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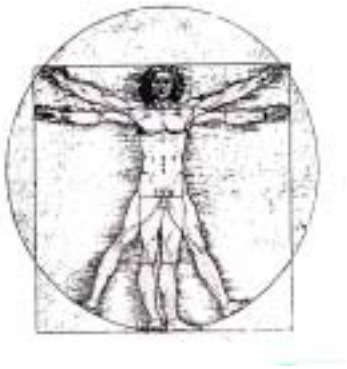

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# LUMBARNI DISKUS HERNII - HISTOLOGIJA NA POSTOPERATIVNO DOBIEN MATERIJAL

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**Izvadok**

**Cel**: da se ispita relacijata me|u tipot na diskus hernija evaluirana so preoperativno ispituvawe so magnetna rezonanca so tkivnata sodr ' ina na herniraniot diskov materijal dobien postoperativno kaj hirur { ki tretirani diskus hernii.

**Materijal i metodi:** trieset pacienti koi bile podlo ' eni na ispituvawe so magnetna rezonanca pred hirur { kiot tretman na diskus herniite bea vklu~eni vo ova studija. Postoperativno dobieniot materijal e ispituvan histolo { ki so boewe so hematoksilin-eozin. Tipot na diskus hernija se evaluira na MR snimkite vo relacija so mor fostrukturnite karakteristiki na postoperativno dobieniot herniran diskov materijal koj histolo { ki se analizira.

**Rezultati:** najgolem del od tkivnata sodr ' ina na tkivoto dobieno posle hirur { ko otstranuvawe na diskus hernijata e tkivo koe pripa|a na fibrozniot prsten, so pomala sodr ' ina na tkivo koe pripa|a na mekoto jadro i kartilaginoznata pokrovna plo~a. Fibrozna i miksomatozna degeneracija na fibrozniot prsten so fokalna nekroza i cisti~ni formacii kako posledica na izrazena miksomatozna degeneracija bea naodi karakteristi~ni za site tipovi diskus hernii. Naod na hijalino-'rskavi~no tkivo be { e voobi~aen za diskus herniite-tip ekstruzii. Neovaskularizacija i fibroblasti~na regeneracija kako histolo { ki naodi bea zna~ajni po~esti kaj diskus hernii-tip ekstruzii. Prisustvo na aglomerati od rskavi~ni kletki be { e isto taka po~est naod kaj diskus hernii-tip ekstruzii.

**Zaklu~oci:** karakteristi~ni naodi pri histolo { ka naliza na postoperativno dobieniot material po hirur { ki tretman na diskus hernii tip ekstruzii bea prisustvo na kapilarna invazija i mali krvni sadovi pridru ' eni so rastreseno svrzno tkivo kako i aglomerati od 'rskavi~ni kletki.

***Klu~ni zborovi:*** lumbarni diskus hernii, histologija, MR imaging

# LUMBAR INTERVERTEBRAL DISC HERNIATIONS - HISTOLOGY OF THE SURGIACALLY OBTAINED MATERIAL

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**Abstract**

**Purpose:** To examine the relationship between the type of disc herniation evaluated with preoperative magnetic resonance (MR) imaging with data for tissue composition of herniated disks in patients after microsurgical removal of herniated material.

**Materials and methods:** Thirty patients who underwent MR imaging before microsurgical removal of lumbar disc herniation material were included in the study. The postoperatively obtained material was histologically analyzed and the sections were stained with hematoxylin-eosin. The type of the disc herniation was evaluated in relation to the morphostructural constitution of the herniated disk material.

**Results:** Tissue composition of the herniated material in all patients was mainly anulus fibrosus with a little amount of nucleus pulposus, and cartilaginous endplate. Fibrous and myxomatous degeneration of the annulus fibrosus with focal necrosis and cysts formations as a consequence of severe myxomatous degeneration were common findings in the herniated material from all types of disc herniations. Hyaline cartilaginous material was an evident finding in the extruded discs. Neovascularisation and fibroblasts regeneration as histological findings were significantly more frequently seen in disc herniations- extrusion type. The presence of hyaline cartilaginous material and agglomerates of cartilage cells was a characteristic finding for disc herniations –extrusions type.

**Conclusion:** In the surgically obtained material from disc herniations-extrusions characteristic histologic findings were a presence of capillary invasion and small blood vessels accompanied by loose fibrous tissue and hyaline cartilaginous material with agglomerates of cartilage cells.

***Key words:*** lumbar disc herniation, histology, MR imaging

**Introduction**

Herniations of the lumbar intervertebral dics can be classified as protrusions (the outer lamellae are intact) or extrusions (the outer lamellae are ruptured). In some indicated cases this condition is treated surgically. The issues that are important for the surgeon are: type of the disc heniation, its localisation and size, compression of the surrounding neurological structures and nature of the herniated disc material. Histologic examination of the surgically obtained material shows that the morphology of all these herniations is very heterogeneous and may include tissue from the nucleus pulposus, annulus fibrosus and cartilaginous endplate. Knowledge of tissue composition is of interest because some authors (1-5) suggest that different tissue compositions may lead to different inflammatory responses. Although the intervertebral disk is generally without a vascular supply, extruded disk material often shows neovascularization, and this is thought to be a part of the natural healing mechanism.

Histologic examination of the herniated disc material can contribute in understanding the process of genesis and development of the disc herniations. The aim of the study was to analyze the relations between morphostructural constitution of the herniated disc material and type of the disc herniations.

**Material and methods**

Thirty patients who underwent MR imaging before microsurgical removal of extruded lumbar disk herniation material were included in the study. Of these patients, 21 were males and 9 females, aged from 29 to 75 years. Histological examinations were conducted on specimens from disc material obtained at surgery (disc excision through hemilaminectomy) from 30 patients, of whom 6 had protruded and 24 had extruded disc. Of the patients with extrusion type of herniation (24), 8 were with complete extrusions. The material was removed as individual piece from the epidural space or the herniated tissue was removed piece-meal from within the intervertebral disc. Immediately after removal, the intradiscal and extruded parts of the removed disk material were placed separately into 5% formaldehyde solution, labeled, and sent for histopathologic examination.

The surgical specimens were brought immediately to the Institute of Pathology in Skopje, where they were embedded in paraffin and completely evaluated. The material was cut with a microtome into 4-ìm-thick slices, which were stained with hematoxylin-eosin to differentiate anulus fibrosus, nucleus pulposus, and hyaline cartilaginous endplate.

**Results**

The results from the histologic analysis of the surgically obtained material from herniated lumbar disc are presented in Table1.

In the patients with disc herniation of extrusion type, in whom the contents of the disc material were completely extruded, and could be obtained as a single large fragment of disc tissue at the time of surgery. Histologically the annulus fibrosus occupied most of the contents of the herniated tissue in all types of disc herniations, or a large amount of the excised tissue was composed of annulus fibrosus. The fibre bundles were separated or the presence of swollen fibers was evident in almost all of the specimens. There was a high incidence of fibrous and myxomatous degeneration of the annulus fibrosus (Fig.1). Focal necrosis and marked cyst's formations as a consequence of severe myxomatous degeneration was observed in 11 specimens. Loose fibrous tissue with capillary invasion and small blood vessels was seen in the disc herniations–extrusions, where the surgically obtained tissue was removed as a single fragment(Fig. 2). Neovascularisation and fibroblasts regeneration as histological findings were evident in disc herniations of extrusion type . Hyaline cartilaginous material and agglomerates of cartilage cells were also a

**Table 1.** Histological findings from the surgically obtained material of herniated lumbar discs from 30 patients

| Histological findings | ProtrusionsN = 6 | ExtrusionsN = 24 | P – value |
|---|---|---|---|
| Swollen fibers | 5(83%) | 15(63%) | 0,3594 |
| Fibrous degeneration | 5(83%) | 16(67%) | 0,4498 |
| Hyalinization | 4(67%) | 18(75%) | 0,6946 |
| Myxomatous degeneration | 1(17%) | 11(46%) | 0,2056 |
| Necrosis | 5(83%) | 14(58%) | 0,2662 |
| Cysts | 2(33%) | 9(38%) | 0,8222 |
| Cartilage cells agglomerates | 2(33%) | 20(83%) | 0,020*Signif. |
| Neovascularisation | 0 | 3(13%) | / |
| Osification | 1(17%) | 2(8%) | 0,5126 |
| Fibroblasts | 0 | 7(29%) | / |

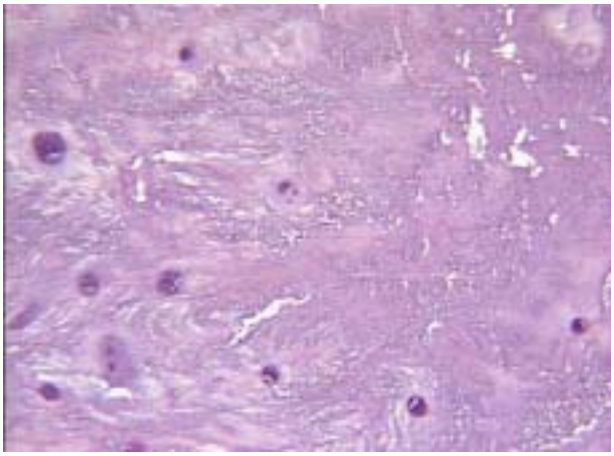

**Fig.1.** Necrosis, myxomatous degeneration and focuses of hyaline cartilage

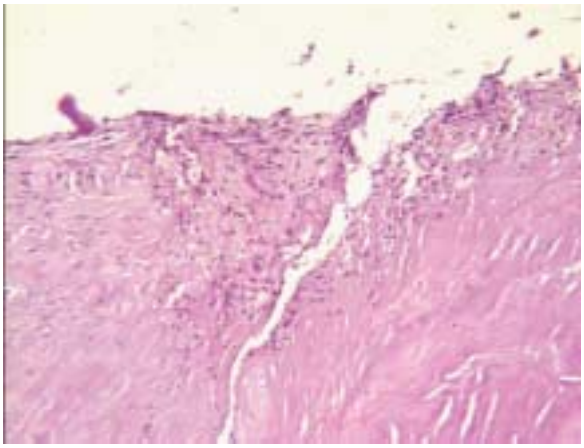

**Fig. 2.** Rupture, mesenchymal proliferation and capillary invasion

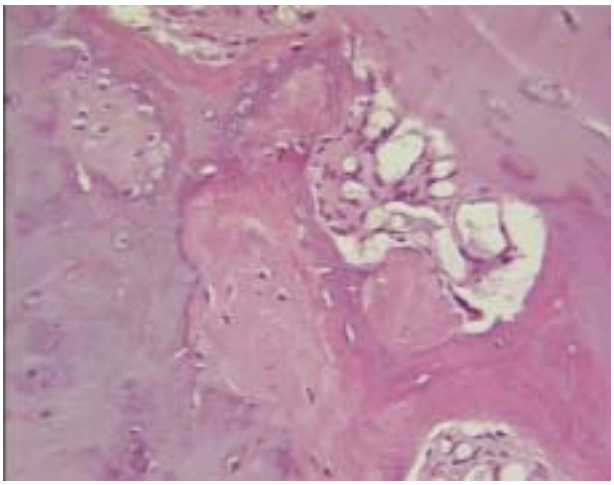

**Fig. 3.** Hyaline cartilaginous material and enchondral ossification

characteristic finding for disc herniations –extrusions (Fig.3).

**Discussion**

Degeneration of the discs with increasing age is characterized by changing the composition of the avascular disc, including a decrease of its proteoglycan content and its hydration and an increase of collagen fibers. The cartilaginous endplate becomes calcified, frequently resorbed and subsequently replaced by bone. The presence of fragmentation and degeneration of the collagen fibers from the annulus fibrosus was evident in the herniated disc material. Tissue composition of the herniated material in all patients in this study was mainly anulus fibrosus, with a little amount of nucleus pulposus, and a cartilaginous endplate. Yasuma et al. (4) reported that where complete extrusion of sequestrated material had occurred, this tissue almost exclusively consisted of anulus fibrosus. All cases exhibited signs of disc degeneration. Pre-existing degeneration of the disc contributes to the formation of disc herniaton, or trauma might not be the exclusive cause of disc herniation without a presence of pre-existing degenerative damage of the disc.The discs may herniate posterioly in three patterns. In the protrusion type of the herniated disc the peripheral layer of the annulus fibrosus remains attached to the vertebral body; in the extusion type of the disc herniations the peripheral annulus fibrosus has become detached from a portion of posterior vertebral body rim. The extrusions can be incomplete or complete, which results from the continuity of the herniated disc tissue with the disc. If the herniated disc tissue is no longer in continuity with the disc, exposed to the epidural space, the extrusion is complete. Tanaka et al. (6) reported that avulsion-type disk herniation predominates in the elderly with cartilaginous endplate rupture. The authors showed that there was only a loose connection between the cartilaginous endplate and the subchondral bone, but there was a strong connection between the inner fibers of the anulus fibrosus and the cartilaginous endplate. Fragments of cartilaginous endplate that apparently had been detached with the annulus fibrosus were found in the surgically obtained material. Our results have shown that there was cartilaginous material in a high proportion of extruded disk herniations. The association of the amount of cartilaginous material with endplate abnormalities supports the theory that avulsion of the vertebral endplate is one source of disk herniation. Authors reported that vertebral endplate marrow signal intensity changes on MR images are indicative of cartilaginous material in the extruded disk herniation material (7).

The other characteristics of the histological findings from the material obtained after surgery of complete extrusions were presence of small blood vessels accompanied with loose fibrous tissue in the marginal regins of the extruded material. Blood vessels are not a characteristic finding in normal intervertebral discs. According to some authors invasion of blood vessels in the intervertbral discs can be considered as a sign of aging

of the intervertebral disc. The blood vessels in the extruded tissue from complete and incomplete type of herniations are newly formed, or these blood vessels invade the intervertebral discs as a result of its degeneration and become extruded together with the disc tissue. There is evidence from experimental studies that blood vessels from the surrounding fibrovascular tissue infiltrate into the herniated annulus fibrosus but not into the herniated cartilaginous endplate (8). The same authors confirmed in an animal study that annulus fibrosus induces vascular sprouting and inflammation with subsequent decrease in size of the annulus material. Even more, when they implanted both annulus and endplate material, neovascularization and inflammation reaction were depressed, possibly as a result of inhibitors of neovascularization found in the cartilage.(9). Indirect confirmation of their findings may be those of other authors (1), who showed that single free fragments, which contain cartilage endplate less frequently than do multiple free fragments, are associated more often with inflammatory granulation tissue around the fragments.

In this study the presence of blood vessels was found to be a characteristic finding for complete extrusions. A limitation of the current study is the little number of specimens from the surgical material obtained after surgical removal of disc herniations-protrusions. It was evident that in some specimens of the extrusion type, a predominant finding was a large amount of hyaline cartilage, while in the other specimens a presence of granulation tissue and neovascularization.

## Conclusion

In the surgically obtained material from disc herniations-extrusions characteristic histologic findings were a presence of capillary invasion and small blood vessels accompanied by loose fibrous tissue and hyaline cartilaginous material with agglomerates of cartilage cells.

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# VRODENI MALFORMACII NA URETERITE KAJ HUMANI FETUSI

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**Izvadok**

Poradi mnoguslo‘eniot embrionalen razvitok na organite odurogenitalniot trakt vrodenite malformacii na ovie organi se edni od naj~estite i zavzemaat okolu 30-40% od site vrodeni malformacii na~ove~kiot organizam.

Celna studijata be{e da se utvrdi distribucijata na vrodenite malformacii na ureterite na fetusite spored stranata na pojavuvawe kajma{kiot i ‘enskiot pol.

Zarealizirawe na postavenata celvo studijata bea analizirani ureterite kaj 300 fetusi so gestaciska starost od III-H lunaren mesec. Od niv, 154 bea ma{ki a 146 fetusi od ‘enski pol.

Analizata so $H^2$test poka‘a deka ne postoi statisti~ki signifikantna zavisnost pome|u pojava na vrodenite malformacii na ureterite kaj ispituvanite fetusi spored polot.

***Klu~ni zborovi***: fetus**,** bubreg, ureter, anatomija, malformacii

# CONGENITAL MALFORMATIONS OF URETERS IN HUMAN FETUSES

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**Abstract**

The organs of urogenital tract have complicated embryonic development and thus, congenital malformations of these organs are more frequent and account for 30-40% of all congenital malformations in the human body.

The aim of this study was to determine the distribution of congenital malformations of ureters in human fetuses according to lateralization in both sexes.

We analyzed ureters in 300 fetuses (154 males and 146 females) with gestational ages from 3rd to 10th lunar month.

The analysis with $X^2$- test has shown that there was no statistically significant dependence in the appearance of congenital malformations of ureters in the examined fetuses according to gender.

***Key words:*** fetus, kidney, ureter, anatomy, malformations

## Introduction

Congenital malformations of the ureter usually appear with malformations of the kidney and they might be on both sides ( they are incompatibility with life).

Malformations of the distribution of ureter are: ureter bifidus and ureter duplex.

Ureter bifidus is a congenital malformation with two kidney pelvis that give rise to two ureters, which then join in to one on different levels. This malformation can be unilateral or bilateral.

Ureter duplex is congenital malformation with two kidney pelvis that gives rise to two ureters, but they are different from ureter bifidus because they enter the bladder with two separate orifices. Ureter duplex is more common malformation in males than in females. The kidney is sometimes normal, sometimes it is more longer, and sometimes it is divided in two parts.

There are cases with two kidney pelvis where one is lower and the other one is upper.Upper pelvis is long and thin. Upper big calyx enters it, bringing urine into the upper third of the kidney. Lower kidney pelvis is more voluminous and it receives the lower big calyx and middle accessory big calyx that birng urine from the lower two tihrds of the kidney. Ureter duplex may be on one side or on both sides .

## Material and Methods

This study included a sample of 300 fetuses:

- Preterm infants and dead born fetuses with gestational ages of the 4th to 10th lunar month
- Of artificial abortions with gestational age of the3rd lunar month. Fetuses were obtained from the University Clinic of Gynecology and Obstetrics in Skopje.

For this investigation we used standard anatomical methods inspection and dissection. Kidney were taken en bloc with suprarenal gland, blood vessels and urinary tract to the bladder (kidney calyxes, kidney pelvis and ureter).

Dissection of the kidney was made in frontal level in order to see the structures. Ureters were analyzed with inspection.

All parameters were statistically processed. Analysis of the relation between attributive statistical series was made with the Pearson is $X^2$-test.

## Results

Ureters of 300 fetuses were analyzed, 146 (48,7%) female and 154 (51,3%) male.

Distribution of congenital malformations of fetal ureters according to the side of appearance in both genders are shown in Table 1 and Fig 1.

**Table 1.** Distribution of congenital malformation of fetal ureters according to the side of appearance in male and female sexes

| **Congenital malformations of ureters** | **male** | | **female** | |
|---|---|---|---|---|
| | **R.K.** | **L.K.** | **R.K.** | **L.K.** |
| ureter bifidus | 1 | 2 | 1 | 1 |
| ureter duplex | 3 | 4 | 2 | 3 |
| Total | 10(6,5%) | | 7(4,8%) | |

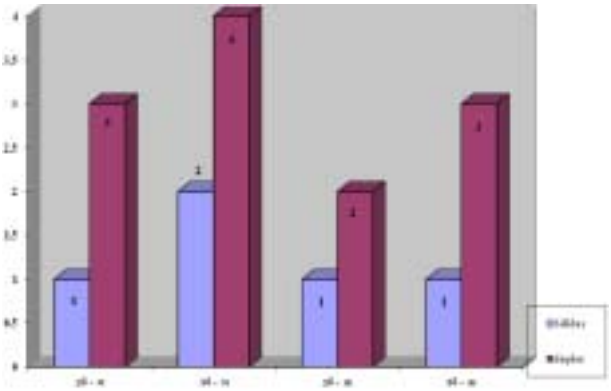

**Fig. 1.** Distribution of congenital malformation of fetal ureters according to the side of appearance in male and female sexes

Congenital malformation of ureters was found in 10 (6,5%) of the total of 154 male fetuses and 7 (4.8%) of the total of 146 female fetuses.

Analysis with $X^2$-test has shown that there was no statistically significant difference between the congenital malformations of ureters in the examined male and female fetuses.The congenital malformations of ureters were more frequent in male fetuses but without statistical significance ($X^2$=o,40 df=1 p=0,5247) (Table .1. and Chart .1a.)

**Fig.1.** Ureter bifidus

**Fig. 2.** Ureter duplex

**Fig 3.** Ureter duplex in left kidney

**Discussion**

The results of our investigation were compared with those of other scientists.

Radivojevic M. (7) found ureter duplex in 5 fetuses of the total of 98. Of them four were male and one female fetuses.

Lonaghan D.(3) in his series of 146 subjects ages 4-18 years, he found 2 cases.the subjects with ureter bifidus.

Ostojic B.(4) found ureter bifidus with a blind-ending branch.

Petrovic S. (6) in his study he found ureter bifidus in 12 cases and ureter duplex in 4 cases out of 340 fetuses.

Petkovic S. (5) examined a series of 146 subjects and he found congenital malformations of the ureter together and of the renal pelvis. Malformations of the ureters were more frequent in male foetuses than in female.

**Conclusion**

Congenital malformations of ureters were registered in 10 (6.5%) of the total of 154 male fetuses and in 7 (4.8%) of the total of 146 female fetuses. The congenital malformations of ureters were more frequent in males, but without statistical significance.

Malformation of ureters in fetal growth and development are persistent after birth, meaning that changes are present in the adults, with or without clinical importance.

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UDK:611.127

# MORFOLO[KI KARAKTERISTIKI NA ATRIOVENTRIKULARNIOT JAZOL

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**Izvadok**

Celta na ovaa studija e da se prika‘at morfolo{kite karakteristiki na atrioventrikularniot (AV) jazol. Analizirani se 100 humani fetusi fiksirani vo 10% formalin, dobieni po autopsii na po~inati od nekardija~ni pri~ini. Za da se ovozmo‘i mikroskopska potvrda na tkivoto na AV jazolot, presecite se boeni so standardnite boewa so hematoksilyn eosin i Van Gieson, a potoa histolo{ki se analizirani. Histolo{kata identifikacija na tkivoto na AV jazolot e pravena spored kriteriumite postaveni od Davice i Pomerance.

