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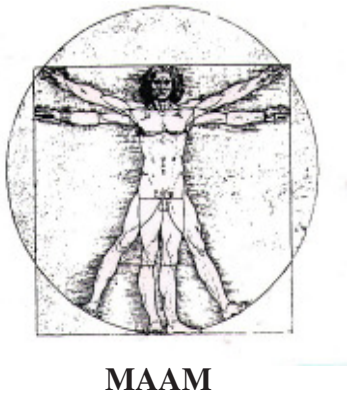

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ACTA MORPHOLOGICA

ПУБЛИКАЦИЈА НА ЗДРУЖЕНИЕТО НА АНАТОМИ И МОРФОЛОЗИ НА МАКЕДОНИЈА

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CONTENT

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LUMBAR MAGNETIC RESONANCE IMAGING CHANGES IN ASYMPTOMATIC INDIVIDUALS OF DIFFERENT AGE

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Abstract

Study Design. A cross sectional study of magnetic resonance imaging changes (MRI) in asymptomatic subjects of different age.

Objective. To examine the prevalence of lumbar spine MRI changes in asymptomatic subjects and their relationship with age.

Background. Previous studies of MRI changes included asymptomatic individuals or patients with low back pain. The influence of age on the prevalence and pattern of intervertebral disc degeneration within the asymptomatic adult and old adult population can provide general insight into the etiopathogenesis of spinal degeneration in order to distinguish early manifestations of a pathologic process from changes due to the normal process of aging.

Methods. Lumbar spine MRIs were obtained in 152 individuals between 35 to 75 years of age. MRI changes such as anular tears (HIZ), disc degenerative changes and disc herniations were evaluated by two independent observers. Degenerative disc changes and herniations were evaluated and graded using the morphologic criteria accepted by the Nomenclature Committee of the North American Spine Society. The severity of degenerative disc changes was calculated by the summation of the degenerative scores for each lumbar level, total disc degeneration score (TDS). The extent of the disc degeneration was determined by the summation of degenerated disc levels, (NDD) as number of degenerated discs.

Results. The prevalence of disc degenerative changes in asymptomatic individuals was increasing progressively to over 90% by the age of 55. The total degenerative score increased with aging. The number of degenerated disc levels also increased with aging. Disc herniations were not associated with aging. There was no significant influence of the age on the prevalence of anular tears (HIZ), disc bulgings, and disc herniations.

Key words: magnetic resonance imaging, lumbar disc degeneration, low back pain

Introduction

The intervertebral joint is a three-joint complex consisting of the endplate-disk-endplate joint of the anterior column and the two facet joints of the posterior column supported by ligaments and muscle groups.

The intervertebral disk and the diarthrodial joints (zygoapophyseal joint or facet joints) interactively degenerate. This degenerative process causes altered stresses on the integrity and mechanical properties of the spinal ligaments, which results in degeneration of the spinal unit as a whole (1,2).

The term degeneration includes any or all of the following morphologic changes: real or apparent desiccation, fibrosis, narrowing of the disk space, extensive fissuring (ie, numerous annular tears) and mucinous degeneration of the annulus, defects and sclerosis of the endplates, osteophytes at the vertebral apophyses. At magnetic resonance (MR) imaging, degenerative changes are manifested by disk space narrowing, T2-weighted signal intensity loss from the intervertebral disk, presence of anular fissures, calcifications within the intervertebral disk, vertebral marrow signal changes and osteophytosis.

There is confusion in the differentiation of changes of the pathologic degenerative process in the disk from those of normal aging, because abnormal MRI findings are also common for asymptomatic individuals. The term degenerative includes all previous mentioned changes (3-5). Previous studies of MRI changes included asymptomatic individuals or patients with low back pain. Thus the influence of age on the prevalence and pattern of intervertebral disc degeneration within the asymptomatic adult and old adult population can provide general insight into the etiopathogenesis of spinal degeneration in order to distinguish early manifestations of a pathologic process from changes due to the normal process of aging.

The aim of the study was to examine the prevalence of lumbar spine MRI changes in asymptomatic subjects and their relationship with age.

Material and Methods

A total of 152 subjects were included in the study (58 females and 94 males). Mean age of the subjects was 46 years (range 35-75 years). All of the subjects were asked whether they have ever had a low back pain. Low back pain was defined in this study as an episode of nonradiating low back pain that had lasted more than two weeks. Criteria for inclusion in the study were no history of low back pain, sciatica or neurogenic caudication. Subjects who met the inclusion criteria entered the study, while the MR imaging examination of the lumbar spine was preformed on the basis of the accepted criteria and patient's consent.

MR imaging and disc abnormalities evaluation

MR imaging examination was preformed with 1,5 T MR unit (Gyroscan T10- NT) with a spinal surface coil. The imaging protocol consisted of a sagittal T1-weighted spin-echo sequence (repetition time msec/echo time msec, 600/14; section thickness, 4 mm; field of view, 280x280 mm; matrix, 240 x 256), sagittal T2-weighted turbo spin-echo sequence (4,700/120; section thickness, 4 mm; intersection gap, 0.8 mm; echo train length of 15), and a transverse T2-weighted turbo spin-echo sequence at one or multiple levels (4,500/120; section thickness, 4 mm; intersection gap, 0.8 mm; echo train length of 15; field of view, 200x200 mm; matrix 240 x 256). Depending on the pathologic condition transverse T1 weighted spin echo was preformed after administration of Gd-DTPA (Magnevist). The obtained MR images were assessed in consensus by two radiologists.

Using a well defined morphologic nomenclature disc abnormalities were evaluated and classified based on the morphologic criteria accepted by the Nomenclature Committee of the North American Spine Society (6). Lumbar intervertebral disc degeneration was evaluated on the basis of signal intensity changes and disc height. Disc degenerative changes were graded employing 3 point graded system, DDS (Disc Degeneration Score). Grade 1 represented slight decrease in signal intensity from the disc, grade 2 represented generalized hypontense disc signal and grade 3 represented generalized hypontense disc signal with narrowing of the disc height. Two additional scores were developed to indicate the severity of degenerative disc disease and the extent of the degenerative disease. TDS (Total Disc Degeneration Score) was derived by summing the scores of all 5 lumbar intervertebral disc levels. A minimum TDS of 0, would mean that all 5 levels were not degenerated, and a maximum TDS of 15, would mean that all 5 levels were severely degenerated (grade 3). TDS < 4 was considered as mild degeneration, TDS ranged from 4 to 7 was considered moderate, while TDS e" 7 was considered as severe degeneration.The extent of the degenerative disc disease was represented by NDD (Number of Degenerated Discs), that was determined as the number of levels with disc degeneration scores (DDS) that were grade 2 or higher.

Disc herniatons were evaluated as disc protrusions, or extrusions. Anular tears were identified as areas of high signal intensity surrounded by a dark rim

Statistical analysis was made with by SPSS 13.0 software. One factor analysis of variance (ANOVA) was used to examine the influence of age on disc degenerative changes and on disc herniations.

Results

A total of 152 subjects were included in this study, divided in four age groups (35-44, 45-54, 55-64 and 65 and older).

Lumbar Disc Degeneration by Age

Figure 1 shows the proportion of subjects with mild, moderate and severe lumbar disc degeneration by age. The percentage of subjects with severe lumbar disc degeneration increased with age. Thirty six percent of subjects in the age group of 35 to 44 years had mild disc degeneration (Total Disc Degeneration Score (TDS)<4), while 57 % of the subjects from this age group had moderate disc degeneration (4<TDS<7). Severe disc degeneration (TDS $\geq$ 7) was seen in 58% of the subjects in the 55 to 64 years age group, and in 80% of the subjects aged 65 and older. Only 20% of old adults had moderate disc degeneration.

Using ANOVA, mean TDS (Total Disc Degeneration Scores) were significantly different between different age groups (F=42,89, p=,00). Mean values of TDS were the lowest in the first age group and the highest in the age group $\geq$ 65 (Fig.2). The differences between the mean values of TDS were the most significant between the third and the other 3 age groups and between the oldest age group and the other 3 age groups.

Disc levels and age

Not only the severity of disc degeneration, but the number of levels with disc degeneration also increased with age (Figure 3). The majority of subjects aged 35 to 44 years had 1 to 2 levels of disc degeneration, 68% of the subjects aged 35 to 44 years had two levels of disc degeneration, whereas 68% of subjects aged 55 to 64 years had four levels of disc degeneration. In approximately 70% of old adults 5 levels of disc degeneration were found.

Using ANOVA, mean NDD (Number of Degenerated Discs) were significantly different between different age groups (F=45,67, p=,00). Mean values of

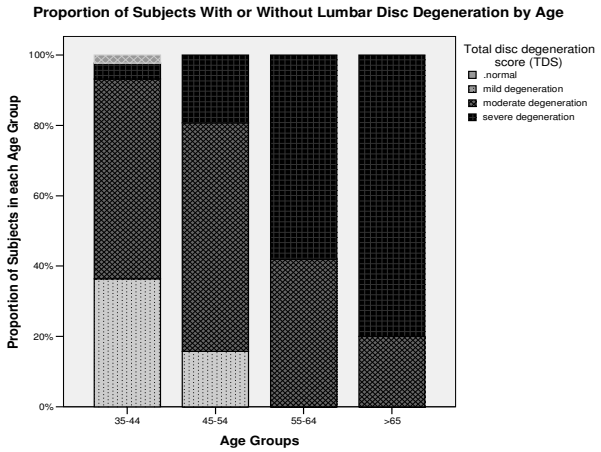

Fig.1. Proportion of subjects with mild, moderate and severe lumbar disc degeneration by age. Subjects without disc degenerative changes were present only in the first age group.

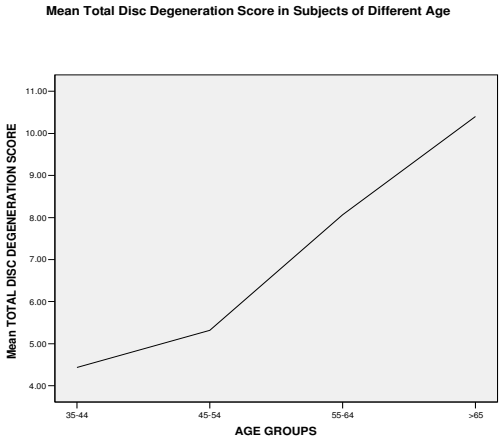

Fig.2. Mean Total Disc Degeneration Score significantly differs between the subjects of different age

NDD were the lowest in the first age group and the highest in the age group ≥ 65. The differences between the mean values of NDD were the most significantlyexpressed between the oldest age groups and the other 3 age groups.

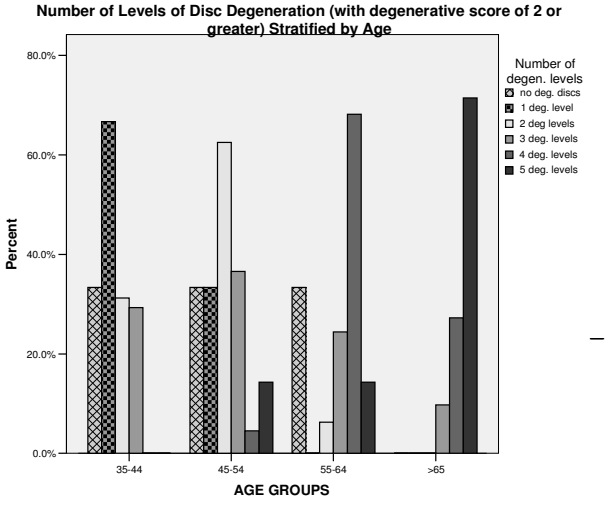

Fig.3. Number of levels of disc degeneration (with Disc Degeneration Score of 2 and greater) stratified by age.

Disc herniations, disc bulges, HIZ and age

Disc herniations (protrusions and extrusions) were found most commonly in subjects aged 45 to 54 years, 33%. Also 26% of subjects aged 45 to 54 yers had disc herniations. Disc herniations were not observed to increase with aging. Disc bulges were found in 38% of subjects aged 45 to 54 years. Disc bulges were not age related findings in adults and old adults (Fig.4). Anular tears were found in 10% of the subjects 35 to 44 years old, but the percent of the subjects with positive MR finding of HIZ slightly decreased to 6,8% in the subjects aged 55 to 64 years. Using ANOVA the number of discs with positive MR findings of HIZ , as well as the number of herniated discs were not significantly different between the different age groups

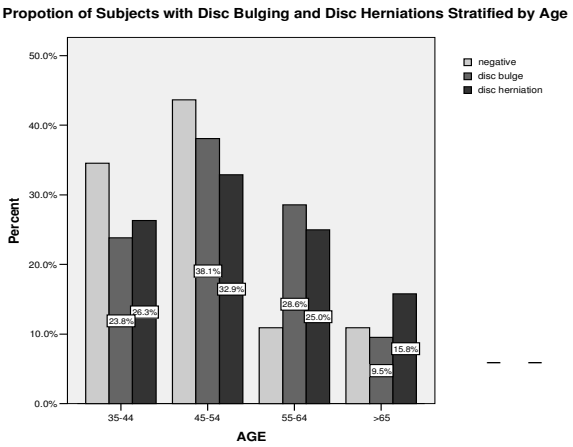

Fig.4. Proportion of subjects with MR evidence of disc bulgings and disc herniations stratified by age.

Discussion

The results of this study confirmed that disc degeneration is increasing with aging. Disc degenerative changes were found with high prevalence in all adult age groups and in old adults. These findings suggested that also other factors than age are involved in the ethiopathogenesis of disc degeneration. Mechanical stresses and nutritional factors are related to disc degeneration process. In addition to mechanical and nutritional causes, a genetic predisposition has been suggested by animal models that consistently develop degenerative disk disease at an early age. Reports of familial osteoarthritis and lumbar canal stenosis in humans also confirmed genetic predisposition (7). Some authors concluded that disk degeneration may be explained primarily by genetic influences and by unidentified factors, which may include complex unpredictable interactions.(8). Individuals with absence of disc degeneration were also evaluated in adults aged 35 to 44 years. Most of adults and old adults have MR scans characterized with lumbar disc degeneration, hence this changes should be analized considering the clinical simptamotology. The study used derived scores in order to quantify the severity of disc degeneration. There was a significant correlation between the aging and the severity of the degenerative disc disease. This method of semiquantitative assessment of disc degeneration have been used in other studies in order to identify predisposing genes for disc degeneration (9, 10). Aging could be an additional factor that contributes in developing the severity of the degenerative process in genetically predisposed individuals. In addition to the changes in the severity of the degenerative process , the number of affected levels also increased with aging. Aging is an additional factor that contributes in spreading the degenerative process, that affect almost the entire lumbar spine in advanced age.

On the other hand, disc herniations had no significant age correlation. Other factors like trauma could have more important role in developing disc herniations. In a study of symptomatic patients, the prevalence of disk herniation in patients with low back pain and those with radiculopathy at presentation was similar (11). Some authors reported a higher prevalence of herniation, 57% in patients with low back pain and 65% in patients with radiculopathy, than the 20%–28% prevalence reported in asymptomatic series (12, 13). Our study showed a prevalence from 25 to 33% in adult asymptomatic individuals.

The limitation of this study were that we couldnot be certain that the subjects involved in this study were representative of the entire population of adults and old adults in terms of the influence of different risc factors, such as smoking, physical activity, proffesion, psychosocial variables that could be additional factors that contribute to the development of degenerative disc process.

Key points

The severity of lumbar disc degeneration is increasing with aging. The number of degenerated disc levels also increases with aging. The prevalences of disc herniations, disc bulging and anular tears (HIZ) are not associated with aging. There is no significant influence of the aging on the prevalence of anular tears (HIZ), disc bulging, and disc herniations.

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CLINICAL IMPORTANCE OF THE ANATOMY OF CORONARY SINUS OSTIUM

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Coronary sinus (sinus coronarius - CS) is the main cardiac vein and it has become a clinically important structure especially for its role in providing access for different cardiac procedures.

Historically, the main topic of the cardiac vascular studies was the coronary artery circulation. Although, the coronary venous system has not been studied as thorough as the coronary arteries, it is very important in many electrophysiological procedures, including ablation procedures of the arrhythmia source, for mapping, biventricular pacing, and for deployment of an array of cardiac devices (1,2,3,4,5,6,7). Anatomical approach to atrioventricular nodal re-entrant tachycardia (AVNRT) ablation based on the triangle of Koch is the standard technique now, with a success rate close to 99%. All these facts make the understanding of CS anatomy necessary (8, 9, 10).

This review will describe the basic gross and developmental anatomy of the CS, its ostium and cardiac venous supply with particular emphasis on specific anatomical aspects that are encountered in invasive and interventional settings.

Anatomical features of the coronary sinus

The coronary sinus - CS is a wide venous channel, about 2.5 - 5cm. in length, which dependent on the site of the drainage of the posterolateral vein (11). It is situated in the left, diaphragmatic part of the atrioventricular (coronary) sulcus, and is covered with muscular fibers from the left atrium. The CS begins proximally at the left atrial auricle, as a continuation of the great cardiac vein, at the valve of Vieussen's, and ends in the right atrium, between the opening of the inferior vena cava (v. cava inferior) and the atrioventricular aperture (ostium atrioventriculare dex.). Its orifice is often guarded by a semilunar valve of the coronary sinus (*valve of Thebesius*).

The diameter of the CS is variable and dependents on the loading conditions, presence and extent of atrial myocardium with the coronary vein, and presence of underlying cardiac disease or prior cardiac surgery. The wall of the CS is covered by the striated myocardium that is continuous with the atria, forming a myocardial sleeve around the venous system.

The CS ostium (ostium sinus coronarii - OSC) is 5–15 mm in diameter and is located on the lower posterior part of the interatrial septum anterior to the Eustachian ridge and valve, and posterior to the tricuspid annulus, forming the base of the triangle of Koch (12).

The CS, like the rest of the cardiac venous system, contains various valves (13, 14).

The valves can be found at various locations. Most common are at the ostium of the CS (Thebesian valve) and at the ostium of the postural lateral vein at the junction of the great cardiac vein and CS (Vieussen's valve). These valves cover different portion of the area of the orifice.

The Thebesian valve is a crescent shaped structure often found guarding the mouth of the CS as it opens to the right atrium. This valve is highly variable and occasionally may present an obstruction during cannulation of the CS. The degree of development of the Thebesian valve varies. There are different clasifications that describe the morphology of the valve. According to Karaca et al. the Thebesian valve can be present or absent. If there is the valve, then it may cover different degree of the ostium (0-100%), and depending on the shape it can be crescent, semilunar or band shaped (15). Of the specimens Karaca et al. examined 31% had crescent, 29% semilunar, 4% fenestrated and 4% band shaped valve. In 1991, Jatene et al. published an anatomic study of the Thebesian valve in 94 human hearts. They described four categories of development of the valve: absent, residual - covering less than 15% of the OSC, partial - covering more than 15% of the OSC and trabecular or double (16). D'Cruz and Shirwany made a classification that combines the shape and the degree of the development of the valve (16, 17, 18). According to them, 45% of the specimens had partial valve that covered more than 15% of the OSC, 31% had residual valve that covered less than 15% of the OSC, and 8% had fenestrated or trabecular valve.

Tributaries of the coronary sistem

The myocardium drains mainly by two groups of veins: the tributaries of the greater and smaller cardiac veins, or the Thebesian veins. Most of these veins open into the coronary sinus. The CS receives blood from the ventricular veins during ventricular systole and empties into the right atrium during atrial systole (19).

The tributaries of CS are the great, small, and middle cardiac veins, the posterior vein of the left ventricle, and the oblique vein of the left atrium, all , except the last one, havevalves at their orifices.

The great cardiac vein (*v. cordis magna; "left coronary vein"*) begins at the apex of the heart and ascends along the anterior interventricular sulcus to the

base of the ventricles. It then curves to the left in the atrioventricular sulcus, and reaching the diaphragmatic surface of the heart, opens into the initial portion of the coronary sinus. It receives tributaries from the left atrium and from both ventricles: one, the left marginal vein (v. marginalis sin), is of considerable size, and ascends along the left margin of the heart.

The small cardiac vein (*v. cordis parva; "right coronary vein"*) runs in the atrioventricular sulcus between the right atrium and ventricle, and opens into the enlarged right part of the coronary sinus. It receives blood from the diaphragmatic surface of the right atrium and ventricle; the right marginal vein ascends along the right margin of the heart and joins it in the atrioventricular sulcus, or opens directly into the right atrium.

The middle cardiac vein (*v. cordis media*) commences at the apex of the heart, ascends in the posterior interventricular sulcus, and ends in the coronary sinus near its right portion.

The posterior vein of the left ventricle (*v. posterior ventriculi sinistri*) runs on the diaphragmatic surface of the left ventricle to the coronary sinus, but may end in the great cardiac vein.

The oblique vein of the left atrium (*v. obliqua atrii sinistri - Marshalli*; *oblique vein of Marshall*) is a small vessel which descends obliquely on the back of the left atrium and ends in the coronary sinus near its left portion; it is continuous above with the ligament of the left vena cava (*lig. venæ cavæ sinistræ vestigial fold of Marshall*), and the two structures form the remnant of the left Cuvierian duct.

The following cardiac veins do not end in the coronary sinus:

1. The anterior cardiac veins**,** comprising three or four small vessels which collect blood from the front of the right ventricle and open into the right atrium; the right marginal vein frequently opens into the right atrium, and is therefore sometimes regarded as belonging to this group;

2. The smallest cardiac veins (*veins of Thebesius*), consisting of a number of minute veins which arise in the muscular wall of the heart; the majority open into the subendocardium of atria, but a few end in the ventricles. The Thebesian veins are composed primarily of endothelial cells, which are continuous with the lining of the cardiac chambers (20). These vessels are found throughout all four chambers of the heart, but are more prominent on the right. This venous system provides drainage to the right appendage as well as a significant portion of the muscular ventricular septum.

Development of the coronary sinus

Development of the CS occurs by differentiation of the sinus venosus During foetal development, a single heart tube separates around the third week giving rise to the primordial atrium and sinus venosus. Initially, the sinus venosus opens into the posterior wall of the right atrium. Around the fourth week, the sinus venosus differentiates into right horn and left horns which through a process of degeneration and involution, give rise to the venous channels entering the right atrium. The right horn persists as the smooth portion of the superior vena cava as well as the area between the vena cava extending into the CS ostium, whereas the left horn undergoes progressive involution until the 10th week. When the left horn persist it gives rise to the CS (21, 22).

Clinical importance

Although various types of congenital cardiac malformations have been well studied, anomalies involving the coronary sinus have received relatively little attention; this is probably due to their extreme rarity and that they can occur without clinical symptoms and without a significant cardiac functional disturbance. However, in some situations, the failure to recognize such venous anomalies may cause a misinterpretation of cardiac catheterization findings (23, 24), affecting the distribution of retrograde cardioplegia in patients requiring a cardiopulmonary bypass (23, 25), and may cause a troublesome hemodynamic alteration during cardiac surgery (23, 26).

Most reported cases of coronary sinus anomalies are diagnosed either during autopsy (25, 27) or by a transesophageal echocardiogram, magnetic resonance imaging (28), and by the use of coronary sinus angiography (28). Multi-detector row computed tomography (MDCT) technology provides a noninvasive alternative to evaluate a cardiac malformation comprehensively. Unroofed CS, engorged CS with atresia of the right atria ostium and coexisting tubular communication to the left atrium, anomalous drainage of the v. cava superior into CS, anomalous pulmonary venous connection to the CS, and atypical coronary course with presence of a valva of Vieussens are among the reported anomalies of the CS. Left superior vena cava (LSVC) (left horn sinus venosus persistens) draining into coronary sinus is not an uncommon anomaly that enlarges the coronary sinus. The enlargement of the coronary sinus distorts the anatomy of triangle of Koch, making slow pathway ablation difficult.

Total upper body drainage via left superior vena cava into coronary sinus (i.e. absent right superior vena cava) is rare anomaly and it further distorts the anatomy of coronary sinus and triangle of Koch.

The OCS is of interest to the electrophysiologist because it provides a useful route for mapping and ablation of left-sided accessory pathways. Nevertheless, pathways that are located close to the mitral valve may be difficult to ablate given their significant distance from the OCS. In addition, the OCS is an electrophysiologically active structure. Previous studies have demonstrated the capability of spontaneous depolarization and slow conduction in the smooth muscle of the CS, providing inherent automaticity (29, 30, 31). Also, the atrial myocardial sleeve that covers the proximal CS provides

the ability for conduction and automaticity, forming an electrical connection between the two atria. Studies have shown that this connection is of clinical importance as it may be a source of arrhythmias such as atrial fibrillation (32). Active myocardium within the CS may serve either as a source for abnormal automaticity generating a focal tachycardia or as a part of a reentrant circuit. In patients with atrial fibrillation, CS myocardium may also initiate recurrent atrial fibrillation. Since the CS is one of the connections between the right and left atria, with Bachmann's bundle forming the other important connection, reentrant tachycardia involving the CS muscle and both atria are sometimes seen in patients with marked atrial enlargement, especially after a surgical maze procedure.

