing practice

Suppositories prepared with valproic acid and lipophile bases have a higer penetration capacity than suppositories prepared with valproic acid and hydrophile bases

After one months it was observed the decrease of penetration capacity and the decrease of stretching capacity for all formulas

Stretching capacity is not influenced by the presence of valproic acid.

Key words: valproic acid suppositories, penetration capacity, stretching capacity.

It is well-known that 2-propyl pentanoic acid which is also called valproic acid, is an agent used against epilepsy and against convulsions, which acts by a physiological mechanism as a metabolic inhibitor on the binding sites of the enzyme which catalyzes the deactivation of γ-aminobutyric acid (GABA), with the result than the brain levels of GABA are increased and this action results in a biochemical control on the mechanisms which give rise to the epileptic crisis.[1,3]

Valproic acid has molecular formula C8H16O2 and molecular weight 144,21. It is a liquid at room temperature (20°C to 30°C) and thus is not suitable for manufacture of solid pharmaceutical dosage forms such as tablets for oral administration.[4,6]

Valproic acid is not absorbed in a uniform manner through the intestin because of the free carboxyl group which is partially in the ionized form.[3]

There are significant differences between countries in terms of the acceptability of suppositories by patients, but, in same populations, rectal drug delivery could represent a convenient, alternative route of anticonvulsivants administration when other routes are not available.[2]

With the use of various adjuvants, the rectal route can provide satisfactory pharmacokinetics and acceptable local tolerance.

We prepared valproic acid suppositories with lipophile base and valproic acid suppositories with hydrophile base by conventional type suppository metods.[5,6]

This study aimed to offer new data concerning the rheological parameters for valproic acid with lipophile and hydrophile bases.

MATERIALS AND METODS

I. Formulation of Suppositories

I.1 Materials

Active drug: valproic acid (Oakaville, Canada)

Lipophile Base:

-Adeps solidus 3 (Massa Estarinum 299) - Huls AG, Trisdorf Germany

-Adeps solidus 50 (Witepsol W 35) - Huls AG, Trisdorf Germany

-Suppocire NAI – Gattefossé France

-Cacao oleum- Nutraceuticals Group, Spania

Lipex 403 - Stéarinerie Dubois, France

Hydrophile base:

-PEG 1500 - Fluka AG.Buchs SG Switzerland;

-PEG 1550 - Fluka AG.Buchs SG Switzerland;

-PEG 1600 – Loba Chemie Fischamend-Österreich

-PEG 4000 Scharlau Chemie, Made in the European Union

All materials had a pharmaceutical grade.

1 Clinical County Hospital Mureș-Pharmacy number 3, Tg Mureș, Romania
2 Ovidius University, Faculty of Pharmacy Department of Pharmaceutical Industry, Constanta Romania

Table I. Composition of the valproic acid suppositories with a lipophile and hydrophilic base

Components	Quantity (g) Formulations								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Valproic acid	0,200	0,200	0,200	0,200	0,200	0,200	0,200	0,200	0,200
Suppocire NA	0,800	-	-	-	-	-	-	-	-
Witepsol W 35	-	0,800	-	-	-	-	-	-	-
Massa Estarinum 299	-	-	0,800	-	-	-	-	-	-
Lipex 403	-	-	-	0,800	-	-	-	-	-
Cacao oleum	-	-	-	-	0,800	-	-	-	-
PEG 1500	-	-	-	-	-	0,800	-	-	-
PEG 1550	-	-	-	-	-	-	0,800	-	-
PEG 1600	-	-	-	-	-	-	-	0,800	
PEG 4000	-	-	-	-	-	-	-	-	0,800

II. Characterization of suppositories

II.1 Materials

9 formulas of suppositories prepared according to Table1.

II.2. Method

II.2.1. Penetration capacity

The analysis was made using an automatic needle penetrator at room temperature (25±5°C) at 1 week and one month after preparation, every 5 seconds by using auto recording for 30 seconds of penetration. The depth of the penetration was read on a mm scale.

The result is the average of 3 analyses.

II.2.2. Stretching capacity

Filter paper was weighted with about 0,5 g suppositories. They were preserved at 37°C for 1 hour in drying oven.

The excesson on filter paper was removed and weighted again.

The stretching capacity was calculated percentage.

The result is the average of 3 analyses.

II.3. Equipments

VEB FEINMESS DRESDEN automatic penetrator

KERN ABJ analytical balance

50 ITM drying stove-Romania

RESULTS AND DISCUSSION

Penetration capacity

Suppositories prepared with valproic acid and lipophile bases have a higer penetration capacity than suppositories prepared with valproic acid and hydrophile bases.

Tables II and III let see that during the conservation of penetration capacity both lipophilic bases and the hydrophore decrease.

The penetration capacity is higher to formulas F5 and F4. The penetration capacity is less to formulas F9 and F8.

The lowest penetration capacity value is observed in F9 (PEG 4000+ VA) for both, preparation and conservation. The phenomenon is explained by the crystallization kinetics of polyethyleneglicols. The polietilenglicols inetracționate by reducing reactions or by esterification reactions, reactions resulted because of the terminal groups of hydroxyl.

The PEG-type of polymers crystallize their forms in two stages: in the first stage they form a crystal nucleon, and in the second stage this crystal nucleon is surrounded by numerous other nucleons, forming all together a structure pattern in microscopic size of the PEG-polymeric-type.

Table II. Penetration capacity for F1-F5

Time (sec.)	Penetration depth (mm) Formula									
	F1		F2		F3		F4		F5	
	1 week	1 month	1 week	1month	1 week	1 month	1week	1month	1 week	1 month
5	113 ±0.96	107 ±0.78	179,4 ±1,16	173,2 ±1.28	101 ±0.86	96,4 ±0.75	197 ±1.23	190,5 ±1.27	246 ±1.78	239,1 ±0.76
10	117,5 ±0.70	110,2 ±0.84	187 ±1.28	179,4 ±1.43	108 ±0.79	101,7 ±0.74	204 ±0.13	193,1 ±0.76	246,1 ±0.99	239,2 ±1.34
15	121,5 ±1.15	114,1 ±0.84	191,4 ±1.29	184 ±1.34	114 ±0.98	107 ±0.85	207,5 ±1.26	196 ±1.24	246,2 ±1.31	239,2 ±1.56
20	124,5 ±1.15	117,3 ±0.94	195,60 ±1.24	189,6 ±1.42	115 ±0.78	108,2 ±0.61	210,5 ±0.45	199,7 ±0.87	246,2 ±1.65	239,3 ±1.24
25	127,5 ±1.05	120,5 ±1.15	199,4 ±1.24	192,3 ±1.15	119 ±0.94	111 ±0.24	212,2 ±1.44	202,6 ±1.26	246,3 ±1.19	239,4 ±1.58
30	128,5 ±0.86	122,4 ±0.74	201,8 ±1.18	195 ±1.24	121 ±1.07	115 ±0.85	212,8 ±1.15	204,7 ±1.42	246,3 ±1.62	239,5 ±1.67

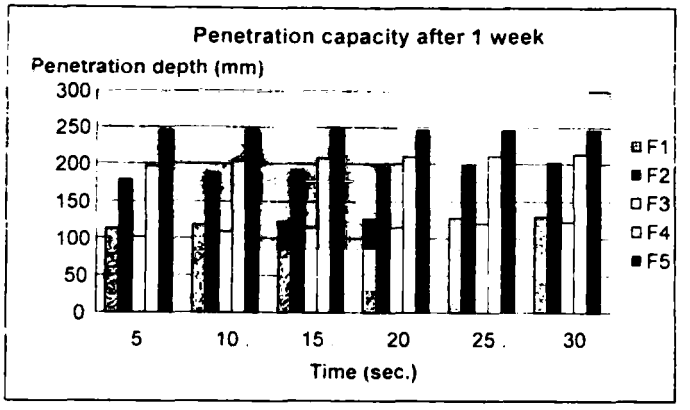

Figure 1. Penetration capacity after 1 week for F1-F5

Table III. Penetration capacity for F6-F9

Time (sec)	Penetration depth (mm) Formula							
	F6		F7		F8		F9	
	1 weeks	1 month	1 week	1month	1 weeks	1 month	1week	1month
5	64	60	61,6	57,8	50,6	46,1±	46±	42,6
	±0.42	±0 45	±0 47	±0.42	±0.35	0.47	0.27	±0.36
10	66,5	62,3	64,6	60,9	53,4	48,5	51	46,9
	±0.26	±58	±0.52	±0.47	±0.38	±0 54	±0,41	±0.44
15	68	65	66,5	62,4	55,6	50,7	54	48,6
	±0.44	±0.35	±0 46	±0.24	±0.41	±0.27	±0.32	±0.34
20	70	68	67,2	63,1	57,6	52,6	55,6	51,3
	±0.51	±0.43	±0.51	±0.42	±0.21	±0.24	±0.23	±0.34
25	71,5	70,2	69,6	64,5	59	54,8	57,2	52,7
	±0.51	±0.47	±0.29	±0.43	±0.24	±0.27	±0.42	±0.24
30	73,5	71,3	70,8	67,3	59,2	55,3	57,6	53,4
	±0.45	±0.37	±0.57	±0.46	±0.35	±0.26	±0.25	±0.42