Histolo{kata analiza na triagolnikot na Koh, kaj site 100 preparati go potvrdi prisustvoto na tkivoto na AV jazolot, lokalizirano vo vrvot na triagolnikot, vo atrijalniot del od atrioventrikularniot septum. Kaj 77 od preparatite AV jazolot ima voobi~aena struktura. Fibrozni promeni se registrirani kaj 10 od preparatite. Kaj 11 preparati e registrirana masna infiltracija na tkivoto na AV jazolot i negovite priodi, a kaj dva preparata registrirani se kalcifikati vo priodite na jazolot.

Voobi~aenite promeni koi se javuvaat vo tek na stareeweto obi~no se vo vid na masna infiltracija, atrofija, hipertrofija, fibroelastoza, kalcifikati i fibroza. Masnata infiltracija na priodite na AV jazolot i negovite priodi, so ili bez fibroelastoza, mo‘e da bide pri~ina za AV blok, junkcionalna ili reentry aritmija.

***Klu~ni zborovi:*** anatomija, histologija, atrioventricularen jazol, triagolnik na Koh.

# MORPHOLOGIC FEATURES OF ATRIOVENTRICULAR NODE

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**Abstract**

The aim of this study was to present the morphological features of atrioventricular (AV) node. The examination was made on 100 human hearts got after autopsies of patients died of noncardiac causes and fixed in 10% formaldehyde. For microscopic confirmation of the AV node tissue, sections were stained with routine hematoxilyn eosin and Van Gieson stains and histologically examined. Histological identification of the AV node tissue was done by Davice and Pomerance criteria.

Histological examination of the tissue of the triangle of Koch, confirmed presence of the AV node tissue in all of the 100 hearts, located near the apex of the triangle, in the atrial part of the atrioventricular septum. The AV node had the usual structure in 77 of specimens. Fibrosis of AV node tissue was registrated in 10 (10%) of the specimens. In 11 specimens (11%) we found fatty infiltration of AV node and its approaches, and in 2 specimens calcification of AV approaches.

Normal aging changes generaly take the form of fatty infiltration, loss of cells with space formation, atrophy, hypertrophy, fibroelastosis, calcifications and fibrosis. Fatty replacement of the AV node and its approaches, with or without fibroelastosis, may give rise to AV block or to varying types of junctional arrhytmias and a reentrant type of arrhytmia at the AV junction.

***Kew words:*** anatomy, histology, atrioventricular node, triangle of Koch

## Introduction

The first histologically supported data about specialised heart muscles organized as an axis conducting electrical impulses through the heart are from the end of the 19th and beginning of the 20th century and are results of the work of the doyens of the cardiac pathology as Tawara, Aschoff, Keith, Flack, His, Kent (1).

In 1893 His and Kent showed muscular connection between atriums and ventricles, which they considered as a bridge conducting cardiac impulses. The same year His confirmed the existence of the atrioventricular bundle, later named by him. In 1906 Tawara linked the atrioventricular axis to a tree, with its roots in atrium as the AV node, its trunk penetrating the fibrous septum as a bundle of His, and its peripheral branches reaching the ventricular myocardium as the ramification of the Purkinje fibbers. Soon after, in 1907, Koch described and illustrated the landmarks of a triangular area in the right atrium (named by him), containing the AV node, seen within the body in the anatomic position (2).

Over the recent years, many revisions of this basic knowledge about the organisation of the specialised cardiac musculature of the conduction system have been made. In spite of this, the authors have been suspicious about their first results; the development of new methods and techniques have made only a few changes in this scientific area.

The triangle of Koch occupies the atrial component of the muscular AV septum, a sloping area that attains its AV location because of the major differences in the levels of the attachments of the leaflets of the tricuspid and mitral valves on either side of the septum (3,4). When viewed from the right atrium, the triangle has discrete and obvious borders. The coronary sinus forms the base of the triangle. Its inferior side is marked by the attachment of the septal leaflet of the tricuspid valve and the superior side by the tendon of Todaro (5). The tissue of the AV node is located subendocardially at the apex of the triangle, above the attachment of the septal leaflet of the tricuspid valve (6) and it can be confirmed only histologically. The AV node is about 6mm long, and 2-3mm high.

**Material and methods**

The examination of the morphological features of the AV node was made on 100 human hearts got after autopsies of patients older than 18 years, died of noncardiac causes. The hearts were removed itact, together with the proximal parts of the graet arteries and veins, and fixed in 10% formaldehyde. The right atrium was opened through an incision between the superior and inferior venous orifices, and then by extanding an incision perpendicular to the first incision along the lateral wall of the atrium into the right appendage. The walls of the atrium could then be reflected to display the triangle of Koch.

For the microscopic confirmation of the AV node tissue, we first identified the membranous part of the heart septum. Then, a block of tissue that extands from anterior border of coronary sinus to the midpoint of the membranous septum was removed. The tissue was cut longitudinaly into 2-3mm wide strips and each strip was processed separately and embedded in paraffin. The tissue 5 ¼ sections were stained with routine hematoxilyn eosin and Van Gieson stains and histologically examined.

Histological identification of the AV node tissue was done according to the following criteria (Davice and Pomerance 1975):

• AV node lies immediately subendocardial above the insertion of the septal leaflet of the tricuspid valve. The deep surface of the AV node abuts into the central fibrous body

• AV node consists of a mass of small loosely arranged myofibrils in an interweaving pattern. Between the muscle fibers there are fine connective tissues, fat and thin walled veins. A central artery is not commonly found.

• The deep zone adjacent to the central fibrous body is composed of small dark stained muscle fibers and gives rise to AV bundle. The more superfitial zone consists of larger, paler muscle fibers that are regarded as transitional between the AV node and atrial muscles.

• At the anterior end of AV node, muscle fibers are arranged in parallel lines and form the beginning of the AV bundle. The penetrating portion of the AV bundle lies within the collagenous central fibrous body and it is closely related to both aortic and mitral valves. The right bundle branch is the direct continuation of the AV bundle.

According to the age of the patients the specimens were grouped in 7 groups: 1. 20-29 years; 2. 30-39 years; 3. 40-49 years; 4. 50-59 years; 5. 60-69 years; 6. 70-79 years; 7. over 80 years.

**Results**

The histological examination of the tissue of the triangle of Koch, confirmed the presence of the AV node tissue in all of the 100 hearts, located near the apex of the triangle, in the atrial part of the atrioventricular septum.

**Table 1.** Distribution of the histological features of AV node.

| Age (years) | Usual structure | Fibrosis | Fat infiltration | Calcification | Total |
|---|---|---|---|---|---|
| 20 – 29 | 2 | / | / | / | 2 |
| 30 – 39 | 11 | / | / | / | 11 |
| 40 – 49 | 12 | 1 | 1 | / | 14 |
| 50 – 59 | 18 | 2 | 4 | 1 | 25 |
| 60 – 69 | 14 | 3 | 3 | / | 20 |
| 70 – 79 | 18 | 3 | 2 | 1 | 24 |
| Nad 80 | 2 | 1 | 1 | / | 4 |
| Total | 77 | 10 | 11 | 2 | 100 |

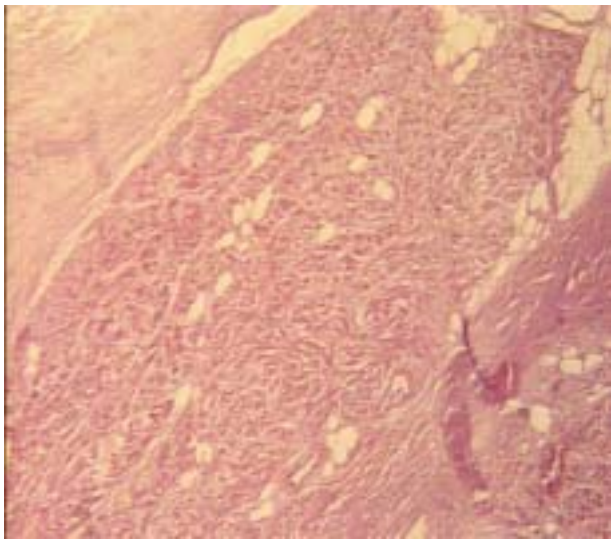

**Fig. 1.** AV node with usual structure ) (hematoxylin eosin x 40).

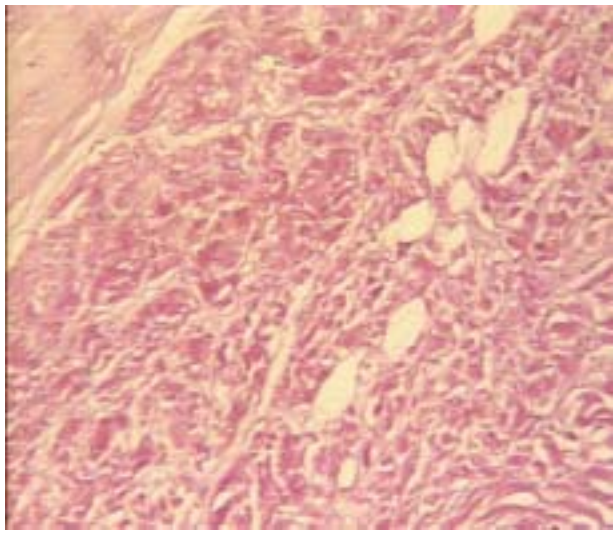

**Fig. 2.** AV node with usual structure consists of a mass of small loosely arranged myofibrils in an interweaving pattern (hematoxylin eosin x 100).

The AV node had the usual structure in 77 of specimens (Fig. 1 and 2): it lied immediately subendocardial above the insertion of the septal leaflet of the tricuspid valve ant its deep surface abuted into the central fibrous body. AV node consisted of a mass of small loosely arranged myofibrils in an interweaving pattern. Between the muscle fibers there were fine connective tissue, fat and thin walled veins. The deep zone adjacent to the central fibrous body was composed of small dark stained muscle fibers and gave rise to AV bundle. The more superfitial zone consisted of larger, paler muscle fibers and it was transitional between the AV node and atrial muscles. At the anterior end of AV node, muscle fibers were arranged in parallel lines and formed the beginning of the AV bundle. The penetrating portion of the AV bundle lied within the collagenous central fibrous body (Fig. 3).

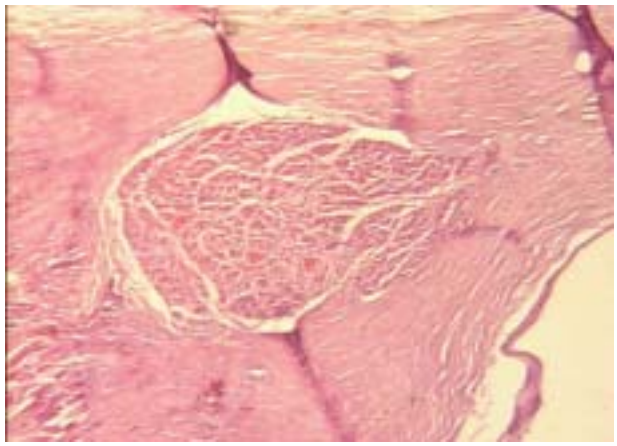

**Fig. 3.** Penetrating portion of AV bundle (A) lies within the collagenous central fibrous body (B) (hematoxylin eosin x 40).

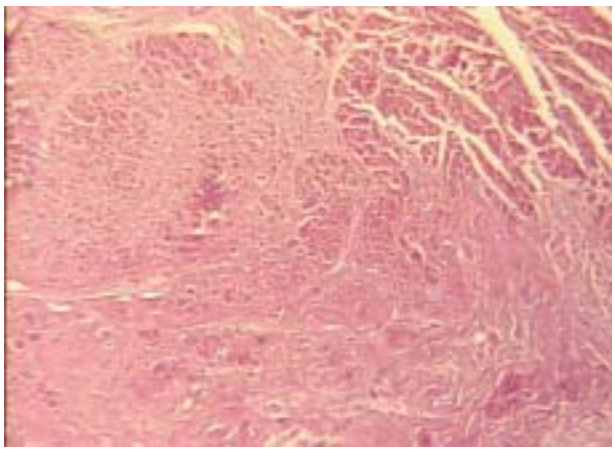

**Fig. 4.** Fibrosis of AV node tissue (hematoxylin eosin x 40).

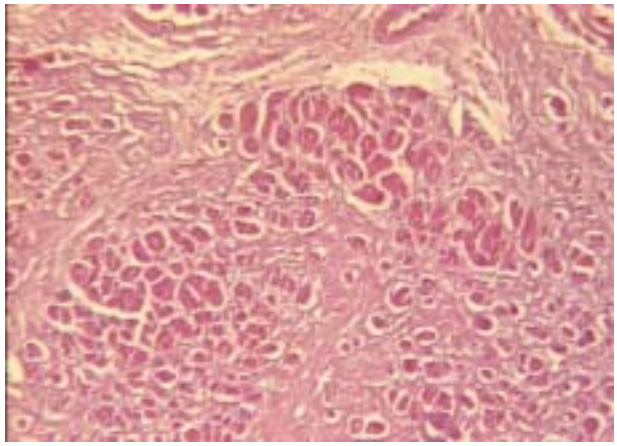

**Fig. 5.** Fibrosis of AV node tissue (hematoxylin eosin x 100).

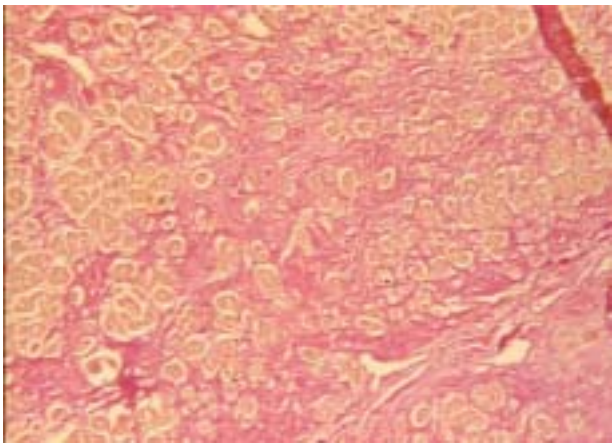

**Fig. 6.** Fibrosis of AV node tissue (Van Gieson x 100).

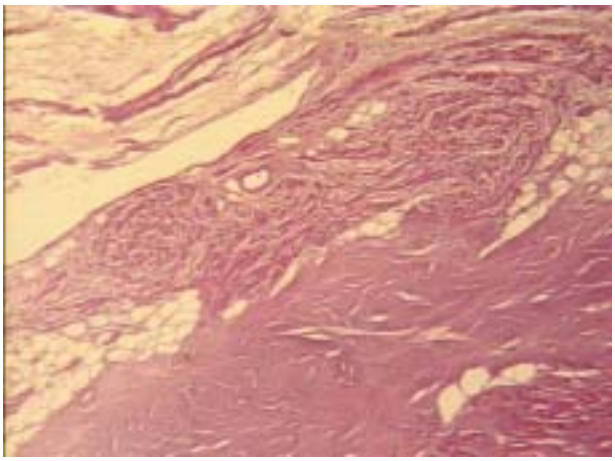

**Fig. 7.** Fatty infiltration of AV node (hematoxylin eosin x 40).

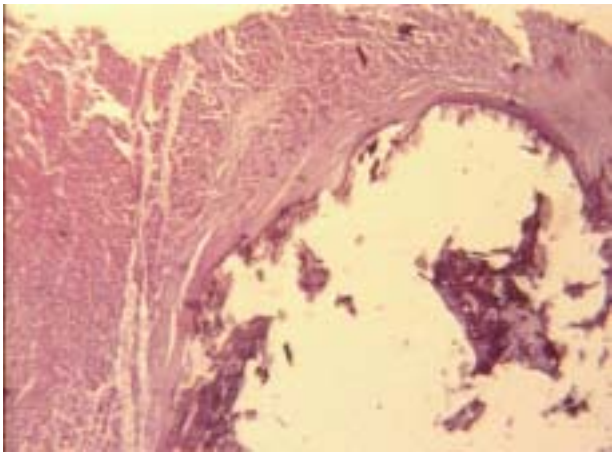

**Fig. 8.** Calcification of the AV node approaches (hematoxylin eosin x 40).

Fibrosis of AV node tissue was registrated in 10 (10%) of the specimens (Fig 4, 5, 6). In 11 specimens (11%) we found fatty infiltration of the AV node and its approaches (Fig. 7), and in 2 specimens calcification of the AV approaches (Fig. 8).

**Discussion**

Histological identification of the AV node tissue was done with the criteria established by David and Pomerance in 1975.

At the electronic microscopic level, there were fewer mitochondria and myofibrils, later arranged in a helter-scelter manner. The sarcoplasmatic reticulum was poorly developed and there was no transverse tubular system. Gap junctions were scare, but desmosomes were frequent (8).

The diameter of AV bundle cells was greater than that of AV node but smaller than that of ventricular cells. Their cytoplasm was stained lighter than that of the cells in the ventricles because there are fewer myofibrils. Striations were present on light microscopy. Mesothelial-like cells and spaces were present between the cells. Electron microscopy revealed more plentiful myofibrils and mitochondria than in the ventricular cells.

Histochemically, conductive cells had a well developed anaerobic oxidative system and poorely developed aerobic one, that is opposite of a contracting myocardial cells. The conduction system contained certain amount of cholinesterase not found in the working myocardium (8, 9).

Normal aging changes generally take the form of fatty infiltration, loss of cells with space formation, atrophy, hypertrophy, fibroelastosis, calcifications and fibrosis. Fatty replacement of the AV node and its approaches, with or without fibroelastosis, may give rise to AV block or to varying types of junctional arrhytmias and a reentrant type of arrhytmia at the AV junction. The fat may involve the AV bundle and the bundle branches, especially the bifurcating part of the AV bundle.

In our study, histological examination showed that 77 of 100 specimens had the usual structure of AV node. In 11 specimens (11%) we found fatty infiltration of AV node and its approaches. The majority of them (4%) were in the age group from 50 to 59 years and from 60 to 69 years (3%). Fibrosis of AV node tissue was registered in 10 (10%) of the specimens (age groups from 60 to 69 and from 70 to 79 years). Fatty infiltration and fibrosis affected not only the AV node tissue, but its approaches and atrial myocardium, as well.

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# TIPOVI NA DEVIJACIJA NA NOSNATA PREGRADA: EVALUACIJA NA KT SNIMKI NA PARANAZALNI SINUSI

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**Izvadok**

Celta na na{ata studija be{e da se napravi klasifikacija na devijacijata na nosnata pregrada i da se prika‘e prevalencijata kaj ispitanici so i bez promeni na sinusnata sluznica.

Vo studijata bea analizirani KT snimki na paranazalni sinusi od 100 slu~ajni ispitanici vo periodot od dekemvri 2006 do januari 2008 godina. Tie bea podlo‘eni na snimawe so KT imaging tehnika na Institutot za radiologija pri Medicinskiot fakultet vo Skopje. Ispitanicite bea na vozrast od 17 do 67 godini. Od niv 49 bea od ‘enski pol i 51 od ma{ki pol. Kaj 54 ispitanici be{e registrirano vospalenie na sinusnata sluznica.

Devijacija na nosnata pregrada (DSN) ja klasificiravme vo 7 tipovi spored klasifikacijata na Rao. Tip 1 go smetavme za normalna postavenost na nosnata pregrada koja ne go naru{uva normalniot protok na vozduh niz nosnata praznina. Tip 2 do tip 6 devijacii bea evaluirani kako prisutni na desnata ili levata polovina od nosnata praznina vo zavisnost na koja strana e konveksitetot na devijacijata.

Najvisoka prevalencija na devijacijata na nosnata pregrada vo odnos na tipot se registrira na tip 5 - levo 15,0 i tip 3 - desno 11.0. Kaj 31% od ispitanicite so vospalitelni promeni na sinusnata sluznica ima{e prisustvo na devijacija na nosnata pregrada bez zavisnost pome|u prisustvoto na septalen defekt i registracija na sinuzitis ($x^2$=1.38 i p=0,2409185).

***Klu~ni zborovi:*** nosna pregrada, anatomski varijacii, paranazalni sinusi

# TYPES OF SEPTAL NASAL DEVIATION: EVALUATION OF CT SCAN OF PARANASAL SINUSES

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**Abstract**

The aim of this study was to make a classification of septal nasal deviation and to show it is prevalence in patients with and without sinusitis.

In this study CT scans of paranasal sinuses from 100 patients were retrospectively analyzed in the period from December 2006 to January 2008. CT scan was performed on the CT scanner Somatom, Volume Zoom, Siemens, multislice 4, at the Institute of Radiology, Medical Faculty, Skopje. Out of the total number of patients, 49 were females and 51 males. The mean age of the patients was 36, ranging from 17 to 67. Fifty-four patients had pathological changes in sinus lining.

Septal nasal deviation (DNS) was classified in 7 types according to the classification by Rao. Type 1 we considered as normal nasal septum which does not disturb the airflow through nasal cavity. Types 2-6 we evaluated as deviation on left or right depending on which side was the convexity of the deviation.

The most present type was type 5 (48%) on left side, and type 3 (61%) on right side of the nasal cavity. 31% of patients with sinusitis had septal nasal deviation without clinical significance ($x^2$=1.38 and p=0, 2409185).