The vein of Marshall may give rise to atrial fibrillation either by virtue of myocardial extensions into the structure or as a result of node like remnants within the vein or by virtue of the rich autonomic innervation that typically encapsulates the structure (30, 31, 32). With regard to left ventricular pacing, atrial electrograms may be sensed by the ventricular lead if it is placed too proximal (basal) within a ventricular branch of the CS.

Cannulation of the CS during interventional procedures may be complicated by obstruction due to Thebesian valves. A three-dimensional, functional anatomy study showed that Thebesian valve morphology varied greatly, and included instances of dynamic obstruction (33). Cannulation would be likely to be more complicated in the living heart during the cardiac cycle. Studies have demonstrated that valves that obstruct a significant portion of the CS ostium may be successfully transversed by use of modern cannulation techniques (i.e. softer-tipped sheath, pre-shaped sheath, and anterior approach) (34).

Our experiance

At the Institute of Anatomy, Medical Faculty in Skopje the postmortem study of the valve of the OCS on 100 human hearts without malformations fixed in formaldehide was made (35). We thought that the most adequate classification to be applied was that of D'Cruz and Shirwany. The results showed the valve was present in 86% of the cases. The valve was moderately developed (residual) in 25% of the specimens, well developed (partial) in 40% and fenestrated in 5.8% of the cases. One specimen had a valve that looked like chordae tendinae, and thin fibers crossed over the ostium, connecting the Eustacian valve with the lower limb of the ostium. The mean value of the diameter of the OSC was 9.33 mm. (36).The postmortem studies made on hearts with cardiac malformations showed the presence of unroofed CS and anomalous pulmonary venous coconnection to the CS (36).

The knowledge of the anatomy of the OSC and its valve as one segment of the right atrium is fundamental for successful realization of therapeutic, diagnostic and invasive methods in cardiology.

Key words:coronary sinus, cardiac veins, anatomy

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RADIOLOGICAL EVALUATION OF ANTERIOR ETHMOIDAL SINUS CELLS

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Abstract

Preoperative radiological-anatomic evaluation of anterior ethmoid sinus cells is important because of their location near to very important structures like nasolacrimal duct and ethmoid infundibulum. Their identification on CT of paranasal sinuses is important to avoid complications while performing functional endoscopic sinus surgery (FESS) and to make association with obstruction of drainage ostia.

In this study we evaluate variations of anterior ethmoid sinus cells on coronal CT scans of 120 examinees of Macedonian nationality at the age of 17 to 70. CT was performed on CT scanner Somatom, Volume Zoom, Siemens, multislice 4, at the Institute of Radiology, Medical Faculty, Skopje. The prevalence of anterior ethmoid cells (agger nasi cell, Haller cell and large bulla ethmoidalis) was evaluated on CT scans as being present separately as unilateral or bilateral.

The most present anterior ethmoid cells were agger nasi present in 86% of patients bilaterally. Haller cells were found in 22% of patients on the left side and in only 17% on right side of nasal cavity. Bulla ethmoidalis left and right was present with 12% and 18%, respectively the difference in percentages of the presence of agger nasi cells, Haller cells and bulla ethmoidalis, according to sex, was statistically insignificant for $p>0.05$.

Key words: paranasal sinuses, anatomical variations, ostiomeatal region, computed tomography

Introduction

Ethmoid paranasal sinus is the most complex of paranasal sinuses and is known as ethmoid labyrinth, localized between orbit and nasal cavity, above the maxilla and below frontal bone. Ethmoidal paranasal cells don't develop until 5 lunar month (1). In newborns they are very small. In the second year in children the pneumatisation of the nasal cavity is toward the future frontal sinus, which appears at the age of 3 (2). They continue to grow until late adolescence, or until sinus wall touch the compact bone. After the age of 9 the pneumatisation of ethmoid cells becomes more extensive and it is not followed by pneumatisation of frontal sinus. That's why sometimes ethmoid cells are not covered with frontal bone and they form the floor of anterior cranial fossa, covered only with dura mater. In such cases one should be careful when anterior ethmoidectomy is performed not to injure the endocranium (2).

Variations of ethmoid cells are due to different extension of ethmoid cells pneumatisation until they touch the compact bone (3). Their clinical significance is very important because they can be the reason for sinusitis and complications and general diseases. There are 10-15 ethmoid cells separated with thin lamellar bone and grouped in anterior and posterior cells. Bone lamella that separates the cells does not allow the sinusitis to be spread from one to another cell. They are called with different names and are very often findings on CT scan of paranasal sinuses. They can be found on the medial wall of the orbit - agger nasi cell and large bulla ethmoidalis, laterally from sphenoid sinus- Onodi cells and on the roof of maxillary sinus - Haller cells. They are normal anatomical variations except in cases where they cause narrowing of the normal drainage openings of paranasal sinuses and cause disturbance of draining sinus secret and sinusitis.

Material and Method

CT scans of 120 examinees of Macedonian nationality at the age of 17 to 70, 59 women and 61 male were evaluated. CT was performed on CT scanner Somatom, Volume Zoom, Siemens, multislice 4, at the Institute of Radiology, Medical Faculty, Skopje. Coronal sections were obtained at 3 mm distance, from anterior wall of frontal sinus to posterior wall of sphenoid sinus.

A total of 120 CT scans were analyzed with normal anatomy of paranasal sinuses. The patients with nasopharyngeal tumour, polyps, mucosal hypertrophy, fractures, earlier operation on the face which interrupt the normal anatomy of sinuses or lead to ostial destruction were excluded from the study. The presence of anterior ethmoid cells: agger nasi cell, Haller cell and large bulla ethmoidalis were evaluated on CT scans as being present on both sides of the nasal cavity, separately as unilateral or bilateral.

Results

We evaluated presence of agger nasi cells, Haller cells and large bulla ethmoidalis on CT scans of paranasal sinuses. The examined group consisted of 61 male, and 59 female patients, at the age of 17 years to 70 years.

The most present variation was agger nasi cell, in 84% of the examinees on the right side and in 86% on the left side of the nasal cavity (Fig.1). Prevalence of unilateral presence of agger nasi cell was 3.0, and bilaterally 86.0.

Agger nasi cells

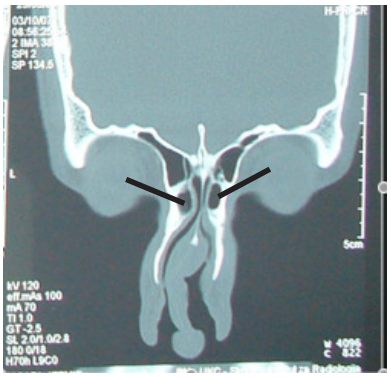

Fig.1. Agger nasi cell present bilaterally, and septal nasal deviation on left side of nasal cavity.

Haller cells

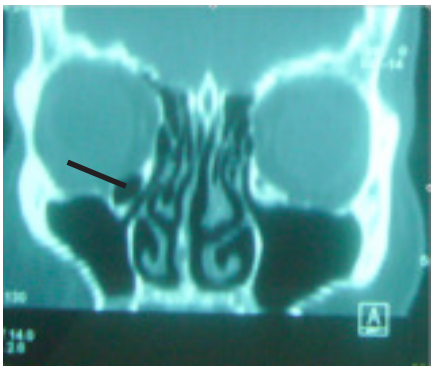

Fig.2. Haller cell on the right side of nasal cavity

Haller cells were present in 22% on the left and 17% on the right side of the nasal cavity. Prevalence of unilateral presence of haller cell was 11.0, and bilaterally 13.0 (Fig.2).

Bulla ethmoidalis

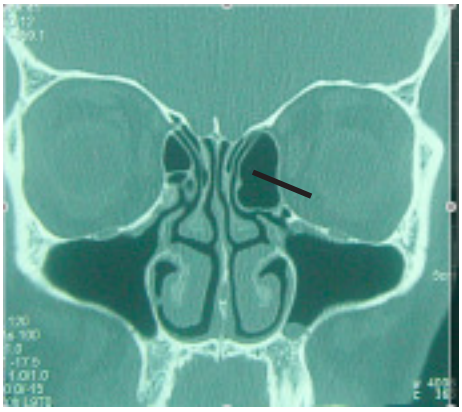

Fig.3. Large bulla ethmoidalis on the left side of nasal cavity (unilaterally)

Bulla ethmoidalis left and right were registered in 12% and 18% of the examinees (Fig.3). Prevalence of unilateral presence of bulla ehtmoidalis was 13.0, and bilaterally 8.0.

Discussion

There are many anatomical variations of ethmoid sinus cells seen on CT scan of paranasal sinuses (4). According to radiological studies, variations are equally present in patients with and without pathology of sinus lining (4, 5, 6). Variations of ethmoid cells are due to different extension of ethmoid cells pneumatisation until they touch the compact bone (3,7). Ethmoid cells pneumatise also frontal bone, maxilla and sphenoid bone and continue with the sinuses in these bones. Variations in developing of ethmoid labyrinth appear in relation to dimensions and position of bulla ethmoidalis, present agger nasi cell, Haller cell and Onodi cell. Individual cell can be isolated in group when it's drainage opening is far from the other cells openings or there is no communication between the cells. Therefore spreading inflammation of sinus lining is important and it's not a rare phenomenon the inflammation of ethmoid sinus to spread to maxillary, frontal or anterior ethmoid cells or not to spread to bulla ethmoidalis which has isolated opening (3). According to Krmpotic-Nemanic et al. it is difficult to perform FESS when there is large bulla ethmoidalis or agger nasi cells (8).

Agger nasi cells are anterior ethmoid cell found below the frontal sinus on the lateral wall of nasal cavity and anterosuperoior of hiatus semilunaris (9). Their clinical meaning was first recognized by Brunner et al. in 1996 whose research showed that excess pneumatisation of the agger nasi cells and it is close relation to frontal recess and lachrymal bone, can cause narrowing of the frontal recess and pneumatisation of lachrymal bone. That is the main and clinically the most important reason for pain in frontoethmoid region, chronic frontal sinusitis, or flow of tears (4). According to Van Alyea they are present in 89% of the examinees (3). K. Dua et al. found presence of these cells in 40% unlike Bolger et al. who found presence of these cells in 88,5 % of patients(10,11). In our study, agger nasi cells were registered in 86% on the right and 87% on the left half of the nasal cavity of our examinees. The prevalence of unilateral presence of agger cells was only 3%, and bilaterally 86%.

Haller cells or the so called infraorbital ethmoidal cells are localized on the roof of the maxillary sinus, or the floor of the orbit. They are found in the area of the opening of the maxillary sinus, in the lower part of lamina papyracea, laterally from processus uncinatus (4). Halle defined these cells in 1765. Their presence varies from 9% to 46%. According to Benjapour they are unilaterally found in 18,2 % of the cases and bilaterally in 14,8%, Bolger et al. found 45,9% Haller cells and Caughely et al. in 27% of the examined group (10,12,13). Our results showed, Haller cells 20% of the examinees had Haller cells, on the left and 17% on the right side of the nasal cavity. According to some authors they are a predetermining factor for maxillary sinus inflammation.

Bulla ethmoidalis is the biggest and the most constant frontal ethmoid air cell which causes prominence of the lateral wall of the nasal cavity. In Latin "bulla" means hollow bone prominence. It is developed by pneumatisation of the second basal ethmoid lamella. The opening of bulla ethmoidalis is located above, under the juncture of the middle turbinate, so there is a smaller possibility, compared to the other cells, that the opening will be narrowed or obstructed by the surrounding structures, except in the presence of concha bullosa (3). It can be enlarged because of hyper pneumatisation and it can be present unilaterally or bilaterally. Filho et al. discovered it in 4%, Bolger et al. in 2,5 % of the examinees, whereas K.Dua et al. in 8% and 6% of the examinees bilaterally (10,11,14). In our study enlarged bulla ethmoidalis left and right was registered in 11% and 18% of the examinees, respectively when performing FESS in this region surgeons should be careful not to harm the orbit through the lamina papyracea (15).The prevalence of the unilateral presence of bulla ethmoidalis is 13%, and bilaterally 8%.

In the last three decades the use of computerized tomography in diagnostics of sinonasal diseases enables very precise visualization of ethmoid pneumatised cells and their extension in near bone structures. Because they are very often present they can be called normal variations, except in cases when they cause narrowing of the drainage sinus openings and in cases when they lead to disturbance in drainage of sinus secrete.

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TORTUOSITY OF THE VERTEBRAL ARTERY

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Abstract

The aim of this study was to analyze the tortuosity of the vertebral artery and to emphasize the clinical importance of tortuosity. The data derived from this study will add important contribution to our anatomical knowledge, but they will also find clinical applications in radiology and surgery.

We examined 40 patients with CT angiography and analyzed the tortuosity of the vertebral artery.

Of the vessels examined, 22 (55%) followed a relatively straight course from their origin to their entry into the transverse foramen. The other 18 (45%) patients showed some form of tortuosity.

Both vertebral arteries showed high incidence of tortuosity. Our study has highlighted the possible role of vertebral artery tortuosity in diagnostic and surgical procedures in order to prevent vertebral artery injury.

Key words: anatomy, vertebral artery, tortuosity, surgery.

Introduction

Vertebral artery is the first and the largest branch of the subclavian artery arising from the posterosuperior aspect of its first part. It runs upwards and backward in the scalenovertebral triangle formed by muscles scalenus anterior and muscles longus colli. The common carotid artery and the vertebral vein are in front of it. It is crossed by the inferior thyroid artery and by the thoracic duct on the left side and the right lymphatic duct on the right side. The seventh cervical transverse process, the inferior cervical ganglion and ventral rami of the seventh and eight cervical spinal nerves lie posterior to the artery. Then the vessel reaches the sixth cervical vertebra to enter into the foramen of transverse processus. It passes through the foramina in the transverse processes of all of the cervical vertebrae except the seventh, curves medially behind the lateral mass of the atlas and enters the cranium via the foramen magnum. At the lower pontine border it unites with its contralateral fellow to form the basilar artery [1].

The vertebral artery is divided into four segments. The segment of the artery from its origin at subclavian artery to its entry into the respective transverse foramen is called the pretransverse, prevertebral or V1 segment. Between its origin and its entrance into the transverse foramen, the segment of the vertebral artery may show some tortuosity which can take a variety of forms and occupy several orientations [2].

The vertebral artery is formed between the 32nd and the 40th gestational day (7-18 mm embryo) from fusion of secondary persistent segments of cervical arteries and the primitive dorsal aortic arch. It is possible that abnormalities in fusion contribute to some tortuosities of the vessel. An abnormal origin is a rare finding; on the other hand, tortuosity is much more common. Whether this is from congenital origin or not, we can only speculate [2].

The aim of this study was to describe the tortuosity of the vertebral artery, to emphasize the clinical importance of tortuosity and to provide basic information for further clinical studies. Before undertaking any surgeries in the cervical region, a thorough knowledge of the surgical anatomy of the vertebral artery is mandatory to avoid a potentially catastrophic injury to a vertebral artery and to take decisions on the appropriate corrective surgery options [3].

Material and Methods

Forty patients from the University Clinic for Radiology in Skopje, R. Macedonia were investigated during a period of 8 months, from February 1st 2010 to September 30th 2010. For the purpose of this study 17 females and 23 males, ranging in age between 19 and 75 years, mean age of 54.1±12 years, were examined.

Anatomical analysis of CTA images realized for medically justified goal was done. Patients were investigated with computed tomography angiography (CTA). CTA was performed with CT scanner Somatom, Volume Zoom, Simens, multislice 4. Contrast material was injected by using intravenous catheter placed in the peripheral vein, a total of 100 ml. at a rate of 3 ml/s with a pressure injector. After the contrast medium was injected, by use of bolus tracking software, scanning was carried out automatically. The data were transferred to a workstation for post-processing. Reconstruction included the following: maximum intensity projection-MIP; four-dimensional CTA with volume rendering; reformatted multiplanar reformation-MPR.

By using the CTA we received 40 high quality images, which met the requirements of our study. Eighty vertebral arteries of the 40 patients were analyzed for tortuosity of the vertebral artery. The path of the vertebral artery was studied in detail, any form of tortuosity was noted; the plane of tortuosity was also examined.

Results

Of the vessels examined, 22 (55%) followed a relatively straight course from their origin to their entry into the transverse foramen (Fig. 1). The other 18 (45%) patients showed some form of tortuosity (Fig. 2). The plane of tortuosity was transverse in 50%, sagittal in 27.7% and

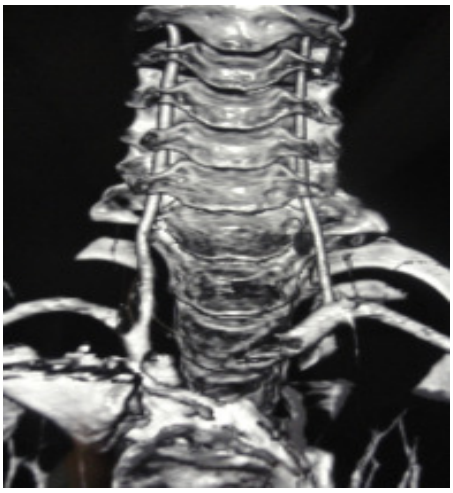

Fig. 1. Straight course of the vertebral artery

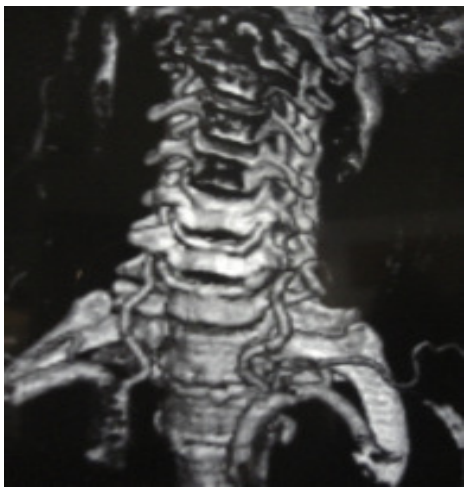

Fig. 2. Tortuous course of the vertebral artery.

frontal in 22.2%. Bilateral tortuosity was encountered in 10 patients and unilateral in 8 patients (5 on the left side and 3 on the right side).

Discussion

From the point of origin toward foramen of transverse processus of the vertebrae, vertebral artery may show different level of tortuosity [4].

Tortuosity of a vertebral artery used to be considered a rare abnormality, but recently its frequency has increased. The left vertebral artery is involved more often than the right one. This may be related to the fact that the left vertebral artery is larger than the right in a higher percentage of individuals. Rarely, bilateral or multilevel tortuosity of the vertebral arteries occurs with corresponding radiologic findings [5, 6, 7, 8].

Matula et al. in their series found that in 61% of the cases the vertebral artery followed non-tortuous path from the origin to the transverse foramen and in 39% followed a tortuous path. Furthermore, they classified tortuosity according to the geometric plane as horizontal (44.9%), sagittal (33.7%), and frontal (21.4%). In 32.5% of the cases the contorted pathway was on the right side, and in 68% of the cases, on the left. [2].

Trattnig et al. in their series found that 52.8% of the examined vessels, followed a relatively straight course from their origin to their entry into the foramen transversarium, but 47.2% showed some form of tortuosity (one coil at most). The main plane of the tortuosity was transverse in 42.5%, sagittal in 30% and frontal in 27.5% plane [4].

Poonam et al. in their series found that a total of 31 vertebral arteries (22.1%) showed the tortuous pathway in 25 cadavers. Bilateral tortuosity was encountered in six cadavers and unilateral tortuosity in 19 cadavers (13 on the left and 6 on the right). Twenty-six vertebral arteries had a single coil tortuosity and five vertebral arteries had a double coil tortuosity. The maximum number of tortuosities was observed in the transverse plane, followed by tortuosity in the sagittal plane [9].

Sastry et al. observed tortuous course in nine cases or 23.7% and in all cases the plane of the tortuosity was horizontal [10].

The results obtained in our study are consistent with the published literature. In our study we found that the vertebral artery had tortuous course in 18 (45%) cases. A relatively high incidence of tortuosity of the vertebral arteries could be explained with the mean age of our patients. There was a high statistically significant difference between the subjects with and without tortuosity according to age ($p<0.01$). A suggested congenital origin is difficult to be assessed since tortuosity has been shown to increase with age.

Clinically, tortuosity of the V1 segment does not have a hemodynamically significant consequence. The presence of a tortuous vertebral artery may go undetected because of the lack of symptoms. However, loops of the vessel have been described to cause several problems. Loops of the V1 segment have been reported to cause radicular symptoms via nerve root compression. Vertebral artery loop formation is one of those infrequent causes which can cause pressure erosion of the adjacent vertebral body, widening of the intervertebral foramen and compress the cervical nerve root causing cervical radiculopathy. Cervicobrachial neuralgia produced by vascular compression presents with paraesthesia and dysaesthesia of the fingers without a triggering factor, the lack of nocturnal symptoms, and the rarity of neurologic deficits. Symptomatic loops may be corrected by a bypass procedure [2, 4, 5, 6, 7, 8, 9, 11].

Cervical spinal fracture has been reported secondary to bony erosion from a V1 loop in contact. Also, a vertebral loop caused by displacement from a mass lesion in the scalenovertebral trigone may be compressed and lead to vertebrobasilar insufficiency. Angiographic or sonographic description of vertebral artery tortuosity in patients with signs and symptoms of vertebrobasilar insufficiency has led to surgical correction [2, 4].

When a tortuous vertebral artery is imaged, clinical correlation is essential to determine whether the

abnormality is responsible for the patient's signs and symptoms. If a cause and effect are not clear, conservative treatment is preferred. However, symptoms have been relieved by surgical intervention in some cases when radiculopathy was caused by nerve root compression due to vascular anomaly [7].

The anterior approach for the decompression of the cervical spinal cord and nerve roots with the anterior interbody fusion is an accepted surgical treatment for cervical radiculopathy. Although the reported results are generally excellent, the procedure has some potential complications, such as vertebral artery laceration [12].

In the presence of an abnormally tortuous vertebral artery, using anatomic landmarks to guide decompression may not prevent iatrogenic injury to the vertebral artery. Failure to recognize this anomaly during preoperative planning can lead to laceration of the vessel, even when decompression is achieved within generally accepted safe limits of lateral decompression [14]. The potentially devastating complications of vertebral artery injury with its supply of the cerebellum, brainstem and spinal cord are well known: brain stem infarction and central respiratory dysfunction [14].

From a surgical standpoint it can be of great importance to know whether the vertebral artery is straight or tortuous in, for example, surgical transposition of the vertebral artery to the carotid artery because of high-grade stenosis at its origin [2, 4].

Conclusion

In conclusion, we have presented an anatomical study of the vertebral artery tortuosity. The authors believe that proper and efficient demonstration and evaluation of vertebral artery tortuosity will add important contribution to the anatomic knowledge. Both vertebral arteries showed a high incidence of tortuosity. Our study has highlighted the possible role of vertebral artery tortuosity in diagnostic and surgical procedures in order to prevent vertebral artery injury.

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STATISTICAL ANALYSIS OF THE AVERAGE VOLUME OF KIDNEY DURING FETAL PERIOD OF GESTATION

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Abstract

Early antenatal diagnostics and importance of genetic consultation are of great interest for echosonographical evaluation of normal fetus anatomy. This study was undertaken in order to analyze the anatomical growth and volume of kidneys during their fetal development (from the IIIrd to the Xth lunar month). The research was carried out on kidney of 300 fetuses of both sexes (154 males and 146 females).

Macrodissection was used to extract both kidneys *en block*. The renal volume was measured by Vernier caliper gauge and calculated from other kidney diameters using the ellipsoid formula:

Volume = length x width x thickness x ð/6.

The analysis of the average volume in the examined series of fetuses has shown statistically significant differences in relation to the lunar month. Average volume of left fetal kidney in the IIIrd lunar month was 0.06 cm^3 and 0.07 cm^3 for the right kidney and continually increased up to 4,920 cm^3 of left and right kidneys in the Xth lunar month.

Key words: fetal kidney, volume, lunar months

Introduction

Renal congenital abnormalities are not uncommon in fetal life and they account for 30-40%. Increasing importance of genetic consultation has great interest for echosonographical evaluation of normal fetus anatomy. By prenatal ultrasound one can find larger than normal kidney measurements in polycystic kidney, fetal hydronephrosis etc., and smaller than normal in dysplasia, hypoplasia, urethrocele etc (1,2,3). For some lethal anomalies, like polycystic kidney, renal dysplasia, bilateral renal agenesis etc., an early detection and termination of the pregnancy may be a better choice. Identification of either one or two kidneys by the ultrasound is possible in 90% of cases from the 17th until 22nd week of gestation, and that is the critical time for genetic consultation. After the 20th week, the kidneys are identified in 95% of cases (4). Unfortunately, diagnosis of renal abnormalities appears relatively late in fetal life or postinatally. Nowadays, ultrasonography is used as the modality of choice for measuring a kidney size.