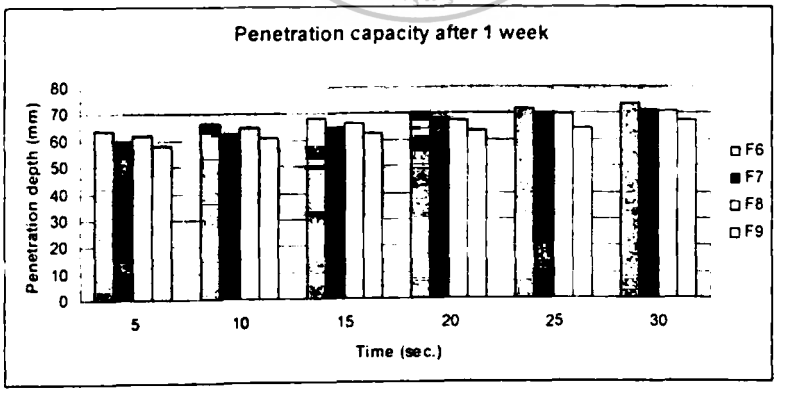

Figure 2. Penetration capacity after 1 week for F6-F9

To be noted that the lipophilic base is the Cacao oleum base, wich has the highest penetration capacity, but during the conservation it decreases. The suppositories reinforcement phenomenon, during preservation, is assigned to the polymorphism of the fat excipients. In case of cocoa butter, the polymorphism is enhanced, because: cocoa butter is a mixture of triglycerides of fatty saturated and unsaturated acids, the hydroxyl index is zero, there is no fixed melting point but a melting range, and triglycerid molecules may be associated in to many ways. Valproic acid association with Cacao oleum will cause

Table IV. Stretching capacity

Formula	Stretching capacity (%)	
	1 weeks	1 month
F1	97.54±0.44	95.33±0.58
F2	97 41±0.51	94.85±0.73
F3	97.35±0.26	95.2±0 2
F4	96.51±0.52	93.93±81
F5	98.43±0.49	97.63±0.47

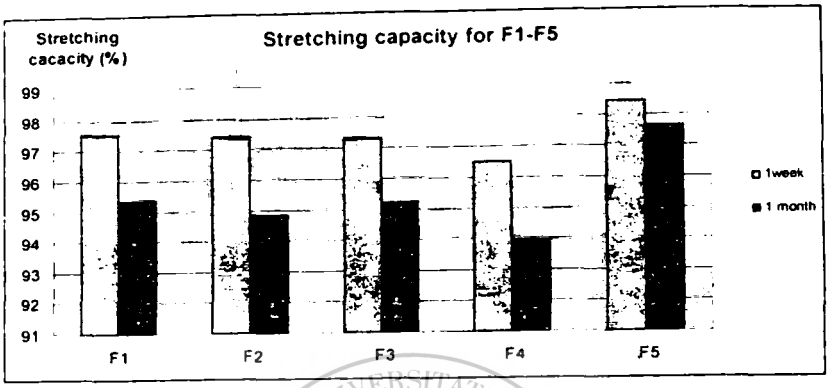

Figure 3. Stretching capacities for F1–F5

a decrease in the melting point of Cacao oleum, and this will lead to difficulties in conservation and administration.

Following association with the AV, all studied lipophilic bases had a good penetration capacity, but it decreases during conservation.

The lipophilic base Witepsol W35 has an increased hydroxyl index that allows a great intimate contact array with the rectal mucosa, which promotes the absorption of AV.

Because Valproic acid is soluble in lipophilic bases, it may have an anticrystallisation effect, so that the suppository have a large penetrating capacity.

Suppositories bases with good penetration capacity provide a good show(array/exhibition)as a film with active principles on the surface of the mucosa, active principles which will arrive in the rectal medium.

Stretching capacity

Based on the measured results we can say that F5 (Cacao oleum + VA) displays best, followed by F1 (Suppocire NAI + VA). The lowest values are obtained in the case of F4 (Lipex 403 + VA) . This phenomenon can be explained by the fact that lipophilic bases with a smaller hydroxyl index display better than the lipophilic bases with a higher hydroxyl index.

The physico-chemical caracter of suppositories bases, influence the stretching capacity. During storage, the triglycerides pass from an amorphous form into a crystall form, form which is no more melting at body temperature. This phenomenon may have adverse consequences on the display and spread of the mass of the suppositories. Being soluble in lipophilic bases, AV may have an anticrystallizing effect and could determine the increase of the scope of capacity or an amount of 200 mg of AV is important (large) for 1g supositorys, and it's responsible for the decrease of m.p. of studied lipophilic bases but maybe in this case stretching capacity is not influenced by the presence of valproic acid.

CONCLUSIONS

The obtained results lead to the following conclusions: suppositories prepared with valproic acid and lipophile bases have a higer penetration capacity than suppositories prepared with valproic acid and hydrophile bases because valproic acid cause an appreciable lowering of melting-point of lipophile bases. After one months it was observed the decrease of penetration capacity and the decrease of stretching capacity for all formulas. Stretching capacity is not influenced by the presence of valproic acid. Physicochemical properties of excipients influence penetration capacity and stretching capacity.

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The comparative study of polarographic behavior of peresthers and nitrones with the view to synthesis through combinatorial electrochemical methods

I.P. Dancs[1], B. Tőkés[2], G. Dónáth-Nagy[2]

As a result of the synthetisation of peresthers and nitrones directly in the polarographic cell we have obtained data which allow us to interpret some of the structure-properties-polarographic behaviour relations, these being-qualitative and quantitative also.

The electrochemical transformations of peresthers of X-C_6H_4-CO-O-O-$C(CH_3)_3$ type have led to interesting results,as for as p-nitro-peresthers are concerned we have observed that the product of electrochemical decomposure is HOOC-C_6H_4-NH-OH and N-substituted-hidroxilamine, which in reaction with carbonilics derivates leads to nitrones, linking these two classes of substances. Close observation allows us to associate these synthetisations to a higher combinatorial character, and because of the electrochemical processes they are included in combinatorial electrochemistry. We foresee a development of these combinatorial electrosynthesis through the diversification of the substituents and their transformation in other functional groups with the view of obtaining some compounds having medication potential respectively.

Key words: peresthers, nitrones, polarograph, combinatorial electrochemistry.

Peresthers represent a family of polarographic active compounds.[5] We studied them and we obtained information about X-C_6H_4-CO-O-O-$C(CH_3)_3$ type compounds which are para- and meta- substituted derivates of tertbutil-perbenzoat.

We have studied the influence of different factors of the polarographic behaviour of these peresthers and the mechanism of their electroreduction. Because of the multitude of possible substituents (X), which appear simultaneously in the polarographic cell, we could consider that these studies also have an combinatorial electrochemical character.

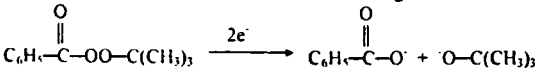
The polarographic study of the peresthers is also a source of information for the deduction of some structure-reactivity relationships.

MATERIALS AND METHODS

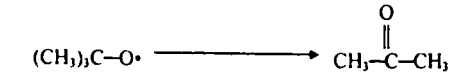
In the preparatory electrolysis the innitial substance was exposed to constant cathodic potential, corresponding to the portion of the limiting current, assuring the conditions identical to those of polarographic studies.

[1]Unirea National College, Târgu-Mureş
[2]University of Medicine and Pharmacy Târgu-Mureş

Starting from known concentration of the depolariser and recording the variation in time of the limiting current, the concentration was measured at the end. The data that were obtained confirm the Deniges reaction. For acetone the reaction was negative.