***Key words:*** nasal septum, anatomical variations, paranasal sinuses

## Introduction

The nasal septum is of fundamental meaning for the development of the nose and paranasal sinuses. For normal functioning of the nose it is important that left and right nostrils are in biological and mechanical balance, which means respiratory lining is normal and without deviation of nasal septum that will disturb the normal airflow through the nostrils. The nasal septum runs down the middle of the nose creating two sides of the nose, each containing a passageway that ends in a nare nostril. The posterior part consists of a bone, in the upper part perpendicular plate of the ethmoid bone, and down and posteriorly vomer, and the anterior part is made of nasal cartilage. Deviation of the nasal septum or nasal septum which is not in the midline where it is supposed to be is one of the most often present variations in nasoethmoid region (1, 2, and 3). These deviations, for the most part, cause no symptoms and require no treatment. However, the septal deviation may be severe enough to obstruct the passage of air through the nostrils. This obstruction may predispose the patient to sinusitis (2, 3, 4, 5). If the septal deviation corrupts the normal breathing, then the septoplasty is recommended, to correct a deformity of the nasal septum. It is sometimes referred to as submucous

resection of the septum (SMR) or septal reconstruction. The usual purpose is to improve nasal breathing, but it may also be performed to allow adequate examination of the inside of the nose for treatment of polyps, inflammation, tumours, or bleeding. When the nasal septum is deformed, there is no medicine that will cause it to be straightened, so surgery is the only solution to this problem. According to Akoglu et al. this anatomical variation is also connected to hypertrophy of inferior nasal turbinate on the opposite side of the deviation and reduction of nasal turbinate and septoplasty is recommended (6). On coronal and axial CT scan of paranasal sinuses localization of nasal septum or variations can be clearly seen . Knowing the relations of nasal septum with other structures in the nasal cavity will help for better performing of surgical treatment without complications. In the literature there are several classifications of septal nasal deviations and we have used the one by Rao et al. from 2005, which is a modification of one Mladina's made in 1987.

**Material and Methods**

Coronal CT scans of paranasal sinuses of 100 examinees were analyzed for septal nasal deviation and their association with inflammatory sinus disease. CT was performed on the 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 and axial sections at 2 mm distance, extension from inferior wall of maxillary sinus to roof of frontal sinus. The examinees were grouped according to sex, age and working diagnosis at the time of performing CT. Patients histories were not taken into account. A total of 100 CT scans were analyzed, with normal anatomy of paranasal sinuses.

In this study we have classified septal nasal deviation in 7 types, according to the classification done by Rao et al. in 2005.

Type 1: midline septum or mild deviation in vertical or horizontal plane, which does not extend throughout the vertical septal length;

Type 2: anterior vertical deviation;

Type 3: posterior vertical deviation (OM and middle turbinate area);

Type 4: "S" septum – posterior to one side and anterior to other side;

Type 5: horizontal spur on one side with or without severe deviation to the opposite side;

Type 6: type 5 with a deep groove on the concave side;

Type 7: combination of more than one type.

In types 2-6, the deviation is marked as left (L) or right (R). In type 4 anterior deviation is marked L or R.

Data were statistically analyzed with Statistics for Windows programme. All continuous variables were expressed as means +/- SD. The mean differences for continual variables were compared using the Student's t-test. Categorical variables were presented as percents, and differences were evaluated by Chi-square test. P value < 0, 05 was considered to be statistically significant.

**Results**

The study comprised 100 CTs of the nasal sinus region. Out of the total number of patients, 49 were females (49%) and 51 males (51%). The mean age of the patients was 36, ranging from 17 to 67. Septal nasal deviation was found in 55% of the patients. Type 1 we considered as normal nasal septum which does not disturb the airflow through nasal cavity. Only 52% of the patients had septal nasal deviation from type 2 to type 7.

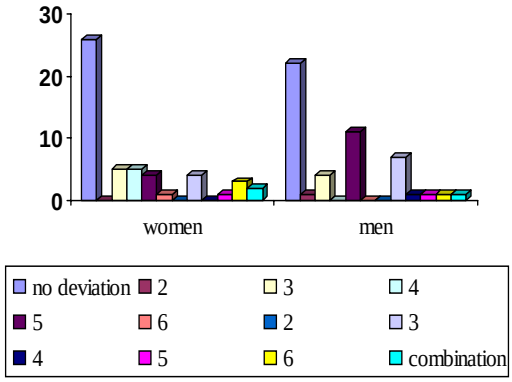


**Fig.1**. Distribution of examinees according to type of septal nasal deviation and sex

According to side of deviation the prevalence on the left was 31,0 and 18,0 on the right side, and combination of more than one type - 3, 0. The most present type was type 5 on the left (48,4%) and type 3 (61,1%) on the right side of the nasal cavity (Table 1).

**Fig. 2.** Type 1 normal nasal septum in the midline, which does not disturb the airflow through the nasal cavity

**Table 1.** Distribution of examinees according to type of septal nasal deviation (SND)

| | **Septal nasal deviation (DNS)** | | | |
|---|---|---|---|---|
| No deviation (type 1) | **number** | **%** | | |
| | **48** | **48.0** | | |
| Type | left | | right | |
| | **number** | **%** | **number** | **%** |
| anterior vertical deviation (2) | 1 | 3.2 | 0 | |
| posterior vertical deviation (OM and middle turbinate area)(3) | 9 | 29.0 | 11 | 61.1 |
| "S" septum – posterior to one side and anterior to other side (4) | 5 | 16.1 | 1 | 5.6 |
| horizontal spur on one side with or without severe deviation to the opposite side (5) | 15 | 48.4 | 2 | 11.1 |
| type 5 with a deep groove on the concave side (6) | 1 | 3.2 | 4 | 22.2 |
| **On both sides** | **number** | | **%** | |
| combination of more than one type (7) | 3 | | 3.0 | |

**Fig. 3.** Type 2 -anterior vertical deviation on the left side of the nasal cavity

**Fig.4.** Type 3- posterior vertical deviation (OM and middle turbinate area)

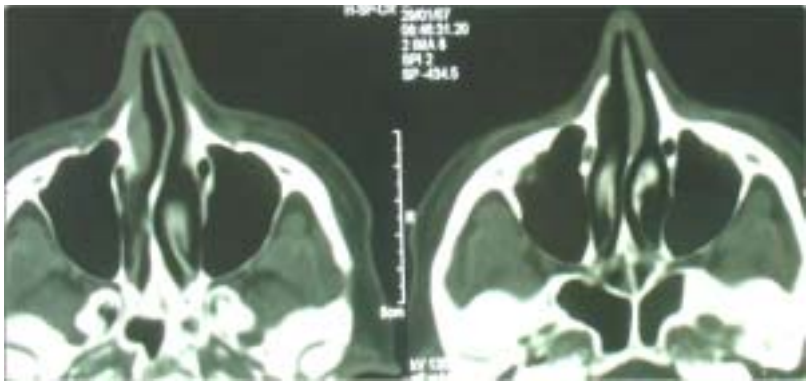

**Fig.5.** Type 4 -"S" septum – posterior curve to one side and anterior to other side (axial CT scan)

**Fig.6.** Type 5 - horizontal spur on the left side of the nasal cavity without severe deviation to the opposite side and hypoplastic middle turbinate on the same side.

**Fig.8.** Type 7- combination of more than one type – DSN type 3 on the right side and DSN type 5 on the left. On the level of type 3 deviation, a paradoxal right middle turbinate is seen

**Fig.7.** Type 6 - horizontal spur on the right side with deep groove on the concave side and hypoplastic middle turbinate on the side of deviation and mucosal changes on the left inferior nasal turbinate and left maxillary sinus lining

According to sex the prevalence of deviations in the period of examination was 25, 0 for men and 28, 0 for women, and in both sexes the prevalence was higher on the left side, 15,0 and 16,0 respectively (Fig 1.) The most present type in men was type 3 and type 4 (16, 1%) on the left side. In women the most prevailing deviation was type 3 on the right side of the nasal cavity (38, 9%).

Radiological detection of mucoperiosteal thickening and opacification of the sinuses were regarded as evidence of sinus disease. 54% of examinees had sinus disease and 46% had no pathological changes in sinonasal mucus. 31, 0% of the patients had septal nasal deviation and sinusitis. No statistical difference was found between septal deviation and registration of sinusitis ($x^2$=1.38 and p=0,2409185).

**Discussion**

Nasal septal deviation or nasal septum which is not located in the midline of nasal cavity and is deviated on the left or right side or has present bone spur, is sometimes associated with inflammatory sinus disease and is one of the most common sinonasal anatomical variants (1,2,3). If deviation is extreme, it can lead to obstruction in ostiomeatal region and can disturb the normal sinus secret drainage causing chronic sinusitis (2,3,4,5). In such a case septoplasty is recommended. In our study we have used the classification by Rao et al. and in 48% of our examinees we considered type 1 or normal nasal septum located in midline of nasal cavity which does not disturb the normal airflow through the nasal cavity. 52% of the examinees had NSD on the left or right side, type 2 to type 7.

The prevalence of deviation on the left side was 31,0 and on the right side 18,0, and combination of more than one type - 3,0. The most prevalent NSD on the left was type 5 (48,4%) and type 3 on the right side (61,1%). Peres-Pinaz et al. found nasal septal deviation in 58%, K. Dua et al. in 44% of their patients and according to them it was the most present variation in nasoethmoid region (2,7). According to sex, the prevalence of deviation was 25,0 in men and 28,0 in women. In both sexes deviations were more present on the left side of nasal cavity; type 3 and type 4 in women and type 3 and type 5 in men. Thirty-one (31,8%) of the patients had septal nasal deviation and sinusitis, 16 (24,2%) had sinusitis but without septal deviation (p=0,6836%). No statistical difference was found between septal deviation and registration of sinusitis ($x^2$=0,166 and p=0, 6836).Stallman et al. reported concha bullosa and contralateral nasal septal deviation as the most present variations in sinonasal anatomy (8). They reported NSD in 65% of examinees but without correlation with sinus disease.

It is very important these variation to be detected on CT scans of paranasal sinuses by the otolaryngologists for better understanding of sinonasal anatomy and pathology, as well as for easier preoperative planning and more safe performing of surgical technique. When diagnosing sinus disease it is important to determine the type of deviation on the left or right side of the nasal cavity, as well as presence of other variations like pneumatised middle turbinate- concha bullosa and paradoxal middle turbinate because they can cause narrowing of infundibulum or disturbed drainage of sinus secret.

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# DERMATOGLIFSKI ISTRA@UVAWA NA TRIRADIUSI KAJ MAKEDONSKATA POPULACIJA OD MA[KI POL

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**Izvadok**

Celi: da se utvrdat plantarnite triradiusi prisutni kaj ma{kata populacija od makedonska nacionalnost; da se ispitaat razlikite me|u dvete stapala vo odnos na zastapenosta na triradiusite, kako i da se sporedat so dobienite rezultati od istra‘uvawata kaj drugi populacii..

Bilateralni otisoci od stapalata na 200 ispitanici od ma{ki pol bea napraveni spored metodata na Cummins and Midlo. Otpe~atocite bea ~itani i klasificirani spored Henri-evata klasifikacija. Ispitani se razlikite me|u dvete stapala i napravena e komparacija so rezultati od istra‘uvawata na drugi avtori.

Na levoto stapalo od osnovnite triradiusi najzastapene d, potoa triradiusot a, pa b, p, e i c triradius.

Kaj dopolnitelnite triradiusi dominira pm, potoa p’, p”, a retko se zastapeni e’ i d’.

Od osnovnite triradiusi na desnoto stapalo najzastapen e b, pa triradiusite a i d, a p, e i c triradiusite se najmalku zastapeni.

Kaj dopolnitelnite triradiusi dominira pm, potoa sledat p”, p’, e’, a d’ e najretko zastapen.

Rezultatite od na{eto istra‘uvawe ja poka‘uvaat zastapenosta na triradiusite kaj makedonskata populacija od ma{ki pol.Analiziranite atributivni parametri pome|u levoto i desnoto stapalo kaj ma‘ite poka‘uvaat deka signifikantni razliki ne se registrirani.Dobienite rezultati ovozmo‘uvaat nivna komparacija so rezultatite od istra‘uvawata sprovedeni kaj drugi nacionalnosti.

***Klu~ni zborovi***: triradius, ma{ki pol, otpe~atoci od stapalo.

# DERMATOGLYPHIC THREE-RADII RESEARCH OF THE MACEDONIAN MALE POPULATION

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**Abstract**

Aims: To determine the plantar three-radii found in male population of Macedonian nationality;to determine bilateral differences of the present three-radii and to compare them with the existing results from other populations.

Bilateral footprints of 200 male examinees were made following the Cummins and Midlo method, read and classified by Henri’s classification. The differences between the two feet were examined and compared with the results of studies carried out by other authors.

The most common of the basic three-radii on the left foot is d, followed by the three-radii a, then b, p, e and c three-radii.

Of the additional three-radii pm predominated, followed by p’, p”, e’ and d’ were very rarely present.

The most common basic three-radius on the right foot is b, followed by three-radius a and d, then p, e and three-radii c is the least common.

Of the additional three-radii pm predominated, followed by p”, p’, e’ and d’ is the least frequent.

The results of our research have shown the presence of three-radii in male population of Macedonian nationality.There aren’t any significant differences in the analyzed relations between the left and the right foot of men.The obtained results were compared with the results as carried among other nationalities.

***Key words***: three-radii, male, footprints.

## Introduction

Dermatoglyphics are patterned traceries of the epidermal ridges on fingers and palms. Palmar and finger dermatoglyphics are formed on the surface of the hand early in intrauterine life (1).

The development of the ridges and three-radii begins with the formation of pads in the fingers, toe and other areas of the embryo’s sole, during the second month of intrauterine life. Epidermal ridges appear on the surface of the sole after the regression of the pads by the end of the fourth foetal month. After this period, dermatoglyphic patterns remain unchanged during the whole life (2).

Three-radius is the centre where three almost parallel lines from three different fields meet. The centre is the point at which three radiants that form a 120º angle of each other meet, thus separating the three adjoining regions. Each of the angles between the radiants has to be larger than 90º, otherwise there won’t be a three-radius (Fig. 1) (3).

Three-radii are the grounds of Galton's system of classification. He describes the terms arc, loop and circle, emphasizing the details for taking footprints as well as following the skin ridges (4).

**Fig. 1.**

At the toe's base there are several three-radii marked with the small letters of the alphabet in tibia-fibular direction a, b, c, d; at the thumb's base e three-radius , and proximally to them an additional p three-radius. The other additional three-radii which can be found proximally to the others are marked with an apostrophe, e.g. p', p'' (5).

The initial interest of dermatoglyphes is based on their usefulness in identification and then to discovery of the biological value of the footprints differencces among nationalities.

Many authors conducted researches of three-radii and analyzed the difference between certain populations.

**Material and Methods**

The study included a sample of 200 male examinees at the age of 16-20 of Macedonian nationality, from the Medical Faculty in Skopje.

The footprints of both feet were taken following the method of Cummins and Midlo (6). Henry's method was used to determine and classify the configuration types noted and expressed in percents.

We analyzed the present digital three-radii a, b, c, and d as well as p lower three-radius according to Wilder, which is proximally placed in relation to the other three-radii (7).

The Spearman's test was used to determine bilateral differences and correlation and the results were compared with those of other authors.

**Results**

The distribution of three-radii on the men's left foot is shown in chart 1.

Of the basic three-radii, a was registered in 180 (90.0%) of the total number of examinees, the three-radius d was most common found in 188 (94.0%) women, b in 176 (88.0%), p in 96 (48.0%), e in 74 (37.0%) and c in 57 (28.5%).

Of the additional three-radii pm predominated and was registered in 92 (46.0%) examinees, p' was found in 38 (19.0%), p" was also found in 38 (19.0%) of the examinees, e' in 29 (14.5%), and d' was rarely found only in 1 subject.

The distribution of three-radii on the men's right foot is shown in chart 2.

Of the basic three-radii, b was the most common one and it was registered in 180 (90.0%) of the total number of examinees, three-radii a was found in 175 (87.5%) and d was found in 178 (89.0%) women, c in 57 (28.5%), p in 106 (53.0%), e in 70 (35.0%).

Of the additional three-radii pm predominated and it was registered in 86 (43.0%) examinees, p' in 41 (20.5%), p" was found in 44 (22.0%), e' in 36 (18.0%), and d' in 2 (1.0%).

Chart 3 shows the differences of the analyzed attributive parameters between men's left and right foot.

No significant differences were registered in the analyzed relations.

Chart 4 shows the relation of the examined parameters between men's left and right foot by using the values of the examined correlation

**Table 1**. Distribution of three-radii- male- left

| Three-radii | present number | % | absent number | % |
|---|---|---|---|---|
| a | 180 | 90 | 20 | 10 |
| b | 176 | 88 | 24 | 12 |
| c | 57 | 28.5 | 143 | 71.5 |
| d | 188 | 94 | 11 | 5.5 |
| e | 74 | 37 | 126 | 63 |
| p | 96 | 48 | 104 | 52 |
| d' | 1 | 0.5 | 199 | 99.5 |
| e' | 29 | 14.5 | 171 | 85.5 |
| p' | 38 | 19 | 162 | 81 |
| p" | 38 | 19 | 162 | 81 |
| pm | 92 | 46 | 108 | 54 |

**Table 2**. Distribution of three-radii- male- right

| Three-radii | | present | | absent | |
|---|---|---|---|---|---|
| | | number | % | number | % |
| basic | a | 175 | 87.5 | 25 | 12.5 |
| | b | 180 | 90.0 | 20 | 10.0 |
| | c | 57 | 28.5 | 143 | 71.5 |
| | d | 178 | 89.0 | 20 | 10.0 |
| | e | 70 | 35.0 | 94 | 47.0 |
| | p | 106 | 53.0 | 94 | 47.0 |
| accesorii | d’ | 2 | 1.0 | / | / |
| | e’ | 36 | 18.0 | / | / |
| | p’ | 41 | 20.5 | 159 | 79.5 |
| | p” | 44 | 22.0 | 156 | 78.0 |
| | pm | 86 | 43.0 | 114 | 57.0 |

**Table 3.** Differences: male left-right (attributive)

| | parameter | U / D | Z | p-level | p | Sig./N.Sig. |
|---|---|---|---|---|---|---|
| Three-radii | a | 19500.00 | 0.432472 | 0.665398 | p>0.05 | N.Sig. |
| | b | 19600.00 | -0.345978 | 0.729360 | p>0.05 | N.Sig. |
| | c | 19943.00 | -0.049302 | 0.960679 | p>0.05 | N.Sig. |
| | d | 19199.00 | 0.692821 | 0.488423 | p>0.05 | N.Sig. |
| | e | 19383.00 | -0.533671 | 0.593570 | p>0.05 | N.Sig. |
| | p | 19000.00 | -0.864945 | 0.387070 | p>0.05 | N.Sig. |
| | p1 | 19700.00 | -0.259483 | 0.795262 | p>0.05 | N.Sig. |
| | p 2 | 19400.00 | -0.518967 | 0.603784 | p>0.05 | N.Sig. |
| | pm | 19400.00 | 0.518967 | 0.603784 | p>0.05 | N.Sig. |

**Table 4**.Correlations male left-right foot (atributive)

| Parameter | Spearman R | |
|---|---|---|
| Three-radii | a | 0.43 |
| | b | 0.39 |
| | c | 0.46 |
| | d | 0.34 |
| | e | 0.35 |
| | p | 0.58 |
| | p’ | 0.23 |
| | p “ | 0.41 |
| | pm | 0.49 |

When it comes to the basic three-radii p (0.58),there is a very strong correlation between left and right foot, for a (0.43), pm (0.49), b (0.39), c (o.46), p”(0.41) there is medium strong correlation between left and right foot, and poor connection of d (0.34), e (0.35) and p’ (0.23). For the last three there is a weak correlation between the two feet.

**Discussion**

The obtained results have shown that in men, the distribution of three-radii on the left foot is as follows: of the basic three-radii d>a>b>p>e>c, and of the additional three-radii pm>p’=p’’>e’>d’.

On the right foot the distribution of the basic three-radii is b>d>a>p>e>c, and of the additional three-radii pm>p”>p’>e’>d’.

The bilateral differences between the two feet in the male population of Macedonian nationality are insignificant.

P three-radius is more present on the right foot than on the left one in both sexes, which is in agreement with the results in the research carried out by Takeya (1936) (8). Fox and Plato (1987) analyzed three-radii in 168 men and 83 women in America and their presence was the same as in our study whereas the additional three-radii were more frequently found in male than in female (9). The obtained results in Flugel, Greil and Sommer (1986) were the same as ours (10).

Although the investigation of sole prints is yet another interesting study, not many investigators have completed their studies and published their findings and the exercises has to be repeated. In order to obtain more precise and reliable results, we need more investigations in our and in other regions.

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# NUTRITIVNI PARAMETRI NA NADLAKTOT VO PROCENA NA NUTRITIVNIOT STATUS KAJ DECA NA VOZRAST OD 8 GODINI

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**Izvadok**

Cel na studijata pretstavuva { e evaluacija na nutritivnite parametri na nadlaktot vo procena na nutritivniot status kaj deca na vozrast od 8 godini.

Vo studijata bea vklu~eni 225 zdravi deca na vozrast od 8 godini (115 ma{ki, 110 'enski), od makedonska nacionalnost, od razli~ni urbani regioni na Makedonija. Merewata bea vr{eni spored IBP so standarna tehnika i oprema za merewe. Izvedeni se i presmetani antropometriski indeksi (te'ina-za-vozrast, visina-za-vozrast, BMI i nutritivni parametri na nadlaktot: TUA-vkupna povr{ina, UMA-muskulna povr{ina, UFA-masna povr{ina i AFI- masen indeks).