Material and Methods

This study was conducted at the Institute of Anatomy, Medical Faculty, Skopje. A total of 300 fetuses were included, as follows:

* premature born and stillborn fetuses with gestational age between IV-X lunar months, in 260 pairs of kidneys "en block";

* artificial abortions in the IIIrd lunar month and 40 pairs of kidneys "en block", obtained from the University Clinic of Gynecology and Obstetrics in Skopje.

Fetal kidneys were grouped according to gestational age and location, left or right kidney. Fetuses with malformations were excluded from the study. Macrodissection was used to extract both kidneys "en block" and they were separated from the surrounding tissue. The renal volume was measured by Vernier caliper and calculated from other kidney diameters using the ellipsoid formula:

Volume = length x width x thickness x ð/6.

All data of interest for this study were proper ordered and statistically analyzed using the following statistical methods:

testing the significance of the difference between three and several arithmetic means was done both with the analysis of variance (ANOVA) and with Tukey's Honestly Significant Difference (HSD) test;

testing the significance of the difference between two arithmetic means was done with the Student's t-tesi.

Data are presented in tables and graphs.

Results

For realization of the aim of the study, a total of 300 fetuses with gestational age from the IIIrd to Xth lunar month were analyzed. Of these, 146 (48.7%) were female and 154 (51.3%) male fetuses. Fetal growth and development of the kidneys (left and right) was monitored by measuring the volume in all kidneys in each lunar month.

Table 1. Mean values in cm^3 of left kidney volume in the examined fetuses according to lunar months. [Analysis of variance (ANOVA)].

Lunar month	N	Mean values of volume of left kidney (cm^3)	±SD
III	40	0.007	0.005
IV	34	0.104	0.070
V	42	0.407	0.107
VI	52	1.538	0.453
VII	28	2.224	0.114
VIII	60	2.850	0.162
IX	27	3.331	0.131
X	17	4.920	0.458

Table 2. Mean values in cm^3 of right kidney volume in the examined fetuses according to lunar months. [Analysis of variance (ANOVA)].

Lunar month	N	Mean values of volume of right kidney (cm^3)	±SD
III	40	0.006	0.004
IV	34	0.104	0.069
V	42	0.499	0.050
VI	52	1.541	0.451
VII	28	2.223	0.115
VIII	60	2.850	0.162
IX	27	3.331	0.131
X	17	4.920	0.461

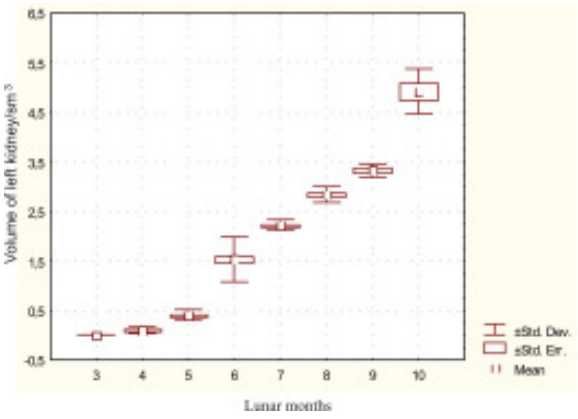

Fig. 1. Mean values in cm^3 of left kidney volume in the examined fetuses aaccording to lunar months.

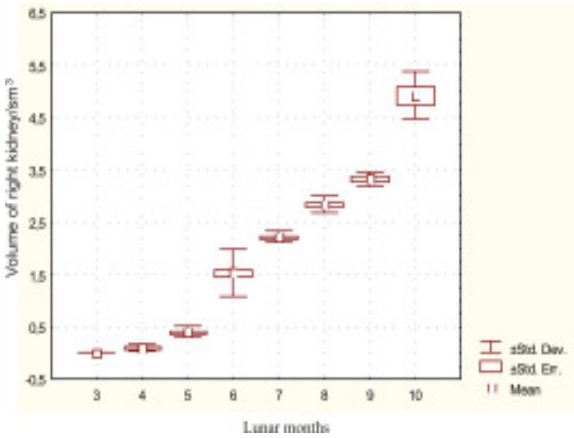

Fig. 2. Mean values in cm^3 of right kidney volume in the examined fetuses according to lunar months.

Table 1a. Significance of difference between mean values of left kidney volume according to lunar months. (Tukey's Honestly Significant Difference (HSD) test).

Lunar month	tukey (HSD) test p
III - IV	0.86759
IV - V	0.00003*
V - VI	0.00003*
VI - VII	0.00003*
VII - VIII	0.00003*
VIII - IX	0.00004*
IX - X	0.00003*

* statistically significant differences

Table 2a. Significance of difference between mean values of right kidney volume according to lunar months. (Tukey's Honestly Significant Difference (HSD) test).

Lunar month	tukey (HSD) test p
III - IV	0.85287
IV - V	0.00003*
V - VI	0.00003*
VI - VII	0.00003*
VII - VIII	0.00003*
VIII - IX	0.00003*
IX - X	0.00003*

* statistically significant differences

Table 3. Significance of difference between mean values of the volume of the left and right kidneys according to lunar months

Lunar months	Left kidney		Right kidney		Student – t test p
	Mean values (cm^3)	±SD	Mean values (cm^3)	±SD	
III	0.007	0.005	0.006	0.004	1.0
IV	0.104	0.070	0.104	0.069	1.0
V	0.407	0.107	0.499	0.050	0.0067*
VI	1.538	0.453	1.541	0.451	0.9352
VII	2.224	0.114	2.223	0.115	0.9149
VIII	2.850	0.162	2.850	0.162	1.0
IX	3.331	0.131	3.331	0.131	1.0
X	4.920	0.458	4.920	0.461	0.9489

* statistically significant differences

The analysis of variance (ANOVA) has shown a statistically significant differences between the mean values of the left kidney volume in correlation with the lunar months (F = 10105,99 p = 0,0001) (Table 1 and Fig. 1).

Tukey honest significant difference (HSD) test has shown the differences between the mean values of the left kidney length in the examined fetuses in the correlation to all examined lunar months separately. The volume of the left kidney significantly increased from one to the next lunar month and all differences were statistically significant. (Table 1a).

Table 2 and Fig. 2 present the mean values of the right kidney volume in all 300 fetuses according to lunar month.

The analysis of variance (ANOVA) has shown statistically significant differences between the mean values of the right kidney volume in correlation with the lunar months (F = 10011,04 p = 0,0001).

Tukey honest significant difference (HSD) test has shown the differences between the mean values of the right kidney length in the examined fetuses in correlation to all examined lunar months separately. The volume of the right kidney significantly increased from one to the next lunar month and all differences were statistically significant. (Table 2a).

Student's t-test has shown that in the IIIrd and IVth lunar month there were statistically significant differences between male and female fetuses in correlation to the volume of left kidney (female fetuses` volume of left kidney is statistically bigger). In all other lunar month the observed differences between the left and right kidney of the examined fetuses were no significant).

Average volume of left fetal kidney in the IIIrd lunar month was 0.06 cm^3 and 0.07 cm^3 of the right kidney and continually increased up to 4,920 cm^3 for both kidneys in the Xth lunar month.

Discussion

To accomplish the aim of this study, we compared the results obtained with the existing knowledge about this issue found in the available literature. Development of the human fetal kidneys goes through a series of continual and mutually dependent anatomical changes during which the kidneys obtain their morphological and functional maturity. Growth and development of the fetal kidney have been of interest to many scientists and researchers (1, 3, 5, 7). In our study we determined the volume of the fetal kidneys by measuring its dimension in the three axes using the ellipsoid forumula. Many authors determined the volume of a child's kidney by measuring its dimension in the three axes and using the ellipsoid formula (5,6,7).

In our study the average volume of left fetal kidney in the IIIrd lunar month was 0.06 cm^3 and 0.07 cm^3 of the right kidney and continually increased up to 4,920 cm^3 for left and right kidneys in the Xth lunar month. However, in the study of Slobodan Vlajkovic (8) the average volume of fetal kidney continually increased from 0.33 cm^3 in the IVth lunar month up to 8.68 cm^3 (left kidney) and 9.53cm^3 (right kidney) in the Xth lunar month. Kidney showed statistically significant ($p<0,05$) increase of volume in the IVth lunar month and VIth lunar month.

The conclusion of Sampaio (5) that male fetuses presented kidney volumes significantly greater than female fetuses (found in 145 pairs of kidneys) is not in accordance with our results where statistically significant difference in kidney volume between male and female fetuses during whole intrauterine life was not found. The ratio: sum of volumes of both kidneys/crown-rump length,

in absence of other better data, shows, in a specific way, the quantity of kidney tissue per unit of fetus largeness. The ratio increases with fetal aging, except in the second half of the Vth lunar month when it stagnates. This ratio displays the highest value in the Xth lunar month, when it clearly shows that kidney is physically prepared for full taking over of all its functions after birth.

Average volume of the left fetal kidney in the IIIrd lunar month was 0.06 cm^3 and 0.07 cm^3 of the right kidney and continually increased up to 4,920 cm^3 for both kidneys in the Xth lunar month.

Conclusion

The analysis of the average volume in the examined series of fetuses has shown statistically significant differences in relation to the lunar month. Average volume of the left fetal kidney in the III lunar month was 0.06 cm^3 and 0.07 cm^3 of the right kidney and continually increased up to 4,920 cm^3 for both kidneys in the Xth lunar month. Measuring of kidney size can help in determination of gestational age, especially in cases where the date of the mother's last menstruation is unknown.

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THE NEW CLASSIFICATION OF DISTAL EXTRAHEPATIC CHOLANGIOCARCINOMA-MORPHOLOGICAL ANALYSIS AND REVIEW OF LITERATURE

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Abstract

Extrahepatic cholangiocarcinoma (EH-CCa) is a malignant tumor that arises from the ductal epithelium of the extrahepatic bile duct and it has been divided into perihilar and distal at the level of the cystic duct . As relatively rare disease perihilar and distal extrahepatic cholangiocarcinoma should be viewed as independent entities because of their distinct biology and management.

The aim of the study was to make morphologic analysis of archive material of diagnosed EH-CCa at the Institute of pathology, Medical Faculty, Skopje in 5-year period and revision of the cases according to 7th edition (AJCC/UICC) TNM staging classification.

We used archive material from the data base of the Institute of pathology, Medical Faculty, Skopje, for 7 cases diagnosed as distal EH-CCA between year 2006 and year 2010.

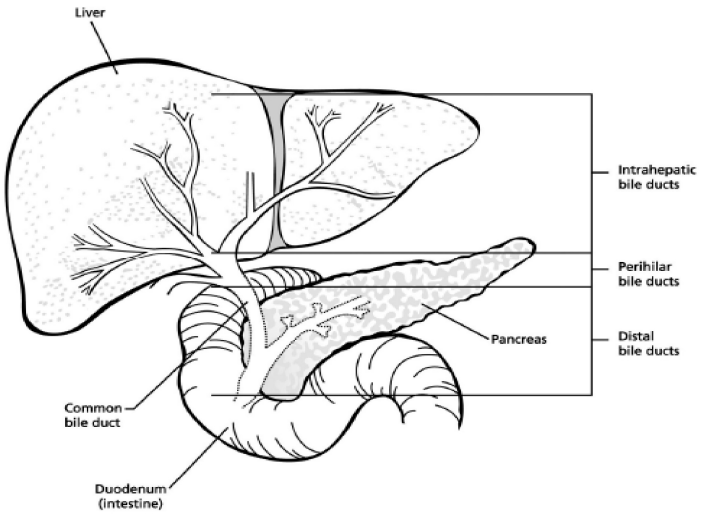
Four analyzed adenocarcinomas showed periductal-infiltrating pattern of growth and 3 cases had mass-forming characteristics. One case revealed pancreatic involvement, 5 infiltrated the periductal soft tissue and 1 was confined to the ductal wall. In 3 cases peri/intraneural invasion was found and 2 cases had vascular tumor embolusses. There were 2 lymph nodes with metastases from 10 examined in only one case, in two cases there was no surgical resection of lymph nodes, and the rest cases were free from metastatic disease. Positive surgical margines we found in 2 cases and 2 were negative. The most of EH-CCA were diagnosed in stage IB, one was in stage IA and one in stage IIB.

The purpose of 7th edition AJCC/UICC TNM staging system is predominantly prognostic and will require additional validation.

Keywords: Extrahepatic cholangiocarcinoma, Distal bile duct carcinoma , Staging

Introduction

An extrahepatic cholangiocarcinoma (EH-CCa) is a malignant tumor that arises from the ductal epithelium of the extrahepatic bile duct and is the most common primary malignancy of the biliary tract. However, cholangiocarcinoma is relatively rare disease, accounting for <2% of all human malignancies. Classically, extrahepatic cholangiocarcinoma has been divided into perihilar and distal extrahepatic cholangiocarcinoma at the level of the cystic duct (Scheme 1). About 60-70% of EH-CCa arise at



Scheme 1. Location of bile duct cancers

the hepatic hilum, 20-30% arising from the distal common bile duct while 5-10% of the tumors may be multifocal. [1, 2]

Perihilar and distal extrahepatic cholangiocarcinoma should be viewed as independent entities because of their distinct biology and management. [1]

Distal extrahepatic cholangiocarcinomas are defined as growing along the common bile duct between the cystic duct and the ampulla of Vater and they are clearly separated from ampullary carcinomas. Histologicaly they are predominantly well-to-moderately differentiated adenocarcinoma. The latest WHO classification, proposes mass-forming, periductal-infiltrating lesion and intraductal macroscopically growing type. EH-CCa is difficult to diagnose due to its silent clinical character, growth patterns, anatomic location, the
low specificity of most diagnostic modalities and lack of definite diagnostic criteria. Most patients present with advanced disease that is not amenable to surgical treatment. The median survival time for these patients varies from 12-24 months.

Identification of the tumor stage and allocation to the optimal treatment approach is important for this aggressive carcinoma. Inaccurate staging can result in inappropriate selection of a treatment option that either deprives patients of a potentially curative procedure or exposes them to unnecessary morbidity and mortality. The 7th edition American Cancer Committee/Union for International Cancer Control (AJCC/UICC) TNM staging classification (2010) is the first classification system that has assignated a separated staging system for distal extrahepatic cholangiocarcinoma. [1, 2]

Aim of the study

The aim of the study was to make morphologic analysis of archive material of diagnosed EH-CCa at Institute of pathology, Medical Faculty, Skopje in 5-year period and revision of the cases according to 7th edition (AJCC/UICC) TNM staging classification (2010).

Methods

We used archive material from the data base of the Institute of pathology, Medical Faculty, Skopje, for 7 cases diagnosed as distal EH-CCA between the year 2006 and 2010. Four were females and 3 were males, mean age 50,3 (ranges 48-60) for men and 60 (ranges 54-67) for women. The performed analysis of the cases was according to tumor growth pattern, histologic type, pancreatic involvement, neural and vascular invasion, surgical margines, lymph nodes metastasis and stage.

Results

The analysis confirmed that all of the cases were adenocarcinomas, 4 of which showed periductal-infiltrating pattern of growth (Figure 1.) and 3 cases had mass-forming characteristics (Figure 2.). All cases showed moderate differentiation, four of which presenting desmoplastic reaction. One case revealed pancreatic tissue

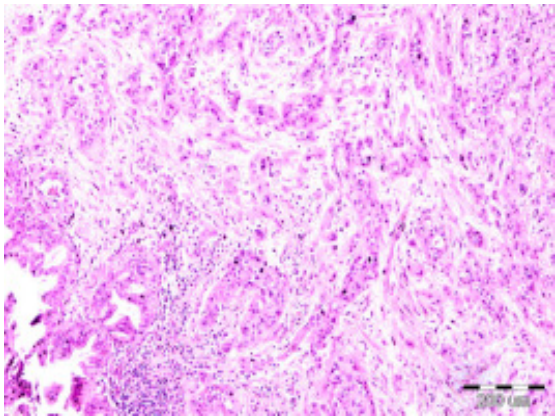


Fig. 1. Periductal-infiltrative growth pattern (H.Ex40)

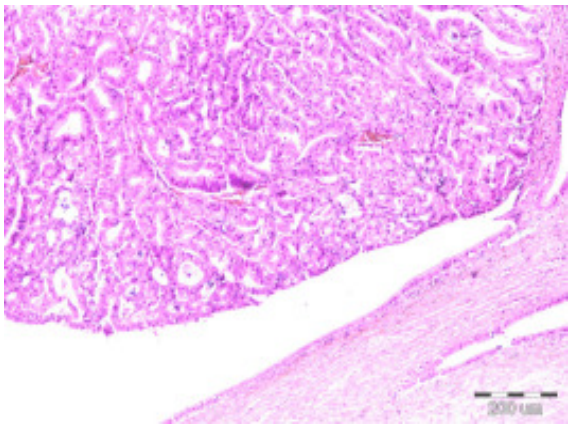


Fig. 2. Mass-forming growth pattern (H.Ex40)

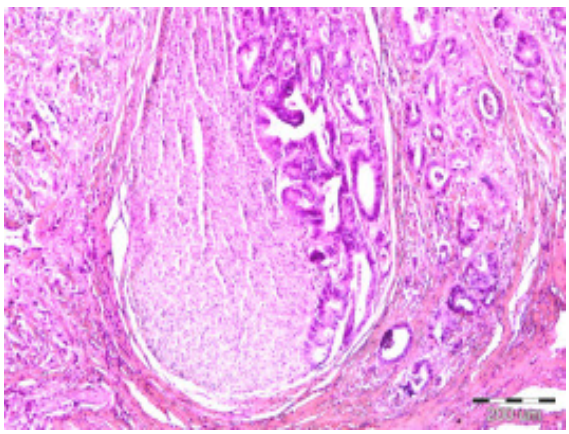


Fig. 3. Perineural and intraneural invasion (H.Ex40)

Table 1. Morphologic analysis of distal extrahepatic cholangiocarcinoma in 5-year period

year	2006	2007	2008	2009		2010	
No of cases	1	1	1	1		3	
histologic gradus	G2	G2	G2	G2	G2	G2	G2
growth pattern	mass-forming	mass-forming	periductal-infiltrative	periductal-infiltrative	periductal-infiltrative	periductal-infiltrative	mass-forming
spread	periductal soft tissue, T2	pancreas, T2	periductal soft tissue, T2	confined to ductal wall, T1	periductal soft tissue, T2	periductal soft tissue, T2	periductal soft tissue, T2
peri/intra neural invasion	/	yes	/	/	yes	/	yes
vascular invasion	/	/	yes	/	no	/	yes
lymph nodes (positive/isolated)	0/1	/	0/2	0/6	0/4	/	2/10
surgical margines	R1	/	R1	/	R0	/	R0
stage*	IB	IB	IB	IA	IB	IB	IIB

* 7th edition (AJCC/UICC) TNM staging classification (2010)

involvement, 5 of them were infiltrating the periductal soft tissue and 1 was confined to the ductal wall. In 3 cases peri/intraneural invasion was found (Figure 3.) and 2 had vascular tumor embolusses (Figure 4.). There were 2 lymph nodes with metastases (Figure 5.) from 10 examined in only one case, in two cases there was no surgical resection of lymph nodes and the rest cases were free from metastatic disease.

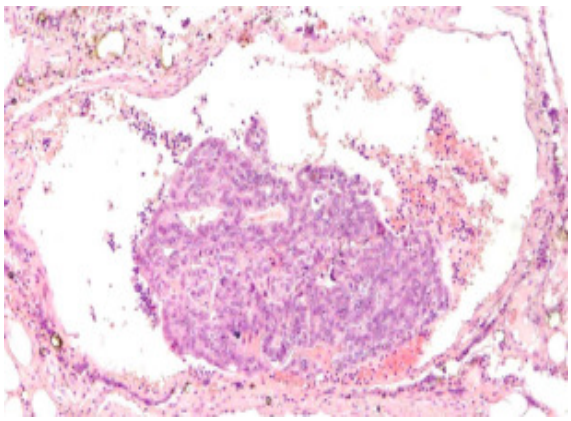


Fig. 4. Vascular tumor embolus (H.Ex40)

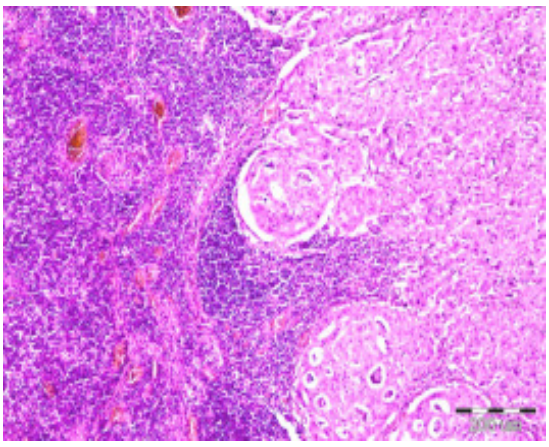


Fig. 5. Lymph node metastasis (H.Ex40)

Positive surgical margines we found in 2 cases and 2 were negative.The analyzed tissues in 3 cases were small resected fragments, so the evaluation of margins was difficult. The most of EH-CCA were diagnosed in stage IB, one was in stage IA and one in stage IIB (Table 1).

Discussion

Extrahepatic cholangiocarcinoma is a devastating malignancy with an increasing incidence. It is difficult to diagnose and frequently presents itself at a late stage, mostly infiltrating the periductal soft tissue as in our study. There is growing acceptance that EH-CCa shoud be treated differently according to their location. The updated 7th edition AJCC/UICC TNM staging classification (2010) is the first classification system that has assignated a separate staging system for distal extrahepatic cholangiocarcinoma. This is an important correction as perihilar and distal extrahepatic cholangiocarcinoma differ in their presentation , natural history, management and molecular signature [1, 2]. For example, lymph node metastatic spread differs between the two types of cholangiocarcinoma and is most commonly observed in distal EH-CCa [3]. It is possible that the observed difference in the rate of regional nodal spread may represent a difference in nodal resection due the anatomic limitations of portal node dissection and pathologic assessment [4].

Murakami,Y et al. (2007) and Yoshida.T et al. (2002) have suggested that number of lymph nodes is an

independent prognostic factor; and more than two metastatic lymph nodes is predictive of worse outcomes [5, 6]. The depth of tumor invasion: confined to the bile duct versus beyond it, or pancreatic invasion-distinguishes by T1 and T2 stage is frequently criticized. [7]. T stages were not found to be predictors of outcomes after resection of these cancers, which indicates that the definition needs to be more precise. Hong S.M. et al (2009) grouped cancers into three groups according to their invasion depth: < 5 mm, 5-12 mm and > 12 mm. They found that this stratification is the strongest predictor for outcome [8]. Most of our cases were classified as T2 (86%). Redifinition of the T stages is also a step in the revision: T4 describing invasion in the celiac axis and superior mesenteric artery and T3 describing invasion of adjacent organs but without invasion of arterial structures. The prior AJCC/UICC distinguished T3 and T4 based upon invasion of different organs; however, it was shown that this definition of T3 and T4 was not able to distinguish prognosis [9]. For distal extrahepatic cholangiocarcinoma, perineural and microscopic vascular invasion as well as negative surgical margines were found to be significant predictors of survival [7, 8,10,11]. The prognostic significance of location within the distal duct appears to be most associated with the ability to resect the lesion and obtain negative margins. After curative resection, tumor free margins can be obtained in about 50% of distal tumor [12]. Our analysis revealed 28% of cases with free surgical margins. According Sakamoto. Y et al (2007) age, gender, tumor histology and histologic grading were not found to be statistically prognostic factors [7].

Conclusion

In summary the purpose of the 7th edition AJCC/ UICC TNM staging system is predominantly prognostic and it will require additional validation. A staging system providing treatment guidance has not been developed and is needed.

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MULTIPLE IMMUNOFLUORESCENCE LABELING IN FROZEN RENAL TISSUE

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Abstract

Multicolor immunofluorescent labeling techniques are used for analysis of co-localization of antigens not only in the same cell, but also in the same cellular compartment.

Objective of this study was to determine the localization of different antigens in a single frozen tissue section.

We present a method of multiple sequential immunofluorescent labeling in renal frozen tissue specimens. For that purpose, we used unlabeled primary antibodies against CD133 and Cytokeratin, fluorochrome labeled secondary antibodies and DAPI as nuclear counterstaining.

The immunofluorescent co-localization study showed that CD133 was localized mainly in the cells of the Bowman capsule, in very few tubular segments in the vicinity of the glomeruli, as well as in the medullary tubular segments.

Key words: multiple immunofluorescent labeling, frozen tissue, antigen co-localization

Introduction

Immunofluorecence has been introduced in the cell biology investigations in the early 1940s when Albert Coons et al. published the method of labeling antibodies with fluorescent dyes (1). Since then, and especially by the turn of the twenty-first century, the method of fluorescent labeling has evolved and its use in the cell biology investigations has become the cause for a revolution in cellular and subcellular imaging.

The idea and necessity of simultaneous visualization of two or more different antigens in the same tissue section, the same cell, or even in the same cell compartment has been challenging researchers since the invention of the immunolabeling method.