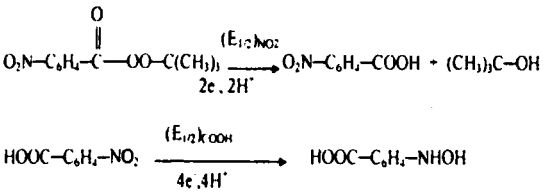
The formation of tertbutoxi radical is excluded, this intern would change into acetone with a remarkable yield after the following reaction:



If one studies the polarographic behaviour of a perbenzoat substituted in the benzenic ring with an active polarographic substitud, the halfwawe potential corresponding to it has to depend on the grouping which results from the reduction of the perestheric function.

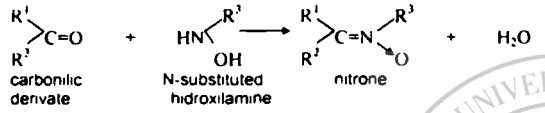
From the size of this effect, the nature of X substitute can be deduced, that is the product of the cathodic reactio studied.

For instance, in the case of nitro-peresthers the electrode process is as follows:



In the case of para- and meta- positions the differences fall in the experimental limit errors, and the orto isomeres differ considerably because of the orto effects.[6]

It can also be noticed that the N-substituted hidroxilamine type of product will establish the connection between the processes that take place in peresthers with the possible syntheses of nitrones[4]:



The polarographic study of the peresthers focused on the explanation of the electroreduction of peroxidic connection of the –O-O- type.

Thus, the following stages were studied: the role of the composition of the solvent on the potentials of halfwawes, the character of the electrode process and the nature of the current, the effect of the structure of the double electric layer over $E_{1/2}$, the kinetic order of the electroreduction reaction, the role of protons in the electrod process.[8]

The products of electrochemical reactions were polarographically identified. On the basis of correlation equations, the halfwawe potential-substitute constant, some particularies regarding the interaction of the reaction center with the substitute were deduced.[1] The polar effect was evidently, because the peroxidic bound interrupt the conjugation. So the resonance effect remains on secondary position, a fact proved within MO[6] calculations too, correllated with the halfwawe potentials.

Chemicals

The used peresthers were synthetised through methods given by chemical literature.[3] The products purity was verified on the basis of active oxygen and their purification was carried out until the [O]≥98% limit was readred. The peroxidic oxygen concentration was determined with iodometric methods. All chemicals were of p.a. purity.

Apparatus

The measurements were carried out with a Heyrovsky sistem LP-55A type polarograph. The electrolysis vessel was an H cell, equipped with a Höppler ultrathermostat.

As reference electrode a normal mercurous sulphate electrod was used (ESN): Hg_2SO_4 / sol. K_2SO_4 0,1 N those potential is +0,395 V higher than the normal calomel electrode. As work electrodes, different Hg dripping ones were used (mercury in distilled water) with characteristics as follows:

$m=1,53$ mg/s; $t_1=4,64$ s; $K=m^{2/3}\cdot t_1^{1/6}=1,72$ $mg^{2/3}\cdot s^{-1/2}$

$m=1,99$ mg/s; $t_1=3,35$ s; $K=m^{2/3}\cdot t_1^{1/6}=1,94$ $mg^{2/3}\cdot s^{-1/2}$.

For the purpose of determining the exchange of electrons a microcell of 0,4-0,6 cm^3 work volume was used. For reduce the electrolysis duration fast dripping capilaries were used: $m=14,96$ mg/s; $t_1=0,59$ s; $K=m^{2/3}\cdot t_1^{1/6}=5,56$ $mg^{2/3}\cdot s^{-1/2}$.

RESULTS

The experimental data are summarised in Tables I, II, III, IV. Comparing the effects of the substitutes with the halfwawe potentials of nitrones and peresthers, a polar and a resonance effect with the nitrones, while the polar effect of the peresthers was evident, because the peroxidic groupe interrupts the conjugation. Therefore the dependence of halfwawe potencial to the substitute nature is less evident.

An important observation is the presence of N-substituted hidroxilamine with carboxylic group, which is in fact a transformable group, and this substance gives the condensation reaction with carbonilic compounds resulting nitrones. Thus a connection can be between

Table I. The separation of polar and rezonance effects in the series of peresthers type $X\text{-}C_6H_4\text{-}COOOC(CH_3)_3$ in ethanol 90% of standard temperature:

X	Position	Effect (mV)	Method		
			Taft	Palm	Swain-Lupton
Cl	Meta	P	28	24	34
		M	-3	0	-4
	Para	P	28	18	31
		M	-8	-1	-13
Br	Meta	P	40	40	36
		M	-10	-1	-4
	Para	P	40	30	33
		M	-30	-2	-14
NO_2	Meta	P	55	40	54
		M	5	7	3
	Para	P	55	30	51
		M	15	14	13

Table II. The comparison of $E_{1/2}$ values calculated of electronic density with those obtained after experimenting in the series X-C_6H_4-COOOC$(CH_3)_3$ in ethanol 90% at standard temperature

X	Q	-$E_{1/2}$ (V; ESN) exper.	-$E_{1/2}$ (V; ESN) calcul.
H	1,000	0,40	0,38
p-Br	1,012	0,39	0,39
m-Br	0,989	-0,37	0,37
o-Br	0,983	-	0,36
p-Cl	0,983	0,38	0,36
m-Cl	1,005	0,38	0,39
o-Cl	0,978	0,36	0,36
p-NO_2	0,948	0,33	0,32
m-NO_2	1,003	0,34	0,38
o-NO_2	0,939	0,32	0,32

Table III. The polarographic levels of reactants, intermediares and products of type X-C_6H_4-NO=CH-C_6H_4-Y nitrones, nitrobensen (1), fenil-hidroxilamine (2), benzaldehyde (3), chemically obtained nitrone (4) and electrochemically obtained nitrone (5)

Substance nr.	Halfwawe potentials, $E_{1/2}$ (V, ESN)		
1	-0,570	-1,280	
2	-1,320		
3	-1,360		
4	-0,340	-1,360	
5	-0,570	-0,840	-1, 360

Table IV. The parameters of $\Delta E=\rho\sigma$ regression equations for type X-C_6H_4-NO=CH-C_6H_4- Y: nitrone

X/Parameter	H	m-COOH	p-COOH
P (V)	0,098	-0,534	0,151
$E_{1/2}$ (V, ESN)	-0,925	-0,817	-0,811
r	0,91	-0,95	0,91

the two classes of substances and it is possible to suggest a combinatorial electrosynthesis of nitrones from peresthers.

DISCUSSIONS

The experimental data show a good concordance with the correlation equations calculated results.[4] The influence of (X) substitute, linked to the benzenic ring, on the halfwawe potencial is evident for both groups of compounds, but is higher with the nitrones, being an extended conjugate system, whereas, in the case of peresthers, the conjugation is limited. The differences noticed in the contribution of the constants of substitutes in the movement of halfwawe potential can be explained in the same way.

CONCLUSION

Through the polarographic method much information obtained about the polarographic behaviour of the substances which contain active polarographic groups and of the qualitative and quantitative relationships of structure-polarographic properties behaviour. The presence of nitroderivates and of hidroxilamines in the electroreduction of peresthers, allows the resulting of nitrones in the same electrolitic cell.

Through the diversifying of the substitutes of basic structure, the above mentioned electrosyntheses belong to combinatorial electrochemistry, whereas the appearance of some common compounds presents new perspectives in the fielf of combinatorial electrosyntheses.

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Determination of the isoniazid and ethambutol by HPLC. Analytical performance of the method

Daniela Gârbuleț, Vasile Dorneanu

The aim of this study was the verification of the analytical performances of an HPLC method for isoniazid (I) and ethambutol (II) determination, by using an Waters e 2695 liquid chromatograph and detection in UV at 270 nm, equipped with a Luna 100-5 C18 (250 x 4 6 mm) column and a mobile phase consisted of a mixture of methanol / buffer acetate = 20 / 80 at a flow of 1 mL/min The method shows a good linearity in the range of 5 - 100 µg/ml, with r^2 = 0.9999 (I) and 0.9999 (II), respectively. The detection limits are 0.05 µg/mL (I) and 0.2 µg/mL (II), the quantification limits are 0.1 µg/mL (I) and 0,5 µg/mL (II). For both substances the system precision (RSD = <2%), the method precision (RSD <5%) and the accuracy were established, the mean recovery being 101,36% in 99.90-102.42% range and 101 16% in 99.82 - 102.60% range for (I) and (II), respectively.