Rezultatite poka'aa postoewe na signifikantna polovo-specifi~na razlika samo kaj ko'nite dipli, masnata povr{ina i masniot indeks vo korist na ispitanicite od 'enski pol. Grani~nite vrednosti za 5$^{ot}$ i 85$^{ot}$ percentil kaj na{ite 8 godi{ni ispitanici od ma{ki pol iznesuvaat: 23.45 kg i 40 kg za te'inata, 122.67 cm i 138.8 cm za visinata, odnosno 14.16 kg/m$^2$ i 21.35 kg/m$^2$ za BMI. Soodvetnite vrednosti kaj 'enskite deca iznesuvaat: 23 kg i 37.5 kg za te'inata, 122.4 cm i 137.9 cm za visinata, 14.6 kg/m$^2$ i 21.45 kg/m$^2$ za BMI. Vrednostite za 50$^{ot}$ percentil na nutritivnite parametri kaj ma{kite ispitanici iznesuvaat: 30.9 cm$^2$ TUA, 22.06 cm$^2$ UMA, 8.65 cm$^2$ UFA, odnosno 30.68% AFI. Soodvetnite vrednosti kaj 'enskite ispitanici imaat vrednosti od: 32.17 cm$^2$ TUA, 21.65 cm$^2$ UMA, 10.9 cm$^2$ UFA, odnosno 34.84% AFI.

Se prepora~uva nutritivnite parametri na nadlaktot da najdat primena vo evaluacija na nutritivniot status kaj deca od 8 godi{na vozrast vo Makedonija.

***Klu~ni zborovi***: antropometrija, detska populacija, nutritiven status, nutritivni parametri

# THE NUTRITIONAL PARAMETERS OF UPPER ARM FOR ASSESSMENT OF NUTRITIONAL STATUS IN THE 8 YEAR-OLD-CHILDREN

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**Abstract**

The aim of the study was the evaluation of the nutritional parameters of upper arm for assessment of nutritional status in 8- year-old children.

In the study were examined 225 healthy 8 year-old-children (115 male, 110 female) Macedonian nationalities, from different regional urban area from Macedonia. Measurements are done using the methodology of the IBP with standard technic of the measurements. A few anthropological indexes were calculated:(weight-for-age, height-for-age, BMI, and nutritional parameters of upper arm (TUA – upper arm area, UMA-upper arm muscle area, UFA-upper arm fat area and AFI arm fat index.

Results showed sex-specific differences only the skin folds, fat-area and fat index in favor of the female examinees. Border values for 5$^{th\ and}$ 85$^{th}$ percentile as a cut off points of our 8 year-old-male examinees are following: 23.45 kg and 40 kg for weight-for-age, 122.67cm and 138.8cm for height-for-age, 14.16 kg/m$^2$ and 21.35 kg/m$^2$ for the BMI. The values of the female examinees are following: 23 kg and 37.5 kg for weight- for-age, 122.4 cm and 137.9 cm for height-for-age and 14.6 kg/m$^2$ and 21.45 kg/m$^2$ for BMI. The values of the 50$^{th}$ percentile for the nutritional parameters of the male examinees are following:30.90 cm$^2$ TUA, 22.06 cm$^2$ UMA, UFA 8.65 cm$^2$ and 30.78% AFI and for the female are 32.17cm$^2$ TUA, 21.65 cm$^2$ UMA, 10.9 UFA cm$^2$ and 34.84 % AFI.

It is recommended that these nutritional parameters of the upper arm should be used for assessment of nutritional status of the 8 year-old-child in Macedonia.

***Key words***: anthropometry, child population, nutritional status, parameters

## Introduction

The global trend of weight gain imposed the necessity of applying anthropometry as a fast, economical, noninvasive and easily applicative method for assessment and evaluation of the nutritional status (1). The anthropometric research used for its assessment gives us the opportunity to monitor hormonal changes in growth and maturation, and to early discover any disruptions in that period. Growth can be especially sensitive to nutritional deficit or surplus, so anthropometry also

provides indexes for the nutritional status as well as detection of possible health risks (2). In order to evaluate the level of nutrition precisely, Frishanko points out that we should determine the muscular and mass area of the upper arm, basing this on the fact that the upper arm and its constituents (muscle, fat and bone) are cylindrical. Knowing the value of triceps skin fold, he constructs so-called nutritional parameters of the upper arm, and also works out standard values (3).

**Aim**

Evaluation of nutritional parameters of the upper arm in the assessment of the nutritional status in 8 year-old-children.

**Material and Methods**

***Subjects***

The study included a sample of examinees defined as a population of 8 year-old-school children of Macedonian nationality from different regional urban area of Macedonia. It was a random choice. The total number (n= 225) of examined children according to the sex criterion was divided in two groups (n=115 male, and n = 110 female examinees).

***Anthropometric procedures***

The following anthropometric parameters were measured: weight, height, circumference of the upper arm and skin folds (scapula, triceps), and they were done using the methodology of IBP (International Biological Programme) with standard equipment and measurement technique. The following standard anthropological instruments were used: anthropometer by ″Martin″ for height with 1 mm reading accuracy; medical decimal scales with 0.1 kg accuracy; elastic plasticized band for circumference with 0.1 mm reading accuracy, caliper John – Bull for the skin folds with 0.1mm reading accuracy.

The following indexes were also drawn: weight-for-age, height for age and BMI (as a relation between the weight and the height of a square) for the corresponding age. The nutritional parameters of the upper arm were also calculated: the total (TUA), the muscle (UMA), the fat area (UFA) as well as the fat index (AFI) using formulas created by Frisanko (3).

TUA = uac $^2$ / 4 X 3,14 ; TUA - the total area of the upper limb (cm$^2$); uac- circumference of the upper limb (cm), UMA = [uac–(tc x 3,14) $^2$/ 4 x 3,14, UMA-muscle area of the upper limb (cm$^2$), tc (mm)-skin fold over triceps, Ufa = tua – uma; Ufa - fat area of the upper limb (cm$^2$), Afi = (ufa / tua) x 100; Afi - fat index of the upper limb.

According to several authors, the following percentile ranks are recommended for defining the anthropometric indicators:

The normal distribution usually corresponds to the percentile rank from the 15$^{th}$ to the 85$^{th}$ percentile; the percentile rank from the 5$^{th}$ – 15$^{th}$ percentile for under average values which still can't categorize the child in the group of underfed; as opposed to this, the values under the 5$^{th}$ percentile with a bigger possibility point to the undernourishment or underweight children; children with values between the 85$^{th}$ and 95$^{th}$ percentile for the parameters weight-for-age and BMI-for-age, are defined as overweight and these children run the risk of becoming obese; the values over the 95$^{th}$ percentile point to the category of obese children. (4-7).

***Statistical analysis***

The gathered data for the relevant variables were analyzed with a descriptive statistics represented by: central tendency measures (average), measures for deviation from the average (standard deviation) and ranges (percentiles). The importance of the differences between two arithmetic series was tested with ANOVA. The values of $p<0.05$ were taken as significant differences.

**Results**

The average values, standard deviations of the examined anthropometric parameters, BMI and the statistical analysis with the ANOVA test are shown in table 1.

The average values of weight and height in 8-year-old male children were 32.41 kg ±7.67 i.e. 131.83cm ±6.69, and in female children 30.98 kg ±6.08 and 130.79cm ± 6.06. In male children the average value of BMI was 18.46kg/m $^2$±3.03, whereas in female examinees it was 18.02 kg/m $^2$±2.78. The statistical analysis with the ANOVA test of the total number of examined male and female children

**Table 1.** Values of the anthropometric parameters for the 8 year-old-children.

| | **Body weight(kg)** | **Body height (cm)** | **BMI(kg/m$^2$)** | **Mid upper *arm circumf (cm)*** | *Skinfolds(mm)* ***Scapula*** | ***Triceps*** |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| **X** | 32.41 | 131.83 | 18.46 | 19.71 | 8.05 | 10.73 |
| **SD** | 7.67 | 6.69 | 3.03 | 2.77 | 3.22 | 3.45 |
| **Female** | | | | | | |
| **X** | 30.98 | 130.79 | 18.02 | 20.13 | 9.43 | 12.08 |
| **SD** | 6.08 | 6.06 | 2.78 | 2.38 | 2.99 | 3.08 |
| **(p)** | P>0.05 | P>0.05 | P>0.05 | P>0.05 | P<0.05 | P<0.05 |

showed that there is a statistically significant difference only with regard to skin folds, in favor of the female examinees. The sex-specific percentiles of the indicators weight-for-age, height-for-age and BMI-for-age are shown in table 2. The cut off points for these indicators were calculated on the 5th and the 85th percentile and they had the following values for our 8-year-old male examinees: for weight 23.45 kg for the 5th percentile, i.e. 40 kg for the 85th percentile, 122.67cm and 138.8 cm for height and 14.16 kg/m$^2$ and 21.35 kg/m$^2$ for BMI. For our 8-year-old male examinees we got the following values: 23 kg and 37.5 kg for weight, 122.4cm and 137.9 cm for height, i.e. 14.6 kg/m$^2$ and 21.45 kg/m$^2$ for BMI.

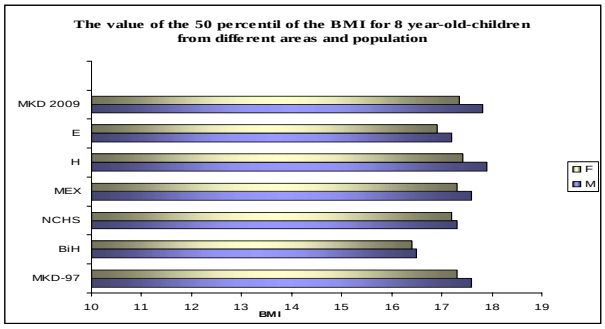


**Fig. 1.** The values of the 50th percentile of the BMI for the 8-year-old-children from different areas and population

**Table 2.** Sex -specific percentiles of the indexes: weight-for-age,height-for-age and Body Mass Index of the 8-year-old children

| | **Percentile** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **MALE** | **5** | **10** | **15** | **25** | **50** | **75** | **85** | **90** | **95** |
| Weight-for-age | 23.45 | 25.00 | 25.00 | 26.25 | 31.00 | 36.75 | 40.00 | 44.00 | 47.00 |
| Height-for-age | 122.67 | 124.30 | 125.34 | 127.03 | 131.80 | 135.88 | 138.80 | 140.55 | 144.31 |
| BMI-for-age | 14.16 | 14.80 | 15.50 | 16.47 | 17.82 | 20.62 | 21.35 | 22.25 | 23.45 |
| **FEMALE** | | | | | | | | | |
| Weight-for-age | 23.00 | 24.50 | 25.00 | 26.00 | 30.00 | 34.50 | 37.50 | 40.00 | 42.00 |
| Height-for-age | 122.40 | 123.10 | 124.50 | 126.45 | 130.50 | 135.30 | 137.90 | 139.10 | 140.05 |
| BMI-for-age | 14.60 | 14.87 | 15.35 | 15.89 | 17.36 | 20.13 | 21.45 | 22.14 | 22.53 |

**Table 3.** Sex specific percentile of the nutritional parameters of upper arm (TUA–upper arm area,UMA-upper arm muscle area, UFA-upper arm fat area and AFI arm fat index) for the 8-year- old children.

| | **Percentile** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | | | | | | |
| | **X** | **SD** | **Med** | **5** | **10** | **15** | **25** | **50** | **75** | **85** | **90** | **95** |
| TUA (cm$^2$) | 31.53 | 8.94 | 30.90 | 18.99 | 20.89 | 22.47 | 24.38 | 30.90 | 36.80 | 40.31 | 42.12 | 48.01 |
| UMA (cm$^2$) | 21.56 | 5.11 | 22.06 | 13.58 | 14.31 | 16.5 | 17.24 | 22.06 | 24.14 | 26.86 | 27.76 | 29.29 |
| UFA (cm$^2$) | 9.97 | 4.39 | 8.65 | 4.93 | 5.48 | 5.90 | 6.68 | 8.65 | 12.40 | 14.66 | 15.95 | 19.18 |
| AFI (%) | 30.68 | 5.93 | 30.78 | 22.02 | 22.97 | 23.80 | 26.63 | 30.78 | 35.1 | 36,23 | 38.01 | 40.63 |
| **Female** | | | | | | | | | | | | |
| TUA (cm$^2$) | 32.7 | 7.63 | 32.17 | 19.88 | 22.47 | 24.38 | 27.25 | 32.17 | 36.80 | 39.24 | 41.39 | 46.44 |
| UMA (cm$^2$) | 21.43 | 3.98 | 21.65 | 15.07 | 15.72 | 16.78 | 18.51 | 21.65 | 23.46 | 24.78 | 26.28 | 27.17 |
| UFA (cm$^2$) | 11.27 | 3.9 | 10.9 | 4.80 | 6.07 | 7.34 | 8.46 | 10.96 | 13.68 | 14.70 | 15.16 | 18.39 |
| AFI (%) | 33.6 | 4.92 | 34.84 | 24.17 | 27.03 | 27.48 | 29.85 | 34.84 | 37.00 | 37.85 | 38.75 | 40.16 |

The average values, standard deviations, median, statistically significant differences and sex-specific percentiles of the nutritional parameters of the upper arm in 8-year-old male and female examinees are shown in table 3.

The average value of TUA – the total area of the upper limb in 8-year-old male examinees is 31.53cm$^2$ 8.94, and 32.7cm$^2$ ± 7.63 in female examinees. The muscle area of the upper limb in male children has a average value of 21.56cm$^2$ ±5.11, and in female children it is 21.43cm$^2$ ± 3.98.

UFA – fat area is 11.27cm$^2$ ±3.9 in female, and 9.97cm$^2$ ± 4.39 in male examinees, and AFI – fat index is 33.6 in female 8-year-old children and 30.68% in male children.

**Discussion**

Several anthropometric parameters were examined in our study, parameters which are used for assessment of the nutritional status in the children population.

The obtained values also provide us with the possibility to compare our own values with the corresponding anthropometric examinations done with children from other regions and populations as well as with children from our region. Namely, Todorovska L. (1997) in her vast anthropometric study of school children at the age of 7-15 includes the age of 8, which was examined in our study. The values on the 50$^{th}$ percentile of the weight and height-for-age indexes in our 8-year-old male examinees are 31 kg and 131.8 cm i.e. 30 kg, and 130.5 cm for female examinees. The obtained values are slightly higher than the values in Todorovska's study, but lower than the values on the corresponding percentile according to NCHS– referent population which are 32.7kg and 132.5 cm for male children, 31.9 kg and 131 cm for girls (4,8). Figure 1 shows the values of BMI on the 50$^{th}$ percentile for children from our study (MKD 2009) compared to the corresponding values of BMI in children from other areas and populations (Bosnia and Hercegovina-BiH, Spain-E, Mexico-MEX, referential NCHS, Todorovska MKD-97, Hungary-H) (4, 9-12). The cut off points on the 85$^{th}$ and 95$^{th}$ percentile for identifying individuals with a risk of becoming overweight and obese in 8-year-old male children in our country are 21.35 and 23.45 and they are higher than the ones published by Cole (18.69 for the 85$^{th}$ and 22.17 for the 95$^{th}$ percentile). Female examinees also have higher values of BMI on the corresponding points (21.45 for the 85$^{th}$ and 22.53 for the 95$^{th}$ percentile) as opposed to 18.76 for the 85$^{th}$ and 22.18 for the 95$^{th}$ percentile (13).

We should mention that when a total evaluation of the nutritional status was conducted, there was a suggestion for additional options with a combination of several more indicators. For that purpose, the nutritional parameters of the upper arm, which represent the peripheral mass component, were also calculated. UMA, or muscle area of the upper arm, should be presented not only with regard to age, but also with regard to height (3,4). Our male examinees have a value of 22.06 for UMA on the 50$^{th}$ percentile, which is lower than the referential 20.89, and in female examinees it is 21.65 unlike 20.34 published by Frisanko (3). For UFA, or fat area of the upper arm, our male examinees have higher value 8.65 than the referential 7.52 (Frisanko), and the female examinees have a value of 10.9 which is slightly higher than the referential 10.42.

These values are just another confirmation of the WHO request for creating our own, clearly defined and precise anthropometric criteria for classification and detection of nutritional problems in children of all age groups, criteria which should arise from measurements conducted on our own population.

**Conclusion**

Based on the results, we can draw the following conclusions:

Male examinees at the age of 8 have slightly higher average values for weight, height and BMI compared to female examinees. Statistically significant sex-specific differences were registered only in skin folds, in favor of the female examinees. Of the nutritional parameters of the upper arm, fat area and the fat index are statistically significantly higher in female, whereas muscular area is slightly higher in male examinees.

The results obtained in this study can be used as anthropometric criteria for assessment and evaluation of the nutritional status of 8 year old children from different regions in Macedonia. They should contribute to recognizing the most common nutritional risks in 8 year old children. They should also signalize certain misbalances and present criteria for selecting individuals for further clinical examinations. By defining the border values as cut off points we should identify children who need nutritional intervention. Undoubtedly, this has huge practical importance for planning certain preventive measures and activities in the field of child nutrition.

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# ULTRAZVU^NI MEREWA NA FETALNITE PARAMETRI

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**Izvadok**

Cel na ovoj trud e da se prika‘e povrzanosta na parametrite dobieni so ultrazvu~no merewe in utero, so istite dobieni ex utero.

Studijata opfa}a merewa na 150 fetusi in utero i 300 fetusi ex utero podeleni vo 5 starosni grupi i toa prva grupa (11-13 g.n); vtora grupa (14-16g.n.); treta grupa (17-19g.n.); ~etvrta grupa (20-22 g.n.) i petta grupa (23-25g.n). Merewata so ultrazvuk bea vr{eni so ATL Ultrasound HDI 1000, a merewata ex utero bea izvr{eni spored metodologijata na Internacionalniot Biolo{ki Program (IBP), na prethodno markirani antropometriski to~ki i nivoa neophodni za merewe.

Izmereni bea slednive antropometriski parametri i toa biparietalen dijametar, obem na glava, dol‘ina na femur, obem na abdomen i transverzalen cerebelaren dijametar. Za taa cel bea sporeduvani vrednostite od prethodno izvr{enite merewa, so ultrazvu~nite vrednosti na istite parametri vo istite gestaciski nedeli. Rezultatite poka‘aa deka merewata dobieni so ultrazvuk i antropometriskite merewa nemaa nekoi pogolemi otstapuvawa vo odnos na dobienite vrednosti osven vrednostite na dol‘inata na femur i obemot na abdomen.

Antropometriskite merewa se korisni za identifikacija na fetalnite anomalii, abnormalniot fetalen rast i se koristat kako dopolnitelni metodi vo procenka na fetalniot rast i razvoj. Ultrazvu~nata metoda e metoda na izbor vo dene{no vreme koja ovie parametri gi meri pri rutinski pregledi i u{te vo ranite gestaciski nedeli t.e. prviot trimestar od intaruteriniot rast, mo‘e da uka‘e na fetalnite abnormalnosti i e korisna za nivna detekcija.

***Klu~ni zborovi:*** fetus, gestaciska nedela, antropometriski parametri, ultrazvuk

# FETAL MEASUREMENTS COMPARED WITH ULTRASOUND

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**Abstract**

The aim of this study is to show correlation between some anthropometrical parameters of fetuses ex utero and compared with fetuses in utero.

The total number of fetuses (n=150) in utero, and (n=300) fetuses ex utero , according to gestational age was divided in five groups. Measurements with ultrasound were done with ATL Ultrasound HDL-100.

Anthropological measurements were done using the methodology of the International Biological Programe with standard technique of the measurements.

Some anthropometrical parameters were analyzed: biparietal diameter, head circumference, femur length, abdominal circumference, body weight and transversal cerebellar diameter.

Results showed that was not a difference between measurements with ultrasound and anthropometrical measurements, except in femur length and abdominal circumference. These anthropometrical measurements are useful in identification of some fetal anomaly and fetal intrauterine retardation in fetal growth. Ultrasonography is a method of choose in the future were those parameters were measurements in routine practice to determine the fetal abnormality in early gestational age in first trimester of intrauterine growth.

***Key words***: fetus, gestational age, anthropometrical parameters, ultrasound.

## Introduction

Gestational age (length of fetal life) is determined with real parameters in modern midwifery, which as routine and technical clinical methods are used in determining gestational age as prenatal estimation. The ultrasound method is one of the recommended methods for estimating gestational age. It provides visualization of the uterus

and the fetal structures within it. The advantage is that the same method enables measurement of individual fetal structures. The values obtained from the corresponding measurements compared to the standard values obtained for individual gestational age are an indirect method for determining the same. Campbell and Dewurst (1) suggested a method of serial echo-cephalometry for measuring the fetal growth. They suggested different intervals between certain measurements of the biparietal diameter for following the growth of the fetal head. A large number of authors agree that this parameter can help diagnose intrauterine stagnation in the growth of the fetus in 50-60% of the cases. The ultrasound as a method of choice in modern diagnostics in obstetrics and gynecology was introduced in 1961. In 1968 in Great Britain, Campbell (1) introduced an original technique for measuring the fetus intrauterine with ultrasound, and after him a number of other authors worked on the value of ultrasound cephalometry (2). Modern ultra-sonogrpahy does not only deal with the fetal head, but also determines abdominal circumference, measures the length of the limbs, anter-posterior diameter of the chest, the function of the internal organs, as well as recognizing possible malfunctions of the fetus on time.

**Aim**

Comparison of the values obtained from previously conducted anthropometric measurements with the ultrasound values of the same parameters in same gestational groups.

**Material and Methods**

The material includes 150 fetuses measured in utero and 300 fetuses measured in ex utero, divided in 5 age groups: first group (11-13 g.w.); second group (14-16g.w.); third group (17-19g.w.); fourth group (20-22 g.w.) and fifth group (23-25g.w). Fetuses without any visible macroscopic malformations served as criteria. The following anthropometric parameters were measured: biparietal diameter, head circumference, abdomen circumference, femur length and transversal cerebral diameter. Ultrasound measurements were conducted with ATL Ultrasound HDI 1000, with trans-abdominal probe of 3.5 MHZ, and ex utero measurements were conducted according to the Methodology of the International Biological Program (IBP), with standard equipment and measurement techniques.

Central tendency and variability were used from the descriptive statistics, SD, X, MED, MIN, MAX. The correlation between certain anthropometric parameters was determined with regressive analysis and correlating coefficients.