The discoveries in many different scientific fields have made the method of multiple immunostaining possible and very valuable for the research work in the cell biology. The synthesis of DAPI (4',6-diamidino-2-phenylindole) in 1971 as a drug for trypanosomiasis, and the discovery in the mid-seventies of the twentieth century of its ability to form fluorescent complexes when bound to DNA (2), was one of them. In addition, the introduction of new fluorescent labels (3), as well as a variety of filter sets for immunofluorescent microscopes, have made their identification possible (4, 5), and the introduction of CCD camera for a high-resolution analysis of biological structures (6) has allowed sophisticated computational image processing and analysis by commercial image analysing software.

Methods for simultaneous detection of triple (7), quadruple (8), and multiple - up to seven (9) antigens have been described in this paper.

Objective

The objective of this paper is to present the method of double immunofluorescence staining in determination of the localization of different antigens (CD133 and Cytokeratin) in a single renal frozen tissue section, and the comparison of the results to those from of immunohistochemical stainings.

Material

We used 20 renal biopsy samples obtained in PBS, frozen in liquid nitrogen and stored at a temperature of -80°C until the analysis. Only those diagnosed as primary glomerulopathy with standard histological and immunofluorescent examination were selected for double immunofluorescent stainings.

As control, we used a tissue from normal adult kidneys obtained for analysis for renal carcinoma which did not affect the surrounding renal parenchyma.

Methods

Reagents

We used mouse monoclonal anti-human primary antibodies against CD133 (clone AC133, dilution 1:10, Miltenyi Biotec, Germany) and against Cytokeratin (clone AE1/AE3, dilution 1:50, Dako, Denmark), and anti-mouse secondary antibodies conjugated with fluorochromes FITC (fluorescein isothiocyanat, emitting green signal when excited at ~460 nm, with peak emission at ~520 nm), and Cy3 (member of a family of synthetic fluorescent cyanine dyes, excited at ~550 nm, with peak emission at ~570 nm, appearing red when seen under fluorescent microscope). For counterstaining of the nuclei, we used DAPI - (4',6-diamidino-2-phenylindole), which emits deep blue signal under fluorescent microscope.

Preparation of tissue sections for storage

The frozen tissue was cut on a cryocut Leitz with 5μm thick sections and mounted on superfrost slides. The sections were airdried for 30 minutes, fixed in cold acetone for 10 minutes and again airdried for 30 minutes. Sections were stored at -20°C until further usage. Before staining, the slides were warmed at room temperature for 30 minutes and washed in PBS 3 times for 5 minutes.

Fluorescent double immunostaining

We performed a sequential staining method with two primary antibodies raised in the same species. The staining was performed at room temperature in a dark humidified chamber.

The whole staining protocol can be divided into the following steps:

1. First blocking step: in order to block unspecific binding of the antibodies, the tissue sections are incubated with 1% BSA in PBS for 30 minutes

2. First primary and secondary antibody: the tissue sections are incubated with the first primary antibody (CD133) diluted in 1% BSA in PBS for 1 hour, followed by incubation with a first secondary antibody, raised in the same species as the primary antibody (mouse), labeled with fluorochrome Cy3 diluted in 1% BSA in PBS for 45 minutes.

3. Washing with PBS 3x5 minutes is performed between the incubation with primary and secondary antibody, as well as after the first secondary antibody.

4. Second blocking step: incubation of the tissue sections with 1% BSA in PBS for 30 minutes

5. Second primary and secondary antibody: the tissue sections are incubated with the second primary antibody (Cytokeratin) diluted in 1% BSA in PBS for 1 hour, followed by incubation with a second secondary antibody raised in mouse, labeled with fluorochrome FITC (which gives green fluorescent signal), diluted in 1% BSA in PBS for 45 minutes.

6. Washing with PBS 3x5 minutes is performed between the incubation with primary and secondary antibody, as well as after the second secondary antibody.

7. Counterstaining of the nuclei with DAPI for 15 minutes.

8. The slides are mounted with anti-fade mounting medium and stored at 4°C.

Immunohistochemistry

Immunohistochemical stainings were performed both on frozen renal biopsy specimens and in control renal tissue, by standard immunohistochemical procedure using Dako EnVision vizualisation kit.

Controls

For positive control we stained frozen sections from normal kidney applying the same immunofluorescent method as described above, as well as immunohistochemically with single antibodies.

Negative control stainings were done on control sections of the same tissue placed on the same slides with the analysed sections, by omitting the primary antibodies during the staining procedure.

Fluorescence microscopy

Fluorescent microscope (Nikon Eclipse 1000) with different optical filters for each fluorochrome and CCD camera with software for image analysis was used for collecting, digitizing, superimposing and storing images. For FITC visualization, we used filter with excitation wavelength between 450 and 490 nm and emission wavelength 510 - 540 nm, for Cy3 filter with excitation range between 510 and 560 nm and emission wavelength between 570 and 590 nm, and the filter for DAPI with excitation wavelength of 350 nm and emission wavelength of 470 nm.

Images of the same view field seen with different filters were acquired and digitally merged in order to compare and see the overlap of the signals.

Results

On the fluorescent microscopy with appropriate filters, the CD133 positive cells were marked with bright red signal (Fig 1a and 1b), the Cytokeratin positive cells were marked with bright green signal (Fig. 2a and 2b) and the nuclei appeared deep blue (Fig.3).

The merged images showed their co-localization. Namely, the CD133+/Cytokeratin+ (Fig. 4a and 4b) cells appeared orange due to the superimposed colors and were found in some of the tubules close to the glomeruli and in many medullary tubular segments. Interestingly, the CD133+ cells in the Bowman capsule showed only a weak signal for Cytokeratin. Furthermore, the analysis revealed some non-epithelial cells positive for CD133 in the fibrotic interstitium between atrophic tubuli.

The same distribution of positive signal for CD133 was found on the immunohistochemical stainings with single antibodies.

Discussion

Immunofluorescence is a common laboratory technique with a broad spectrum of application in biology, both for research and for routine analyses. There are two basic immunofluorescent methods such as the direct labeling (where the antibody is conjugated to a fluorescent dye – fluorochrome) and the indirect labeling (where the primary antibody is unconjugated and the secondary antibody, which amplifies the signal, is labeled with a fluorochrome).

In histopathology practice, these analyses can be performed in fresh and in fixed tissue samples (10), both of which have advantages and disadvantages.

There are plenty of advantages of immunofluorescent staining techniques performed on frozen tissue are many: shorter time of tissue processing, better preservation of the antigenic content, less autofluorescence than in paraffin embedded tissue, while the paraffin embedded tissue takes longer time to prepare, sometimes needs antigen retrieval technique, but gives much better tissue and cell morphology. On the other hand, the obtained staining signal is permanent in the paraffin embedded tissue and fading in time in frozen tissue specimens.

Both immunofluorescence and immunohistochemical techniques can be used for multiple labeling and co-localization studies. However, immunohistochemistry is better applicable for antigens located in different tissue segments (11), where the single colors can be easily be appreciated and distinguished from each other. On the contrary, if the targets are co-localized within the same cell, the effect of the mixture of both colors

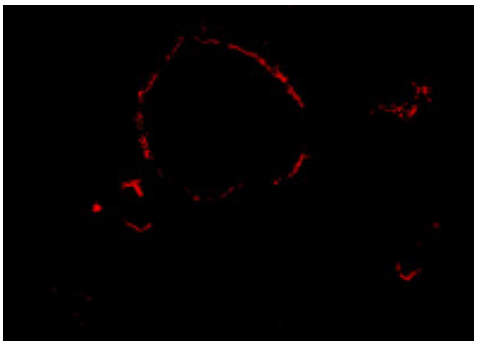

Fig. 1a. CD133 positive cells in cortical area - Bowman capsule and in the vicinity of the glomerulus (x20)

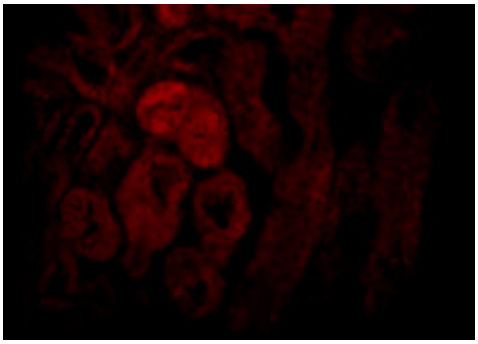

Fig. 1b. CD133 positive cells in medullary tubular segments (x20)

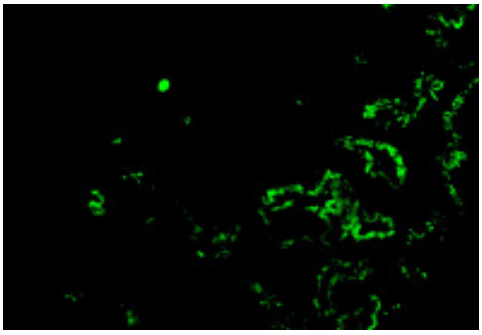

Fig. 2a. Cytokeratin positive cells in the cortical area (x20)

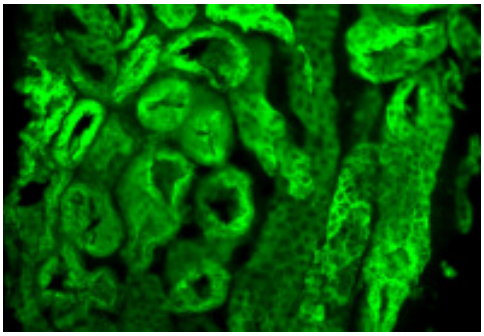

Fig. 2b. Cytokeratin positive cells in medullary tubular segments (x20)

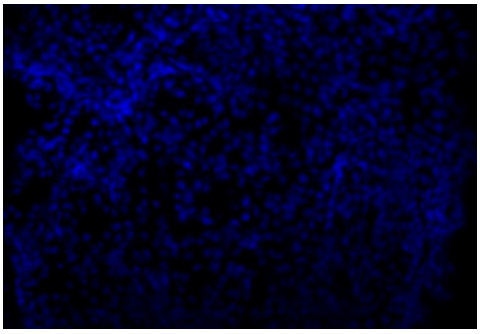

Fig. 3. DAPI positive nuclei (x20)

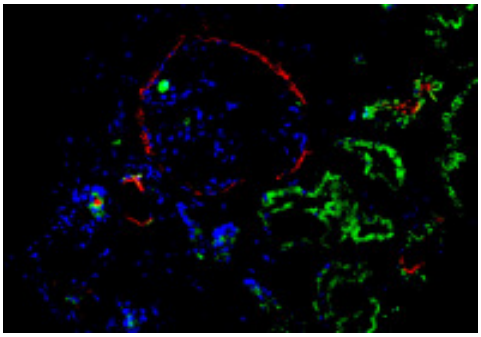

Fig. 4a. Merged image showing CD133+/Cytokeratin+ cells in the cortical area (x20)

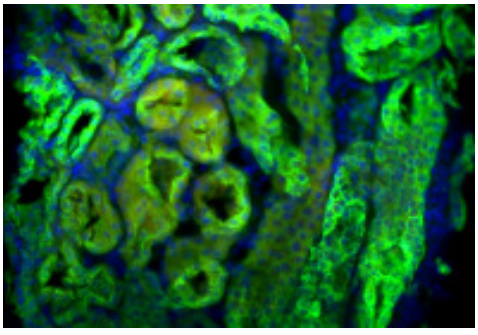

Fig. 4b. Merged image showing CD133+/Cytokeratin+ cells in the medullary area (x20)

creates interpretation difficulties and can be easily misinterpreted if one of the colors overwhelms the other (12), the cells are tightly packed or have only scant cytoplasm, like lymphocytes (13).

The method of double immunofluorescent labeling has the advantage of digital image analysis where each fluorescent dye is separately viewed and stored, and the acquired images are digitally merged (14). Consequently, this method enables better determination of intracellular co-localization, but lacks the possibility of deeper morphological analysis.

There are two basic approaches to multiple fluorescent immunolabeling the simultaneous and sequential approach. During the simultaneous method, the tissue is first incubated with a mixture of two different primary antibodies, and then with mixture of appropriate two different secondary antibodies. In order to avoid cross-reactivity, this method requires the used primary antibodies, as well as the appropriate secondary antibodies, to be raised in different species (e.g., combination mouse-rabbit, mouse-goat, etc.).

We present a sequential method of step-by-step incubation with a single primary antibody, which is followed by incubation with appropriate single secondary antibody, a procedure which is repeated with the second primary and secondary antibody. The advantage of this method is that both primary antibodies (and thus, the appropriate secondary antibodies, too) can be raised in the same species. During this method, in order to avoid unspecific binding of the antibodies, the first step is blocking by incubation with 1% BSA, or alternatively, with 10% serum from the species in which the secondary antibody was raised in, followed by thorough washing with low pH buffer is performed between each step in order to remove the immunoreagents from the previous staining sequence and prevent cross-reactivity (15). Control experiments are necessary for safe interpretation of results obtained with this type of double staining. The so called "Half-double staining" experiment has also been proposed, i.e. omitting the primary antibodies (12). Another control for the quality of the method is the comparison to the single stained slides. We have chosen to do this control with the immunohistochemical method applying the same antibodies, in order to visualize the surrounding tissue components and total tissue morphology, which otherwise can not be appreciated on the dark field microscopy.

CD133 is a transmembrane glycoprotein selectively expressed on populations of hematopoietic stem and progenitor cells, on stem cells in a variety of adult non-hematopoietic tissues (kidney, prostatic epithelia, muscle tissue, liver and cornea), on cancer stem cells of a variety of cellular and tissue origin, as well as in human fetal tissue (brain, skin, neural tube, gut, kidney and liver).

Cytokeratin is a protein of keratin-containing intermediate filaments in the cytoskeleton of the epithelial cells, and is therefore used for their identification in normal tissue and for detection of origin of cancer tissue.

The presented study and the evident co-localization of these two antibodies in certain resident kidney cells encourages further analysis with a broader panel of antibodies in order to identify the stem cells and determine the sites of their localization in normal and pathologically altered kidney tissue.

In conclusion, the method of double immunofluorescent labeling is a valuable and mighty tool in the researcher's hands, but only when planned thoroughly and used with proper controls. Therefore the combination of antibodies must be well studied and planned in order to avoid cross-reactivity, controls must be included, and ancillary analyses of tissue morphology must be performed as well.

Acknowledgements

We thank Prof. Claudia Mueller from the Medical University Clinic and Center of Medical Research, Tuebingen, Germany for providing the protocols and most useful advices, as well as crucial technical support. We also thank Sanja Cirovic from the Institute f Pathology, School of Medicine, Belgrade, Serbia for technical assistance.

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MEDULLARY THYROID CARCINOMA, HISTOTYPE FREQUENCIES, KI-67 AND BCL-2 EXPRESSION

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Abstract

Medullary thyroid carcinomas (MTCs) are rare neoplasms comprising not more than 2-10% of all thyroid malignancies. It has been noted that some MTC cases present an indolent, and some, an aggressive clinical course. We re-evaluated archive specimens from 20 cases of MTCs and performed additional immunostainigs with anti-Ki-67 and anti-Bcl-2 antibodies. The re-evaluation included: histological subtype, type of tumor margin (expansive or infiltrative), expression of Bcl-2 and Ki-67. We also analyzed the age and sex distribution, tumor greatest diameter, presence of regional lymph node and/or distant metastases at the time of operation, the stage of the disease, and the clinical history of the patients. According to the clinical parameters, patients had been clinically divided into two groups designated as "group 1" - indolent disease course and "group 2" - aggressive disease course. The ethnical ratio of macedonian vs. non-macedonian patients (70:30) is quite similar to the overall ratio in the general population, suggesting absence of specific ethnicity-related factors influencing the incidence of MTC in our population. Most of the patients (n = 11) were in stage II by the time of operation, and the mean tumor diameter at operation was 4,2 cm. Tumor histotype was not correlated to the investigated parameters. Ki-67 expression was low (6,55%). We found significantly higher Ki-67 expression in women, compared to men ($p < 0,05$). This difference had no influence on the disease course, and there were no correlations to the other parameters. Bcl-2 expression was high (68,3% of tumor cells), with no male:female differences, neither correlations to other investigated parameters. The aggressiveness of the disease was correlated to the type of tumor growth, regional lymph node status and the stage of the disease at operation. The infiltrative type of tumor margin was correlated to more aggressive clinical course, compared to the expansive type of tumor margin ($p<0,05$).

Key words: Medullary thyroid carcinoma, histopype frequency, Ki-67, Bcl-2

Introduction

Medullary thyroid carcinomas (MTCs) are rare neoplasms comprising not more than 2-10% of all thyroid malignancies (1, 2). Most of them appear as sporadic (75-80%), and the reminder are familial and MEN2 related cases (1, 3, 4). According to the US National Cancer Institute the incidence of MTCs in the US range between 500-1000 cases per year (in a population of 300 mil.), or 2-3% of all thyroid cancers which incidence is 37300 cases per year. According to the statistics from France and USA 43% and 44% of MTCs, respectively, are familial cases, and frequently associated with MEN2 syndrome, while in Sweden, this percentage is much lower – around 26%. According to the NCI estimations, the same percentage should be applicable to Europe in general (2). Having in mind the aforementioned proportions, data from the Macedonian Institute for Public Health, the Institute of Pathology and the Institute for Radiotherapy and Oncology in Skopje, there should be 2-3 newly diagnosed cases of MTC in Macedonia (population of approx. 2 mil.) per year. However, in the last 15 years we have diagnosed only 22 cases, and none of them was MEN2 related, neither a case of Familial Medullary Thyroid Cancer (FMTC).

It has been noted that some MTC cases present an indolent, and some an aggressive clinical course (1, 4, 5).

According to the WHO, 12 histologic variants of MTC are recognized (Tab.1), although there are no exact frequency data, neither data about possible population

Table 1 Variants of medullary thyroid cancer (modified from the WHO)

Variant	Variant designation	Histologic characteristics
Type 1	Papillary or psaudopapillary	Presence of true papillae or artefactual pseudopapillae, caused by tissue fragmentation
Type 2	Glandular (tubular or follicular)	Presence of tubular or follicular structures lined with neoplastic cells
Type 3	Giant cell	
Type 4	Spindle cell	
Type 5	Small cell and neuroblastoma-like	
Type 6	Paraganglioma like	
Type 7	Oncocytic cell	
Type 8	Clear cell	
Type 9	Angiosarcoma-like	
Type 10	Squamous cell	
Type 11	Melanin-producing	
Type 12	Amphicrine	

related differences, nor data about the correlation of the specific histotypes with the clinical behaviour of the tumors. In general, addressing the issue of Ki-67 there are reports about very low Ki-67 expression in all MTCs (6), with documented exeptions (7), whereas for the Bcl-2, high expressions have been reported in majority of the cases, and some studies have shown that Bcl-2 expression has a positive prognostic value (8).

Aims

To evaluate the clinical and pathohistological features of MTCs arising in Macedonian population, primarily the histotypes of the tumors, possible different expression patterns for Ki-67 and Bcl-2, and their correlation to the clinical behavior of the tumors.

Material and Methods

We re-evaluated retrospectively archive specimens from 20 cases of MTCs on standard HeEo stainings, and performed additional immunostainigs (LSAB+, DAKO) with anti-Ki-67 antibody (clone MIB-1) and anti-Bcl-2 antibody (clone 124) both from DAKO. The dilution for the anti-Ki-67 antibody was 1:25, and for the anti-Bcl-2 antibody 1:50. The microscopic re-evaluation included the following parameters: the histological subtype, the type of tumor margin (expansive or infiltrative), as well as, expression of Bcl-2 and Ki-67. Microscopic re-evaluation was performed by at least two pathologists independently, and equivocal parameters were solved with consensus. We also analysed the age and sex distribution, tumor greatest diameter, presence of regional lymph node and/or distant metastases at the time of operation, and the stage of the disease. Furthermore, we evaluated the clinical history of the cases using the clinical data obtained from the University Clinic for Thoracovascular Surgery, and the Institute for Pathophysiology and Nuclear Medicine in Skopje. We included data for the elapsed time from onset of the first symptoms/signs until the operation, time from the primary operation until the eventual recurence and/or appearance of local lymph node and/or distant metastases, family history and presence of a possible MEN2 syndrome. According to the clinical parameters (as mentioned previously), patients had been clinically divided into two groups designated as “group 1” containing patients with indolent disease course and “group 2” consisted of patients with aggresive disease course.

Results

Sixty percent of the patients were women, and the mean age of the patients was 53,7 years with normal age distribution (fig. 2) (min. 19; max. 76; med. 55,5; St. dev. 14,3), with no significant age difference between men (mean age 55,8) and women (mean age 52,2) (p>0,05) (Figs. 1-2).

Fig. 1. Distribution according to gender

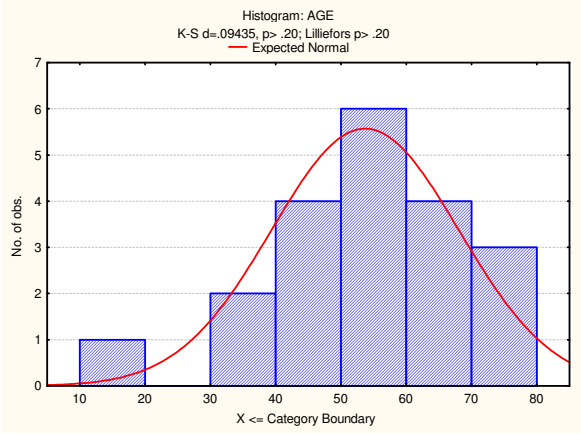


Fig. 2. Age distribution of the patients

According to the ethnicity, 70% of the patients were macedonians, and 30% of non-macedonian ethnicity (fig. 3).

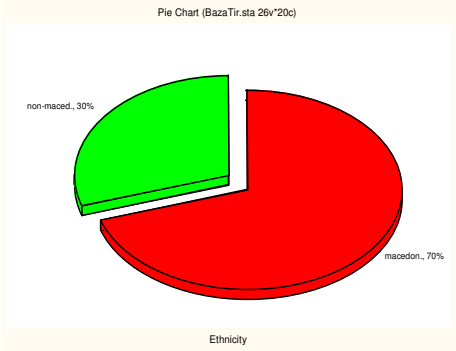


Fig. 3. Ethnical distribution of the patients

Eight of the patients had been clinically designated in the first group, indolent/intermediate disease

course (Gr. 1), while 12 of them had an aggressive disease (Gr. 2). None of the patients had FMTC or MEN2.

The mean tumor diameter at operation was 4,2 cm (Min 2,5 cm; Max. 9,0 cm; Med. 3,8 cm; St. dev 1,74). There was no significant correlation between the tumor diameter and the age of the patients (Spearman Rank Order Correlation R=-0,02) (p>0,05), and we also did not find any differences between the tumor diameter in females vs. males (Mann-Whitney U Test U=41,0; Z=-0,084; p>0,05) (Fig. 4).

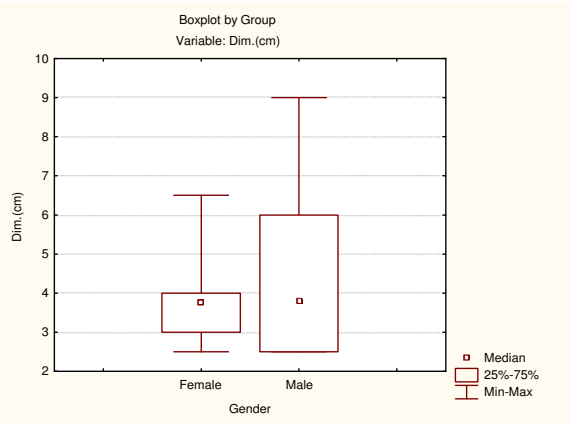


Fig. 4. Tumor diameter in female and male patients

Eleven of the patients were in Stage II, 7 in stage III, 2 in Stage IV, and there were no patients in Stage I disease (Fig. 5).