Key words: isoniazid, ethambutol, HPLC

Antitubercular chemotherapics act as bactericide or bacteriostatic on the *Mycobacterium tuberculosis* and some atypical *mycobacteria*, especially *M. kansasii*. The initial treatment and consolidation the treatment of the obtained results are done using major primary or first-choice antitubercular chemotherapics, category to which isoniazid and ethambutol belong. The isoniazid acts as a bactericide, and the ethambutol bacteriostatically on the populations rich in tubercular bacilli, which multiply quickly at the level of the caverns wall, in a neuter environment. The association ethambutol – isoniazid is advantageous, because it acts on all these bacterial populations. Sometimes, it is desirable to add 1-2 more additional chemotherapics, in order to enhance the efficiency and to avoid the consequences of the primary or acquired resistance.

The prophylaxis of the active tuberculosis can be done by isoniazid in the selected cases, in the high-risk people: tight contact with the diseased suffering from an active tuberculosis, the presence of the inactive infection signalled by the positive reaction of the test to tuberculin, the diseased with ancient tuberculosis and a reactivation risk (infecting contact, surgical procedure, immunodepressive treatment).[4]

Considering the importance of the isoniazid and ethambutol in the treatment of tuberculosis, as well as the presence of the two drugs in the oral combined forms, we have elaboration and validation of a method of quantitative determination of them by a high-performance liquid chromatography. The validation of the method has been carried out in according to the specialty literature in the field.[1-11]

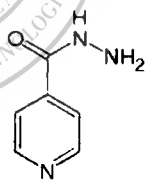

Figure 1. Isoniazid structure

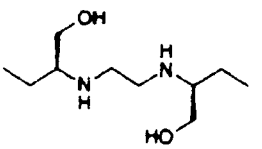

Figure 2. Ethambutol structure

MATERIAL AND METHOD

Materials:

-Reference substances: ethambutol and isoniazid, according to the European Pharmacopoeia[16];

-purity methanol HPLC (Merck);

-Ammonium acetate, copper acetate (Merck);

-MilliQ water (resistivity 18.2 MΩ/cm^3) ;

-HPLC Waters e 2695 provided with a detector UV - Waters 2489.

Chromatographic conditions:

-chromatographic column Luna 100-5 C18, 250 x 4,6 mm, lot:5291-77 (Phenomenex);

-UV detection at the wavelength of 270 nm;

-mobile phase - methanol / acetate buffer pH=5,0 in relation to 20 /80;

-solvent : Milli Q water / methanol = 80/20;

- flow 1 mL/min;

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- the volume of injected solution 20 µL;
- temperature of the column =25°C;
- temperature of the samples = 4°C.

Standard solutions:

Standard stock solutions containing isoniazid and ethambutol of a concentration of 1 mg / mL in the solvent (Milli Q water / methanol = 80/20);

From the standard stock solutions working standard solutions were prepared by dilution (5 ml of each standard stock solution) and completion at 25 mL with solvent; a mixture with a concentration of 0,2 mg/mL for each component was prepared.

RESULTS AND DISCUSSIONS

In order to carry out the separation, the mixed working standard solution has been injected (20 µl) using several mobile phases, with a detection at the wavelength = 270 nm. In the end, the mixture methanol/acetate buffer pH=5.0 was in a volumetric ratio of 20 / 80, obtaining symmetric peaks (symmetry factor = 1.07 for isoniazid, and 1.11 for ethambutol. The composition of the mobile phase has been established also depending on the resolution, so for found mobile phase composition, a resolution of 15.7 (Figure 3) was obtained.[1,5,6,7,8]

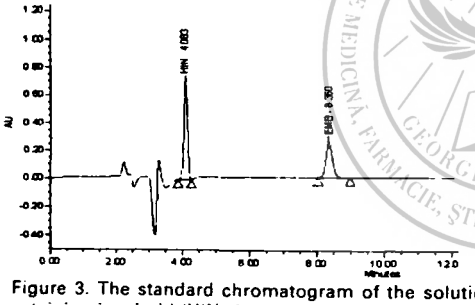

Figure 3. The standard chromatogram of the solution containing isoniazid (HIN) 0.2 mg/ml; Rt =4.08 ±0.03 min. ethambutol (EMB) 0.2 mg/ml; Rt = 8.36 ± 0.04 min, detection 270 nm; mobile phase methanol/acetate buffer mixture pH=5.0, 20 / 80 (v/v), flow 1 ml/min

Determination of the linearity

For the determination of the linearity a series of nine solutions have been prepared from the standard working solution, containing isoniazid and ethambutol in the concentration range 5 - 300 µg/mL. Each of these solutions has been analyzed 3 times each, measuring the area of the corresponding peaks. The average values obtained are given in table I.

Considering the fact that we are taking into account the possibility to apply the method for the quantitative determination of the isoniazid and ethambutol not only from raw materials or various pharmaceutical preparations but also in biological environments, where it is found in small concentrations, we have studied especially the interval 5 - 100 µg/mL. On this interval, the data obtained have been evaluated statistically, obtaining the following results:

-For isoniazid, the equation of the linear regression is $y = 19828 x - 278.7$; the determination coefficient $r_1 > 0.9999$;

-For the ethambutol, the equation of the linear regression is $y = 13086.63 x - 1903.6$; the determination coefficient $r_1 > 0.9999$;

From the statistic data obtained we can notice that on the concentration field studies, the linearity is very good, the values of the correlation coefficients are higher than 0.9999.

The detection limit (LOD) and the quantification limit (LOQ)

The detection and quantification limits have been calculated using the ratio signal/noise of 3/1 for LOD and 10/1 for LOQ.

The calculated values of the two limits are:

Isoniazid – LOD = 0.05 µg/mL, LOQ = 0.2 µg/mL

Ethambutol – LOD = 0.1 µg/mL, LOQ = 0.5 µg/mL

Determination of the precision

The precision is the degree when the method is repeated applied of performance of the operations, instruments and methods used in order to get a result. For the study of the precision, the system precision, the precision of the analysis method and the intermediary precision have been determined.[1,5,8,12,11]

For the determination of the system precision, a sample has been analysed for 6 times, containing isoniazid, and ethambutol in a concentration of 200 µg/mL.

Table I. Linearity study areas of the peaks corresponding to the isoniazid and ethambutol

No of det.	Isoniazid		Ethambutol	
	Concentration (µg/mL)	Average area (n=3)	Concentration (µg/mL)	Average area (n=3)
1	5.00	98593	5.00	56234
2	10.00	198200	10.00	112235
3	20.00	396370	20.00	226388
4	30.00	594600	30.00	337832
5	50.00	991020	50.00	570321
6	100.00	1952372	100.00	1205643
7	200.00	3985627	200.00	2534516
8	250.00	5012465	250.00	2879850
9	300.00	6120350	300.00	3456821

Table ... Experimental data regarding the precision of the system

Area of the isoniazid peak (AU)	Area of the ethambutol peak (AU)
5332349	3407741
5347880	3403533
5328449	3396866
5323591	3385502
5325446	3391155
5333676	3391772
Average = 5331898.6	Average = 3396094.9
SD = 8733.0	SD = 8326.9
RSD = 0.2%	RSD = 0.2%

Table III. Experimental data regarding the precision of the method and the intermediary precision for isoniazid determination

Theoretical concentration (µg/mL)	Precision of the method			Intermediary precision		
	Peak area	Calculated concentration (µg/mL)	%	Peak area	Calculated concentration (µg/mL)	%
	396854	19.98	99.90	390125	19.85	99.25
20	394581	19.96	99.80	389654	19.96	99.80
	390153	20.21	100.05	389059	18.80	99.00
	991205	50.06	100.12	989562	49.89	99.78
50	989562	49.98	99.96	985462	49.85	99.70
	990561	50.10	100.20	980532	49.30	98.60
	1585632	81.03	101.28	1579860	79.86	99.82
80	1580123	80.82	101.02	1570025	78.95	98.68
	1583012	81.06	101.32	1535641	78.64	98.30
Statistical data		SD =	0.617		SD =	0.592
		RSD (%) =	0.617		RSD (%) =	0.597

Table IV. Experimental data regarding the precision of the method and the intermediary precision for ethambutol determination

Theoretical concentration (µg/mL)	Precision of the method			Intermediary precision		
	Peak area	Calculated concentration (µg/mL)	%	Peak area	Calculated concentration (µg/mL)	%
	226384	19.78	98.90	220124	19.68	98.40
20	225645	19.76	98.80	218154	19.60	98.00
	226021	19.85	99.25	225642	20.35	101.75
	570235	49.96	99.92	568451	50.03	100.06
50	569451	49.83	99.66	567845	49.86	99.72
	568975	49.75	99.50	560124	49.92	99.84
	912356	80.02	100.02	911235	80.20	100.25
80	913564	80.10	100.12	910256	79.98	99.97
	919564	80.30	100.37	909568	80.06	100.07
Statistical data		SD =	0.548		SD =	1.0824
		RSD (%) =	0.550		RSD (%) =	1.085

The experimental values obtained are given in Table II.