**Results**

Results show that weight, as a basic characteristic and indicator of the physical growth, is of great practical importance because it supplies information whether the

**Table 1.** Values of some anthropometrical parameters measured with ultrasound in different gestational weeks

| (11-13 g.w) | BPD(cm) | Head circumf (cm) | Femur lenght (cm) | Abdom circumference | TCD(cm) |
|---|---|---|---|---|---|
| X | 2.0 | 2.46 | 0.96 | 6.3 | 1.85 |
| SD | 0.33 | 0.23 | 0.23 | 1.0 | 0.28 |
| MIN | 1.6 | 2.1 | 0.8 | 5.2 | 1 |
| MAX | 2.4 | 2.9 | 1.1 | 7.4 | 2.5 |

**Table 2.** Values of some anthropometrical parameters measured with ultrasound in different gestational weeks

| (14-16 g.w) | BPD(cm) | Head circumf (cm) | Femur lenght (cm) | Abdomcircumference | TCD(cm) |
|---|---|---|---|---|---|
| X | 3.16 | 3.93 | 1.8 | 9.53 | 2.08 |
| SD | 0.43 | 0.3 | 0.35 | 1.0 | 0.38 |
| MIN | 2.8 | 3.4 | 1.5 | 8.4 | 1.8 |
| MAX | 3.6 | 4.6 | 2.2 | 10.6 | 2.9 |

**Table 3.** Values of some anthropometrical parameters measured with ultrasound in different gestational weeks

| (17-19 g.w) | BPD(cm) | Head circumf (cm) | Femur lenght (cm) | Abdomcircumference | TCD(cm) |
|---|---|---|---|---|---|
| X | 4.2 | 5.36 | 2.76 | 13.3 | 2.15 |
| SD | 0.46 | 0.33 | 0.46 | 1.50 | 0.42 |
| MIN | 3.9 | 5.0 | 2.5 | 12.0 | 1.5 |
| MAX | 4.5 | 5.7 | 3.0 | 14.0 | 3.3 |

**Table 4.** Values of some anthropometrical parameters measured with ultrasound in different gestational weeks

| (20-22 g.w) | BPD(cm) | Head circumf (cm) | Femur lenght (cm) | Abdomcircumference | TCD(cm) |
|---|---|---|---|---|---|
| X | 4.93 | 6.4 | 3.43 | 16.3 | 2.55 |
| SD | 0.43 | 0.36 | 0.56 | 1.83 | 0.49 |
| MIN | 4.7 | 6.1 | 3.2 | 15.1 | 1.9 |
| MAX | 5.2 | 6.8 | 3.7 | 17.6 | 3.8 |

**Table 5.** Values of some anthropometrical parameters measured with ultrasound in different gestational weeks

| (23-25 g.w) | BPD(cm) | Head circumf (cm) | Femur lenght (cm) | Abdomcircumference | TCD(cm) |
|---|---|---|---|---|---|
| X | 5.36 | 7.9 | 4.53 | 19.9 | 3.18 |
| SD | 0.56 | 0.41 | 0.46 | 2.0 | 0.45 |
| MIN | 5.0 | 7.6 | 4.3 | 18.6 | 2.5 |
| MAX | 8.0 | 8.2 | 4.8 | 21.2 | |

fetus's weight is normal for the gestational week or there are some deviations which lead to an abnormal development of the fetus. By comparing the values of the measured parameters obtained with ultrasound and the ones obtained from the anthropometric measurement, we see that there aren't any bigger deviations. By measuring the biparietal diameter (a parameter which is measured from the 12$^{th}$ gestational week until delivery) we notice progressive increase in gestational weeks, which is explained with the increases pace of growth of the head where the middle values of the anthropometric measurements in all examined groups were compared to the ones measured with ultrasound (x =3.28; x=3.73; x=3.98; x=5.05 and x=6.02). The values of the head circumference which is measured together with the biparietal diameter didn't show bigger deviations compared to the obtained results. The values, by gestational weeks, were (x=12.05; x=14.72; x=14.64; x=18.41; x=21.70). The length of femur- biometric parameter, which is considered to be very valuable, in the second and third trimester of pregnancy increases linearly with gestational age. The obtained values show small deviation in favor of anthropometric measurements due to musculature thickness and subcutaneous fat of the lower limb, while the values of the abdomen circumference show higher values than the anthropometric ones especially in the fifth age group. Transversal cerebral diameter is highly correlated with gestational age as well as with abdomen circumference and its values increase correspondingly. Table (1,2,3,4 and 5).

**Discussion**

The information about the research of some of the characteristics of the intrauterine growth of the fetus show that most studies on this topic agree that changes in the fetal period are relatively slow and prolonged and can be actively determined with quantitative methods.

The ultrasound method is one of the methods for estimating gestational age as well as for following some parameters which are measured routinely during the entire pregnancy, like biparietal diameter, head circumference, length of the limbs, abdomen circumference etc. In recent years William J and D.W.Gauthier (3), have emphasized the transversal cerebral diameter (TCD). From the latest research we can draw a conclusion that this parameter is stable, dependent of gestational age and can be used for early detection of fetal abnormalities. Fetal measurements show high correlation between the transversal cerebral diameter and gestational age TCD/GA; as well as between TCD/AC (4). This parameter is already routinely measured in almost all European countries and wider (5). Standard ultrasound measurements of certain fetal parameters show increased values that correspond to the increase in gestational age which was shown in our results. The correlation between the anthropometric parameters and the parameters measured with ultrasound and gestational age allow us to get standard values of the corresponding

parameters for each gestational week by using statistic data processing. We get the standards of the intrauterine growth based on data for a wider or narrower area of population. Its use is clinically very useful and based on the obtained results we can easily notice eutrophy, hypo or hypertrophy in intrauterine growth (6). Measurements of a femur length and abdomen circumference give acceptable results in those cases where the head of the fetus has some deviations during the corresponding gestational week (7). The differences in the values of certain parameters measured with anthropometric measurements and the same measured with ultrasound are due, among other things, to fetal anthropological characteristics. It is proved that depending on the population group, the correlation between the body and the limbs, the distribution of fat and musculature is different in the fetal body. This was noticed by Campbell and it was proved in a number of other researches (8).

**Conclusion**

By comparing the values of the anthropometric parameters obtained with ultrasound and the ones obtained from our anthropometric measurement, we notice that they are only slightly different because these two ways of measuring are closest to each other compared to the orientation points of a given anthropometric parameter. The parameters measured with ultrasound allow estimation of the gestational age of the fetus, early discovery of fetal anomalies, determination of chromosome abnormalities in the second trimester, which points to the importance of using ultrasonic fetal biometric parameters specific for a certain population, as well as using anthropometry as an additional in everyday clinical practice.

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UDK:616.153.1:577.152.231(497.7)

# DISTRIBUCIJA I KONCENTRACIJA NA HOLESTEROL ESTER TRANSFER PROTEINOT (CETP) VO PLAZMA KAJ ZDRAVI MAKEDONCI

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**Izvadok**

Holesterol ester transfer proteinot (CETP) u~estvuva vo recipro~niot transfer na esterificiran holesterol i triacilgliceroli pome|u lipoproteinite so visoka gustina (HDL) i lipoproteinite bogati so triacilgliceroli. CETP ja regulira plazma koncentracijata na HDL holesterolot i goleminata na HDL partiklite i ima klu~na uloga vo reverzniot transport na holesterolot (RTH). RTH e klu~en proces za namaluvawe na rizikot od pojava na ateroskleroza.

Glavna cel na ovaa studija be{e odreduvawe na plazma koncentracijata i distribucijata na CETP kaj zdravi normolipemi~ni Makedonci i odreduvawe na korelacijata pome|u koncentracijata na CETP so lipidite, lipoproteinite i apolipoproteinite. Plazma koncentraciite na CETP, lipidite, lipoproteinite, i apolipoproteinite bea odredeni kaj 50 normolipemi~ni, zdravi individui, oddvata pola, na vozrast od 28 do 63 godini.

CETP koncentracijata be{e odredena so enzyme-linked immuno-sorbent assay (ELISA). Srednata vrednost na plazma koncentracijata na CETP be{e 1.84 (± 0.69 SD) μg/ml. Referentnite vrednosti za koncentracijata na CETP vo plazma vo na{ata populacija se 0.46 - 3.22 g/ml.

Nema{e statisti~ki zna~ajna razlika vo CETP vrednostite pome|u ma'ite (1.86 ± 0.69 μg/ml) i 'enite(1.82 ± 0.70 μg/ml).

Postoe{e statisti~ki signifikantna korelacija pome|u koncentracijata na CETP i koncentracijata na apoA1 (r=0.31, p=0.0349). Ne postoe{e statisti~ki zna~ajna korelacija pome|u koncentracijata na CETP i drugite lipidni parametri.

***Klu~ni zborovi:*** CETP, HDL, ateroskleroza

# DISTRIBUTION AND CONCENTRATION OF CHOLESTERYL ESTER TRANSFER PROTEIN IN PLASMA OF HEALTHY MACEDONIANS

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**Abstract**

Cholesteryl ester transfer protein (CETP) facilitates the reciprocal transfer of cholesteryl ester (CE) and triglycerides between high density lipoprotein (HDL) and triglyceride-rich lipoproteins. Since CETP regulates the plasma levels of HDL cholesterol and the size of HDL particles, CETP is considered to be a key protein in reverse cholesterol transport (RCT), a protective system against atherosclerosis.

The primary objective of this study was to determine the CETP concentration and distribution in plasma of healthy normolipidemic Macedonians and to examine the relationship with lipids, lipoproteins and apolipoproteins. Plasma levels of CETP, apolipoproteins, lipids and lipoproteins were measured in 50 normolipemic, healthy subjects of both sexes between 28 and 63 years of age.

Plasma CETP concentration was determined using a double – antibody sandwich ELISA.

The mean plasma CETP concentration was 1.84 ± 0.69 μg/ml. Reference values were 1.84 ± 1.38 μg/ml (being the mean ± 2SD) ranged between 0.46 to 3.22. The standardized skewness value of 0.899 and standardized kurtosis value of -1.33 were within the range expected for data from a normal distribution.

There was no statistically difference in CETP values between females (1.82 ± 0.70 μg/ml) and males (1.86 ± 0.69 μg/ml).

There was a statistically significant relationship between CETP levels and apoA1 (r=0.31, p=0.0349). There was no correlation between CETP concentration and other lipid parameters.

***Key words:*** CETP, HDL, atherosclerosis

**Introduction**

The cardio protective role of high density lipoproteins (HDL) is widely accepted [1]. The anti-atherosclerotic effect of HDL is commonly ascribed to its role in reverse cholesterol transport (RCT), i.e. the process by which cholesterol is removed from peripheral cells and transported to the liver for metabolism and excretion in the bile [2].

Cholesteryl ester transfer protein (CETP) is an important factor in RCT, functioning as a facilitator of the reciprocal transfer of cholesteryl ester (CE) and triglycerides between HDL and very low and low density lipoproteins (VLDL and LDL) [3]. This lipid transfer protein reduces the cholesteryl ester content of large, spherical HDL in exchange for a gain of triglycerides. Thereafter, size of HDL particles is further decreased when triglycerides are hydrolysed by hepatic lipase. Consequently, CETP plays an important role in HDL remodeling and metabolism, together with hepatic lipase, phospholipid transfer protein and lecithin: cholesterol acyltransferase [4]. On the other hand, in triglyceride-rich lipoproteins, the cholesterol content is increased and the generation of small dense LDL particles is enhanced [4].

In normolipidemia, the concentration of triglyceride-rich lipoproteins provides a driving force for net cholesteryl ester transfer, and the cholesteryl ester transfer rate is determined by the triglyceride-rich lipoprotein concentration. In severe hypertriglyceridemia, in contrast, the amount of active CETP becomes rate limiting [5].

Despite intensive research, the potential impact of CETP on cardiovascular disease is still debated. On the one hand, the CETP-mediated cholesteryl ester transfer process contributes to an atherogenic lipoprotein profile [6]. On the other hand, the CETP-mediated cholesteryl ester transfer process provides an additional route for delivery of HDL-derived cholesteryl esters to the liver via VLDL and LDL [7].

More clinical data are needed to understand the relationship of CETP and atherosclerosis.

A reliable method to measure plasma CETP concentrations is therefore primarily important.

CETP activity and concentration measurements are comparable. The coefficient of correlation for the two methods is 0.85 ($P$<.0001). This agreement extended to dyslipidemic and normolipidemic samples. The phenotype of dyslipidemia had no effect on the relation of CETP mass and activity [8].

The MAbs against CETP have allowed the development of an enzyme linked immunosorbent assay (ELISA) for the determination of CETP levels [9].

The primary objective of this study was to determine the CETP concentration and distribution in plasma of healthy normolipidemic Macedonians and to examine the relationship with lipids, lipoproteins, apolipoproteins, glucose and other risk factors (BMI, alcohol and cigarette consumption) for coronary heart disease.

**Material and methods**

Fifty healthy individuals of both sexes (25 men and 25 women) between 28 and 63 years of age were included in the study. Information regarding the use of antihypertensive, blood glucose lowering and lipid lowering drugs, smoking and alcohol consumption was obtained using a check-list. Individuals who were receiving lipid lowering therapy were not included in the study.

Clinical characteristics of healthy subjects are presented in Table 1.

**Table 1.** Baseline clinical characteristics of healthy individuals (n=47)

| | |
|---|---|
| **Age (years)** (mean ± SD) | 52 ± 8.3 |
| **BMI (kg/m$^2$)** | |
| >30.16 (%) | 56 |
| <30.16 (%) | 44 |
| **Cigarette smokers (%)** | 52 |
| **Alcohol users (1U per day, %)** | 54 |
| **Hypertension (%)** | 40 |

**BMI –Body mass index**
**1U -14 grams of ethanol**

Fasting venous blood was collected into EDTA-containing glass tubes. Plasma concentration of glucose, total cholesterol (TC) and triglycerides (TG) were measured enzimatically shortly after blood sampling.

The plasma HDL-cholesterol concentration was measured after precipitation of apolipoprotein B-containing lipoproteins with dextransulfate and magnesium sulfate. Plasma LDL-cholesterol was calculated according to the equation of Friedewald et al. Plasma levels of apolipoprotein A1 (apoA1) and apolipoprotein B (apo B) were measured by turbidimetric immunoassay.

The samples were stored at -80$^0$C until determination of CETP concentration.

Plasma CETP concentrations were measured by an ELISA with two different monoclonal antibodies (AplcoDiagnostics, Salem, NH, USA).

The CETP concentration of the normolipidemic human plasma was determined using a 1:80 sample dilution.

Briefly, test wells are coated with anti-CETP MoAb (3-11D). CETP in the sample is captured by the antibody in the first incubation which lasts for 2 hours. After the first incubation and washing to remove all of the unbound material, HRP-labeled anti-CETP MoAb (14-8F) is added. Incubation lasts for 1 hour.

After the second incubation and subsequent washing, substrate solution (o-phenylenediamine) is added. Next, stop reagent ($H_2SO_4$) is added. The intensity of color that develops is read by a microplate reader at 492 nm. The absorbance is proportional to the concentration of CETP in the sample.

The concentrations for the unknown samples were read from the standard curve.

Statistical analysis:

SPSS-X (release 4) software (SPSS) was used for data analysis. Data are expressed as mean ± SD. Relationships between variables are presented as Pearson correlation coefficients. A value of $p < 0.05$ was accepted as statistically significant.

**Results**

No CETP was detected in plasma samples of 3 individuals because no increase of absorbance was observed over a 10 to 100-fold range of plasma dilution.

Plasma CETP concentration in our study group is presented in Table 2.

There was a statistically significant relationship between CETP levels and ApoA1 at the 95% confidence level (r=0.31, p=0.0349). There was no correlation between CETP concentration and other lipid parameters.

**Discussion**

CETP is one of the major potentional determinants of plasma lipoprotein profile by its action in transferring cholesteryl esters among lipoproteins [3].

Availability of monoclonal antibodies for CETP is of primary importance for determination of CETP [9].

**Table 2**. Plasma CETP concentrations in males and females

| | CETP (μg/ml) mean ± SD | Median(μg/ml) | Min-Max(μg/ml) |
|---|---|---|---|
| Males (n=24) | 1.86 ± 0.69 | 0.81 | 0.89-3.21 |
| Females (n=23) | 1.82 ± 0.70 | 1.75 | 0.75-3.1 |
| Total (n=47) | 1.84 ± 0.69 | 1.76 | 0.75-3.21 |

The mean plasma CETP concentration of 47 normolipemic subjects was 1.84 ± 0.69 μg/ml. Reference values were 1.84 ± 1.38 μg/ml (being the mean ± 2SD) ranged between 0.46 to 3.22.

Of particular interest was determination of standardized skewness and standardized kurtosis which can be used to determine whether the sample comes from a normal distribution. The standardized skewness value of 0.899 and standardized kurtosis value of -1.33 were within the range expected for data from a normal distribution.

There was no statistically significant difference in CETP values between females (1.82 ± 0.70 μg/ml) and males (1.86 ± 0.69 μg/ml).

No statistically significant differences were found in CETP levels between smoking and non-smoking ($p=0.27$), obese and lean individuals ($p>0.05$) (data not shown).

Table 3 shows correlation of lipid and apolipoprotein parameters with plasma CETP concentration.

Using ELISA technique with two different mAbs, 3-11D and 14-8F, we have measured the CETP concentration in plasma samples of our study subjects.

The present study is the first that focused on CETP distribution and concentration in healthy individuals from R. Macedonia.

There was no difference in plasma concentration of CETP between males and females included in our study which is in agreement with Marcel et al. who have reported slightly higher CETP concentration in female as compared with male subjects (1.92 ± 0.52 μg/ml vs. 1.50 ± 0.26 μg/ml) [10].

In our study, CETP has showed positive correlation with plasma ApoA-1 concentration and no correlation with the HDL cholesterol. Tato et al. [8] reported a significant inverse association between CETP activity and HDL-C in 47 patients with combined hyperlipidemia but not in study groups with normolipidemia.

Among 79 normolipidemic subjects Mcpherson et al. [11] observed significant relation between CETP and

**Table 3**. Correlation of CETP concentration (1.84 ± 0.69 μg/ml ) with plasma lipids and apolipoproteins in plasma of healthy individuals

| Parameter | Mean ± SD | p |
|---|---|---|
| Total cholesterol (mmol/l) | 4.9 ± 0.8 | 0.22 (NS) |
| Triglyceride (mmol/l) | 1.3 ± 0.4 | 0.18 (NS) |
| HDL-cholesterol (mmol/l) | 1.3 ± 0.2 | 0.32 (NS) |
| LDL –cholesterol (mmol/l) | 3.1 ± 0.7 | 0.63 (NS) |
| Apolipoprotein A-1 mg/dL | 146.0 ± 16.8 | 0.0349* |
| Apolipoprotein B mg/dL | 106.5 ± 16.4 | 0.29 (NS) |

*p<0.05

ApoA-1 concentration which is in agreement with our findings.

The significant correlation between CETP and apoA1 in normolipidemic circumstances suggests that the concentration of circulating CETP is a function of ApoA1 availability for CETP binding in agreement with their documented affinity for each other. In univariate analysis of the normotriglyceridemic group, CETP showed a strong direct association with apoA-I but not statistically significant relationship with HDL-C; these findings have been reported by Marcel et al [10] and Foger et al. [12].

Apart from the situation of CETP deficiency due to defects in the CETP gene [13,14], the inverse relationship between CETP and HDL-C is not readily apparent. Several studies have investigated whether the common fluctuations and/or variations of CETP concentrations in healthy, normolipidemic subjects on unrestricted diets affect their HDL-C levels [15]. Most of the studies observed no significant relationship [16,17].

We did not find any relationship between LDL cholesterol and CETP concentration. Sasai found positive relationship between LDL-C and CETP [9].

Patients with various forms of hyperlipidemia often have relatively high activities or levels of CETP. This observations raises the possibility that an increased activity of CETP contributes to elevated cholesterol levels in lipoproteins containing apoB [18].

Many other metabolic factors may influence the plasma CETP concentration independently. Chronic alcoholic intake reduces CETPT activity which may account for the increase of HDL [19]. Effect of cigarette smoking is controversial [20]. Reports are still controversial about correlation of CETP activity and body mass index [21].

Most clinical data are needed for understanding the role of CETP in atherogenesis. The CETP concentration should be analyzed more carefully in clinical trials, taking into account the heterogeneous background of the plasma CETP concentration.

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# ODREDUVAWE NA KONCENTRACIJATA NA LECITIN HOLESTEROL ACIL TRANSFERAZA KAJ NORMOLIPEMI^NI INDIVIDUI

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**Izvadok**

Lecitin holesterol acil transferaza (LCAT) e klu~en enzim odgovoren za esterifikacija na slobodniot holesterol i dobivawe na esterificiran holesterol. Esterifikacijata na slobodniot holesterol zavisi od plazma koncentracijata na aktivniot enzim i od koncentracijata na triacilglicerolite vo plazma.

LCAT ima klu~na uloga vo metabolizmot i remodeliraweto na lipoproteinite so visoka gustina (high density lipoproteins -HDL).

Vo na{ata studija bea odredeni plazma koncentraciite na LCAT, apolipoproteini, lipidi i lipoproteini kaj 50 zdravi, normolipemi~ni individui, od dvata pola, na vozrsst od 28 do 63 godini.

Za prv pat vo Republika Makedonija be{e koristena enzyme-linked immuno-sorbent assay (ELISA) metodata za kvantitativno odreduvawe na koncentracijata na LCAT vo plazma.

Srednata vrednost na plazma LCAT koncentracijata iznesuva{e 4.07 (± 0.58 SD) μg/ml. Minimalnata i maksimalnata odredena koncentracija na LCAT kaj na{ite ispitanici be{e 3.21 i 5.2 μg/ml.