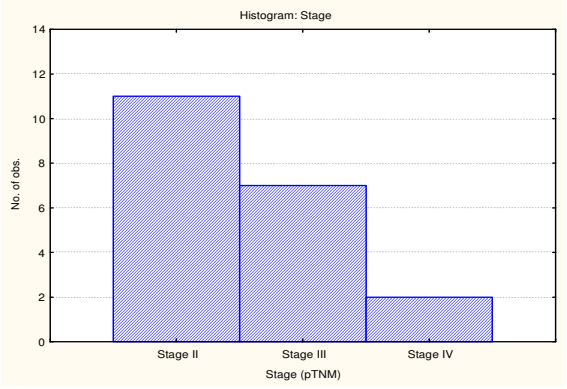
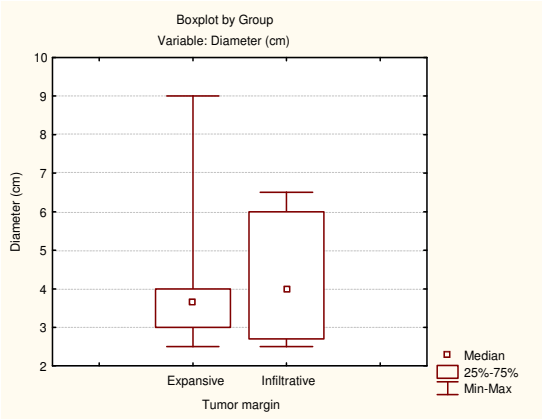


Fig. 5. Frequency table: Distribution according to the disease stage

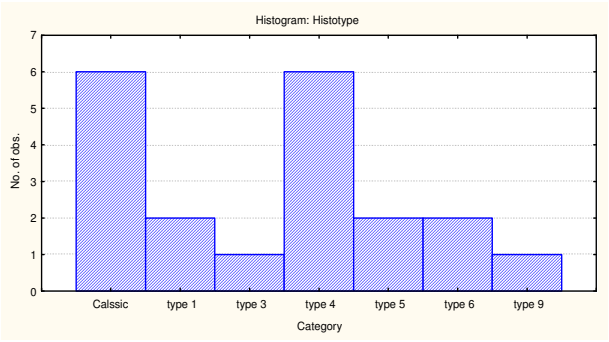
The stage was only correlated to the parameters already comprised in it (i.e. pTNM parameters), and also with the type of the tumor margin (R = 0,54; p<0,05), although there was no direct correlation between the type of the tumor margin and the tumor diameter, neither a significant differences of the tumor diameter between cases with infiltrative vs. expansive tumor margin (Fig. 6).



Mann-Whitney U Test: U = 40,5; Z = -0.29; p>0,05

Fig. 6. Mean diameter in tumors with expansive vs. infiltrative tumor margin

According to the tumor histotype, most of the tumors were of "classical" (n = 6) and "spindle cell" appearance or type 4 (n = 6), followed by "papillary/ pseudopapillary" – type 1, "small cell and Neuroblastoma-like" - type 5, and "Paraganglioma-like" - type 6 variants – all of them represented by 2 cases each, and the least frequent were "Giant cell" - type 3 and "Angiosarcoma-like" – type 9 tumors – represented by a single case each (fig. 7, 8). The tumor histotype was not correlated to any of the investigated parameters, neither had any significant influence on the tumor behavior, regarding the aggressiveness of the disease, or the stage of the disease.



*See tab.1

Fig. 7. Frequency of observed tumor histotypes

For Bcl-2 expression we found that majority of the tumors had high Bcl-2 expression, averaging 68,3% of tumor cells (max. 95%; min. 1%; St.dev. 33,7). There was no significant difference in Bcl-2 expression pattern

between males and females (p>0,05) (fig. 8, 9). Bcl-2 expression was not correlated to any of the investigated parameters, neither had any significant influence on the tumor behavior, regarding the aggressiveness of the disease.

other of the investigated parameters, neither had any significant influence on the aggressiveness of the disease.

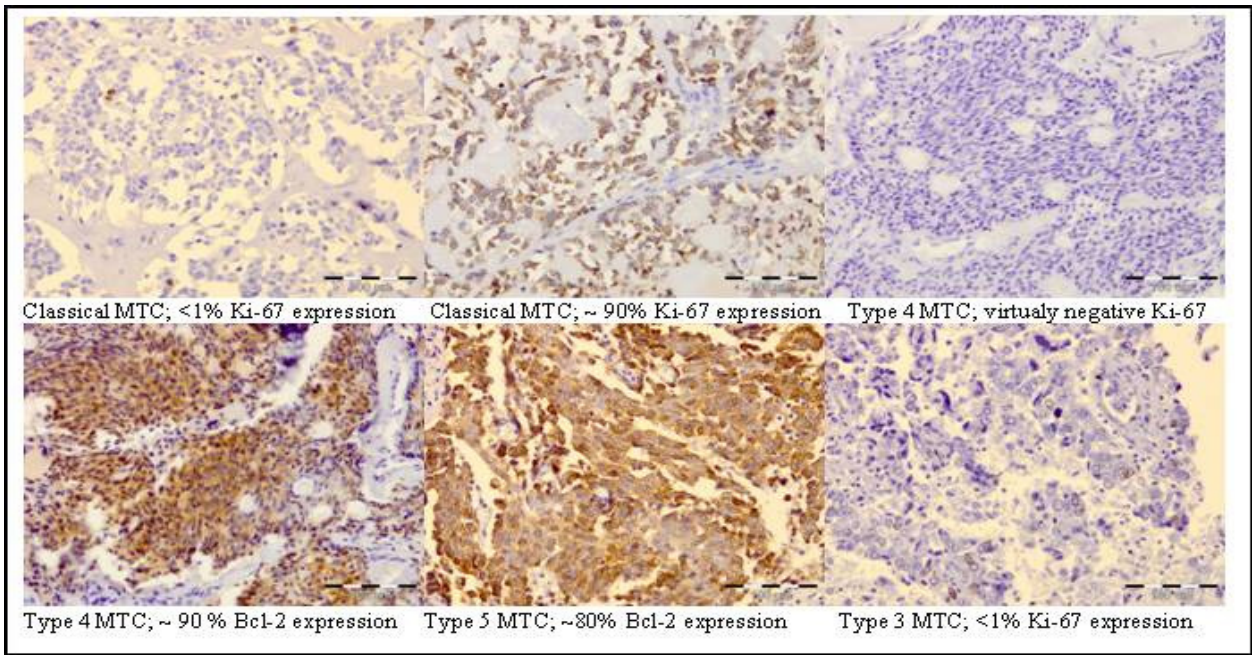


Fig. 8 Histotypes and staining patterns for Ki-67 and Bcl-2

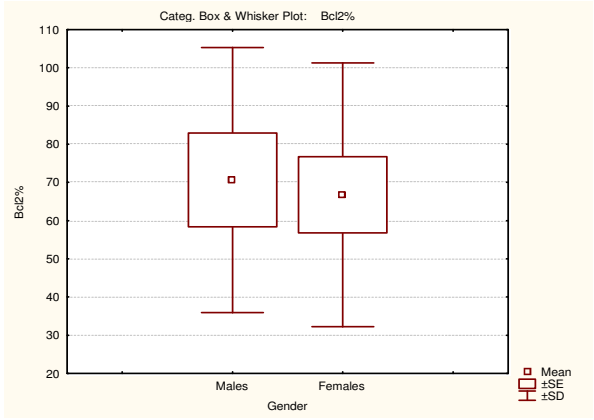
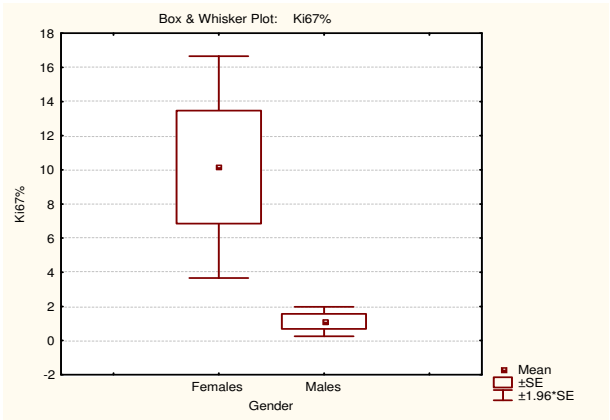


Fig. 9. Bcl-2 expression in males and females



T-test: 2,2; df = 18; p<0,05

Fig. 10. Ki-67 expression in males and females

For Ki-67 we found that majority of the tumors had low expression (fig. 8) with mean value of 6,55 % of tumor cells (max. 30% , min. 0%; St. dev. 9,86). However, there was a significant difference between males and females for Ki-67 expression (p<0,05) with Ki-67 averaging 10,2% of tumor cells in females (max. 30%; min. 1%; St.dev. 11,5) and only 1,1% in males (max. 5%; min. 0%; St. dev. 1,24) (fig. 10). Ki-67 expression was not correlated to any

In cases where metastases appeared after the operation, during the follow up, the mean time for the appearance of the nodal metastases was 38 months (observed in 2 males and 2 females) (fig. 11), with three of the cases presenting with regional lymph node metastases, and one of the females with a liver metastasis. One of the males and one of the females were in stage II

(postoperative staging) and evolved into Stage IV with the appearance of the metastases, and the other couple shifted from stage III (postoperative staging) into Stage IV (during follow up).

	Valid N	Mean	Min	Max	Std.Dev.
t(m) to MS	4	38.00000	19.00000	51.00000	15.38397

*2 females and 2 males

Fig. 11. Time elapsed from operation until the appearance of metastases.

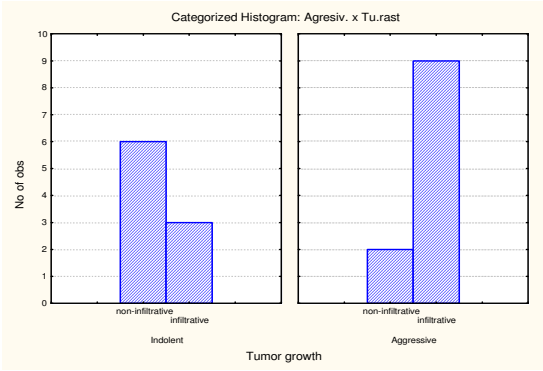
Because of the low number of cases, we were not able to perform any analytical statistics for this parameter.

The attribute of aggressiveness was correlated to the type of tumor growth, regional lymph node status and the stage at operation (fig. 12).

	Valid	Spearman	t(N-2)	p-level
Aggress. & Tu.growth	20	0.49	2.4	< 0.05
Aggress. & pN	20	0.70	4.2	< 0.01
Aggress. & Stage	20	0.79	5.53	< 0.01

Fig. 12. Parameters correlated to the disease aggressiveness

We tested the significance of differences between the aggressive disease cases and the indolent ones for the above mentioned parameters (figs. 13-15).

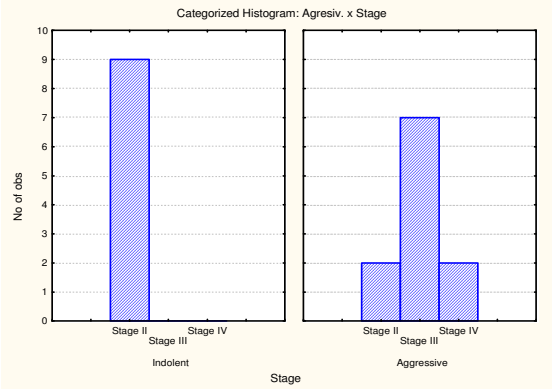


Chi-Square (df = 1) 4,85; p < 0,05
Fisher exact (one-tailed); p<0,05

Fig. 13. Distribution of aggressive and indolent clinical course cases in respect to the lymph node status at operation.

Chi-Square (df = 1) 9,9; p < 0,01
Fisher exact (one-tailed); p<0,01

Fig. 14. Distribution of aggressive and indolent clinical course cases in respect to the lymph node status at operation.



Distribution of Stage 2 vs.stage 3 patients according to the agressiveness of the disease:
Chi-Square (df = 1) 11,45; p < 0,01
Fisher exact (one-tailed); p<0,01
Distribution of Stage 2 vs.stage 4 patients according to the agressiveness of the disease:
Chi-Square (df = 1) 8,18; p < 0,01
Fisher exact (one-tailed); p<0,05

Fig. 15. Distribution of aggressive and indolent clinical course cases in respect to the stage at operation.

We found that there was a significant difference in case distribution according to the clinical behavior in respect to the tumor growth, lymph node metastases and the stage of the disease at operation. Six (of nine) of the indolent cases presented with non-infiltrative growth pattern in contrast to only 2 (of 11) of the "aggresive" cases (p<0,05) (fig. 13). Most of the aggressive cases (n = 9) have already had lymph node metastases by the time of operation, compared to only 1 of the "indolent clinical course" cases (p<0,01) (fig. 14). Similar situation was

observed in respect to the stage of the patients. All indolent cases were still in stage II at operation, while in the "aggressive" group only 2 patients were in stage II, while most of them were in stage III (n = 7) and two were in stage IV (fig. 15).

There was a rather peculiar phenomenon concerning the ethnicity of the patients. We observed significantly higher frequency of higher pT parameter in non-macedonian ethnicity compared to the macedonian patients who presented more frequently lower pT parameter (p<0,05). Eleven of 14 macedonians had tumors designated in pT2 category, but only 1 of 6 non-macedonians had tumors designated in pT2 category, with larger tumors (pT3) being found more frequently in non-macedonian patients (fig. 16).

Pearson Chi-square 6.706349; df=1; p< 0,01
Yates Chi-square 4.375000; df = 1; p<0,05

Fig. 16. Tumor diameter (pT) in respect to the patients' ethnicity.

However, ethnicity of the patients was not directly correlated to none of the other investigated parameters. Furthermore, we tested the differences in mean tumor diameters in macedonian vs. non-macedonian patients, and we came up with a non-significant difference. The mean tumor diameter in macedonian patients was 3,68 (min. 2,5; max; 6,0; St. dev. 1,18), while in non-macedonian patients the mean diameter was 5,28 (min. 2,7 max. 9,0; St. dev. 2,34) p>0,05 (Fig. 16), suggesting a statistically biased distribution of the pT parameter for the observed absolute tumor diameters.

t = -2,02; df = 17; p>0,05

Fig. 17. Mean tumor diameter (cm) in macedonian vs. non-macedonian patients

Discussion

The age and gender distribution of the analyzed group is in accordance to other previously published analyses (4, 6) where slight female predominance has been obseved, which in our case was 1:1,5 (male to female ratio), and mean age 50, similar to our mean age of 53,7.

The ethnical ratio of macedonian vs. non-macedonian patients (70:30) is quite similar to the overall ratio in the general popullation (9), suggesting absence of specific ethnicity-related factors influencing the incidence of MTC in our population. We encountered quite unexpected ratio between the cases presenting an indolent disease course (n = 8) and those with an aggressive disease course (n = 12), taking into consideration that indolent disease course is expected in patients suffering from FMTC and those with MEN2B syndrome (4, 5, 6), but not so frequently in patients with sporadic MTC, as our patients actually were. We exclude the possibility of MEN2 since all of the patients have been checked thoroughly for clinical and biochemical signs, although the possibility stays that some of them could be in fact cases of FMTC with poorely analysed disease - specific family history or some of them have not given correct anamnestic data (deliberately or unvolountarily). This possibility goes along with the fact that at least 20% od MTCs in Europe are expected to be familial cases (1, 2, 3, 4, 6). Nevertheless, these results suggest further, extended (at least anamnestic) evaluation of the patients' relatives and more precise anamnestic re-evaluation of the patients, and possibly random ultrasound and/or biochemical screening of the relatives of our younger patients – since it has been shown that familial cases tend to appear approximately 10 years earlier than sporadic do (2, 4, 6). Having in mind that we were already missing at least 8 MTC patients (according to the incidence statistics for Europe), there is another, rather unlikely possibility that those missing cases would be FMTC cases with indolent disease course that have remained unrecognnized or have died of disease- unrelated cause before correct diagnosis could have been established.

Most of the patients (n = 11) were in stage II by the time of operation, and the mean tumor diameter at operation was 4,2 cm (min 2,5 cm; max. 9,0 cm; med. 3,8 cm; St. dev 1,74), which we consider a rather large tumor, although comparable to other studies (6). However, it is quite unsatisfactory that none of the patients had been operated in stage I, although this could be easily explained by the fact that none of the patients were actually aware of being at an increased risk for MTC (i.e. positive familial history), which is on the other hand closely dependent on our ability to"recognize" such families.

The notion of the most prevalent histotypes of MTC in our series (the classical and spindle cell type; comprising for 60% of cases) have merely a differential diagnostic significance, since the histotype was not correlated to none of the investigated parameters including the clinical behaviour of the tumor. We also couldn't find any relevant data about the influence of the histologic variants on the clinical course of the disease. The general Ki-67 expression was low (6,55% of tumor cells) as expected and as published in other studies (6). However in our series we encountered a rather odd phenomenon of significantly higher Ki-67 expression in women (mean 10,2% of tumor cells) compared to men (mean 1,1% of tumor cells) ($p < 0,05$). We have no plausible explanation for this phenomenon, and we haven't found similar published results in the reviewed literature. However, this difference had no influence on the disease course, and there were no correlations to the other investigated parameters. Maybe further studies about the influence of sex hormones would provide a satisfactory explanation. Opposite to the Ki-67 expression, which was generally low, we found high Bcl-2 expression averaging 68,3% of tumor cells, but with no significant male to female differences, neither correlations to other investigated parameters, including the disease course. Our data generally correlate with the results from Cobanoglu et al. and Viale et al. (7, 8) who also found high Bcl-2 expression. However, our average Bcl-2 expression (68,3%) is lower that that observed by Cobanoglu et al. who reported 90% positivity. This discrepancy has emerged because we had 4 patients in our series with (extremely) low Bcl-2 expression (1%, 5%, 10% and 20% respectively). Viale et al. found that 26 out of 33 MTC cases had Bcl-2 expression in more than 25% of the cells. Although they have reported longer survival in Bcl-2 positive cases, we were not able to find any correlations between the Bcl-2 expression to the clinical parameters. The aggressiveness of the disease was correlated only to the type of tumor growth, regional lymph node status and the stage of the disease at operation (fig. 11). Since the stage of the disease includes the regional lymph node status (as one of the pTNM parameters), it is obvious that it can not be observed as an independent predictor of the tumor aggressiveness. However, the type of the tumor margin (infiltrative vs. expansive) appears to be a distinct predictive factor for the aggressive tumor behaviour (at least in our series). This parameter has not been included in the prognostic parameters by the WHO (6). The most similar parameter recognized by the WHO as a predictive factor is the "extent of the local tumor invasion", which is clearly different from the "type of the tumor margin" that we have analyzed. As far as the aggressiveness of the disease is concerned, there are a lot of studies (and controversy) dealing with the influence of different ret proto-oncogene mutations (1, 3, 6, 10), yet relatively few studies analyzing the morphologic parameters that influence the disease course (6, 11, 12). Many of the latest studies suggest genetic testing for ret mutations in all cases of MTC, especially when diagnosed in younger patients, and in cases of suspected FMTC or MEN2 syndromes, thus lowering the chances of having unrecognized familial cases, as probably happened in our series.

Conclusions

1. In the last 15 years there are fewer diagnosed MTCs compared to the predicted incidence and the European average.
2. In our series there are missing FMTC and/or MEN2 patients, that should have been diagnosed according to the predictions based upon US and European statistics.
3. Further analyses are needed to confirm or rule-out the aforementioned statistical discrepancies, and probably applying more detailed anamnestic questionaires, ultrasound and/or biochemical screening at least of the younger patients' close relatives.
4. Genetic testing for ret mutations in MTC cases should be taken into serious consideration having in mind the latest trends, and the problems we are facing regarding the FMTCs and MEN2-related MTCs.
5. The infiltrative type of tumor margin is correlated to more aggressive clinical course, compared to the expansive type of tumor margin ($p<0,05$).
6. Bcl-2 and Ki-67 immunohistochemical expressions are not correlated to the clinical behaviour of MTC.
7. The most frequent MTC histologic variants in our population are the "classical" and the "spindle cell variant" comprising 60% of cases, followed by the "Papillary/pseudopapillary", "Small cell and Neuroblastoma-like", and "Paraganglioma-like" variants – 10% each; and the least frequent are the "Giant cell" - and "Angiosarcoma-like" variants – 5% each.
8. Histologic variants of MTC are not correlated to the clinical behaviour of the tumors, neither to the rest of the invesigated parameters, but knowing them well is probably important for solving differential diagnosis in some cases.

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MORPHOLOGICAL ABERRATIONS OF THE WIDTH OF ATTACHED GINGIVA AND THEIR INFLUENCE ON GINGIVAL HEALTH AND ATTACHMENT LOST

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Abstract

Since many years, a great number of studies have been discussing the role of attached gingiva in marginal periodontal behavior, either accepting that a minimum of 2.0 mm of width is required to maintain marginal periodontal health or suggesting that width is negligible if excellent oral hygiene is performed, yet areas with narrow width of attached gingiva seem to be prone to periodontal attachment loss. Therefore the main aim of our study was to determine the influence, if any, of different widths of attached gingiva on attachment loss.

The survey was carried out on 209 lower incisor due to extraction.

Prior to extraction plague index, index of gingival bleeding upon probing, and gingival inflammation (Sillnes Loe) were noted for each tooth. Afterwards the width of attached gingiva in lower vestibule was measured.

After extraction the periodontally involved part of the root was carefully scaled and cleaned in running water and then stained for 10 seconds in 0.1% toludine blue to facilitate the measurement of attachment loss. Loss of attachment was then measured along the long axis of the tooth from cemento–enamel junction to the most coronal level of the stained periodontal ligament on the vestibular, distal and lingual surface.

Obtained data were divided into two groups according to the previously measured width of attached gingiva.

-Group1 teeth extracted from regions with attached gingiva equal or more than 2 mm (n=115)

-Group2 teeth extracted from regions with attached gingiva less than 2 mm. (n=94)

Obtained data showed statistically significant higher values of plague and gingival indices for the second group. Attachment loss was significantly higher in regions with inadequate width of attached gingiva.

Key words: attached gingiva, width, attachment loss, gingival inflammation.

Introduction

The width of attached gingiva is defined as the distance between the mucogingival junction and the projection on the external surface of the bottom of gingival sulcus or the periodontal pocket. It is not synonymous with keratinized gingiva because the latter also includes the free gingival margin. The width of attached gingiva varies in different individuals and on different teeth of the same individual, but generally appears to be accepted as an inevitable part of the aging process. Many adult patients may have areas of a narrow band of attached gingiva that are stable and do not require a specific intervention. However, in some patients the same condition may lead to exposure of an unacceptable amount of root surface causing esthetic problems, dental hypersensitivity, potential root caries, as well as mucogingival problems.

Several studies have been undertaken to find the relationship between amounts of attached gingiva, or its absence and gingival inflammation.[1,2] An adequate band of attached gingiva is considered that amount that is sufficient to maintain periodontal health. [3,4]

The study by Lang and Löe (1972) regarding the significance of an adequate zone of attached gingiva for maintaining periodontal health concluded that 2 mm of attached gingiva is adequate to maintain gingival health; and this expression has been widely quoted as a definition as to what constitutes an adequate width of gingiva for the maintenance of periodontal health [1].

It is debatable that an adequate zone of attached gingiva is an essential prerequisite to maintain periodontal health. The validity of the assumption that the presence of attached gingiva reduces the risk of further loss of attachment appears to be questionable [5].

Therefore the main aim of our study was to determine the influence, if any, of different widths of attached gingiva on attachment loss.

Material and Methods

The survey was carried out on 209 lower incisor due to extraction. The preextaction history was of no significant interest for our study. Patients were selected according to previously set criteria

-Age between 20-40 years of age

-no previous history of any disease or medications that have been taken

-the teeth in the examined region should have no extensive caries destruction or destruction that was under the marginal gingiva.

– the teeth should have an intact cemento–enamel junction, no dental restoration or caries extending beyond cemento–enamel junction and there was no visible damage from extraction procedure.

-patients with mucogingival aberrations such as high frenulum insertion, and morphological abnormalities of teeth such as root grooves, enamel pearl were excluded from the study.

- Patients in the examined region did not have any prosthetic appliances especially implants or crowns on the examined teeth.

Prior to extraction plague index, index of gingival bleeding upon probing, and gingival inflammation according to criteria proposed by Sillnes and Loe [6] were noted for each tooth. Afterwards the width of attached gingiva was measured.

After extraction the periodontally involved part of the root was carefully scaled and cleaned in running water and then stained for 10 seconds in 0.1% toludine blue to facilitate the measurement of attachment loss. Loss of attachment was then measured along the long axis of the tooth from cemento–enamel junction to the most coronal level of the stained periodontal ligament on the vestibular, distal and lingual surface.

Obtained data were divided into two groups according to the previously measured width of attached gingiva.

-Group 1 consisted of 115 teeth extracted from regions with attached gingiva equal or more 2 mm

-Group2 consisted of 94 teeth extracted from regions with attached gingiva less than 2 mm.

Results were statistically processed using the computer program Statistic 6. Mean value of the measured attached gingiva, and attachment lost was calculated for both groups. Significance of difference of values for the gingival indices and attachment lost between both groups was tested by student t test.

Results

The examined group was formed of 138 male and 71 female (total 209) patients with mean age of 32, 2 years. Results for the mean value for the width of attached gingiva for each group were;

Group 1 – (n=115) attached gingiva equal or more than 2mm (3, 25±0,637mm)

Grup2 – (n=94) attached gingiva less than 2mm (1, 87±0,785mm)

Results for plague and gingival index values for both groups are shown in Tab 1. Values for plaque and gingival indices were significantly higher in the second group.