The experimental data obtained, after the statistic processing, lead to the conclusion that the working system is precise, the values of the relative standard deviation obtained for the two compounds are very low.

For the determination of the precision of the method standard and samples have been analyzed in a range of concentration of ±40% compared to the interest concentration which has the value of 50 µg/mL. For each value of the concentration, a number of three determinations have been carried out. From the experimental values of the peak areas, the concentration of the sample has been calculated by using the equation of the linear regression. The precision is expressed by the relative standard deviation (RSD) of the obtained values. Two sets of determination have been carried out, on different days, in order to evaluate also the intermediary precision tables III and IV.

According to the data given in tables III and IV we can see that the precision of the method is very high (RSD% = 0.61 for isoniazid and RSD% = 0.59 for ethambutol), and also the intermediary precision (RSD% = 0.55 for isoniazid and RSD% = 1.08 for ethambutol).

Table V. Experimental data regarding the exactitude of the method of isoniazid and ethambutol determination

Theoretical concentration (µg/mL)	Peak aria	Isoniazid Calculated concentration (µg/mL)	Recovery (%)	Peak area	Ethambutol Calculated concentration (µg/mL)	Recovery (%)
20	387564	20.15	100.75	215465	19.98	99.90
	385642	20.09	100.45	225641	20.42	102.10
	382154	19.98	99 90	232015	20.52	101.78
50	985642	51.20	102.40	588945	50.89	100.86
	975642	50.86	101.72	564512	50.43	102.40
	986451	51.21	102.42	585015	51.20	100.83
80	1598654	80.68	100.85	945124	80.67	99.82
	1604512	81.34	101.67	925641	79.86	100.18
	1654512	81.72	102.15	939854	80.15	
Statistical data		Average = 101.36 Minimum = 99.90 Maximum = 102.42			Average = 101.16 Minimum = 99.82 Maximum = 102.60	

The accuracy

The exactitude (accuracy) of the method represents the likeliness degree of the results obtained with the real value. The accuracy refers to the quality of a result and it is different from the method precision, which refers to the quality of an operation by which that result is obtained.[5,8,9]

For the evaluation of the exactitude of the method, samples have been analyzed in a range of concentration of ± 40% compared to the interest concentration. A number of three determinations have been carried out for each value of the concentration. From the experimental values of the measured areas, the concentration of the sample is calculated by using the equation of the linear regression. The exactitude of the method is expressed by the percentage in which the quantity of substance taken in the study Table V is found.

Analyzing the data in table V, we can notice that on the concentration field studies (20 - 80 µg/mL), the average recapture is 101.36 (minimum 99.90% and maximum 102.42%) for isoniazid, and 101.16% (minimum 99.82% and maximum 102.60%) for ethambutol. These values prove the fact that the method of analysis proposed is accurate enough.

CONCLUSION

To sum up, the analysis method proposed is linear, precise and accurate; in a simple and fast way, it can be applied for the determination of the isoniazid and ethambutol in various types of samples, after further performances analysis, such as specificity against sample matrices and recovery after sample treatment.

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Applatisation-plication for pulmonary abscesses - case report

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IINTRODUCTION: Surgery procedure applatissation-plication in the treatment of pulmonary abscesses is highly recommended on patients with critical state which do not tolerate a major lung resection.
MATERIAL AND METHOD: We present a 35 old patient, treated conservative for 2 months for purulent cough, thoracic pain, fever (left pneumonia). In evolution she developed a left giant pulmonary abscess and she is admitted to our Clinic for surgical treatment. We performed abscess sequential applatisation-plication, Botianu procedure, without direct closure of the bronchial fistulae.
RESULTS: Postoperative evolution was uneventful with complete symptoms resolution and with general state improvement.
CONCLUSIONS: Without extensive pulmonary resection the anesthetically and surgical risks are reduce, this procedure being in concordance with principles of modern thoracic surgery: respect for the pulmonary parenchyma.
***Key words**: applatisation-plication, pulmonary abscess, bronchial fistulae*

Surgery procedure applatisation-plication (Figure 1) in the treatment of pulmonary abscesses is highly recommended on patients with critical state which do not tolerate a major lung resection. This procedure is enough end economical, we resects only the pulmonary tissue that is functionally compromised in the suppurated process. The procedure is performed in free pleural space, using oro-tracheal anesthesia, preferably selective intubations of the lungs (prevention of the purulent secretion leakage into the healthy lung).[1,3,6]

After abscess identification we perform the following steps:

1. Abscess applatisation: abscess cover removal and pulmonary compromised tissue resection until the reach of the normal pulmonary tissue.
2. Remnant cavity wipe
3. Remnant cavity plication with absorbable stitches (glycolic acid 2.0), without direct closure of the bronchial fistulae.

In the first 24-48 hours, bronchial fistulae are used as a natural drainage of the remnant cavity. When the cavity becomes dry, the bronchial fistulae will close.[1,2,3]

CASE REPORT.

We present a 35 old patient which was conservative treated for a period of 2 months (Ampicillin, Gentamicin and Ceftriaxonum for the past 2 weeks) for left pneumonia, without any improvement of the general state and in evolution with the development on chest x ray of a hydro-aeric image in the left lower lobe: left pulmonary abscess (Figure 2). She was referred to our Clinic for surgery. At presentation the patient accused cough, thoracic pain, severe dyspnoea and fever.

Pulmonary examination revealed a matity at the left side on percussion and a diminished breath sounds at auscultation.

Laboratory examination indicate moderate anemia (Hgb: 8.9 g/dl, Htc: 28.6%) and leukocytosis (L: 17500 /mml). Bronchoscopy (obligatory examination in every lung suppuration - deferral criteria for a suppurated pulmonary neoplasm) revealed acute laryngitis with acute bronchitis. Functional respiratory tests indicate a severe respiratory dysfunction - VC 51%, FEV1 47.3%, criteria for avoiding a major pulmonary resection.

After a minimal preoperative equilibration (anemia correction and antibiotic treatment) surgical approach was made trough a postero-lateral standard thoracotomy. Due to local severe pachypleuritis we need to perform a Fraser Gourd decortication. After the lung liberation from the adherences we visualize the pulmonary lesions: a giant pulmonary abscess of the basal pyramid and Fowler segment and a secondary abscess of the lingular segment. Bacteriologic examination from the puss, prelevated intraoperative, marks out *Methiciline-Resistant Staphylococcus aureus* and *Acinetobacter spp.* (multidrug resistance bacteria –

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Figura 1. Radiografie toracica PA și profil drept la prezentare – imagine hidro-aerică lob inferior drept

Figura 2 Aplatizarea abcesului

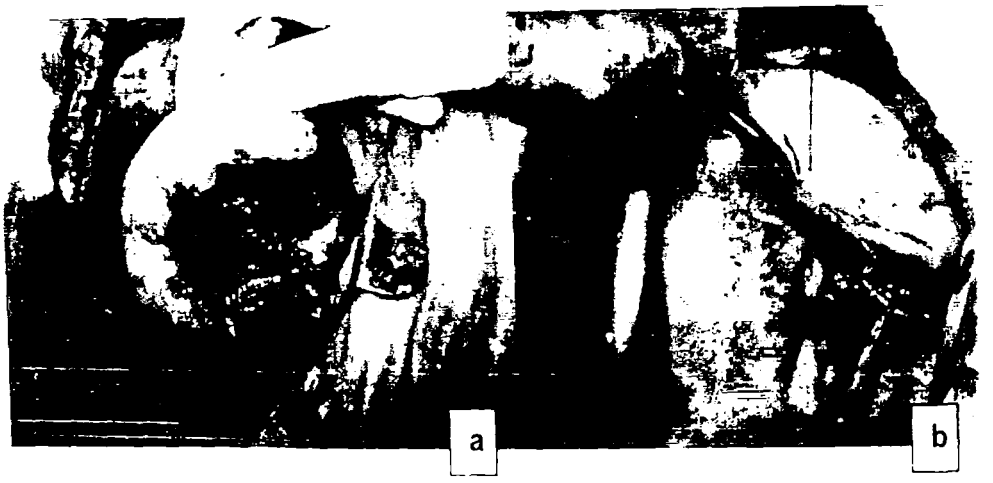

Figura 3 Plicaturarea cavității restante fără sutura directă a fistulelor bronșice (a,b)

Figura 4. Radiografie de control PA și profil drept la 3 săptămâni postoperator, la nivelul locului plicaturii se mai mentine o opacitate pulmonară, clinic fără acuze

probably secondary to delay of surgery and antibiotics abuse).