Poradi nejzinata visoka senzitivnost, ELISA metodata e soodvetna za kvantitativno odreduvawe na LCAT vo plazma i serum kaj normolipemi~ni i hiperlipemi~ni individui,

***Klu~ni zborovi:*** LCAT, HDL, normolipemi~ni individui

# DETERMINATION OF LECITHIN CHOLESTEROL ACYLTRANSFERASE LEVELS UNDER NORMOLIPIDEMIC CIRCUMSTANCES

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**Abstract**

Lecithin:cholesterol acyltransferase (LCAT) is the key enzyme responsible for esterification of free cholesterol to cholesteryl esters. The cholesterol esterification reaction in plasma is determined by the amount of active LCAT in plasma, as well as by the plasma triglyceride concentration.

LCAT plays a major role in HDL metabolism and remodeling. As such, LCAT may serve as an indicator for circulating HDL.

Plasma levels of LCAT, apolipoproteins, lipids and lipoproteins were measured in 50 normolipemic, healthy subjects of both sexes between 28 and 63 years of age.

For the first time in the Republic of Macedonia, a two-step sandwich method of enzyme-linked immuno-sorbent assay (ELISA) was utilized for quantitative determination of plasma LCAT levels.

The minimum – maximum detection limit for LCAT in plasma was 0.14 -35 μg/ml. LCAT values in our sample size ranged from 3.21-5.2 μg/ml.

The mean plasma LCAT concentration of 50 normolipidemic subjects was 4.07 ± 0.58 μg/ml.

Because of its high sensitivity, this method is suitable for quantitation of LCAT in plasma and serum of normolipidemic and hyperlipidemic subjects.

***Key words:*** LCAT, HDL, normolipidemic

## Introduction

Lecithin:cholesterol acyltransferase (E.C. 2.3.1.43) (LCAT) is synthesized by the liver in mammals and it is responsible for esterification of unesterified cholesterol (UC) in plasma [1]. This plasma glycoprotein plays an important role in reverse cholesterol transport (RCT) [1,2]. LCAT performs a central role in high density lipoprotein (HDL) metabolism by catalyzing the transfer of a sn-2-fatty acid from phosphatidylcholine (PC) to the 3-hydroxyl group of the cholesterol which results in formation of lysolechitin and cholesterol ester. The LCAT reaction, which requires apoA-1 as cofactor, results in the

generation of cholesteryl ester molecules that by their hydrophobic nature are retained in the core of HDL particles [3].

The cholesterol esterification reaction in plasma is determined by the activity of LCAT, i.e. the amount of active LCAT in plasma [4]. It has been proved that LCAT mass was highly correlated with the molar cholesterol esterification rate. As the plasma esterification rate approaches zero, so does the plasma LCAT mass. This suggests that all immunodetectable LCAT is active in "normal plasma" [5].

Two classes of genetic deficiencies of human LCAT are known: familial LCAT deficiency (FLD) [6] and fish eye disease (FED) [7]. FLD is caused by either null or missense mutations; in Class 1 defects, null mutations cause total loss of catalytic activity and virtual absence of LCAT mass, whereas in Class 2, missense mutations are characterized by loss of activity and either normal, reduced, or absent LCAT mass. FED is caused by missense mutations only; these mutations affect either LDL or HDL activity in Class 3 defects, and LCAT mass is reduced. In Class 4 defects, the missense mutations are associated with partial loss of activity against HDL only, and reduced LCAT mass.

Direct measurement of the enzyme mass and activity may contribute to the differentiation of LCAT defects.

For the first time in the Republic of Macedonia, the two-step sandwich method of enzyme-linked immuno-sorbent assay (ELISA) was applied for the measurement of LCAT in normolipidemic subjects.

**Material and Methods**

Fifty healthy individuals of both sexes (25 men and 25 women) between 28 and 63 years of age were included in the study. Information regarding the use of antihypertensive, blood glucose lowering and lipid lowering drugs, smoking and alcohol consumption was obtained using a check-list. Individuals who were receiving lipid lowering therapy were not included in the study. Written informed consent was obtained from all of them.

Fasting venous blood was collected into EDTA-containing glass tubes. Plasma glucose was measured enzimatically shortly after blood sampling.

The samples were stored at -80$^0$C until analysis. Plasma total cholesterol (TC) and triglycerides (TG) concentrations were determined by enzymatic methods.

The plasma HDL-cholesterol concentration was measured after precipitation of apolipoprotein B-containing lipoproteins with phosfomolibdenic acid and magnesium chloride.

Plasma LDL-cholesterol was calculated according to the equation of Friedewald et al [8].

Plasma LCAT concentrations were determined utilizing a two-step sandwich method of enzyme-linked immuno-sorbent assay (ELISA) (AplcoDiagnostics, Salem, NH, USA).

The LCAT concentration of the normolipidemic human plasma was determined using a 1:100 sample dilution.

Briefly, test wells are coated with anti-LCAT monoclonal antibody (MoAb (36486)) which binds LCAT in the sample. After the first incubation which lasts for 2 hours and washes to remove all of the unbound material, horseradish (HRP) peroxidase -labeled anti-LCAT MoAb (36487) is added. Incubation lasts for 1 hour at room temperature.

After the second incubation and subsequent washing, the antibody/lcat/enzyme complex is incubated with a substrate solution (o-phenylenediamine) and terminated with a stop reagent (7.7% $H_2SO_4$). The intensity of color that develops in the enzyme reaction is measured by using a microplate reader at 492 nm. The absorbance is proportional to the concentration of LCAT in the sample.

The calibration curve was constructed from a set of seven dilutions of the stock calibrator (concentration 37.0 μg/ml). A calibration curve for each assay was constructed with seven duplicate dilutions of standards. A curve was fitted to the data points,and LCAT concentrations were calculated with data analysis software. There was a good linear relation between the dilution ratio and concentration of LCAT detected by the ELISA.

The minimum – maximum detection limit for LCAT in plasma was 0.14 -35 μg/ml.

Statistical analysis:

Data are expressed as mean ± SD. Pearson's correlation coefficient *r* was used to show the degree of linear association between the different variables. $P < 0.05$ was considered significant.

**Results**

The study population consisted of 50 healthy individuals, of whom 50% were men. Mean age was not significantly different between males and females (p>0.05).

Clinical characteristics of healthy subjects are presented in Table 1.

**Table 1.** Baseline clinical characteristics of healthy individuals (n=50)

| | |
|---|---|
| **Age (years)** (mean ± SD) | 52 ± 8.3 |
| **BMI (kg/m$^2$)** | |
| ≥30.16 (%) | 56 |
| <30.16 (%) | 44 |
| **Cigarette smokers (%)** | 52 |
| **Alcohol users (1U per day, %)** | 54 |
| **Hypertension (%)** | 40 |

**BMI –Body mass index**

Table 2 shows laboratory characteristics of 50 individuals included in the study.

**Table 2**. Plasma lipids, lipoproteins, apolipoproteins and LCAT concentration in healthy individuals

| Parameter | Mean ± SD | Range |
|---|---|---|
| Total cholesterol (mmol/l) | 4.9 ± 0.8 | 3.5-6.2 |
| Triglyceride (mmol/l) | 1.3 ± 0.4 | 0.6-2.1 |
| HDL-cholesterol (mmol/l) | 1.3 ± 0.2 | 1.0-1.6 |
| LDL –cholesterol (mmol/l) | 3.1 ± 0.7 | 1.4-4.1 |
| Apolipoprotein A-1 (mg/dL) | 146.0 ± 16.8 | 111-173 |
| Apolipoprotein B (mg/dL) | 106.5 ± 16.4 | 82-134 |
| LCAT (μg/ml) | 4.07± 0.58 | 3.21-5.2 |

**Table 3**. Frequency distribution of LCAT concentration in 50 study subjects.

| Class | Lower Limit | Upper Limit | Midpoint | Frequency | Relative Frequency | Cumulative Frequency | Cum.Rel Frequency |
|---|---|---|---|---|---|---|---|
| at or bellow | 3.1 | | 0 | 0.0000 | 0 | 0.0000 | |
| 1 | 3.1 | 3.44286 | 3.2714 | 9 | 0.1800 | 9 | 0.1800 |
| 2 | 3.4429 | 3.7857 | 3.6143 | 9 | 0.1800 | 18 | 0.3600 |
| 3 | 3.7857 | 4.1286 | 3.9571 | 7 | 0.1400 | 25 | 0.5000 |
| 4 | 4.1286 | 4.4714 | 4.3 | 6 | 0.1200 | 31 | 0.6200 |
| 5 | 4.4714 | 4.8143 | 4.6429 | 15 | 0.3000 | 46 | 0.9200 |
| 6 | 4.8143 | 5.1571 | 4.9857 | 3 | 0.0600 | 49 | 0.9800 |
| 7 | 5.1571 | 5.5 | 5.3286 | 1 | 0.0200 | 50 | 1.0000 |
| Above 5.5 | | | | 0 | 0.0000 | 50 | 1.0000 |

**Table 4**. Correlation of plasma LCAT concentration (4.07 ± 0.58 μg/ml) with lipids, lipoproteins and apolipoproteins in normolipidemic individuals

| Variable | r | p |
|---|---|---|
| Total cholesterol (mmol/l) | -0.0045 | 0.7539 |
| Triglyceride (mmol/l) | 0.089 | 0.5400 |
| HDL-cholesterol (mmol/l) | -0.016 | 0.9139 |
| LDL –cholesterol (mmol/l) | 0.1713 | 0.2342 |
| Apolipoprotein A-1 mg/dL | -0.1108 | 0.4438 |
| Apolipoprotein B mg/dL | 0.078 | 0.5912 |

LCAT values in our sample size ranged from 3.21-5.2 μg/ml.

15 individuals (30%) had LCAT values between 4.47 and 4.81μg/ml with midpoint of 4.64 μg/ml (Table 3).

The mean plasma LCAT concentration of 50 normolipidemic subjects was 4.07 ± 0.58 μg/ml.

Relationships between variables are presented as Pearson correlation coefficients in Table 4. No significant correlation was found between LCAT and TC, TG, HDL-C, LDL-C, ApoA-1 and ApoB. Inverse relationship, but not statistically significant was found between LCAT and HDL.

**Discussion**

LCAT role in the reverse cholesterol transport has been considered "antiatherogenic" as the cholesterol esterification is the prerequisite for the formation of mature HDL particles and may create a gradient necessary for the flow of unesterified cholesterol from tissues to plasma. The protective or potentially atherogenic role of LCAT depends on the quality of HDL and concentration of LDL [9].

LCAT is required for the generation of cholesterol esters for HDL particles, and in the presence of hypertriglyceridemia, when there is abundant unesterified cholesterol present on TGRLP, the availability of LCAT may be one factor that influences the amount of cholesterol ester carried in HDL particles. It is possible that variable LCAT activities have a greater influence on HDL-cholesterol levels in hypertriglyceridemic patients than in normolipidemic individuals [4].

Since a high LCAT activity may raise HDL-cholesterol levels and a high CETP activity reduces the level, the balance between the two activities could be a

determining factor for HDL-cholesterol levels. In normotriglyceridemic patients, there is a positive correlation between CETP and LCAT levels, which may have contributed to the maintenance of normal HDL-cholesterol levels [4]. This correlation did not exist in hypertriglyceridemic patients, which could play a role in keeping HDL-cholesterol levels [4].

Highly specific monoclonal antibodies provide a sensitive and specific analytical system for measurements of LCAT protein [10].

Using ELISA technique with two different mAbs, (L86 and L87), we have measured the LCAT concentration in plasma samples of our study subjects.

Participants in the study were selected on the basis of fasting normal lipid levels, good health and absence of lipid modifying medication.

We did not find statistically significant relationship between LCAT and cholesterol. Contrary, Albers et al. report that LCAT concentration is significantly correlated with plasma cholesterol [10].

In our study, LCAT concentration was not significantly related to HDL-cholesterol which is consistent with the study of Albers et al. who reported that LCAT concentration was not significantly correlated with HDL cholesterol, apolipoproteins A-I and A-II [10].

Earlier studies suggested that plasma cholesterol esterification rate is positively correlated with cholesterol, triglyceride and percent ideal body weight and negatively correlated with HDL cholesterol [11,12]. The inverse relationship observed between HDL cholesterol and LCAT appears to be primarily related to the inverse relationship between triglyceride and the HDL cholesterol (data not shown), since partial correlation analysis keeping triglyceride constant indicated that HDL cholesterol is no longer significantly negatively related to the esterification rate (r=-0.085) [10].

Thus in normal subjects there seems to be a direct relation between very low density lipoprotein and LDL lipid concentration and molar LCAT rate but no relation between HDL lipid concentration and LCAT rate [11].

In healthy normolipidemic men, both smoking and drinking affect HDL-C levels, but do not affect plasma LCAT levels [14].

Univariate regression analysis demonstrated that in healthy subjects plasma LCAT activity was positively correlated with waist circumference (r=0.31,p=0.004), CRP (r=0.22,p<0.004), TG (r=0.55 ,p<0.001), non-HDL cholesterol (r=0.56,p<0.001), and Apo B (r=0.45, p<0.001) and negatively correlated with HDL cholesterol (r=—0.33,p = 0.043) [15-18].

In our study, univariate regression analysis showed an inverse relation between HDL cholesterol and plasma LCAT activity in our study which is in agreement with Dullart et al. [19,20].

The mean plasma LCAT concentration of 50 normolipidemic subjects in our study was 4.07 ± 0.58 μg/ml. Healthy individuals in the study of Calabresi et al. had LCAT mean values of 4.47±0.21 with range of 3.1-6.7 μg/ml [21].

Measurement of LCAT concentration and activity along with plasma cholesterol esterification rate will permit differentiation of abnormalities of enzyme from qualitative or quantitative substrate or cofactor abnormalities.

It should be stressed that all immunodetectable LCAT in plasma is active in normal subjects, so measurement of LCAT concentration will clarify different lipid abnormalities.

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# ARTROSKOPSKA EVALUACIJA I TRETMAN NA LEZIITE NA ZGLOBNATA RSKAVICA NA KOLENOTO

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**Izvadok**

***Voved:*** Artikulira~ka (zglobna) rskavica e specijaliziran organ koj gi pokriva zglobnite povr{ini na koskite. Toa e mazna, sjajna i elasti~na hijalina struktura koja ima zada~a da go namali trieweto, pritisokot a voedno i da go amortizira cikli~noto optovaruvawe bez nejzino o{tetuvawe.

***Cel na trudot:*** Da se poka'at prednostite na artroskopskiot naod vo odnos na naodot na magnetna rezonanca vo evaluacija na leziite na artikulira~kata rskavica na koleniot zglob.

***Materijal i metodi:*** Ovaa studija be{e prospektivna vo period od tri godini (2007-2009). Artroskopija be{e izvedena kaj 173 pacienti, a vo studijata bea vklu~eni samo 83 od niv kaj koi bea dijagnosticirani i tretirani lezii na zglobnata rskavica. Be{e koristena klasifikacija na o{tetuvawata na rskavicata po ICRS. Pacientite bea podeleni vo tri vozrasni grupi. Naodite od magnetnata rezonanca (1 Tesla) gi analizira i interpretira iskusen kvalifikuvan radiolog i istite se notiraat vo formularot od ICRS.

***Rezultati i diskusija***: Incidenca na javuvawe na lezii na zglobna rskavica e 47.98%, so dominacija na leziite na medijalniot kondil od 67%, so prose~na dimenzija na leziite od 10.68mm i signifikantna razlika me|u vozrasta i goleminata na leziite i stepenot na o{tetuvawe.

***Klu~ni zborovi:*** artroskopija, 'rskavi~ni lezii, MRI

# ARTHROSCOPIC EVALUATION AND TREATMENT OF THE KNEE ARTICULAR CARTILAGE LESIONS

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**Abstract**

*Introduction.* Articular cartilage is a specialized organ that covers articulating surface of the bones. It is elastic, hyaline tissue which provides resistance to shear forces, to compressive stress and high elasticity.

*Methods*. This is a 3-year prospective study (2007-2009) performed by the same surgical team . Arthroscopy was done on 173 patients , but only 83 were included with diagnosed and treated lesions of knee articular cartilage. Chondral lesions were classified according to the ICRS classification and all were included in a database with MRI-reports. The MRI reports were performed by a qualified radiologist in all cases.

*Purpose.* To determine the advantages of arthroscopy versus magnetic resonance findings in evaluation of the knee articular cartilage lesions .

*Results and Discussion.* Incidence of chondral lesions was 47.98% , most of them located on medial femoral condyle 67%, with average dimension of the lesion 10.68 mm significant correlation between patient age and the size of the lesion (p=0,001).

***Key words:*** arthroscopy, chondral lesion, MRI

## *Introduction*

Articular cartilage is a specialized organ which covers the articulating surface of the bones (1). It is a smooth, elastic, hyaline structure which provides resistance of shear forces, resistance of compress stress , low friction and lubrication and is also responsible for the mechanism of shock- absorption (3). This is due to collagen architecture of the cartilage which consists of big amount of comprised water attracted by proteoglycans Morphologically , articular cartilage can be divided into four zones. Each zone has unique chondrocyte orientation, biology and structural organization (2,4). Superficial zone consisting of chondrocyte elongated along the articular surface. High tensile integrity is produced by horizontally oriented fibres and from the thin collagen structure called –lamina splendid. Hydration of this zone is bigger than in other. Chondrocytes in transitional zone are in round-shape form columns having the same direction of the collagen fibres with highest concentration of proteoglycans (5,6). In the deep zone chondrocytes are organized in vertical columns and the collagen is perpendicular to subchondral bone (14,17). 65 - 85% from the mass of the cartilage is water.(21) More than 70 of the dry cartilage is collagen represented in 95 % by collagen type II. This type is the triple helix structure where a single molecule is folded with two others like net who provide the strength of the cartilage.

Concept between duration, intensity and frequency of applied force on the articular cartilage is definition of the mechanism of cartilage lesion (10,11).

**Material and Method**

This is a prospective study from 2007 to 2009 performed by same surgical team. Arthroscopy was done on 173 patients, but only 83 were included with diagnosed and treated lesions of knee articular cartilage. According the International cartilage repair society classification (5) Patients were divided in three groups-

1. group with lesions –first degree,
2. group : lesion –second and third degree
3. group : fourth degree lesions.

Magnetic resonance reports (1 Tesla) were performed by a qualified radiologist in all cases and were included in the database.

Description of the lesion was made according to their location, type and dimension.

According to the age and sex, patients were divided in male and female group and three age groups: 15-30 years; 30-45 years and 45-60 years.

Arthroscopic evaluation on the articular cartilage was done on femoral, tibial and patellar articular surface using the probe hook. In the study we used standard arthroscopic equipment and instruments.

Purpose:

1. Accuracy of advantages of arthroscopic evaluation versus magnetic resonance findings of cartilage lesions.
2. Determination of the incidence of chondral lesions.
3. Distribution of the chondral lesion by location, type, dimensions according to age and sex.

**Results**

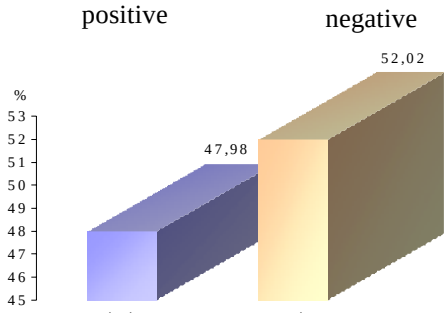


**Fig. 1.** Incidence of chondral lesions

**Table 1.** Isolated /combined lesion

| | No | % |
|---|---|---|
| Isolated lesion | 40 | 48.19 |
| Combined | 43 | 51.81 |
| Summary | 83 | 100 |

All the patients are divided in three age groups: age group between 15 - 30 years -22(26.5%) patients, 28 patients (33.7%) are in the middle group 31 - 45 years, and most of them 33(39.8%) are in the oldest age group ( 46 - 65y).

**Table 2 .** Distribution according to age

| Age | Number | % |
|---|---|---|
| 15-30 | 22 | 26.51 |
| 31-45 | 28 | 33.73 |
| 46-65 | 33 | 39.76 |
| Summary | 83 | 100 |

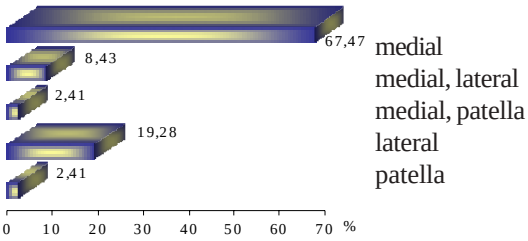


**Fig. 2.** Lesion location

Most affected was medial condyle at 56(67.5%) patients, lateral condyle was affected at 16(19.3%) and the isolated patellae lesion was found only in 2(2.4%) .Isolated cartilage lesion is diagnosed in 69(83.1%) patients instead of 69(83.1%) multiple lesions.

**Table. 3**. Isolated versus multiple lesion

| **Parameter** | No | % |
|---|---|---|
| Isolated | 69 | 83.13 |
| Multiple | 14 | 16.87 |
| Summary | 83 | 100 |

According to the grade of the lesion patients are divided into three groups according to the ICRS classification: the first group of 16 patients (19%) with grade one lesion ,the second one which is dominant with 54 (65.1%) patients with second and third degree of lesion and the last group with 13 patients (15.7) with fourth degree lesions.

Magnetic resonance was done to all patients before the arthroscopy and 53 (63.9) patients were with no pathological findings , only in 30 (36.1) patients MRI detected chondral lesions.