Measured mean values for attachment loss on all four surfaces of teeth were also significantly higher for the second group with attached gingival less than 2m (Tab 2). It is worth mentioning that vestibular surfaces showed bigger lost of attachment (5, 82±1, 92 mm) than the other surfaces. (Tab.2)

Discussion

The general concept of our study was to identify the influence of the width of attached gingiva on periodontal attachment lost. This is not a new problem in Periodontology and had been periodically discussed for decades. For some periodontologist it is a question of academic character and has no practical appliance, but still the width of attached gingiva needed to maintain periodontal health and unaltered attachment levels is a controversial topic and no conclusive evidence have been presented up to now. [3, 4]

It has been discussed that periodontal health can be maintained in areas with minimal zones of attached gingiva as long as the patient can maintain the area and there are no restorations in the area of the gingival margin. [7, 8, 9] Maintenance of optimal oral hygiene in areas of narrow attached gingiva especially in the lower vestibule is more than an impossible task. Therefore the theory of disregarding the width of attached gingiva in assessment of periodontal risk for the patient is sustainable only in

Table 1. Plaque and gingival index values for Group 1(width of attached gingiva more than 2mm) and Group 2(width of attached gingiva less than 2mm)

	Plague	gin.inflamation	bleeding upon probing
Group 1	0, 45	1, 40	1, 56
Group2	1, 89	2, 46	2, 76
t	10,34	6,71	11,30
p	<0,001	<0,001	<0,001

Table 2. Mean values for attachment loss on different surfaces of teeth for Group 1 and Group 2

	mezial (mm)	distal(mm)	vestibular (mm)	lingual(mm)
Group1	1,8±0,837	2,03±0,96	3,25±0,23	2,02±0,25
Group2	3,61±1,79	4,83±0,75	5,82±1,92	3,96±0,62
t	5,72	10,30	5,26	6,71
p	<0,001	<0,001	<0,001	<0,001

ideal conditions such as regularly preformed professional plague removal.

The problem even escalated over the past few years when new and expensive technologies were introduced in dentistry. Dilemmas impose in everyday practice such as: should one apply such dentures or implants at periodontal risk sites? If so should we demand from patients to visit us each day for professional plague removal for the rest of the lifetime they have this implants?

There have been suggestions of authors [10] that when restorative procedures enter the gingival crevice, a minimum of 5 mm keratinized tissue is required (2 mm of free gingiva and 3 mm of attached gingiva). So some width of attached gingiva is needed to maintain plague control. The results from our study speak in favor of this concept. Regions with less than 2 mm of width of attached gingiva showed nearly two fold higher values of plague index than the first group (1,89 for the second and 0,45 for the first group). Results obtained for gingival inflammation and bleeding upon probing are with accordance with elevated plague accumulation in areas with width of attached gingiva under 2mm.Our results are supported by published results of other authors. [1, 2, 3]

The main aim of our study was to verify that these areas are prone to greater attachment loss. Dorman [11] have reported that after surgically aggravating the width of attached gingiva there was no significant gain of attachment therefore the width of attached gingiva will have no influence on periodontal health or attachment loss. Our results speak otherwise. In the first group loss of attachment was approximately 3, 25±0,23mm and in the second group was significantly higher 5, 82 ±1,29mm ($p>0,001$) measured on the vestibular surface.

The keratinized and attached gingiva as well provides a protective fibre barrier against inflammation and attachment loss. The results obtained in this study have shown that areas with narrow width of attached gingiva seem to be prone to periodontal attachment loss, which could result in recession of the gingival margin in the presence of risk factors. Lang, Löe [1] have demonstrated that such areas show clinical signs of inflammation even in the absence of dental plaque, as evidenced by an increase in gingival crevicular fluid (GCF) flow rate, which suggests that tissue behavior is mainly associated to pathological than to physiological processes under these conditions [12].

Gingival tissue fluid transudates rather through gingival sulcus than through alveolar mucosa in areas presenting at least a 2mm attached gingiva width, while areas presenting less than 2mm of attached gingiva width shows transudation of tissue fluid by alveolar mucosa. These findings suggest that the protective role [13] and homeostatic response exerted by GCF is compromised in areas with a narrow width of attached gingiva.

Furthermore, wider area of attached gingiva favors physiological behavior of the gingival sulcus by a better dissipation of GCF; a closer proximity of gingival margin and alveolar mucosa influence the dissipation of tissue fluid through alveolar mucosa, which is more permeable and mobile, impairing primary defense of gingival sulcus by the concentration of GCF [14, 15, 16].

Conclusion

The obtained data from our study speak in favor of the concept that areas with width of attached gingiva less than 2mm are periodontaly risk sites for plague accumulation, inflammation and attachment lost. Areas with width of attached gingiva less than 2mm are difficult to maintain with regularly home care oral hygiene procedure. Therefore while planning any kind of prosestetic appliances in such regions the amount of attached gingiva should be seriously considered.

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ANATOMICAL PERSPECTIVE OF CLINICAL APPLICATION OF MICRO-IMPLANTS FOR TEMPORARY SKELETAL ANCHORAGE IN ORTHODONTICS

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Abstract

During the orthodontic treatment anchorage control is the most important factor of successful treatment.

Micro-implants are new stationary anchorage; they are osseointegrated implants, implanted in the jaw of the patients. Skeletal anchorage system allows direct connection between screw-implant and target teeth that we need to correct orthodontically; provides absolute anchorage and adequate tooth movement. Orthodontic treatment, using a skeletal anchorage system, is not only effective, but it also offers a variety of treatment alternatives in challenging cases where traditional mechanics cannot be used.

The orthodontic treatment with micro-implants is independent of patient's cooperation; it takes shorter time; enables harmonious occlusion and gives functional and aesthetic individual optimum.

Keywords: skeletal anchorage system, micro-implants, micro-screw, temporary anchorage device

Introduction

Multi-bracket appliance such as edgewise appliance has generally been used in orthodontic treatment for alignment of maxillary and mandibular teeth. During the orthodontic treatment anchorage control is the most important factor of successful treatment. The issue of anchorage has been one of the most problematic in the field of orthodontics since all the obvious anchors, teeth and even molars, move in a response to orthodontic forces.The control of anchorage is one of the most critical factors in orthodontic treatment [2].

The commonly used anchorages could be divided into two categories, intraoral and extraoral. Intraoral anchorage such as palatal bar, lingual arch, and the Nance holding arch, cannot provide sufficient anchorage. Clinicians could not expect maximum anchorage using extraoral anchorage, such as a headgear, because, it totally depends on the cooperation of the patient.

There have been many studies [11,12] presenting maximum anchorage, independent of patients' cooperation, using dental implants, surgical mini-implants, mini-screws, and micro-screws [8]. The skeletal anchorage system is temporarily implanted in the maxilla or the mandible as orthodontic anchorage and it is a new stationary anchorage implanted in the jaw - osseointegrated implant. Miniature screw is relatively less traumatic and frequently used in orthodontic treatment when maximum anchorage is required, hence, they can be used where conventional anchorage cannot be applied [3]. Different types of head of micro-screws had deferent designs that are currently available on the market, and their use depends on the particular situation [19].

Seth [35] reported that these devices have been called by various names and some of the popular ones are: mini implants - MI, micro implants-MI, skeletal anchorage system-SAS, temporary anchorage device - TAD.

Implantologists use the term mini-implant, which is a kind of temporary implant to make temporary crown during osseointegration of implant. The term temporary is true for all anchorage devices. Since the primary goal of retention of most micro-implants is a mechanical lock within the bone, they require a tight fit to be effective. Their stability depends almost entirely on the quality and quantity of the available cortical and trabecular bones [12]. Mini-implants can be fully loaded immediately after placement, and they are removed at the end of the treatment. They are easy to insert, safe, cause little or no discomfort, and are simple to remove [16].The diameter of prosthodontic implants is a little bit larger than the orthodontic one.

This paper presents a critical review of the existing literature referring to type of teeth movement with force. It is usually achieved by one or two implants in the palatal

or vestibular area or by a combination between fixed appliances and micro-implants. The aim of this study was to describe skeletal anchorage for single active tooth movement, especially for correction of the upper incisors, since it gives good result for correction of deep bite and a gummy smile. If the patient has skeletal and dental transversal and vertical discrepancies, unilateral or bilateral crossbite mini-implants are used. In case the patient has a mandibular shift from centric to habitual occlusion, resulting in facial asymmetry, mini-implants can be used as the temporary anchorage device. Mini-implants can also be used for class II correction, molar mesialization or distalization, molar intrusion, or molar uprighting.

The advantages of micro-screws

Mini-implants can be implanted into any area of bone in the mouth, including radicular alveolar bone between the posterior teeth [27], usually between the roots of the teeth (Fig.1). Micro-implants can be implanted as well as other locations nearby the roots, it can be put in zygomatic arch, maxillary tuberosity, palate, chin, and retromolar area; it can provide anchorage for every kind of tooth movement. [18].

Skeletal anchorage system has been developed in our orthodontic treatment using a various type of osseointegrated implant, titanium miniplate and mini-screw. They can be used in preprosthetic treatment for uprighting of the maxillary or mandibular molars, particularly in the mandible, mesialization or distalization of some irregular teeth in dental arch, space closure, intrusion in the mandible and maxilla on groups of teeth, and alignment of wisdom teeth.

Treatment with micro-implants offers passive stabilization for correcting a Class II, preventing protrusion of incisors, anchorage in lingual technique, and anchorage of dentitions with a reduced number of teeth.

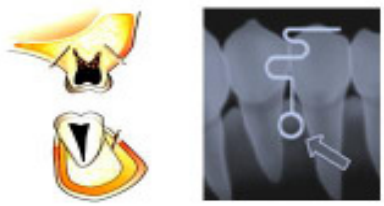

Fig. 1. Micro-implants between the roots of the teeth

The mini crew has a specific design: small size allowing placement in any area of the alveolar bone, simple to place and remove, low cost, inexpensive and short interval between implantation and orthodontic force application. Pioneers in micro-implant technology are AbsoAnchor developers-Korean orthodontists: JH. Sung [19], I. Kim [18], HM. Kyung [19], Bae, [2], HS. Park [30].

AbsoAnchor implants are made of high quality titanium alloy, they feature a variety of head types, and accept all orthodontic systems, including wires, ligatures, elastomers, and springs. Different designs of microimplants have appeared in the market for different purposes. Almost all of them have a hole in the head to attach accessories, and others have different kinds of slots or round heads. Several types of AbsoAnchor microimplants are available for different tasks and sites (Fig.2). Different types of head structures can be chosen depending on kinds of elastomers, biomechanics, sites of placement and individual preference. In bracket head type, two kinds of screws are designed depending on the driving directions. Left-handed screw should turn counter clockwise direction during driving. Depending on the direction of moment, orthodontists can choose right- or left-handed screws[16].

Fig. 2. AbsoAnchor types of micro-implants from Korean orthodontists

Classification of skeletal anchorage

Skeletal anchorage devices can be classified into two main categories. The first category is micro-implant which is osseointegrated with the bone [34]. The second category finds its origin in the surgical mini-implants, such as the one used by Creekmore [11], and those described later by Kanomi [16].

Classification of implants used for orthodontic anchorage suggested by Labanauskaite [21] is the following:

- According to the shape and size: conical (cylindrical), mini-screw implants, palatal implants, prosthodontic implants, mini-plate implants, disc implants;

- According to the implant bone contact: osseointegrated, nonosseointegrated;
- According to the application: used only for orthodontic purposes (orthodontic implants), used for prosthodontic and orthodontic purposes (prosthodontic implants).

Selecting the proper implant site can be an important factor in the overall success of this treatment approach. The length of screw portion ranges from 6 mm to 12 mm. One should always consider the depth of soft tissue when choosing proper length of micro-implants and should have in mind that palatal mucosa may be very thick [40]. If the tissue is very thick, then a mini-screw of 6mm is recommended to be inserted into the bone. Longer micro-implants (10 mm and 12 mm) are designed to be stronger for placement in denser bone; they lead to better mechanical stability of dental prosthetic implants, but they might damage anatomical structures, such as roots, maxillary sinus and nerve [3].

Depending on the inter-radicular distance, quality of bone and site of placement, orthodontists can choose different diameters of microimplants. The diameters of screw, ranging from 1.2 mm to 2 mm and can be placed anywhere in the mouth. The C-shaped implant is a unique titanium device (Fig.3 a, b) that provides absolute orthodontic anchorage, mainly from osseointegration [8]. Each implant is packed in an aseptic vial and blister pack. The C–micro implants, with conical or cylindrical head (Fig 3a) design, are usually placed in the interdentally spaces between the second premolars and first molars, or between the first and second molars [9]. This is a new concept for temporary skeletal anchorage in orthodontic treatment, for active tooth movement or passive stabilization. It is of special design as are the other products [4].

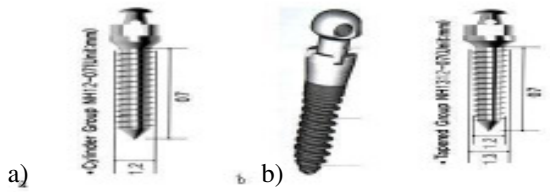

Fig. 3. a) Cylinder and b) Tapered C-micro implant

Common sites of placement (Fig.4a) in maxilla are: infrazygomatic crest area, tuberoses area, between the 1-st and 2-nd molars buccally, between the 1-st molar and 2-nd premolar buccally, between canine and premolar buccally, between incisors facially [27], mid-palatal area (Fig. 4c).

Common sites of placement (Fig.4b) in mandible are**:** retromolar area, between the 1-st and 2-nd molars buccally, between the 1st molar and 2nd premolar buccally, and between canine and premolar buccally [38]

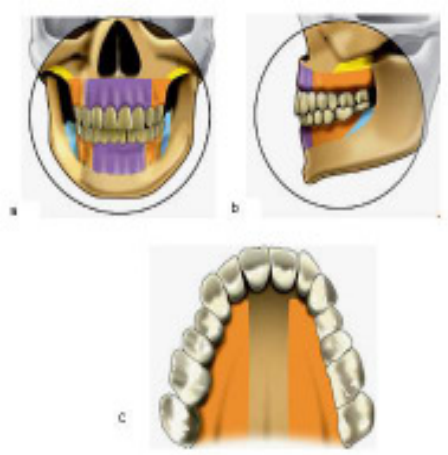

Fig. 4. Common sites of placement in a) maxilla b) mandible and c) mid-palatal area.

Mini-screws are modern systems that are sterile. Each implant is packed in an aseptic vial and blister pack [23]. Depending on the treatment goal, the different indications can be divided into three groups: active tooth movement, passive stabilization, or preprosthetic treatment.**.**

Treatment with application of a skeletal anchorage system

One of the primary goals of orthodontic treatment is functional stability with optimum esthetics. Therefore, micro-implants are the best and easiest way to get absolute anchorage for orthodontic treatment because, the problem of anchorage has been one of the most problematic in the field of orthodontics [21]. Skeletal anchorage was originally developed as an adjunct to tooth-bone anchorage for intermaxillary fixation after orthognathic surgery [34]. Mini-screws used for active tooth movement can correct: mandibular molar inclination (Fig.5), uprighting of the maxillary or mandibular molars [31], asymmetric occlusal plane, supraposition of lower anterior teeth (Fig.6), asymmetric Class II malocclusion with "en-masse" retraction for correction of this class malocclusion using sliding mechanics [22], supraposition of upper anterior teeth [28], and their uprighting (Fig.7). Micro-implants are used for distalization and derotation of the first maxillary molar (Fig.8), for space closure in the class

I occlusion [20], or for correction of impaction of maxillary canine with distalization and uprighting of these teeth (fig.9). All illustrations are taken from the Dentaurum Illustrated Atlas of Skeletal Anchorage, 2006, provided by: OA Dr. Benedict Wilmes, Dusselforf, Germany

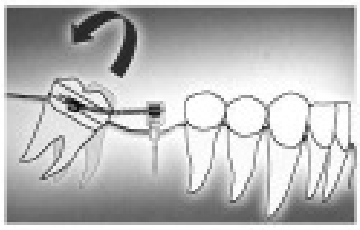

Fig. 5. Micro screw for mandibular molar uprighting

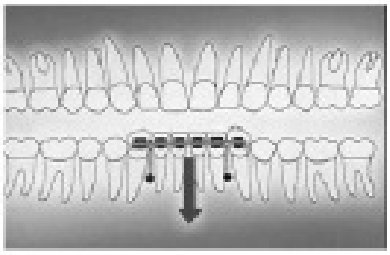

Fig. 6. Micro implants for uprighting en masse intrusion of lower anterior teeth

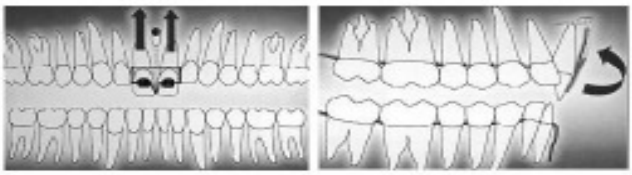

Fig. 7. Correcting the supraposition of upper anterior teeth and their uprighting

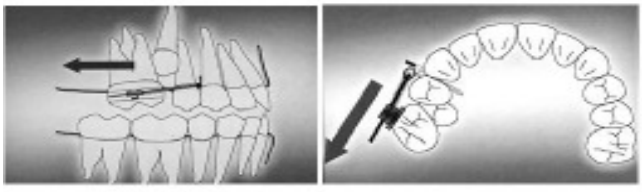

Fig. 8. Micro implants for distalization and derotation of the first maxillary molar

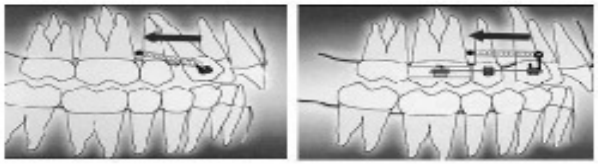

Fig. 9. Micro implants for distalization and uprighting of the maxillary canine

Ohnishi [28] suggested that micro-implant can be used for intrusion of some teeth, especially of upper incisors, because it gives good result for correction of deep bite and gives a gummy smile, very simply and efficiently. If the patient has skeletal and dental transversal and vertical discrepancies mini-implants can be used for correction of skeletal crossbite. In case the patient has a mandibular shift from centric to habitual occlusion, temporary anchorage device can be used widely [25].

Lee [22] described this micro implants as being anchorage in lingual technique for correcting a Class II malocclusion, and for preventing protrusion of incisors.

Skeletal anchorage system developed in our orthodontic treatment [12] utilizes various types of osseointegrated implant, titanium miniplate and mini-screw. They can be used in preprosthetic treatment of mandible, mesialization or distalization of some irregular teeth in dental arch, space closure, intrusion in the mandibular or maxillar groups of teeth [6,41], and alignment of wisdom teeth.

Thicker micro-implant results in greater mechanical retention, but in greater possibility for root contact, too. Thicker micro-implants do not always guarantee higher success rate, although there are reports that micro-implants of smaller diameter have shown higher success rate than thicker ones. Also, thicker micro-implants may be hard to remove due to osseointegration. In general, it is always more practical to choose the smallest diameter. Some mini-screws have small heads, which can ligate and various springs can be applied in order to move the teeth more easily (Fig. 10) [19].

Fig. 10. Mini-screw with delta coil springs

Different designs of micro-implant

There are various applications of screw in anterior and posterior area. We are presenting some of them and we are going to describe how teeth can be more easily and precisely controlled using indirect anchorage of mini-implants.

Usually, orthodontists can use many basic types of titanium anchor plates. These are: Dentaurum TOMAS micro implants, T- shaped; C shaped; Y shaped, I shaped; Quattro micro-implants, Rocky Mountain Orthodontics RMO-Dual top anchor system, Stryker Corporation Leibinger micro-implant universal anchorage system,Vector TAS micro-implant.

Chung, Kim and Kook [8] have described two components of micro-screw in the upper arch with 1.8 mm in diameter and 8.5 mm, 9.5 mm, or 10.5 mm in length. C-Implant has a head with measures are 2.5 mm in diameter and 5.35mm, 6.35mm, 7.35mm in height.

Applied forces can range from 50 to 200 grams, depending on the quality of the bone and the orthodontic movement desired. Kyung [18] stated that even smaller 1.2 mm and 1.3 mm micro-implants could withstand as much as 450 g force. The surface of each plate which will be attached to the bone is sandblasted so that it becomes osseointegrated more readily. This force system controls only the mode and direction of tooth movement.

Park [31] used micro-implants having dimensions of 1.2 mm in diameter, 8 mm in length for maxillary arch, 6 mm for mandible arch, and 10 to 12 mm in length for maxillary palate. In order to reduce possible damage of root during implantation procedure, micro-implants are implanted 30-40 degrees in maxillary arch and 10 – 20 degrees in mandibular arch bone.

Misch [25] explained temporary anchorage device (TAD). It is a titanium-alloy mini-screw, ranging from 6 to 12 millimeters in length and 1.2 to 2 mm in diameter that is fixed to bone temporarily to enhance orthodontic anchorage. Placement is minimally invasive and often completed using only topical anesthetic (Fig.11). They can be inserted directly through the gingival tissue into bone with a hand driver. In regions of thick soft tissue and dense cortical bone, a mucosal punch and pilot hole may be placed to help guide insertion. Stationary anchorage is achieved by gripping mechanically to cortical bone, rather than by osseointegration.

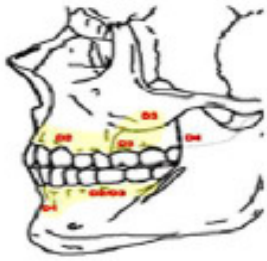

Fig. 11. Regions of bone density as classified in Misch

Region mark with D1 is bone with the highest density. D4 bone has the lowest density and is not recommended for placement. Temporary anchorage devices (TADs) can be placed in D1 to D3 bone with a 70 to 90 percent success rate. The maxillary mid-palatal region contains D1 and D2 bone. TADs placed in the retromolar pad or zygomatic region may require a flap; patients requiring such placement should be referred to a surgeon.

Insertion technique of temporary anchorage devices

For patient's safety and biomechanical control Carano [4] suggests proper angle of cortical insertion anchorage. In the posterior area in maxilla, the angle of insertion should be 30 to 45 degrees to the occlusal plane. In the anterior and posterior area, in the maxilla, Kravitz [17] suggests the angle of insertion to be approximately 90 degrees to the occlusal plane parallel to the paranasal sinus floor because such position minimizes perforation of the sinus. In the mandible, the angle of insertion should be 30 to 45 degrees to the occlusal plane to increase the surface area contact between the minicrew and the thicker cortical bone [4].

Risks and complications of micro-implants placement

Cheng [7] described the risks of using micro-implants in orthodontic treatment. There are potential complications common to all implant procedures, including: damage to anatomic structures such as nerves, vessels, loss of a screw during placement or loading, breakage of a screw within the bone during insertion or removal, inflammation around implant sites. Root trauma to the periodontal ligament or dental root may lead to loss of tooth vitality or ankylosis. If the patient is in pain, it usually may be derived from the contact of the implant with the root. In such cases, micro-implant should be

immediately removed and the condition of the teeth has to be checked.

Another common complication is failure of the mini-implant [7]. Miyawaki [26] described micro- implants failure. Mini-screws become mobile, and they will not regain stability and may need to be removed and reinserted. Inadequate primary stability on initial placement likely is a result of inadequate cortical bone thickness. Soft-tissue irritation TADs placed in loose alveolar mucosa may result in soft-tissue irritation, tissue overgrowth and minor aphthous ulceration.

Branemark [3] **described** sinus complication, and perforation of small part of the paranasal sinus floor (<2 mm). Perforations will heal by themselves without complications and should not affect mini-screw stability. Larger perforations may result in sinusitis or chronic oroantral fistula.

Another common complication is relapse. Sugawara [37] described relapse extrusion of intruded molars. The average relapse rate for the first and second molar intrusion is approximately 30%.

Micro-implant has a failure rate of 5-25% depending on the dentists' technique, patients' type, insertion sites, and usually failure occurs more often on mandible rather than maxilla [7,36]. However, many orthodontists are still hesitating to use orthodontic micro-implants, because they are afraid of surgical intervention and post-surgical complications.

Conclusion

Micro-implants have become very popular in the orthodontic community in recent years for providing anchorage. Micro-implants are especially useful in adult patients.Orthodontic treatment has become an important aspect of dental practice because the number of adult patient who receive treatment has increased. Other advantages include their relatively small size which results in minimal anatomical limitations; they are user-friendly; have immediate loading potential, adaptability to biomechanics in effecting orthodontic and orthopedic forces; high success rate, low cost and most importantly patient acceptability.

Small-sized orthodontic micro-implants allow easy placement with minimal anatomic limitations. The criteria of micro-implant success are: no inflammation of the soft tissues surrounding the micro- implant, no clinically detectable mobility, and anchorage function sustained until the end of the purpose for which the implant was used.

Advantages of mini-screws are: applicability at any stage of development, interceptive therapy, shorter treatment time, since there is no need to prepare dental anchorage; independence of patients' cooperation, patients' comfort and low cost.

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OCCULT BREAST LESIONS - PERCUTANEOUS PREOPERATIVE NEEDLE LOCALIZATION

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Abstract

Mammographic examination of the clinically normal breast can identify some breast masses before they become palpable. In order to remove them successfully a surgeon requires the help of some localization procedure. The only method recommended is preoperative needle localization under control of mammography or ultrasound.