We performed applatisation-plication of the abscess situated in basal pyramid and in Fowler segment, with maximal resection of the compromise pulmonary tissue. On remnant cavity we found several bronchial fistulae which were covered with a Tachosil patch without direct closure of the bronchial fistulae. We also performed a non-anatomical resection of the lingular segment with remnant cavity plombage with adjacent pulmonary parenchyma. At the end we perform plication of the remnant cavity with Tachosil patch tie in plication stitches (Figure 3).

Postoperative evolution was uneventful with complete symptoms resolution and with general state improvement. Chest X ray at 2 weeks after surgery revealed a minimal opacity on the place of the plication, with cavities complete resolution. (Figure 4). The patient was discharge at 3 weeks after surgery. At late follow up, the patient present a good general biological status, being able of normal physical activity, without complains, with improvements of the functional respiratory tests CV 71%, FEV1 67.2%.

DISCUSSIONS

Surgical treatment for lung abscess is usually reserved for complications such as massive hemoptysis, bronchopleural fistula, and empyema. It is also used in the setting of fulminant infection and in those patients who fail medical therapy. Approximately 10% of lung abscesses require surgical intervention. Prognostic factors which are associated with medical treatment failures include recurrent aspiration, large cavity size (>6.0 cm), prolonged symptom complex prior to presentation, abscess associated with an obstructing lesion, abscesses with thick walled cavities, and serious comorbidities such as advanced age, neoplasm, and other chronic medical conditions.

Very important to remember is that in any pulmonary suppurations surgical evaluation is mandatory – avoidance of extensive lung resection. Antibiotic treatment is considered to have failed if fever and other symptoms continue after 10-14 days of treatment; if chest X rays indicates that the abscess is not shrinking; or if the patient has pneumonia that is spreading to other parts of the lung. Data literature considers that after 2 weeks of ineffective medical treatment, begins sclerosis processes of the pulmonary tissue, which worsen the prognostic and increase the surgical risks.[5]

CONCLUSIONS

Our procedure, trough its operative steps, realizes:

-Applatisation - resection of the anatomical and functional compromised pulmonary tissue

-Plication – assemble pleural borders, prevention of the recontamination of the pleural cavity

-Bronchial fistulae – postoperative natural way for drainage of the remnant cavity

Applatisation-plication advantages are:

-This procedure is fitted in patients with critical general state, with severe respiratory dysfunction (frequent present in patients with pleuro-pulmonary suppuration), where pulmonary resection is contraindicated.

-Technical vascular risk present in pulmonary resection for chronic suppuration (pulmonary tissue fibrosis), bronchial blunt fistulae and resection lodge empyema risks are minimal because we do not approach pulmonary hilum in our procedure

-This procedure resolves the problem of the bronchial fistulae – we use bronchial fistulae as a natural way of drainage of the plicated cavity.

-Being performed in free pleural space, we also may perform associated procedures as decortication, thoracoplasty, etc.

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Intrafamilial phenotypic expression of autosomal dominant polycystic kidney disease. Case report and a review of the literature

Carmen Căldăraru, G. Dogaru, Mirela Gliga

Phenotypic expression at the time of first presentation and also in the evolution of autosomal dominant polycystic kidney disease may differ considerably among patients who belong to the same families.
The paper presents four member of the same family with autosomal dominant polycystic kidney disease. The ages at onset of first symptoms, the type of first symptoms, the progression to end stage kidney disease were very different between family members.
Moreover, two cases were misdiagnosed until end stage renal disease was confirmed.
In the paper, a brief discussion about the clinical manifestations of autosomal dominant polycystic kidney disease and about the differential diagnosis of renal cysts is provided.
Key words: autosomal dominant polycystic kidney disease, clinical manifestation, cystic disease of the kidney

Case report 1

MR, female, 66 years old, has the first manifestation of a disease in january 2002 when she was addmitted in a urology clinic with renal colic. At addmitance creatinine 4,4 mg/dl, serum urea 75 mg/dl, Hgb 8,7 g/dl, Htc 28,7%, sistolic blood pressure 160 mmHg and diastolic blood pressure 100 mmHg. The stone passed spontaneous 24 hours after the onset of symptoms; after that the patient was referred to the nephrologist.

Three of his siblings (her mother, a brother and a sister) was known with renal disease, one of them (his brother) has renal stones.

Renal ultrasound showed kidneys of about 90 mm, with bilaterally renal cysts, and no evidence of liver cysts.

Case report 2

OD, male patient, 73 years old, was referred to the neprologist in june 2002, with a rapid increase of serum creatinine after unstable angina with hypotension. At addmitance serum creatinine was 7,5 mg/dl, blood urea 178 mg/dl, hemoglobin 8,6 g/dl, hematocrit 24,3%, serum potassium 4,2 mmol/l. Urinalysis was positive (>100000 UCF/ml) for Pseudomonas aeruginosa.

Renal ultrasound was suggestive for small kidneys (left kidney 75 mm, right kidney 85 mm) with reduced renal parenchima, with bilaterally renal cysts, no liver cysts and no evidence of cysts in other abdominal organs. Small liver cysts and the bilaterally kidney cysts were found on computer tomography, with no evidence of cysts in other organs.

His medical history was suggestive for multiple renal colic and hematuria episodes with left kidney stone. In this setting, he was admitted for 5 times in a urological clinic between 1987 – 2002 with the diagnosis: Renal stone with renal colic. Polycystic kidney.

In 1987 – a open surgery procedure was performed with the aim of relieving a right kidney renal stone. In 1999 and 2000 extracorporeal shock wave lithotripsy (ESWL) procedure was performed for left kidney renal stone. In 2000 a Pseudomonas aeruginosa bacteriuria was confirmed together with a small increase of serum creatinine (2,5 mg/dl).

The cause of death of his parents was known (father skin cancer, mother renal disease), he had three daughters aged between 44 and 56 years old (one of them had hypertension, the others had no medical history). The patient had two sisters with renal disease, one of them with kidney stone.

Case report 3

PD, 23 years old, with no medical problems. He was addmited in a medical clinic for nausea, vomits, hypertension (180/100 mmHg), with a serum creatinine of 15 mg/dl, serum urea 230 mg/dl, hemoglobin 7 g/dl, hematocrit 21,5%.

Renal ultrasound was suggestive for big kidneys with bilateral cysts, with multiple liver cysts.

Hemodialysis was initiated and five months later a renal transplantation was made. The renal graft was received from his father; his mother was also tested but

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on ultrasound she presented bilaterally renal cysts and she was considered also ill and unable to be a kidney donor.

The cases presented are all siblings. Case 1 and 2 are sister and brother. Case 3 is the nephiew of case 3 and his mother is one of the two daughters of case 2 without medical records.

They have autosomal dominant polycystic kidney (ADPKD) disease and the family described is a clear example of the difficulties in making such a diagnosis especially when the positive family medical history is no evident from the beginning

In three of cases the clinical picture was very „noisy", but renal cysts was the most important feature for all patients.

Renal cysts are non unique to ADPKD.

The differential diagnosis includes genetic diseases (autosomal dominant: autosomal dominant polycystic kidney disease, tuberous sclerosis, medullary cystic disease and autosomal recessive: autosomal recessive polycystic kidney disease, nephronophtisis) and nongenetic (aquired: simple cysts, aquired renal cystic disease, hypokalemia related cysts and developmental: medullary sponge kidney, multicystic dysplastic kidney).

Autosomal recessive polycystic kidney disease is a rare genetic disorder with recessive inheritance and with a incidence of approximately 1 in 55000. Children have hepatic and renal involvement. The liver will show hepatic fibrosis in all children by the time they reach late adolescence; there may also be biliary stasis, splenomegaly (a result of portal hypertension secondary to the hepatic fibrosis).