Correlation between patient age and lesion degree we examine with Spermanov-koef. Corelation The result of $R=0.28$ and $p<0.05$ confirm correlation between these two parameters in direction of positive correlation, due to age increasing of patients with chondral knee

**Table. 4**. Isolated versus multiple lesion –patients according age group

| Isolated /multiple | | age group 1 | age group 2 | age group 3 | Summary |
|---|---|---|---|---|---|
| | No | 21 | 25 | 23 | 69 |
| | % | 25.30 | 30.12 | 27.71 | 83.13 |
| | No | 1 | 3 | 10 | 14 |
| | % | 1.20 | 3.61 | 12.05 | 16.87 |
| | No | 22 | 28 | 33 | 83 |
| | % | 26.51 | 33.73 | 39.76 | 100 |

Chi-square=7.39 df=2 p=0.025

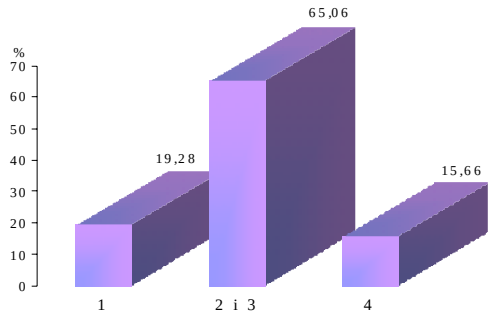


**Fig. 3.** Lesion degree

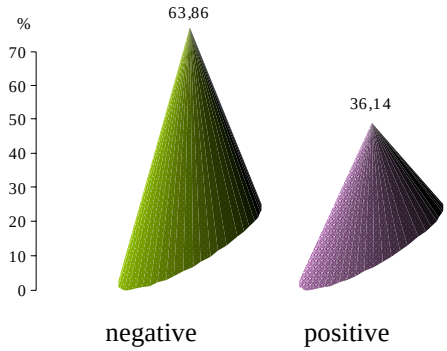


**Fig. 4.** MRI- findings

**Table 5.** MRI Findings

| MRI Findings | Broj | % |
|---|---|---|
| negative | 53 | 63.86 |
| positive | 30 | 36.14 |
| Summary | 83 | 100 |

**Table. 6.** Lesion degree –according patients age group

| Lesion degree | | 15 e”30year. | 31 e”45 year. | 46 e”65. | Summary |
|---|---|---|---|---|---|
| 1 | No | 7 | 7 | 2 | 16 |
| | % | 8.43 | 8.43 | 2.41 | 19.28 |
| 2,3 | No | 9 | 21 | 24 | 54 |
| | % | 10.84 | 25.30 | 28.92 | 65.06 |
| 4 | No | 6 | 0 | 7 | 13 |
| | % | 7.23 | 0 | 8.43 | 15.66 |
| | No | 22 | 28 | 33 | 83 |
| | % | 26.51% | 33.73 | 39.76 | 100 |

Chi-square=14.88 df=2 p=0.048

lesions shows bigger number of lesions which according the ICRS classification are in higher degree of cartilage degeneration .

Average dimension of the lesions is statistically significant bigger in female patients. T-test confirms that the discrepance between average dimension of the male patients 10.1±2.8 mm versus female 11.7±3.4 mm is statistically significant for $p<0.05$

**Table. 7.** Lesion dimension -according to sex

| Dimension of the lesion | N | Mean | t-value | p | Std.dev |
|---|---|---|---|---|---|
| male | 45 | 10.10 | | | 2.79 |
| female | 38 | 11.68 | | | 3.43 |

**Discussion and Conclusion**

This prospective study shows that arthrosccopy is the accurate method for evaluation of cartilage lesions. The relatively high incidence-47% of the cartilage lesions and low MRI sensitivity-36% confirm that. There is no significant distinction between each knee: but there exists between the condyles –dominant medial condyle with 67%;(literature-46%(29)). The answer to this is probably in late detection of varus knee in the population. (12) Isolated carilage lesion is diagnosed at 40 patients , so more than 50 % are combined cartilage, meniscal and ligament lesions with average dimension of 10.8mm.(9). Second and third degree cartilage lesions according the ICRS classification are dominant within 54 patients (65%): (82%-Friemert publication).(31)

Sensitivity of MRI (1T) in detection of cartilage lesions due to arthroscopy as a gold standard was 36.14% , specification of 64.44% positive predictive 48.39 % and negative predictive issue was 52.25%.

According the arthroscopic findings patients were treated with several methods of treatments:

1. Lavage and debridman
2. Bone marrow stimulating techniques (microfracture, abrasion, drilling)
3. Osteochondral autograft
4. Autologous chondrocyte transplantation
5. High tibial osteotomy

According to the high incidence of chondral lesiones (47%) and relative low sensitivity of magnetic resonance (36%) every arthroscopist should know how to detect, evaluate and treat non-diagnosed chondral lesions. Exact arthroscopic evaluation can predict the type of treatment for preventing the joint function and to prevent or even prolong osteoarthritis as an end stage of cartilage degeneration.

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# MINIMALNO INVAZIVNA PERKUTANA OSTEOSINTEZA SO PLO^KA VO TRETMANOT NA SKR[ENICITE NA DISTALNATA TIBIJA

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**Izvadok**

**Cel:** Cel na trudot e da go prika‘ e stepenot na zadovolstvo na pacientite tretirani so novata operativna tehnika, preku VAS-FA koeficientot. So ova smetame deka bi se dobil odreden stepen na kvantifikacija na edno relativno subjektivno ~uvstvo t.e. ~uvstvoto na zadovolstvo na pacientite koi se tretirani so odredena operativna tehnika.

**Dizajn na studijata:** Prospektivna randomizirana klini~ka studija.

**Mesto na izveduvawe:** Pacientite se tretirani vo klini~ka bolnica od sekundarno nivo.

**Metodi:** Vo ova studija, }e prika‘ eme heterogena grupa od 30 pacienti, so skr{enica na distalnata tibija (18 ma‘ i, 12 ‘ eni), klasificirani spored AO/OTA klasifikacijata kako (43A1, 43A2 i 43A3 tipovi na skr{enici), a tretirani so minimalno invazivna perkutana osteosinteza so plo~ka (MIPPO).

**Rezultati:** Site skr{enici zarasnaa vo zadovolitelni granici. Merkite na ishod se odreduvaa spored Vizuelnata Analogna Skala za Stapalo i Glu‘ d (VAS-FA). So nivnata statisti~ka analiza (Ednonaso~na ANOVA analiza, T-test, SD i SEM)se poka‘ a deka statisti~ki zna~ajna razlika postoi pome|u vkupniot rezultat na VAS-FA za grupata na frakturi A1 i A3, kako i pri sporedbata na grupite A2 i A3.

**Zaklu~ok:** Metodot na minimalno invazivna perkutana osteosinteza so plo~ka poka‘ uva pomal procent na komplikacii, a vo isto vreme dava i relativno visoki koeficienti na zadovolstvo kaj pacientite, so zabele{ka deka visinata na rezultatite zavisi i od tipot na skr{enicata.

**Klu~ni zborovi:** skr{enica na distalnata tibija, MIPPO, AO/OTA-klasifikacija, VAS-FA.

# MINIMALLY INVASIVE PERCUTANEOUS PLATE OSTEOSYNTHESIS OF THE FRACTURES OF DISTAL TIBIA

Clinical Hospital “SISTINA” – Skopje [2]- City Surgical Clinic “St. Naum Ohridski” - Skopje
Nastov Nebojsha[1], Vuckov S †, Mitev K[2], Ivanovski M[1] **Abstract**

**Abstract**

**Aim:** Aim of this study is to show the patient satisfaction rate, in the group of patients treated with the new operating technique, through the VAS-FA ratio. We assume that with this ratio we can quantify one relatively subjective feeling i.e. patient satisfaction.

**Study design:** Prospective randomized clinical trial.

**Setting:** All patients treated in clinical hospital of secondary level.

**Methods:** We will present heterogenous group of 30 patients with fracture of the distal tibia (18 male, 12 female), randomized according to the fracture type in three groups (AO/OTA 43 A1, 43A2, 43A3), treated with minimally invasive percutaneous plate osteosynthesis.

**Results:** All fractures united within acceptable limits. Outcome measures were calculated with the VAS-FA (Visual Analogue Scale Foot and Ankle) score. Using the statistical analysis (One way ANOVA testing, T-test, SD, SEM) of this score we have found statistical significance when comparing the results between the groups A1-A3, and A2-A3.

**Conclusion:** Method of minimally invasive percutaneous plate osteosynthesis is showing lower complication rate, and at the same time relatively high satisfaction rate, with notion that results differ depending on the fracture type.

**Key words:** fracture of distal tibia, MIPPO, AO/OTA-classification, VAS-FA.

**Introduction**

Fractures of the distal tibia (Fig. 1,2) are not the most common fractures but, depending on their location, are one of the most complicated in terms of soft tissue handling. Compared with other fractures, fractures of the distal tibia are associated with a higher incidence of wound infection, postoperative ankle stiffness and swelling.

In addition to fracture type, the soft tissue status is very important to the outcome of treatment. Overlying soft tissues must be checked for possible wounds and assessment of the degree of soft tissue injury. This assessment can be partially achieved through direct visualization of the skin, looking carefully for areas of contusion or abrasion, or can be performed indirectly through evaluation of the fracture, which provides an estimate of the gradations of energy delivered during the injury (1). Tscherne outlined the importance of soft tissue coverage, with the development of soft-tissue injury classification for closed fractures (2).

Fracture management objectives should encompass both biological and mechanical goals. The biological goal is to maintain viability of the bone by preserving its soft-tissue attachments and blood supply. The mechanical goal is to obtain axial and rotational alignment with stable fixation of the fracture. The the individual fragments and to achieve improved fracture healing. The main methods utilized are indirect reduction (5) and bridge plating (6). Indirect reduction (7) and closed fixation are technically much more demanding than an open procedure; thus, accurate preoperative planning is needed to choose the appropriate implant size and length, shaping of the plate, number, position and order of insertion of the screws (8).conflict between the need for absolute anatomical reduction and mechanical stability and the desire for soft tissue preservation is analogous to the saying “wash me but don’t get me wet” (3). The concept of minimally invasive percutaneous plate osteosynthesis (MIPPO) (4) refers to the conservation of the vascularity of the bone, especially the small fragments, during surgical procedures, to ensure the continued vitality of

**Patients and Methods**

Thirty (30) patients with closed, unstable, fractures of the distal tibia classified as Types 43A1, 43A2 and 43A3 were prospectively followed for an average period of 28 months (range, 18-40 months). In all patients, the standard protocol for fracture treatment was applied. Preoperative x-rays were analyzed for prior classification, according to the Comprehensive Classification of Fractures of Long Bones (9). After obtaining informed consent, patients were included in the study. X-rays were taken immediately after surgery and evaluated for malalignment in the sagittal and coronal planes. Further x-ray investigations were performed after one month, 3, 6 and 12 months, for evidence of union. Postoperative complications, time to union, time to partial and full weight bearing were recorded, as the outcome measures for the ankle joint according to the Visual-Analog-Scale Foot and Ankle (VAS FA) (10).

All 30 patients underwent minimally invasive percutaneous plate osteosynthesis within six hours of the injury. The patient was placed in the supine position on a radiolucent table. The entire lower limb was prepared and draped in the usual sterile fashion. The tibia was approached anteromedially through two small (1-2 cm) incisions Fig. 3. Prior to the application of the plate into the subcutaneous tunnel, one or two lag screws were placed, if possible, to add a stability to the construct. After preparing the tunnel, a previously precontoured 4.5 mm LCDCP plate Fig.4, was placed in the tunnel. Plate length was calculated using the plate span ratio. The position of the plate and alignment of the fragments was checked with an image intensifier in the A-P and lateral views. Fig.5,6

**Results**

Thirty patients were treated with MIPPO between November 2003 and July 2007. Time from injury to surgery ranged from 3-8 hours (average, 5.5 hours). All fractures healed within acceptable limits of alignment (less than 5 degrees varus, less than 10 degrees valgus, less than 5 degrees procurvatum/recurvatum). No patient had evidence of delayed union, delayed wound healing, wound dehiscence or deep infection.

Statistical analysis with GraphPad InStat Version 3.10, 32 bit for Windows with One-way ANOVA testint, t-test, p-value, SD and SEM was performed.

Time to partial weight bearing with crutches averaged 5 weeks (range, 4-6 weeks) (Table 1).

**Table 1.** Partial weight bearing with crutches (distribution according to fracture types - days)

| No. | A1 | A2 | A3 |
|---|---|---|---|
| **1** | 30 | 36 | 41 |
| **2** | 29 | 32 | 40 |
| **3** | 28 | 34 | 38 |
| **4** | 29 | 36 | 39 |
| **5** | 28 | 34 | 41 |
| **6** | 29 | 32 | 42 |
| **7** | 28 | 33 | 40 |
| **8** | 30 | 34 | 43 |
| **9** | 29 | 33 | |
| **10** | 30 | | |
| **11** | 31 | | |
| **12** | 31 | | |
| **13** | 30 | | |
| **Mean** | **29.385** | **33.778** | **40.500** |

Summury statistic results for SD (standard deviation) and SEM (standard error of mean) showed in table:

| | A1 | A2 | A3 |
|---|---|---|---|
| **Mean** | 29.38461 | 33.77778 | 40.5 |
| **Standard deviation (SD)** | 1.044 | 1.481 | 1.604 |
| **Sample size (N)** | 13 | 9 | 8 |
| **Std.error of mean (SEM)** | 0.2895 | 0.4938 | 0.5669 |
| **Lower 95% confidence limit** | 28.754 | 32.639 | 39.159 |
| **Upper 95% confidence limit** | 30.015 | 34.916 | 41.841 |

Bartlett's test suggests that SD differences exist, but without any statistical significance, with p-value of 0.3944, and Bartlett's statistic (corrected) of 1.861.

Further analysis suggests that variation among column means is significantly greater than expected by chance. All of this is confirmed with the calculation of the q-values which are higher than 3.509, and p-value smaller than 0.05, according to the Tukey-Kramer Multiple Comparisons Test.

| Comparison | Mean Difference | q-value | p-value |
|---|---|---|---|
| **A1 vs A2** | -4.393 | 10.676 | p<0.001 |
| **A1 vs A3** | -11.115 | 26.065 | p<0.001 |
| **A2 vs A3** | -6.722 | 14.578 | p<0.001 |

| | | 95% Confidence Interval | |
|---|---|---|---|
| **Difference** | **Mean difference** | **From** | **to** |
| **A1 - A2** | -4.393 | -5.837 | -2.949 |
| **A1 - A3** | -11.115 | -12.612 | -9.619 |
| **A2 - A3** | -6.722 | -8.340 | -5.104 |

All this results suggests that differences in beginning with the partial weight bearing is statistically signifficant, and in proportion with the comminution of the fractures.

Time to full unprotected weight bearing averaged 11 weeks (range, 9-12 weeks) and was determined as time to clinical union of the fractures.

Corrected Bartlett's statistics in ths case is 0.2958, where p-value is 0.8625, which suggests that SD of the groups are not equal, but in the same time they are not statistically significant.

Again, it was suggested that differences in the mean values between tree types of fractures , are statistically significant, what was expected, because of different degree of comminution of the fractures. Also it was confirmed that largest mean and individual difference was observed when comparing A1 and A3 types of fractures.

**Table 2.**

| No. | A1 | A2 | A3 |
|---|---|---|---|
| **1** | 65 | 76 | 78 |
| **2** | 68 | 72 | 79 |
| **3** | 64 | 75 | 78 |
| **4** | 67 | 77 | 78 |
| **5** | 64 | 74 | 81 |
| **6** | 65 | 72 | 83 |
| **7** | 63 | 73 | 81 |
| **8** | 67 | 75 | 84 |
| **9** | 68 | 77 | |
| **10** | 64 | | |
| **11** | 70 | | |
| **12** | 69 | | |
| **13** | 65 | | |
| **Mean** | **66.077** | **74.556** | **80.250** |

Summary statistics for the SD and SEM, presented in table:

| | A1 | A2 | A3 |
|---|---|---|---|
| **Mean** | 66.07692 | 74.55556 | 80.25 |
| **Standard deviation (SD)** | 2.216 | 1.944 | 2.375 |
| **Sample size (N)** | 13 | 9 | 8 |
| **Std.error of mean (SEM)** | 0.6146 | 0.6479 | 0.8399 |
| **Lower 95% confidence limit** | 64.738 | 73.062 | 78.264 |
| **Upper 95% confidence limit** | 67.416 | 76.050 | 82.236 |

| Comparison | Mean difference | q-value | p-value |
|---|---|---|---|
| **A1 vs A2** | -8.479 | 12.668 | p<0.001 |
| **A1 vs A3** | -14.173 | 20.434 | p<0.001 |
| **A2 vs A3** | -5.694 | 7.593 | p<0.001 |

| | | 95% Confidence Interval | |
|---|---|---|---|
| **Difference** | **Mean difference** | **from** | **to** |
| **A1 - A2** | -8.479 | -10.827 | - 6.130 |
| **A1 - A3** | -14.173 | -16.607 | -11.739 |
| **A2 - A3** | -5.694 | - 8.326 | - 3.063 |

Morning stiffness was found in eight (26%) patients and swelling around the ankle joint in four (14%).

Table 3 shows the overall VAS FA postoperative score which was 95.33 (range, 88-100). Pain scores were the lowest with overall score of 92.16 (range, 84-100), while the best results were found in the other complaints group of questions with an overall score of 97.5 (range, 94-100).

**Table 3.** Patient data regarding VAS FA

| Patient No. | Age/Sex | Mechanism of injury | Type of fracture | VAS FA Pain | VAS FA Function | VAS FA Other | VAS FA Overall |
|---|---|---|---|---|---|---|---|
| 1. | 48/M | RTA | 43A2 | 93 | 95 | 98 | 95,3 |
| 2. | 35/M | RTA | 43A1 | 97 | 98 | 99 | 98 |
| 3. | 55/F | Fall | 43A3 | 91 | 94 | 97 | 94 |
| 4. | 26/M | RTA | 43A1 | 98 | 100 | 100 | 99,3 |
| 5. | 29/M | Fall | 43A2 | 97 | 100 | 100 | 99 |
| 6. | 15/F | RTA | 43A1 | 100 | 100 | 100 | 100 |
| 7. | 49/F | Fall | 43A2 | 92 | 94 | 97 | 94,3 |
| 8. | 64/F | Fall | 43A3 | 87 | 95 | 95 | 92,3 |
| 9. | 57/M | Fall | 43A3 | 92 | 95 | 97 | 94,6 |
| 10. | 25/M | RTA | 43A1 | 98 | 98 | 100 | 98,6 |
| 11. | 68/F | Fall | 43A3 | 93 | 87 | 97 | 92,3 |
| 12. | 44/M | RTA | 43A2 | 91 | 95 | 96 | 94 |
| 13. | 35/F | RTA | 43A1 | 96 | 99 | 99 | 98 |
| 14. | 27/M | RTA | 43A2 | 98 | 100 | 100 | 99,3 |
| 15. | 48/M | RTA | 43A1 | 92 | 95 | 95 | 94 |
| 16. | 54/M | Fall | 43A2 | 91 | 95 | 98 | 94,6 |
| 17. | 62/F | Fall | 43A3 | 85 | 85 | 95 | 88,3 |
| 18. | 67/F | Fall | 43A3 | 85 | 87 | 94 | 88,6 |
| 19. | 36/F | RTA | 43A1 | 98 | 95 | 98 | 97 |
| 20. | 49/M | Fall | 43A1 | 94 | 97 | 99 | 96,6 |
| 21. | 24/F | RTA | 43A1 | 99 | 100 | 99 | 99,3 |
| 22. | 37/M | RTA | 43A3 | 95 | 97 | 98 | 96,6 |
| 23. | 46/F | RTA | 43A2 | 91 | 98 | 97 | 95,3 |
| 24. | 55/M | RTA | 43A2 | 92 | 95 | 97 | 94,6 |
| 25. | 38/M | Fall | 43A1 | 96 | 99 | 100 | 98,3 |
| 26. | 63/M | Fall | 43A1 | 89 | 94 | 95 | 92,6 |
| 27. | 67/M | RTA | 43A3 | 84 | 87 | 93 | 88 |
| 28. | 51/F | Fall | 43A2 | 90 | 93 | 96 | 93 |
| 29. | 38/M | RTA | 43A1 | 95 | 98 | 98 | 97 |
| 30. | 49/M | RTA | 43A1 | 96 | 98 | 98 | 97,3 |

In the process of statistical analysis of the overall VAS FA results, we've found statistical significance when comparing the A1-A3, and A2=A3 types of fractures, but no statistical significance was found in comparing the A1-A2 fracture types.

| Comparison | Mean difference | q-value | p-value |
|---|---|---|---|
| **A1 vs A2** | 1.896 | 2.509 | p>0.05 |
| **A1 vs A3** | 5.547 | 7.084 | P<0.001 |
| **A2 vs A3** | 4.312 | 4.312 | p<0.05 |

| | | 95% Confidence Interval | |
|---|---|---|---|
| **Difference** | **Mean difference** | **From** | **to** |
| **A1 -A2** | 1.896 | -0.7559 | 4.547 |
| **A1 - A3** | 5.547 | 2.799 | 8.295 |
| **A2 - A3** | 3.651 | 0.6801 | 6.623 |

Bartlett's statistic (corrected) is 1.931, while p-value is 0.3808, which again confirms hat SD are not equal, but at the same time, they are not statistically significant.

| | A1 | A2 | A3 |
|---|---|---|---|
| **Mean value** | 97.38461 | 95.48889 | 91.8375 |
| **Standard deviation (SD)** | 2.092 | 2,189 | 3.232 |
| **Sample size (N)** | 13 | 9 | 8 |
| **Sample size (N)** | 0.5802 | 0.7296 | 1.143 |
| **Std.error of mean (SEM)** | 96.120 | 93.806 | 89.135 |
| **Lower 95% confidence limit** | 98.649 | 97.171 | 94.540 |

**Fig. 1.** AP view of 43-A1.2 fracture of the right distal tibia with simple f-re of the distal fibula

**Fig. 2.** Lateral view of the same fracture

**Fig. 3.** Two small incisions with finished subcutaneous tunneling on the anteromedial side of the tibia using Long curved Pean clamp.