The aim of this study was to show our experience in percutaneous preoperative needle localization.

Our experience with 69 clinically occult breast lesions is presented. The localization of most clinically occult, mammographically detected lesions was done by using mammographic guidance. We used perforated compression plate, local anesthesia and Bard hook wire.

The mean age of patients was 52.33 years. The major indications for localization were microcalcifications (37.68%).

The overall incidence of malignancy observed was 47.83% and of benign lesions 50.72%. We used single wire in 62 patients and two wires in 7 patients. Mammography guided localization was done in 81.16% of patients and ultrasound was used in 18.84%.

Percutaneous needle placement is a simple method of localizing occult lesions found by mammography.

Key words: occult lesion, hook wire, localization, breast cancer

Introduction

Breast cancer is the most common cause of women death in Macedonia. The war against breast cancer is being won by detecting small cancers. Our imaging tools are mammography and ultrasound. Mammography can detect clinically occult breast cancer. However, minimal or no physical findings of the lesion can be quite difficult to be detected by a surgeon. Microcalcifications can be detected only by mammography. When there is a nonpalpable cancer in a dense breast, clustered microcalcifications are on the primary x-ray mammographic findings and sometimes they are the only findings (1).

Localization of nonpalpable, mammographically and ultrasound detected breast lesions is a well established technique which we believe to be safe, rapid and accurate method for small and highly curable breast cancers.

The methods developed for preoperative localization of breast lesions may be grouped into those using external and internal markers. External markers are located on the skin based on coordinates determined at mammography. It might be difficult to apply this method for deep lesions since the lesions seen during surgery might be different from what was seen on mammography. Using internal markers involve the insertion of a needle into or near the nonpalpable lesion (2). This lesion is marked by inserting a hooked wire with a needle so that the surgeon can localize it more easily.

Material and Methods

This retrospective study reports the results of 69 outpatient procedures using needle localization during the period of 2009-2011 at the University Clinic of Radiology in Skopje.

We make use of the mammomat Hologic Inspiration and ultrasound Aloka with linear array 7.5MHz - 10MHz. Mammography was the initial procedure applied in all asymptomatic and symptomatic women over the age of 40. It was followed by sonography. The selection of

which modality to be used for guidance depends on: (1) the adequacy of visualization of the lesion by the modality used, (2) the position of the lesion, (3) the ease of positioning the patient, (4) the need to reduce the radiation exposure, (5) the skill of operator, (6) the overall patient condition, (7) size of the lesion. All lesions were localized using a Bard-hook wire technique. We approached all lesions with the needle parallel to the chest wall, using a superior approach for lesions in the upper two thirds of the breast and inferior approach for lesions in the inferior one third of the breast. Dedicated mammographic equipment should be used to obtain high quality images. Mammograms should always be interpreted on dedicated high - luminance mammographic view boxes and magnifying glass should be used routinely. We always compare current mammograms with prior mammograms to determine the stability of any calcifications detected. Local anesthesia (lidocain 1%) is administered in the skin and subcutaneous tissue. The specimen is radiographed in all cases.

Results

Table 1 presents the mean age of the patients and Table 2 presents the age distribution of the patients with benign and malignant lesions.

Of the 69 cases, 34 were positive for cancer and 35 were benign breast lesions (Figure 1).

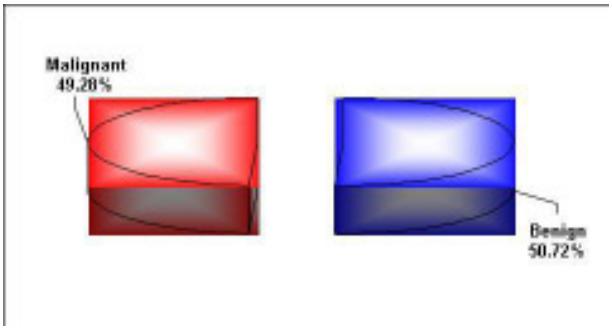


Fig. 1. Distribution of malignant and benign lesions

The major indications for localization were microcalcifications in 26/69 (37.68%) patients and a mass without calcifications in 22/69 (31.88%) patients.

Forty-seven of the patients had nonpalpable, but mammographically suspicious lesion and twenty- two were clinically indeterminate.

In 56 cases (81.16%) localization was done by mammography and in 13 (18.84%) by ultrasound . We used a single wire in 62 patients and two wires in 7 patients.

The spectrum of dominant morphologic characteristics of the benign masses is outlined in Table 3.

The distribution of malignant histopathologic findings is shown in Table 4.

Table 1. Age of the patients

Descriptive Statistics								
Age	Valid N	Mean	Confidence-95%	Confidence+95%	Min.	Max.	Std.Dev.	Stand.Error
years	69	52.33	49.98	54.68	30.00	74.00	9.77	1.17

Average age for patients is 52.33±9.8 years

Table 2. Age distribution of benign and malignant lesions

t-test for independent samples									
Age	Meanbenign	Meanmalignant	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.
years	51.17	53.52	-1.0	67	0.32	35	34	10.8	8.5

Average age for patients with benign lesion is 51.17±10.8 years;Average age for patients with malignant lesion is 53.5±8.5 years;There is no significant statistical difference between two groups of patients.

Table 3. Distribution of benign histopathological findings

Benign Histopathological Findings	N	%
Mastopathia	14	40
Fibroadenoma	6	17,14
Mastopathia fibrocistica	3	8,57
Blunt duct adenosis	1	2,86
Fat necrosis	1	2,86
Sclerosing adenosis	7	20,0
Papilloma intraductale	3	8,57
Total:	35	100

Table 4. Distribution of malignant histopathological findings

Malignant Histopathological Findings	N	%
Carcinoma ductale invasivum	21	61,77
Multicentric carcinoma	1	2.94
Carcinoma ductale in situ	4	11.76
Carcinoma tubulare	4	11,76
Carcinoma lobulare invasivum	3	8.82
Carcinoma papiilare invasivum	1	2.94
Total:	34	100

There were no complications that resulted from preoperative hook localization in our study.

We present some interesting cases in Figure 2, 3 and 4.

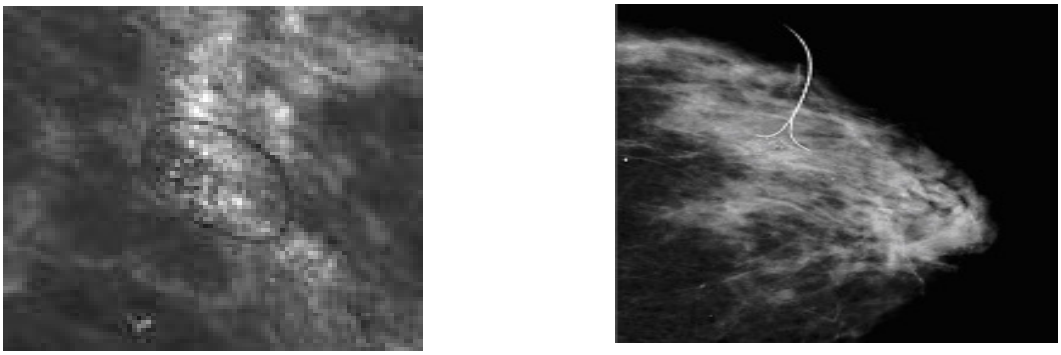
Discussion

Widespread use of mammography has resulted in detection of an increasing number of suspicious, but nonpalpable lesions. The tumors that are not palpable in the breast because of their location carry the same risk as palpable cancers of the same size and histological type. Screening programs employing mammography, designed to detect breast cancer in this pre-palpable stage are encountered as a means of uncovering a higher proportion of such cancers at an earlier stage in their natural histories (3).

Percutaneous needle placement is a simple method of localizing occult breast lesions found by mammography (4).

The mean age of our patients with malignant disease was 53.5 years. In Marrujo's study, cancers tended to occur in younger women, average age of 52 years (5).

The most frequent benign histopathological finding was mastopathy (fibrocystic and displasia) and malignant finding was ductal carcinoma. The incidence of malignancy in our study was 47.83%, which was in



A. Magnification view of calcification

B. Hook-wire localization of calcifications

Fig 2. Mammografic presentation of ductal carcinoma in situ.

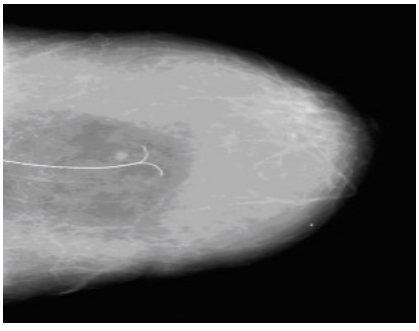


Fig. 3. Mammografic presentation of small carcinoma

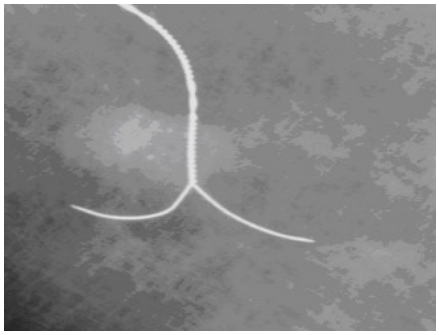


Fig. 4. Magnification of hook-wire localization of small carcinoma

agreement with literature. Schwartz et al. reviewed 469 cases with malignancy rate of 32.2% (6).

Rosenberg reported 27% of malignant lesions and Leis 38.2% (7, 8). Higher percent of malignancy detected in our patients can be described with the fact that the University Clinic of Radiology is the unique public center for needle localization in our country.

In the reported literature series involving needle localization technique, the predominant pathological cancer type was the ductal one (9, 10). We identified only one case of multicentric disease.

In our study 81.16% of the lesions were localized under mammography control because we consider it to be more precise when the lesion is smaller and the breast is bigger.

We reported 50.72% of lesions located in upper and lateral quadrant, which can be compared with those in literature (11, 12).

There were no complications during performing the procedure, while literature consulted had reported 0-0.2% complications (13).

Surgeons are urged to resect nonpalpable breast lesions only detected by mammography and ultrasound. The precision and success are related to the radiologists' experience in marker placement.

We found the verbal communication between radiologists and surgeons to be particularly helpful with reference to the position of hook wire and target lesion. Relevant mammograms should also be available in operating rooms.

Conclusion

In order to remove occult lesion successfully, a surgeon requires the help of a localization procedure. Preoperative wire localization is an integral component in the early detection of breast carcinoma.

The technique is easy and painless. Its precision and success relate to close cooperation between the radiologist, surgeon and pathologist.

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EVALUATION OF MANNHEIM PERITONITIS INDEX IN PATIENTS WITH SECONDARY AND TERTIARY PERITONITIS

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Abstract

Prognostic evaluation of patients with peritonitis is needed in order to program correct medical treatment, to plan more aggressive therapeutic procedures and to predict the outcome.

This study was performed to evaluate the value of MPI in patients with secondary and tertiary peritonitis for the prediction of surgical outcome.

Clinical, imaging and laboratory data of 36 patients with secondary and tertiary peritonitis were analyzed.

MPI was calculated and outcome was predicted in two categories: ≤ 26 and > 26 points.

Indicators of severity of the disease were correlated to outcome and MPI.

Inflammatory palpable mass, constipation and stop of flatus, arterial blood preasure, oliguria <20 mL/h, urea>167 μmol, creatinine >177 μmol, pO2 < 50 mmHg, pCO2 > 50 mmHg and radiographically hydroaeric levels were in correlation with the fatal outcome of the patients. There was a strong correlation between MPI and death and among MPI and above mentioned parameters.

We conclude that MPI is a useful tool to determine the outcome in patients with peritonitis which is easily to calculate and has good accuracy.

Key words: Peritonitis, Mannheim index, Mortality predictor

Introduction

Peritonitis is one of most urgent and important infectious problem in every day surgeon's practice. Despite the progress in treatment of the patients with peritonitis, the mortality rate is still high, ranging from 5% to 50%, depending to the severity of the disease (1-5).

The prognosis in these patients is strongly influenced by the previous health status, many risk factors and the appropriate management of the disease, including: controlling the infective source, elimination of microorganisms and toxins, maintaining of systemic organ function and controlling the inflammatory process (6-10).

The management of peritonitis requires a competent care, minimizing of risks, urgent decision making and aggressive surgical intervention. The reoperation if needed, have to be made immediately to achieve benefit for patients (8-12).

Prognostic evaluation of patients with peritonitis is needed in order to program correct medical treatment, to plan more aggressive therapeutic procedures and to predict the outcome (13,14).

There are many scoring systems for the prediction of outcome in patients with peritonitis.

More of the scoring systems are based on elements provided by patient's clinical signs, systemic signs of sepsis and complications that influence organs function. These systems are useful in predicting the outcome of patients with severe illness admitted in Intensive Care Units, and in patients with peritonitis especially because their outcome becomes poor in a phase when a multiple organ dysfunction develops, despite the evident achievements in medical and surgical treatment (15,16).

Mannheim Peritonitis Index (MPI) was developed by Wacha and Linder in 1983, based on analysis of 1253 patients with peritonitis, in which 20 elements were analyzed. Eight of them proved to be relevant and were incorporated in MPI, classified according to their predictive value (15-17).

The effectiveness of MPI was confirmed by investigations made in several European surgical units and some authors consider MPI as more practical than the other scoring systems. It has an acceptable specificity and sensitivity (13, 18,19).

This study was performed to evaluate the value of MPI in patients with secondary and tertiary peritonitis for the prediction of surgical outcome.

Patients and Methods

This clinical study was done during a period of 3 years, from January 2008 to January 2011 and 36 patients (20 female and 16 male) with secondary (SP) and tertiary peritonitis (TP) were included. Patients younger than 18 years and older than 80 years were excluded. The younger patients than 18 years were excluded because of their treatment in another surgical department and the older of 80 years were excluded because of their severe comorbidity due to the age. The youngest patient had 26 and the oldest 79 years and overage age was 54,2 year.

Every patient was worked-up as follows: Clinical history, physical examination, blood, serum and urine investigation, abdominal X-ray and abdominal ultrasound. A CT scan was performed for some of the patient, if the clinical doctor asked for it. Operation was performed trough a midline laparotomy incision, when diagnose of acute abdomen was made. All 36 patients underwent laparotomy, elimination of the sources of infection, aspiration of intra-abdominal exudates, debridement, a lavage with 4-5 liters normal saline and insertion of abdominal drains. A nasogastric tube was placed to all patients. They all received intravenous fluid and antibiotics. Ceftriaxone (Na) 2gr/day was infused in two separate doses 5-7 days. The patients with genitourinary source of infection and those who underwent anastomosis, got an additional therapy of metronidazole 3x500 mg/day IV, 7days. If needed, antibiotics were changed, according to the microbiological findings. Analgesics were administered as required.

The following data were examined: pain, vomiting, distension, constipation, stop of flatus, inflammatory palpable mass, tenderness, muscle rigidity, blood pressure, pulls, fever, oliguria <20mL/h, ileus >24h, coma, neurtophils, urea>167 μmol, creatinine >177 μmol, pO2 < 50 mmHg, pCO2 > 50 mmHg (oliguria, urea, creatinine, pO2 and pCO2 according to MODS - Multiple Organ Dysfunction Syndrome). Radiographs were analyzed for hydroaeric levels and free liquid or gas, and ultrasound findings were analyzed for free liquid, peristalsis and collection of pus. Complications were considered as leakage, dehiscence, wound infection, abdominal compartment syndrome and sepsis.

MPI was calculated and outcome was predicted in two categories: $\leq$ 26 and > 26 poens.

Data were analyzed using SPSS software and normality distribution test (Kolmogorov-Smirnov), mean value, frequencies and Spaerman's coefficient of correlation r, were done.

Table 1. Mannheim peritonitis index

Risk factor	Points
Age>50 y	5
Female gender	5
Organ failure	7
Malignancy	4
Preoperative duration of peritonitis >24h	4
Non colonic origin of sepsis	4
Diffuse generalized peritonitis	6
Exudates	
Clear	0
Purulent	6
Fecal	12

Results

Of 36 patients with secondary peritonitis 6 patients (16,6%) developed tertiary peritonitis. Six patients (21,4%) died in hospital after medical treatment, 3 of them with secondary peritonitis and 3 with tertiary peritonitis. Three of them developed multiple organ failure, 2 died due to sepsis and 1 of the patients died due to respiratory failure. Eight patients (22,2%) developed local

complications including wound infection (5), abscess (1), suture dehiscence (3). One of the patients developed MRSA infection.

Intra-hospital treatment last from 3 to 37 days, a mean of 11,19 days; 2 patients spent more of 30 days in hospital.

All of them had variable feeling of pain, distension, tenderness and objectively some level of muscle rigidity.

The statistical analysis showed that all of the parameters shown in table 2 were in correlation with the fatal outcome of the patients. There was a strong correlation between MPI and death and among MPI and above mentioned parameters.

Source of peritonitis, its type and outcome of patients are shown in table 3.

Relation of patients MPI to fatal otcome is shown in table 4. The non-survivor patients MPI range was 30-40 points, a mean of 34, and the survivors MPI range was 15 - 26 points, a mean of 23.

Discussion

Peritoneal infections may be primary, secondary (SP) and tertiary (TP).

Primary peritonitis or spontaneous bacterial peritonitis (SBP) occurs without evident intra-abdominal source of infection. It is an uncommon disease often associated with urinary or hepatic pathology and almost exclusively observed in patients with ascites due to chronic liver disease. Primary peritoneal infections were not an object of this analysis (21).

Secondary peritonitis most often is a result of bacterial contamination of sterile peritoneal cavity due to a perforation of a hollow organ, transmural infection or external source. Sterile chemical irritants as bile, blood, gastric juice in the first several hours, ischemic injuries or latrogenic trauma (endoscopy procedure) may also be a cause of SP. The incidence of SP varies from less than 2% in patients with elective abdominal operations (leakage, anastomosis dehiscence, intestinal injury) to 10% in patients with inflammatory disease and 50% in patients with hollow organ perforation and gangrenous bowel disease (21).

There were 6 (16,6%) postoperative peritonitis in our study due to local complications and 16 (44,4%) cases of peritonitis due to hallow organ perforation.

Tertiary peritonitis occurs as a persistent or recurrent peritoneal infection following adequate therapy of SBP or SP, most often without the original visceral organ pathology. It is often seen in patients with significant preexisting comorbidity or in immunocompromised patients and is usually presented as an abscess or phlegmon inflammation. Mortality rate in patients with TP ranges from 50% to 70% (21).

In our study TP developed in 5 cases (13,8%) after proper treatment of SP due to hollow organ perforation and in 1 case (2,7%) of postoperative peritonitis.

Clinical manifestations of peritonitis are abdominal pain (first dull, than severe), anorexia, nausea, vomiting, abdominal tenderness, rebound tenderness, increased abdominal wall rigidity, fever with temperatures, paralytic ileus. With time tachycardia, hypotension, decreased urine output and septic schok occur (15,21).

Despite the progress in antimicrobial agents and the advances in intensive care procedures and surgical techniques intra-hospital mortality rate of peritonitis continuously remains high. Therefore the peritonitis remains one of the most serious problems for surgical treatment (9,13,14,16,22).

Many different factors as age, duration and extent of peritonitis, site of perforation and delayed surgical intervention influence the prognosis and outcome of peritonitis (9,16).

Parameters like pulse, oliguria, level of creatinine and urea, pCO2, pO2 are considered to be indicators of severity of peritonitis (23).

We found out inflammatory palpable mass, constipation and stop of flatus, arterial blood preassure, oliguria <20 mL/h, urea>167 μmol, creatinine >177 μmol, pO2 < 50 mmHg, pCO2 > 50 mmHg and radiographically hydroaeric levels to be indicators of severity of the disease, also.

The outcome therefore is difficult to predict and depends upon early surgical intervention, source control, adequate intaoperative peritoneal lavage and complete intensive care (9,16).

Early objective grading system of severity of peritonitis is needed for appropriate medical and surgical

Table 2. Statistically significant correlation to fatal outcome

Parameter	No of cases	%	p	r
Inflammatory palpable mass	10	27,7	p<0,05	0,331
Constipation and stop of flatus	26	72,2	p<0,05	0,331
Arterial blood pressure	8	22,2	p<0,05	-0,678
Oliguria <20 mL/h	4	11,1	p<0,01	0.661
Urea>167 μmol	6	16,6	p<0,01	0,837
Creatinine >177 μmol	8	22,2	p<0,01	0,679
pO2 < 50 mmHg	9	25	p<0,01	0,617
pCO2 > 50 mmHg	8	22,2	p<0,01	0,679
Hydroaeric levels (Rtg)	12	33,3	p<0,05	0,378
MPI	36	100	p<0,01	0,836

Table 3. Cause of peritonitis, outcome and type of peritonitis

Cause of peritonitis	N° cases	Outcome	Type of peritonitis
Duodenal perforation	1	ex	SP
Gastric perforation	1	-	SP + TP
Gastric perforation	2	-	SP
Small intestine perforation	4	-	SP
Small intestine perforation	2	-	SP+TP
Small intestine gangrene	4	-	SP
Morbus Chron	3	-	SP
Colonic perforation	5	-	SP
Colonic perforation	2	ex	SP+TP
Genitourinary tract perforation	1	ex	SP
Tubo-ovarian abscess	3	-	SP
Postoperative peritonitis	1	ex	SP + TP
Postoperative peritonitis	1	ex	SP
Postoperative	4	-	SP
Carcinomatosis	2	-	SP

Table 4. MPIcalculation and relation to death

MPIcalculation	Nº cases	%	ex	%
≤ 26 points	28	77,7	0	0
> 26 points	8	22,3	6	75

The non-survivor patients MPI range was 30- 40 points, a mean of 34, and the survivors MPI range was 15 - 26 points, a mean of 23.

treatment, planning aggressive surgical procedures and predicting the outcome. It is needed for programming the most adequate therapeutic plan and selecting the high-risk patients for reoperation. The scoring systems also help in evaluating the risks, comparing the effectiveness of different treatment procedures and assuring quality of care (6,9,16,)

Many prognostic scoring systems are used for appropriate prediction of outcome of the disease: score of Elebute and Stoner, SSS (Sepsis Severity score) , PIA (Peritonitis Index Altona), MOF (Multiple Organ Failure), APACHE II (Acute Physiology and Chronic Health Evaluation), SAPS (Simplified Acute Physiology Score), MPI (Mannheim Peritonitis index) (13,15-19,23,24).

The score of Elebute and Stoner and SSS developed as the earliest scoring systems and are considered as scores with limitations (24).

PIA is based upon history, physiological data, clinical examination and intraoperative findings. MOF score is based upon dysfunction of pulmonary, cardiovascular, hepatic, renal, gastrointestinal, nervous and hematological system. APACHE II system is based upon physiological findings and has a large range of scores that enable risk calculation and correlate to the mortality. MPI is based upon preoperative and operative data and is designed specifically for peritonitis (13,15,24).

We evaluate the value of MPI in patients with secondary and tertiary peritonitis for the prediction of surgical outcome as a scoring system designed specifically for peritonitis.

Many authors reported MPI as a qualitative predicting score for patients with peritonitis (13-19,21,22).

Pacelli et al (1996) calculate prognostic significance of several variables including APACHE score II and MPI in patients with intra-abdominal infections (IAI) and found out that both score systems correctly graded IAI severity and were strongly and independently associated with the outcome. The MPI was easier to apply (10).

Notash at al (2005) compared efficiency of MPI and MOF in patient with peritonitis and concluded that MPI has been shown to be an appropriate objective prognostic factor in patients with peritonitis to predict their outcome. The author commented that accuracy of MPI was comparable or slightly superior to that of other sepsis classification systems, including APACHE II (13)

Our results correspond to the findings of other authors who considered MPI as a specific score with good accuracy that in easy way provides prediction of individual prognosis of patients with peritonitis (13-19,21,22).

We conclude that MPI is a useful tool to determine the outcome in patients with peritonitis which is easily to calculate and has good accuracy.

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SURGICAL TREATMENT OF PRESSURE ULCERS

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Abstract

Pressure sores or bedsores are injuries to the skin and underlying tissues that result from prolonged pressure on the skin. Bedsores most often develop on areas of skin that cover bony parts of the body, such as the heel, ankles, hips or buttocks. These wounds most often present themselves in immobilized or bedridden patients on parts of the body that are compressed between the skeleton and underlying surface. The treatment is often long lasting, time consuming and management is difficult process that demands enormous financial and human resources.

The aim of this paper is to present our experience with the surgical treatment of the deep pressure sores. Our study presents a cohort of 30 patients that were surgically treated at the University Clinic for Plastic and Reconstructive Surgery, in Skopje, Republic of Macedonia, during a fifteen year period from 1996 to 2010. Emphasis of the surgical treatment was placed on harvesting and transplanting cutaneous and myofasciocutaneous flaps in order to cover the deep soft tissue defects. Results include statistical analyses of the group, systematization of the pressure ulcers according to the regions affected, utilized operative techniques as well as the average hospitalization stay. The youngest patient is a 10 years old child with a scalp pressure ulcer following a neurosurgery operation. The oldest patient is 83 years old male with a decubitus in the sacral region. Mean hospitalization stay is 26 days. Surgical treatment of the decubital ulcers, adequate and on time applied, in combination with appropriate pre- and post-operative care, gains satisfying functional and aesthetic results. That applies especially in selected patients where primary illness as reason for pressure ulcer is prone to successful rehabilitation.