The renal sonographic features includes multiples small cysts in a normal sized-kidney, increased cortical echogenicity and loss of corticomedullary differentiation.[25]

Nephronophthisis (NPH) is rare, the cysts are not a feature till late in the disease and are typically corticomedullary. ESRD occurs in adolescence or early childhood. Extrarenal involvement occurs in 20% of NPH cases: the most frequent feature is retinitis pigmentosa. Other rare manifestations include liver (hepatomegaly, hepatic fibrosis), bone (cone-shaped epiphysis), and central nervous system (mental retardation, cerebellar ataxia) abnormalities, quite often in association.[19]

Unlike nephronophthisis, in medullary cystic kidney diseases (MSK) cysts are restricted to the renal medulla and typically first appear in adulthood. In addition to the typical clinical features of nephrocalcinosis and urolithiasis, patients with MSK show tubular function defects. Metabolic acidosis secondary to distal tubular defect in H^+ excretion is frequently observed in patients with MSK. Medullary sponge kidney is a disease with prevalence of 1:5000, familial clustering being uncommon.[11]

Simple renal cysts and acquired renal cystic disease are two common causes of nonsyndromic cystic disorders that may be confused with ADPKD. Simple renal cysts are common and increase with age in the general population. Aquired polycystic disease occurs in end stage renal disease (ESRD) patients undergoind peritoneal dialysis or hemodialysis. The incidence of multiple cysts formation increases with the length of time on dialysis.[32]

Tuberous sclerosis (prevalence 1:10000) is a disease with autosomal dominant inheritance. Skin lesions (facial angiofibromas, etc), angiomyolipomas, mental retardation, seizures, retinal hamartomas are common.[33]

Deletion of PKD1 results in polycystic kidney disease in infancy or childhood with ESRD. On ultrasound the kidney can be normal or slightly enlarged, with small renal cysts precalyceal and without liver cysts.

ADPKD is the most common of all inherited renal diseases with about 6 million cases worldwide. It occurs in 1 in 800 live births and is the reason for hemodialysis in 7 to 10 percent of patients.

ADPKD is due to mutations in one or two genes, PKD1 and PKD2, which code for the linked transmembrane proteins polycystin 1 and polycystin 2, found on the primary cilium present on almost renal tubular cells. Ninety per cent of families have the gene located on the short armof chromosome 16 (PKD1) the second gene is on chromosome 4 (PKD2). Spontaneous mutation („second hits") can occur before the cyst develop explaining the variability between clincal presentation and the lack of history in some families; they are somatic mutations in an individual tubular cell that inactivates to varying degrees the unaffected gene from the normal patient.[5,12,34,38]

There are two types: type I is caused by mutations in the PKD1 gene and accounts for 85 to 90 percent of cases, and type II is caused by mutations in the PKD2 gene and accounts for 10 to 15 percent of cases.[17]

Although ADPKD is characterized by kidney cysts and renal failure, it should be regarded as a systemic disease.

ADPKD is fully penetrant, meaning that virtually 100% of individuals who inherit a mutated PKD gene will develop renal cysts that can be detected sonographically by age 30.[29]

The diagnosis is based on family history and imaging. Where the family history is unknown or negative, the diagnosis is supported by the presence of bilaterally enlarged cystic kidneys, hepatic cysts and the absence of extrarenal manifestations that suggest a different renal cystic disease.[6]

The Ravine ultrasonographic criteria for the diagnosis of ADPKD are based on the patient's age, family history, and number of cysts:

-at least two cysts in one kidney or one cyst in each kidney in at-risk patients aged < 30 years

-at least two cysts in each kidney in in at-risk patients aged 30-59 years

-at least four cysts in at-risk patients aged >60 years

This criteria has a sensitivity of 97-100% in PKD1 patients after 30 years old, but the false negative rate could be higher in younger PKD2 patients. [35]

Computer tomography and MRI are not routinely used for the diagnosis, but they can be helpful in some

particular instances (cyst infection, cyst hemorrage, in differentiating symple cyst from renal cell carcinoma, etc).

The two types of autosomal dominant polycystic kidney disease have similar pathological and physiological features, but type II disease has a later onset of symptoms and a slower rate of progression to renal failure, thus, patients have a longer life expectancy (69.1 years) than those with type I disease (53.0 years).[5]

Cysts usually develop from the collecting duct, they generally increase in size and number throughout, resulting in nephromegaly and replacing normal renal tissue, causing macrophage infiltration, neovascularization, progressive fibrosis and a slow deterioration in renal function.

The development of renal insufficiency is highly variable in ADPKD. Renal failure has been reported in children, and, conversely, individuals with the condition may live a normal life expectancy without knowing that they have the disease. The increase in cyst volume correlates best with the decline in renal function.[15,23]

Some authors found that the presence of at least one affected family member who developed ESRD at age <55 was highly predictive of a PKD1 mutation (positive predictive value 100%; sensitivity 72%). In contrast, the presence of at least one affected family member who continued to have sufficient renal function or developed ESRD at age >70 was highly predictive of a PKD2 mutation (positive predictive value 100%; sensitivity 74%).[2]

Tremendous cystic enlargement of both kidneys is characteristic of autosomal dominant polycystic kidney disease. The cysts cause discomfort and pain by putting pressure on the abdominal wall, and back, by impinging on neighboring organs, by bleeding into the cysts, and by the development of kidney stones or infected cysts.

Gross hematuria is often the first presenting sign of ADPKD but it can be also a presenting symptom of common, but unrelated, disorders that may occur coincidentally.[7,14,22]

Kidney stones occur in patients with ADPKD with a frequence greater than in general population. Computer tomography scanning is sensitive for identifying stones, and management generally involves the same strategies as in patients without autosomal dominant polycystic kidney disease.

Hypertension is a frequent and early finding and is considered an important risk factor for cardiovascular disease. Increased blood pressure has beent attributed to activation of the renin angiotensin system, but the mechanism of hypertension in ADPKD patients is not well understood. [9,25]

ADPK patients have polyuria, and flank pain and are prone to recurrent urinary tract infections. The later seems to be more frequent because of the stagnant urine.[13]

Almost two thirds (64%) of people with adult polycystic kidney disease also develop microscopic or macroscopic haematuria. Gross hematuria after trauma is a common feature of the disease.[7]

Most episodes are due to urinary tract infections and renal cyst rupture that relate to the underlying anatomical abnormalities.[15]

Clinically significant cysts are also common in the liver (their incidence increases in the second through fifth decades of life), pancreas, and intestine. Unlike renal cysts, hepatic cysts do not usually affect liver function. The severity of polycystic liver disease parallels that of PKD but exceptions to this rule are common. One genetically distinct form of autosomal dominant polycystic liver disease without kidney involvement exists.

Symptoms, when they occur, are caused by the mass effect of the cysts, the development of complications (cyst torsion, cyst hemoragy, cyst rupture, cyst infection, etc), or rare associations of polycystic liver disease. [7]

Other locations of cysts include the spleen, pancreas, arachnoid membranes, and seminal vesicles.[7]

Patients have an increased risk of diverticular disease and heart-valve defects. [7,24,27]

ADPKD patients may also have aortic and intracranial aneurysms, with an increased risk compared with the general population for sudden death from ruptured intracerebral aneurysms. The prevalence of intracranial aneurysms in ADPKD depends on the age of the patient and the family history of intracranial aneurysms.[16,21]

Uncontrolled hypertension is a key factor in the rate of progression of kidney disease in ADPKD.[1,8,26,36]

Pericardial effusion occurs with an increased frequency in patients with ADPKD, possibly as a result of increased compliance of the parietal pericardium.[2,31]

The ability to monitor disease progression not only will improve the ability to demonstrate efficacy of potential therapies but also may allow targeting treatment to those most at risk for progression.[4]

Renal function can remain normal for decades but the evolution is toward end stage renal disease. The management of chronic kidney disease and its complications should be undertaken according to accepted guidelines for CKD.

The patient with ADPKD can present many therapeutic challenges. There is no cure for ADPKD, but the physicians must know that slowing the progression of renal failure, managing disease complication and managing the consequences of chronic kidney disease are of great importance.

The autosomal dominant inheritance must be described to the family members, at-risk individuals must be informed on the consequences of establishing the diagnosis.

CONCLUSION

The ultimate diagnosis in cystic disease of the kidneys requires clinical, genetic, radiological, and pathological information. Errors can arise when insufficient information are available at presentation and when the patient complaints are not monitored by one physician who knows all the features of the disease. In our case report, the second patient was addmited more times for hematuria, kidney stone, with or without urinary tract infection, but he was never asked about his family history. He had hypertension but he was treated in ambulatory care by the cardiologist who

knows only about renal stones but who had never view the medical documentation of the patient. Moreover, the ultrasonographic aspect was not tipically for autosomal dominant polycystic disease: the kidneys were small-sized and the liver had no visible cysts, so in the presence of high serum creatinine it should raise the suspicion of aquired cystic disease. [39]

That is why the complete diagnosis was never made before the nephrologist. Knowing that more members of his family had renal disease and also that one of the family members have had kidney transplantation for end stage renal disease were features who favoured making the final diagnosis.