Key words: decubitus, reconstruction, flap

Introduction

Decubital ulcer, pressure sore, pressure ulcer, bed sore or simply decubitus are all synonyms that refer to wound occurring due to long-lasting excessive pressure over the tissues which are compressed between the bone prominences and external objects. These objects could be anything that creates compression over the soft tissue like beds, mattresses, wheelchairs etc. On the other hand, the bone prominences usually are sacral, ischial, trochanteric, calcanear, spine processi, calvaria and other parts of the skeleton comprising the most affected regions. (1,2)

The term decubitus originates from the Latin word "decumbere", which means "to lay", "to lie on" inducing the fact that these wounds have been seen initially in patients lying immobile for longer time. As a medical problem, pressure ulcers seem to be as present as the civilization, as J. Thompson Rowing in 1961 has described a decubital ulcer in Egyptian mummy. Sir James Paget in 1873 was first to explain the pathophisiology of these ulcers. Namely, the main reason for decubital ulcers is the pressure exerted over the tissues which can withstand a short termed high pressure, but not prolonged. When this compression rises to level higher than the capillary filling pressure, which is approximately 32mm Hg, a process of hypoxia and ischemia occurs leading to cell death and tissue necrosis. Furthermore, uninterrupted and prolonged pressure causes thrombosis of the small arteries and veins, additionally contributing to tissue necrosis and subsequent inflammation. This common pathway initiates the magic circle towards pressure ulcer and can occur after as little as 2 hours of unrelieved overpressure. Tissue resistance to pressure and hypoxia differs among distinct cell types regarding their dissimilar oxygen needs, as muscle cells are more amenable to ischemia than the overlying skin which can bear as long as 12 hours over-pressed without appearing any signs of ischemia. This is the reason for wider and deeper necrotic areas than expected when seen on initial patient inspection. Time needed for pressure ulcer to develop also depends on patient weight, blood supply of the tissue, concomitant local infection, maceration and other contributing factors (1-3).

Any patient with impaired mobility is prone to pressure ulcer occurrence such are comatose or sedated patient, patient with quadriplegia/ paraplegia, heavily or terminally ill patient, burned or polytraumatised patient. While immobile, they can't assist when needed in order to inhibit long-lasting over pressure thus being susceptible to pressure sores unless medical stuff prevent it. Muscle

atrophy seen in prolonged immobilizations or neurological impairments, is the reason for de-bulking the bone prominences. The lack of sensibility in spinal cord injuries makes skin thinner and more vulnerable to friction and traction forces, which break down skin barrier, resulting in macerations that adhere to clothes and injure the skin additionally. Also, insensibility means painless, thus annulling the main symptom of injury, the pain. On the other hand, wound presence postpones the rehabilitation program which is of great importance for these patients.

Furthermore, other local factors and comorbidities do contribute. Local factors comprise bad hygiene, urinary or fecal incontinence, moist and wet environment, lack of effective dressings, infections. Comorbidities like anemia and malnutrition are of great importance, due to the fact that both decelerate wound healing and aggravate catabolism. Vascular abnormalities as seen in diabetics stress out the probability for sore appearance (3, 4, 5).

Out of many staging classifications, the one of Shea modificated by National Pressure Ulcer Advisory Panel is widely used. Comprising 4 stages of ulceration, this system describes the depth of the sore. First stage is presented as non-bleachable erythema, induration, pale color, but still an intact skin. Second stage presents a partial thickness skin loss that involves epidermis and possibly dermis, usually seen as blister or superficial ulcer. Stage III refers to a full thickness skin loss and sub dermal tissue necrosis with an unaffected fascia. Stage IV represents the deepest ulcer involving fascia, muscle, tendons, joint capsule and probably bone. At this stage, osteomyelitis is often seen as well as deep undermining sinus tracts that destroys the tissue in the vicinity. A practical staging system divides pressure ulcers in shallow ulcers (stage I and II), deep ulcers (stage III and IV) and ulcers where the depth cannot be evaluate at the moment of inspection due to present necrosis that need to be debrided (6).

The complex treatment of pressure ulcers is time consuming, demanding and slow going process that employs lot of resources and efforts. Modern approach of the treatment comprises adequate handling of the primary illness, prevention with all means especially usage of anti-decubital beds and mattresses that eliminates overpressure, correction of comorbidities like anemia and hypoproteinemia, employing optimal wound healing environment and finally, surgical treatment. Shallow ulcers are treated conservatively whereas deep ulcers conservatively or/and surgically.

Interest of this paper is the surgical treatment of deep pressure ulcers i.e. pressure ulcers of stage III and IV. Surgical treatment is applied when the general condition of the patients allows operation. By using various reconstruction methods the intention is to close the ulcer as soon as possible, but as well to shorten the hospital stay and accelerate the rehabilitation process. Pressure ulcer surgery includes following basic principles when utilized: ulcer and surrounding scar excision, removing of the underlying pressure point bone (ostectomy) which is seldom infected and lastly, reconstruction of the defect, covering dead space, restoring contour with similar and stable tissue by using various flap transfers. Prior reconstruction, radical necrectomy is a must and includes removing not only of the dead tissue, but also the surrounding scar and even the granulations out from the wound bed. The resulting defect is thus far larger and primary closure is practically impossible to conduct. Sutures have to be placed without any tension, on contrary the wound will dehiscence and the reconstruction is doomed to fail. Inclusively, usage of flaps, local or distant, is obligatory concerning the fact that primary closure is infeasible, expect maybe in few cases with smaller and narrow sores (2, 7).

Patients where good result with minor recurrence risk is expected are enrolled for surgery. This means patients with good general condition preoperatively assessed, patients with complimentary social resources (social support, anti-decubital beds, adequate home medical care, wheelchair mattresses etc.), compliant attitude and knowledge to prevent recurrence. To minimize operative risk, as well as to enhance the success rate, all comorbidities should be corrected, especially anemia, hypoproteinemia (obtaining serum albumin level > 35g/l), glycemic level in diabetics. Good overall health and mental states, younger age, sufficient nutritional status, negative smoking history and absence of urinary or/and fecal incontinence indicate satisfying outcome. Postoperative infection is minimized if bacterial load is less than 10^6/g tissue from decubital site. To accelerate wound cleansing and to promote healing after necrectomy, modern dressing technologies (impregnated alginates, hydrocolloids, autolytic gels, adhesive barriers etc.) are used more frequently as they absorb much discharge, provide adequate moisture environment, stimulate necrolysis but also decrease odor often present in combination of daily whirlpool and high pressure saline irrigation as well as vacuum assisted closure. Yet another modus in treatment is hyperbaric oxygen therapy which stimulates granulation tissue growth by obtaining optimal local oxygen level (3, 7, 8).

Material and Methods

The study comprises all operatively treated patients on the University Clinic for Plastic and

Reconstructive surgery, Medical faculty in Skopje, Macedonia, in the period of last fifteen years, from 1996 to 2010. Out from 30 patients, 21 were males (70%). The average age is 58.6 years (range 10 – 83). The youngest patient is a 10 years old boy presented with an occipital pressure sore, due to previous brain surgery and prolonged comatose state. The oldest patient is an 83 years old male with a decubital ulcer on sacral region due to a lasting immobility. Inclusion criteria are pressure ulcer stage III and IV, conscious patients, good general health status, adequate social support with expected sufficient rehabilitation and postoperative prevention of decubital recurrence, serum albumin level > 35g/l, wound bacterial contamination less than 10^6 CFU/gr. wound tissue, absence of anemia. Exclusion criteria are pressure ulcer stage I and II (where conservative treatment is treatment of choice), infections, hyperpyrexia, sepsis, concomitant injuries and severe diseases of other nature, quadriplegia as well as patient without social support and affected mental status wherein no postoperative rehabilitation process could be followed and recurrence is expected. Age is not criteria, but younger patients are advanced.

Etiologically, most of the patients were spinal cord injured with paraplegia, comprising 24 patients (80%). Three patients (10%) had the ulcer as a result of prolonged unconsciousness after neurosurgery. Two (6.66%) had the ulcer due to hospitalization in the intensive care unit for longer time. One patient (3.34%) was immobile for few months at home after hip replacement surgery and that's our oldest patient.

According to pressure ulcer localization, patients are classified into regions as following: sacral region pressure ulcer in 15 patients (50%), trochanteric region in 7 patients (23%), occipital region in 4 patients (13%) and other regions affected in 4 patients (13%). On inspection, prior operation, sores are measured and staged, using the Shea decubitus staging system (modification by National Pressure Ulcer Advisory Panel). Stage III is present in 19 patients (63%), whereas stage IV in 11 patients (37%). Majority of the cases emphases decubital ulcer with dimension ranged from 5cm^2 to 10 cm^2. Data relating decubitus distribution in the group according to etiology, localization, stage and dimension is summarized in Table 1.

Good nutritional status is obtained by hypercaloric, rich in protein diet, for optimizing albumin blood level more than 35g/l. Occasionally, parenteral or enteral nutritional solutions are administrated prior surgery. Preoperative blood samples are taken in order to reveal any abnormalities. Correction of anemia is also imperative. Additional supplements, vitamins and minerals are used as well. While preparing for surgery, it is necessary to clean and dress the wound bed in order to reduce bacterial load which is measured by frequent tissue biopsy or/and microbiology swabs. Necrectomies, dressings and saline irrigations are done ambulatory, thus preparing the wound bed as soon as possible. By using hydro gels that speed up local autolytic processes and high-absorbent pads, like alginates, silver alginates and hydrocolloid barrier dressings, we enhance cleaning faster than by using the traditional gauze dressings.

Operation in general anesthesia is planned at the most appropriate time for the patient, when general health parameters and local wound characteristic allow aggressive treatment. Operative technique includes radical excision of whole necrotic tissue with the surrounding scar and granulations, thus reaching the bone prominence. When necessary, bone prominence is ablated after which

Table 1. Distribution of pressure ulcers in the group according to etiology, localization, stage and dimension

Localization		Etiology		Stage	Dimension	
Sacral region	15	Spinal cord injury	24	III stage –		
	50%	with paraplegia	80%	19	>10 cm²	7
Trochanteric	7	Brain injury and	3	63%		33%
region	23%	comatose patients	10%			
Occipital	4	Prolonged intensive	2	IV stage –		
region	13%	care unit admittance	6.66%		< 10 cm²	23
Others	4	Immobilization	1	11		77%
(ischiadic, heels)	13%		3.34%	37%		

This table shows that sacral region is most affected, whereas patients with spinal cord injury are most frequent. Decubitus with III stage is in predominance as well as pressure ulcer smaller than 10cm^2 in surface area.

Table 2. Distribution of flap types used in reconstruction of pressure ulcers

Myocutaneous flaps	m.tensor fasciae lata flap		m.gluteus maximus flap			
Total:30 (100%)	1 (12.5%)		7 (87.5%)		8 (27%)	
Cutaneous flaps	Transposition flap	Unilateral rotation flap	Bilateral rotation flap	Bipedicular flap		Total:30 (100%)
Total: 22 (100%)	10 (45%)	8 (36%)	2 (9.5%)	2 (9.5%)	22 (73%)	

This table shows the frequency of the flaps we've utilized in our group and it points out that cutaneous flaps, out of which transposition flap as subtype, are the most used.

designing and rising the flap for covering the defect follows. Local flapping is the most appropriate type for reconstruction dealing with the depth and location of these defects. Concerning the different region and magnitude of the defect to be reconstructed as well as the quality of the nearby skin, which is often damaged, it is impossible to use one uniform flap pattern in each defect. Thus, designing the flap is of great importance for successful operation whereto we pay great attention. Whether adjacent muscle is to be included in flap design or not, depends mostly on wound depth and necessity for optimal bone padding. If bone is ablated and wound is not that deep, cutaneous flap is rather used. Vice versa, if bone is not affected, (ulcer stage III), not ablated, in thin patients with deep wounds, than musculocutaneuos flap is rather utilized. That is why we apply variety of flap patterns. Cutaneous flaps are used in 22 patients (73%), whereas myocutaneous in 8 (27%). In one trochanteric decubitus, m.tensor fasciae lata myofasciocutaneous flap is designed whereas in 1 trochanteric,1 ischiadic and 5 sacral pressure ulcers, myocutaneous m.gluteus maximus flap with different pivotal point is conducted. When cutaneous flaps are used, various transfer methods regarding directions of tissue movement are patterned. These comprise transposition flaps (used in 10 patients), unilateral rotation flaps (used in 8 patients), bilateral rotation flaps (in 2 patients) and bipedicular flaps (in 2 patients). Transposition is a kind of pivotal flap where pedicle with the donor site flap is transposed in order to cover the adjacent defect. When a rotation flap is planned, we raise flap from the surroundings and bring it by means of rotation directly in the defect. Bipedicular flap has two pedicles, large enough to reconstruct defect. At the end of operation, loose dressing is applied over the base where blood supply comes from and on contrary, compressive dressing over the flap body in order to prevent any hematoma formation that will compromise flap viability. Vacuum drainage is employed compulsory in each case. First redressing follows on the ward after 3-5 days when the drain tube is taken out. Sutures are pulled out after 10-16 days. Patient has to avoid pressure over operative field while lie on bad for at least 4-6 weeks postoperatively; frequent patient repositioning and anti-decubital mattresses are conducted on regular base. Subcutaneous low-weight heparin (enoxiparin) administration follows for to prevent any vascular compromitation of flap blood supply.

In one patient, two decubital ulcers on distinct sites are present. While the one on sacral region is to be treated with flap, the other on the trochanteric region is sutured directly after the usual preoperative ulcer preparation. Although not advisable, in selected cases when dealing with small but deep ulcers, with good adjacent skin quality and appropriate local environment, direct closure can be utilized safely. Otherwise, among other due to suture tension, suture line will dehiscence and reconstruction is doomed to failure.

Follow up after hospital discharge is advised on weekly basis first month, than monthly first half year and gradually increasing the interval. Written advices for prevention of recurrences are issued on discharge day.
Flaps used in reconstruction of decubital ulcer are summarized in Table 2.

Results

Operations underwent satisfactory and each patient took it well. When performing this surgery, it is anticipated complications to occur. These can be divided into early and late complications seen on follow ups. Early complications occur on the ward in first two - four weeks after surgery and these include:

- hematoma formation due to suction failure of the drainage system in 5 patients (16.7%);
- infection in operative site in 3 patients (10%), all in cases with hematoma;
- partial dehiscence in 5 cases (16.7%), three in cases with postoperative hematoma;
- marginal flap necrosis in 4 cases (13.3%).

These complication are overlapping in same patients and all in all occurred in totally 9 patients (30%), out of which 6 were reconstructed with myocutaneous flaps. This rate, although as high as nearly one third of

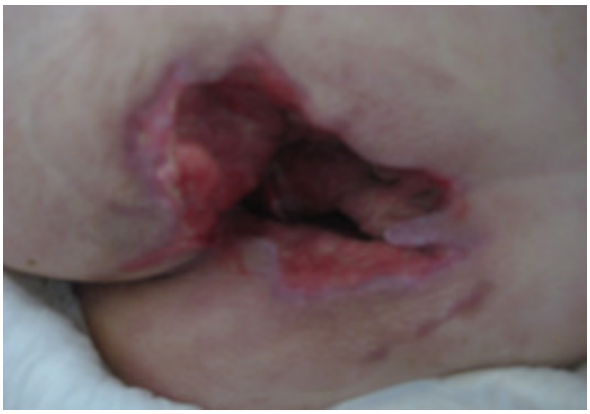

Fig. 1. Deep pressure ulcer, stage IV with dimension over 10cm^2, in sacral region, in paraplegic 39 old female, with concomitant diabetes mellitus, with clean wound bed without any necrosis and infection, ready for operation

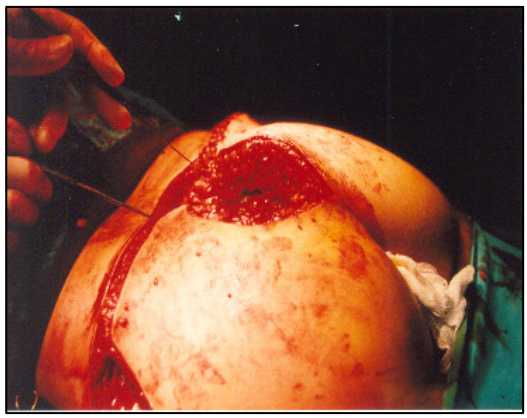

Fig.2. Surgical treatment of the same patient, reconstruction of the defect with two rotational flaps

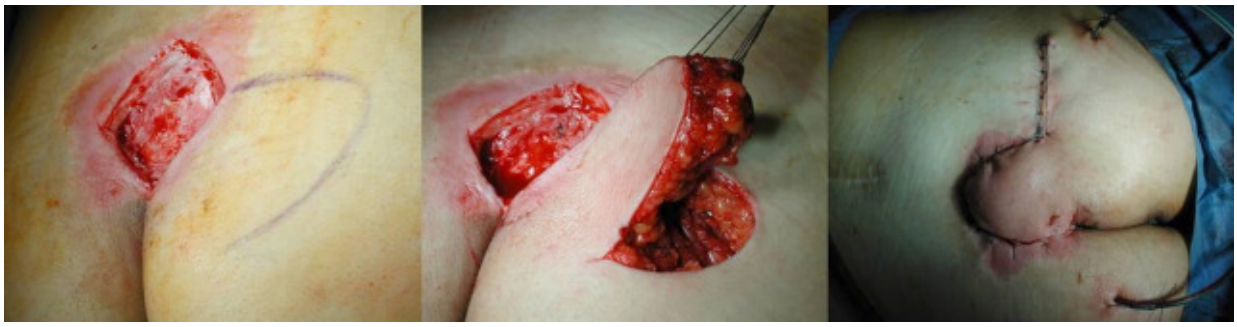

Fig 2. Transposition flap used for covering sacral post-excision pressure ulcer defect

the cases, is reasonable, bearing in mind the sensitivity and fragility of the patients treated. Hematoma was drained properly through suture line holes. Infection resolved by antibiotics, locally and systematically administrated, as well as by evacuation. Partial dehiscence and marginal flap necrosis were let to sanate by second intention for a period and delayed suture applied. Wounds in uncomplicated cases healed regularly in 14-18 days. Average hospitalization stay was 26 days (range 15 – 58 days) and more the complications occurred, longer the hospital admittance.

Discussion

Operative treatment of decubital ulcer is needed in order to achieve good functional and aesthetic results in patients, to improve patients' quality of life and gain fast wound healing in those who demand rehabilitation process. Flap surgery is the mainstream for reconstruction (9). Skin grafting can be used just as a temporary coverage on clean granulated wound bed. Grafts' lacking of resistance to shearing and friction forces make them prone to easy damaging, no matter how thick they are.(2) On the other hand, flaps comprise health, thick tissue with their own blood supply and innervation, thus resistance to injury is as high as the normal tissue, promoting them as best solution for defect reconstruction in decubitus patients. Flaps might content different tissue types, with or without muscle included and their bulkiness is what we need when treat patient with pressure sore. The same effect can be achieved by ablation of bone prominence. Regarding the tissue composition, flaps can be fasciocutaneous and musculocutaneous. Type of the flap to be used, depends on the region, the gross of the defect and its depth. In the sacral region, flaps to be considered as an option are unilateral or bilateral rotation fasciocutaneous, musculocutanous of m. gluteus maximus or transversal lumbo-sacral flaps. Musculocutanous flaps with m.gluteus maximus, m. semitendinosus, m. semimembranosus or m. biceps femoris or just fasciocutanous flaps might be used when

reconstruction of ischiadic pressure ulcer is planned. In trochanteric region, m.fascia lata musculocutaneous flaps as a rotation flap could be in mind, but also random vascularisation rotational or transposition cutaneous flaps. Free skin grafts are usually applied for covering donor site defects (10). Comparing to musculocutaneous, local fasciocutaneous flaps as less aggressive reconstruction method, seem to be more feasible in these patients considering their often present malnutrition and anemia thus less affecting their overall health condition. Ablation of bone prominence is advised in order surgically to prevent recurrence after reconstruction if bulky flap is not to be used. Bulky flap should always include muscle. If myocutaneos flap and bone ablation are combined, the chances for postoperative ulcer recurrence are by far decreased. This two tools cannot be always combined. In patients with pressure sores, that are often critically ill, with limiting primary illness, operative risk higher that others and present comorbidies, less aggressive and shorter surgery is warranted. In that case, bone ablation with an usage of local cutaneous flaps insures adequate and satisfying results still more that myocutanous flaps are burdened with higher complication rate as seen in our study. When utilized cutaneous flap, corrective bone ablation of the site of ulcer is compulsory. When closing the donor site, directly or with skin grafts, must be paid attention to bone ablation of the donor site if present. For instance, when using transposition flap, flap base has to be over the tuber ischii cause that is the bulkiest part, or if not, tuber ischii has to be ablated. On contrary, we form iatrogenic locus resistentio minoris for subsequent sciatic ulcer development (2, 10, 11, 12).

Surgical treatment of pressure ulcer is complimentary with other options for medical treatment although its place is usually a final event. As already mentioned, this treatment bears lot of efforts, engage huge resources, it is complex, time-consuming and often unpredictable with the results. It affects not only the patient, but his/her family as well and has health, social and ethical impact. Once occurred, it resolves slowly, despite all measures employed, has high recurrence rate and incidence constantly increasing with unfavorable cost-effectiveness. Ulcer recurrence as late complication is most devastating incident that could happen. Usually it occurs after few months after operation. Bearing in mind the complexity of treatment, all measures that will prevent recurrence following successful closure are combined starting immediately after transferring the patient from the operating table to bed. Specialized support surfaces, weight bearing devices, frequent self- hand lifting and repositioning at least every 2 hours by medical stuff, daily skin care and usage of skin protecting barrier ointments, soft underneath pads etc. are some of them. Advices for patient is of great importance and they have to be trained to deal with preventive methods as well as to inspect skin on daily basis for any impending necrosis. (9, 10) Ulcer recurrence occurred in 4 patients (13%). When present, it is harder to reconstruct; health tissue already used and lacking donor site for new flap. As luck would have it, our recurrences were superficial ulcers that cured conservatively although very slowly.

It is estimated that just in USA, total medical care treatment costs are about $11 billion, cardinal reason being that decubitus is one of the most underrated medical problem. Still, hospitalized patients have unreasonable high development risk, among which the intensive care units patients the highest. In many countries, hospital - acquired stage III/IV decubitus is declared as adverse patients safety event, and if not presented prior hospital admittance, its treatment is not a subject to reimbursement. That is why, much more attention is payed on prevention of pressure ulcers with a lot of comprehensive prevention programs being implemented. First step is to determinate who has over the average risk thus needing greater support. Braden and Bergstrom designed the widely used Braden scale which outflanks risk factors such are sensory perception, mobility, activity, nutrition, moisture and friction/share (13). According to risk, this scoring system, divides patients into 4 groups: patients with mild, moderate, high and very high risk. Many will agree that this system is not comprehensive enough and doesn't asses other predictive factors, such are advanced age, hypoproteinemia, prolonged intensive care unit stay, severity of illness, low arteriolar pressure, diabetes mellitus, sepsis, vascular abnormalities and other comorbid conditions. Prevention continues with implementing many other medical measures and devices that will reduce or eliminate the main cause, overpressure on the surface. These includes specialized support surfaces (antidecubital air-flowed mattresses), repositioning of the patient every 2 hours despite usage of specialized beds, daily skin care by keeping skin clean and free of urine/ feces, application of barrier skin ointments, frequent skin quality evaluation, sufficient protein intake and good nutritional status with diet supplements added, optimized wound dressing if necessary. Unfortunately, despite all measured utilized, pressure ulcer do develop. While stage I/II ulcer, in general, heal spontaneously with adequate non-surgical medical methods, stage III/IV ulcer do need surgery and consultation with plastic surgery, who perform pressure sore reconstruction, is necessary (4, 9, 13).

Conclusion

Holistic and multidisciplinary approach includes involvement of variety specializations, like surgeons (general surgeons, neurosurgeons, plastic surgeons, urologists), rehabilitation medicine specialists, social workers, psychologists and psychiatrists as well as geriatricians and internists, all in order to improve patients' general health, to prevent and treat pressure sores and to support progress and attitude as much as possible. If unnoticed and untreated, complications as infection, sepsis, malnutrition, amyloidosis, malign degeneration and squamous cell carcinoma (Marjolin ulceration) can follow and finally, decubitus can lead to death.

Surgical treatment of the decubital ulcers, adequate and on time applied, in combination with appropriate pre- and post- operative care, gains satisfying functional and aesthetic results. That applies especially in selected patients where primary illness as reason for pressure ulcer is prone to successful rehabilitation.

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