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Familial dental agenesis - molecular diagnosis

Noemi Meszaros[1], Nicoleta Andrescu[1], Monica Stoian[1], D. Belengeanu[2]

Tooth agenesis is the one of the most common anomaly of the human dentition. The etiology is genetic and clinically heterogeneous. Two genes have been identified as causing this condition namely, MSX1 *and* PAX9, *both have been associated with two different types of tooth agenesis. The MSX1 (muscle segment homeobox 1) gene is a member of MSX family and has a critical role in odontogenesis and craniofacial skeleton. Mutations in* MSX1 *coding regions cause tooth agenesis affecting preferential premolars, mutations in* PAX9 *coding regions causes preferential tooth agenesis of molars.*

The aim of our study was to assess whether a mutation in MSX1 *is responsible for the lower premolar hypodontia affecting an 11-year-old boy and his 35-year-old mother both from a family with autosomal dominant inheritance model of dental agenesia.*

Key words: dental agenesis, MSX1 gene, Sequencing

Experimental and animal studies, as well as genetic mutations in man, have indicated that the development of dentition is under the control of several genes. Tooth agenesis is a clinically heterogeneous disorder affecting various teeth at different rates. Hypodontia is one of the most common alterations of the human dentition.

Evidence confirms the role of genetic heterogeneity in this common dental anomaly, and alterations in two genes have been identified as causing this condition namely, *MSX1* and *PAX9*. The two genes have been associated with two different types of tooth agenesis, indicating that different genetic pathways determine the development of differently shaped teeth.

PAX9 is expressed in the neural-crest-derived mesenchyme of the maxillary and mandibular arches, and contributes to palate and tooth formation. Mutations in *PAX9* coding regions or a *PAX9* deletion causes preferential tooth agenesis of molars.

Mutations in *MSX1* coding regions cause human tooth agenesis of various types of teeth, preferentially premolars[1] The MSX1 gene is located on the short (p) arm of chromosome 4 between positions 16.3 and 16.1. More precisely the MSX1 gene is located from base pair 4,912,292 to base pair 4,916,563 on chromosome 4. It contains two exons and an intron. MSX1gene play an essential role in teeth and nail development.

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10 to 25% of the Caucasian population present tooth agenesis most frequently involving third molars. The incidence of missing permanent teeth, excluding third molars, varies from 2% to 10%. Approximately 80% of tooth agenesis cases in Caucasian population involve one or two teeth.

The primary objective of our study was to assess whether a mutation in *MSX1* is responsible for the premolar hypodontia affecting an 11-year-old boy and his 35-year-old mother both from a family with autosomal dominant inheritance model of dental agenesia.

CASE REPORT

Detailed family and medical histories were taken based on a custom questionnaire and panoramic (Figure1) and retroalveolar radiographs (Figure 3) and/ or intraoral photos (Figure2, Figure 4) were provided. Clinical evaluation of the two patients and their parents included a documentation of the teeth present.

MSX1 Mutation Screen and Sequencing

Informed consent was obtained from the patient and his mother. 3 ml of blood was drawn and stored in EDTA anticoagulation tubes. Genomic DNA was prepared from the obtained material using standard protocols for DNA extraction. 8 overlapping primer pairs spanning the two MSX1 exons were amplified and screened for single-strand conformation polymorphisms.

Fragments that involve the mutated nucleotide were amplified from the genomic DNA of the patients. The PCR products were subjected to direct sequencing.

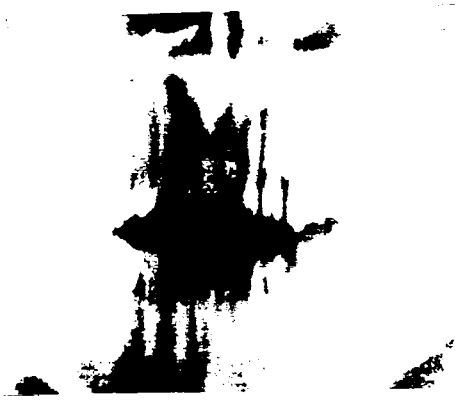

Figure 1. Panoramic X-ray of case1

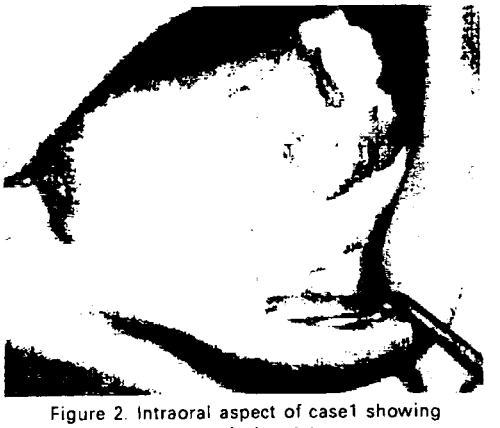

Figure 2. Intraoral aspect of case1 showing the missing 4 4

Figure 3.Retroalveolar radiograph of case 2 confirming dental agenesis

Figure 4.Intraoral aspect of case2 showing the missing 3.4 and the present 7.5

Each sample contained 10 ng f genomic DNA, 10 pmoles of each primer, 25 µM of dNTP, 2.5 mM MgCl2, Taq buffer and 0,01 U of Taq polymerase. After an initial denaturation step (at 96 °C), the samples were prepared for sequencing (96°C for 30 seconds, 50°C for 15 seconds and 60°C for 4 minutes - 25 cycles). Then samples of the amplification products were separated by electrophoresis in a 2% agarose-gel. Amplified DNA fragments were subjected to direct cycle sequence analysis. The results were compared to the published sequence in NCBI database. Positive controls consisting of known *MSX1* variants were also loaded on the gel for comparison with observed shifts in the tooth agenesis samples.

Clinical Diagnosis

A review of the medical history did not reveal significant medical problems in affected individuals or their family (figure 5). No abnormalities of the toenails, fingernails, hair, or sweat glands were found in these patients. We examined dental radiographs to confirm the diagnosis of oligodontia for the two affected individuals. Figure 5.

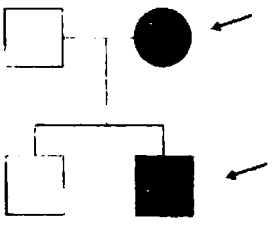

Figure 5 Family pedigree, arrows indicate the affected members.

MSX1 Mutation Screen and Sequencing

No new variants were observed in subjects in any portion of the coding region of *MSX1*. Direct sequencing of PCR product of the patient showed a heterozygous T-to-A transition mutation at nucleotide 620 (figure 6), leading to a missense mutation of the MSX1 gene Figure 6.

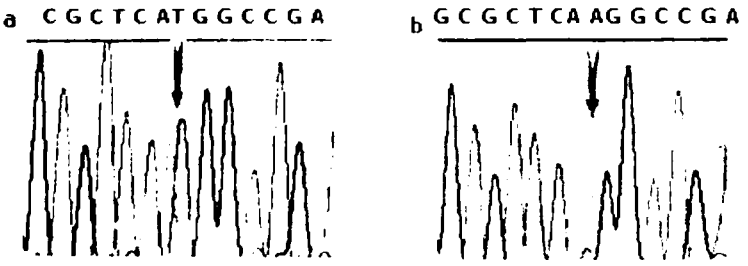

Figure 6. MSX1 mutation and sequence. (a) Arrow indicates nucleotide 620 in MSX1 sequence of unaffected individual (b) sequencing traces showing the missense mutation T to A for nucleotide 620.

DISCUSSIONS

Tooth agenesis occurs in syndromic and nonsyndromic forms and is classified as a genetically and clinically heterogeneous condition affecting various combinations of teeth. Non-syndromic familial tooth agenesis is a common developmental anomaly of dentition characterized by one or more congenitally missing teeth. The unavoidable dental consequences include malocclusion because of improper position of the teeth, deficient growth of the alveolar processes associated with the missing teeth and excess space within the dental arches.

The MSX1 (muscle segment homeobox 1) gene is a member of MSX family and has a critical role in development of teeth and craniofacial skeleton. MSX1 is strongly expressed in the dental mesenchyme and is eliminated from the dental epithelia during the bud, cap, and bell stages of tooth development[3]. The hypodontia pattern observed with *MSX1* mutations suggests that a threshold level of MSX1 function is vital in the development of only selected teeth and that MSX1 functions to pattern the dentition[4].

CONCLUSIONS

Our results thus confirm the importance of *MSX1* in odontogenesis and support the hypothesis that similar patterns of tooth agenesis may arise from different mutations in the same gene. We conclude that *MSX1* mutations result in a specific pattern of inherited tooth agenesis.

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