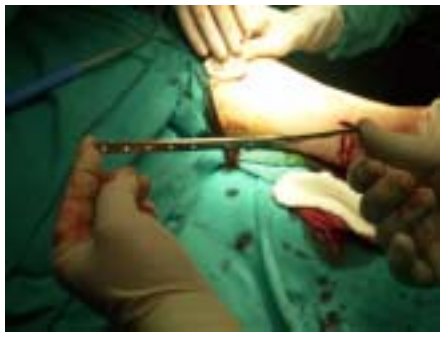

**Fig. 4.** Precontoured plate before insertion into the tunnel.

**Fig. 5.** Postoperative AP radiograph showing the position of the plate and two interfragmentary screws

**Fig. 6.** Postoperative lateral view.

**Discussion**

From the presented results, it can be seen that the only statistically significant difference is present when comparing the final outcomes of the higher degrees of comminution of the fracturtes (10). Also very much predictible was the fact that the worst results related with the pain will appear in the higher comminution group.Of exceptional clinical relevance is the fact that beginning with weight bearing is proprtional to the degree of the comminution of the fracture. The same trend is present both in partial and full weight bearin groups. This is very important because the time of full weight bearing is considered to be the time of clinical bone healing. Differences in the gender distribution of the type of the injury did not show statisticall signicance.

All the injuries were result of two different mechanisms, i.e. Road traffic accident and fall on flat surface. Morning stiffness as well as the swelling around the ankle joint were found in 26, i.e. 14% respectively, that also presents statistically significant percentage.

As an overall estimation, we can stress the fact that all the fractures treated with this type os osteosynthesis united in an optimal period of time, and also with satisfactory results. Our results did not differ much from already published data in other series (1,3,7) where other types of fixation were used. According to our opinion complication rate in our series is lower when comparing to other fixation methods, but further studies in that direction should be performed to prove this opinion.

**Conclusion**

On the end, we would like to conclude that with the analysis of the results, we have noticed some significant differences between the different groups, especially when discussing about beginning of the partial and full-unprotected weight bearing. Nevertheless, some parameters show less difference (overall VAS FA, third category of questions regarding VAS FA) that gives us hope for optimistic thinking related with the treatment of this type of fractures. Good functional outcome and patient satisfaction are the primary goals of the treatment of this type of injuries.

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UDK:616.77-006.6

# STAPKA NA INCIDENCA NA BCC (BASAL CELL CARCINOMA) NA O^NIOT KAPAK VO R.MAKEDONIJA

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**Izvadok**

Basal Cell Carcinoma (BCC- BCK) e voobi~aeno najzastapeniot malignom kaj lu|eto i prestavuva pribli‘no 80% od site ne-melanoma ko‘ni karcinomi. Incidencata na BCK na o~niot kapak poka‘uva varjabilnost vo svetot, godi{nata incidenca na 100.000 ‘iteli na BCK na o~niot kapak se dvi‘i od 0,3 do 3. Stapkata na incidenca vo studijata se dvi‘i 1,47 do 1,76 na 100.000 ‘iteli vo ispituvaniot period 2005-2008g.. Op{tata stapka na incidenca poka‘uva tendencija na zgolemuvawe. Vo ispituvaniot period vo izrabotenata studija, se registrira povisoka stapka na incidenca kaj ma{kiot pol od 1,66 do 2,04 na 100.000 ‘iteli, dodeka stapkata na incidenca kaj ‘enskiot pol e poniska i e od 1,28 do 1,47na 100.000 ‘iteli, stapkite na incidenca spored polot poka‘uvaat tendencija na zgolemuvawe. Pomalata incidenca na BCK na o~niot kapak kaj ‘enite mo‘e delumno da se objasni so toa da tie delumno so svojata kosa vr{at za{tita, po~esto koristat {e{iri i o~ila za za{tita od ultravioletovite zraci.

***Klu~ni zborovi:*** Basal Cell Carcinoma, o~en kapak, stapka na incidenca, pol

# INCIDENCE RATE OF BASAL CELL CARCINOMA (BBC) OF THE EYE LID IN R. MACEDONIA

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**Abstract**

Basal Cell Carcinoma (BBC) has been commonly the most represented malignant tumor in humans and account for approximately 80% of all non-melanoma skin carcinomas. The incidence rate of BBC of the eye lid shows variability in the world, the annual incidence rate ranges between 0,3-3%/100 000 population. The incidence rate in our study ranges from 1,47 to 1,76/100 000 population. General incidence rate shows a tendency to increase. In the analyzed period of the performed study, a higher rate was registered in male gender, ranging from 1,66 to 2,04/100 000 population, while the incidence rate of the female gender is lower, ranging from 1,28 to 1,47/100000 population, the incidence rates according to gender show a tendency to increase. The lower BBC incidence in women can be partly explained with the fact that the women make some protection with their hair, use hats and eyeglasses more often for protection from the ultraviolet rays.

***Key words:*** Basal Cell Carcinoma, eye lid, incidence rate, gender

## Introduction

Basal Cell Carcinoma (BCC- BCK) has been commonly the most represented malignant tumor in humans (1-3) and represents approximately 80% of all non-melanoma skin carcinomas (4). That percent varies from 84% in Singapore (5), 90% in France (6), 90,8% in the investigation of Cook in Minnesota (7), 65,1% in Taiwan (8). It is registered typically in parts of the human body exposed on the sun (9) and periocular region as a place of predilection. The fact is that it is registered even in ancient time (10), the first detailed description of BCK was as a tumor of the eye lid (11) (rodent ulcer, Jacobs 1827). BCK of the eye lid has a gradual growth and rarely gives metastases (12) but it causes significantly local destructions and disfragmentations if it is disregarded or treated inadequately, but with adequate therapy, the prognosis is excellent.

The BCK incidence rate of the eye lid shows a geographical variability, In Australia it is the highest in the world with 884/100.000 (13), while according to other studies the incidence is 823/100.000 (14,15). In Hawaii, Reziner et al. registered the highest incidence rate form the counties consisting the USA, from 422/100.000 (16). In Sweden, with the low UVR exposition almost every year are registered 15.000 cases in 9 millions, i.e. 166/100.000 population (14). In USA, half of the all registered carcinomas fell to the skin cancer, 407 BCK cases of the eye lid in 100000 population were males, and 212/100000 population to females (17). The incidence rate in the world has been increasing for 10% each year, and it is 300-600 cases/100000 population (18,19). In Australia, the BCK rate of the eye lid in persons younger than 60 years is stable, the period 1985-2000, due to the performance of the successful preventive programme (13).

**Aim:** To determine the BCK incidence rate of the eye lid in the 3-year period.

**Material and Methods**

The investigation comprised the period from the year 2005-2007. A retrospective method with prospective approach was used for realization of this aim. Data for the number of patients with BCK of the eye lid registered in R Macedonia were collected from the Institute for Public Health. Data of the population according to gender 2005-2007 were taken from the Bureau of Statistics of R Macedonia, estimation of the population in 30.06 and 31.12.

Data are presented tabelary and graphically. The incidence rate was calculated with adequate mathematical formula.

**Resultats**

The incidence rate of BCK of the eye lid within the period 2005-2007 ranged from 1,47/100.000 population in 2005; 1,71/100.000 population in 2006; and 1,76/100.000 in 2007 (Table 1-2, Graphs 1a-2a). In the analyzed period, a higher rate was registered in male gender, going from 1,66 to 2,04/100 000 population. The incidence rate of the female gender was ranging from 1,28 to 1,47/100000 population (Table 1-2; Graphs 1-2b).

**Table 1.** Distribution of the total number of cases with BCK of the eye lid in the territory of R Macedonia, within a period of three years

| year | total number | gender | number |
|---|---|---|---|
| 2005 | 30 | male | 17 |
| | | female | 13 |
| 2006 | 35 | male | 17 |
| | | female | 18 |
| 2007 | 36 | male | 21 |
| | | female | 15 |

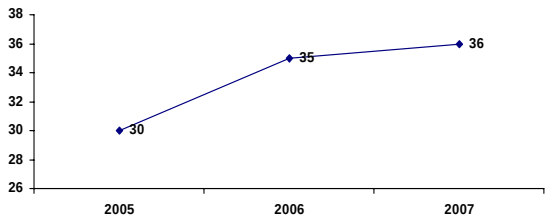


**Fig. 1 a**.

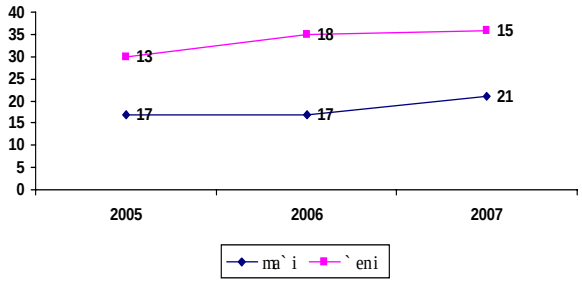


**Fig. 1 b**.

**Table 2.** Distribution of the incidence rate of registered cases with BCK of the eye lid in the territory of R Macedonia, within a period of three years

| **years** | **Incidence/100.000** | **gender** | **Incidence/100.000** |
|---|---|---|---|
| 2005 | 1,47 | male | 1,66 |
| | | female | 1,28 |
| 2006 | 1,71 | male | 1,66 |
| | | female | 1,76 |
| 2007 | 1,76 | male | 2,04 |
| | | female | 1,47 |

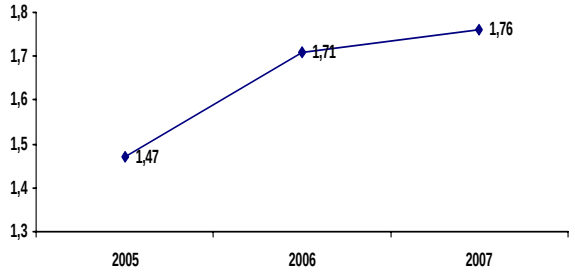


**Fig. 2 a**.

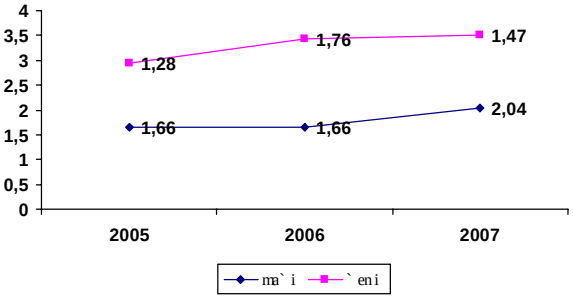


**Fig. 2 b**.

**Discussion**

The incidence of BCK of the eye lid shows shows a geographical variability in the world. Cooke et al. (7) in Minnesota (USA) showed that in the period of 15 years, registered an annual incidence rate between 1,37 to 0,08/100.000 population. In the period of 28 years, Lee et al. (5) made an investigations in Singapore, recording an incidence 6/1000000 population. The incidence showed a rise in the investigation performed in Taiwan of 1,5/1000000 in 1979 to 5,1/1000000 in 1999 (8). The incidence of BCK of the eye lid varied in the Finnish investigation within the period 1953-1997, between 0,7 to 3,0/100.000 population annually for men and 0,5 to 2,8/100.000 population for women (20). The annual incidence rate on 100.000 population with BCK of the eye lid in South-West Finnland showed statistically significant increase from 0,82 (1977-79) to 2,88 (1995-97) (21). The incidence rate in our study ranged from 1,47 to 1,76/100.00. General incidence rate showed a tendency to increase.

According to performed investigations of the skin cancers and periorbital region in Croatioa by Vuchic M. Et al. (22) within the period 1998-2002, from 286 registered, 62,65 fell to BCK, and 62% of them on the eye lid.

Representation of the gender in registration of BCK of the eye lid was variable. Cooke et al (7) in their investigation registered equal representation of the affected men/women, the incidence of men was 16,9/100.000 and 12,4/100.000. Higher incidence in relation to the gender was registered in Singapore (5), 6,5/1000000 men and 5,5/1000000 women. Higher incidence of BCK of the eye lid was registered by Paavilainen et al. (20) in men 2,1 and 1,9/100.000 population. Approximately, the same results Malhotra found in his investigation performed in Australia (23). In the investigation period of our study, a high incidence rate was registered in men, from 1,66 to 2,04/100.000 population, while the incidence rate in females was lower, ranging from 1,28 to 1,47/100.000 population, the incidence rates according to the gender showed a tendency to increase. Smaller incidence of BCK of the eye lid in women could be partly explained by the fact that the women partly make protection with their hair, use more frequently sunglasses for protection of ultraviolet rays.

Particular motive for making this epidemiological analysis, i.e. determination of the incidence rate in our country and the comparison with the incidence rates from available literature has been the disturbed ecological condition in the world, registration and enlargement of the ozone hole, as well as the increased UV radiation in the world and also in ours country. On this fact the location of the R Macedonia has been connected. The Republic of Macedonia is situated in South Europe, between $40^0$ 50' and $42^0$ 20' northern geographical latitude, and $20^0$ 27' and $23^0$ 0,5' eastern geographical longitude. It takes the central part of the Balkan peninsula, with a surface of 25.713 $km^2$. Because of the geographical location and the relief of the territory, Macedonia is under the influence of several climate types: from south and west the influence of the Mediterranean climate, from the west the winds, and from north and north-east enters the continental climate. As a consequence of these influences, three basic climate types are present on the territory of R Macedonia: altered Mediterranean, mountainous, and moderately continental climate with registration of above 250 sunny days within on calendar year.

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# AKTUELNI PU[A^I NA CIGARI I NIVNIOT RIZIK ZA POJAVA NA LARINGEALEN KARCINOM

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**Izvadok**

**Cel** na studijata e da se sogledaat eventualni kauzalni asocijacii me|u navikata za pu{ewe cigari i nastanuvaweto i distribucijata na laringealniot karcinom.

Istra‘uvaweto e case-control studija. Vo nea se vklu~eni 185 lica zaboleni od laringealen karcinom i identi~en broj lica bez maligno zaboluvawe, koi pretstavuvaat kontrolna grupa (KG). Preku presmetuvawe na rizicite so stapki na predimstvo (Odds ratio-OR) se definirani faktorite na rizik, koi imaat uloga vo nastanuvaweto na bolesta, a so intervali na doverba e definirana statisti~kata zna~ajnost na ispituvanite varijabli kako faktori na rizik.

Me|u ispitanicite so karcinom na larinks kategorijata aktuelni pu{a~i (AP) e zastapena so 79%. Vo KG e re~isi dvojno pomal procentot na AP (40,5%). Univarijantnata analiza poka‘a deka, aktuelnite i porane{nite pu{a~i zaedno imaat 16,03 (95% CI, 6,25-41,12), pati signifikantno pogolem rizik da zabolat od karcinom na larinks, sporedeno so nepu{a~ite. Pove}e od polovinata zaboleni pu{at 21-40 cigari na den (c/den) (54,8%). AP koi pu{at < 20 c/den imaat 10,49 (95% CI, 3,87-28,45), dodeka onie, koi pu{at > 20 c/den, imaat 45,6 (95% CI, 16,55-125,67), pati signifikantno pogolem rizik da zabolat od karcinom na larinks, sporedeno so nepu{a~ite. Koga se zemaat vo kombinacija brojot na dnevno ispu{eni cigari i dol‘inata na pu{a~kiot sta‘, rizikot za pojava na laringealen karcinom e ~etiri pati (95% CI, 2,35-7,88), signifikantno pogolem kaj ispitanicite, koi pu{at > 20 godini > 20 c/den, sporedeno so onie, koi istiot vremenski period pu{at < 20 c/den.

Rezultatite od studijata ja potvrduvaat ulogata na pu{eweto kako najzna~aen faktor na rizik za nastanokot na laringealniot karcinom.

***Klu~ni zborovi:*** laringealen karcinom, pu{ewe cigari.

# CURRENT CIGARETTE SMOKERS AND THEIR RISK FOR OCCURRENCE OF LARYNGEAL CANCER

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## Abstract

The aim of this paper was to analyze the eventual causal associations between the habit of cigarette smoking and onset and distribution of laryngeal cancer.

This was a case-control study. It comprised 185 patients with laryngeal cancer and matched controls with no malignant diseases. By calculating the odds-ratios, the risk factors that play a role in the disease onset, have been estimated. Statistical significance of the examined variables as risk factors has been defined with confidence intervals.

Among the interviewees with laryngeal cancer, the category of current smokers (CS) was represented with 79%. In control group (CG) the percent of CS was two-fold smaller (40,5%). Univariate analysis demonstrated that both current and former smokers had 16,03% (95% CI, 6,25-41,12), times significantly higher risk to become ill from LarC, compared to non-smokers. More than a half of the diseased have been smoking 21-40 cigarettes/day (c/day) (54,8%). CS who were smoking less than 20 c/day had 10,49 (95% CI, 3,87-28,45), while those who were smoking above 20 c/day, had 45,6 (95% CI, 16,55-125,67), times significantly higher risk to become ill from laryngeal cancer, compared to non-smokers. When a combination of the number of daily smoked cigarettes and the length of the smoking habit are taking into account, the risk of occurring LarC has been four times (95%, CI, 2,35-7,88), significantly higher in the interviewees who are smoking longer than 20 years, more than 20 c/day, compared to those, who in the same time period, smoke less than 20 c/day.

The results obtained have confirmed the role of smoking as the most important risk factor for the onset of laryngeal cancer.

***Key words:*** larynx cancer, smoking.

**Introduction**

Cancer represents a particular problem in highly developed industrial countries. In these countries, a great percent of general population belongs to older age categories, in which the risk of occurrence of this disorder is higher. In developed countries, cancer is on the second most frequent cause of death, after the cardiovascular system diseases and is responsible for one quarter of all deaths (1, 2).

The risk for those cancer locations being related with alcohol consumption (upper respiratory and digestive tracts), is increasing in several European countries, especially in Germany, Denmark and the East Europe countries, and further stays high in countries with the greatest risk for these locations, such as France and India. Highest incidence rates of laryngeal cancer (LarC) are noted in Brazil, India and USA (3, 4).

In the Republic of Macedonia the laryngeal cancer notes an incidence of increase in male gender. Within the period 1998-2005, this disease is on the fourth place among the ten most primary malignant neoplasms. Majority of ill persons were registered in 2005, with the incidence rate of 17, 9/100 0000 population. The least diagnosed persons with this disease was in 2002, when their number was 163 and the incidence rate 16, 1/100 000 population. LarC was significantly poorer represented in female persons and was not among the first ten most frequent primary localizations (5).

According to many studies, several risk factors are brought in connection with LarC. The most significant and generally accepted is alcohol consumption and the habit of cigarette smoking. Beside these, significant is the role of the professional exposition, particularly those on asbestos, inorganic acids, cement and the free crystal silicon (6). According to the International Agency for Research of Cancer (IARC), malignant tumors of lung, larynx, oral cavity, pharynx, esophagus, stomach, pancreas, kidneys, and bladder, are caused by cigarette smoking and are termed as tobacco-related cancers. According to many evaluations, cigarette smoking habit caused about 30% of all cancers, due to which it represents the most significant risk factor for occurrence of these disorders in humans (7).

The **aim** of this paper was to analyze the eventual causal associations between the habit of cigarette smoking and onset and distribution of laryngeal cancer.

**Material and Method**

This was a case-control study. It comprised 185 patients with laryngeal cancer (Investigated group – IG = 185) and matched controls with no malignant diseases (Control group – CG = 185). The questionnaire of the examines was conducted in the period between 01.V.2005-01.V.2007. Data was obtained from the Clinic of Otorhinolaryngology, Institute of Oncology and Clinic of Rheumatology. Only interviewees with pathohistologically verified laryngeal cancer were included in this study.

*Statistical analysis*

- Structure percents were determined in series with attributive characteristic;

- The risk factors were quantified through calculation of risks with Odds ratio (OR), having a role in occurrence of the disease, and with the Confidence intervals (CI – 95%) the statistical significance at error level less than 0, 05 (p) was defined.

**Results**

Among the interviewees with laryngeal cancer, the category of current smokers (CS) was represented with a high percent, being 79%. Former smokers (FS) were represented with 18,3%. Only five cases, representing 2,7% of the diseased, did not smoke and do not smoke cigarettes. In CG the percent of CS was two-fold smaller and was 40,5%. The group of FS comprised 28,7%, while there were many more non-smokers in relation to the diseased persons (57 of 30,8%).

Univariate analysis demonstrated that both current and former smokers had 16,03 (95% CI, 6,25-41,12), times significantly higher risk to become ill from LarC, compared to non-smokers. When only CS were taken into consideration, the risk was even higher, being 22,19 (95% CI, 8,53-57,71).

In the group diseased from LarC the percent was high for those who started with this habit before their age of 20 years, and was 82,8%. A 65,4% belonged in this category in CG. Only one interviewee with LarC started to smoke at the age over 40 years.

Current smokers with LarC, in average, started with this habit at the age 17,04±6,27 years, while the CG members, somewhat later, at the age 19,95±6,57 years.

**Table 1.** Distribution of interviewees according to number of cigarettes smoked daily (cigarettes per day – c/day)

| Number of cigarettes per day – c/day | Investigated group | | Control group | |
|---|---|---|---|---|
| | N | % | N | % |
| ≤ 10 | 2 | 1.4 | 12 | 16.0 |
| 11-20 | 44 | 30.1 | 38 | 50.7 |
| 21-40 | 80 | 54.8 | 22 | 29.3 |
| > 40 | 20 | 13.7 | 3 | 4.0 |
| Total | 146 | 100 | 75 | 100 |

The risk of LarC occurrence was 2,57 (95% CI, 1,35-4,88), times significantly higher in interviewees who started smoking before the age of 20, compared to those who did it somewhat later.

More than a half of the diseased have been smoking 21-40 cigarettes/day (c/day) (54,8%). Contrary to them, in CG members this percent was 29,3%. A total of 13,7% LarC interviewees smoked more than 2 boxes cigarettes per day, and only 3 (4,0%) from CG (Table 1).

The research realized showed that the CS, who were smoking less than 20 c/day had 10,49 (95% CI, 3,87-28,45), while those who were smoking above 20 c/day, had 45,6 (95% CI, 16,55-125,67), times significantly higher risk to become ill from laryngeal cancer, compared to non-smokers. Interviewees belonging to the group of the so called "passionate smokers" (persons who smoked > 40 c/day), had 3,81 (95% CI, 1,09-13,26), times significantly higher risk to become ill compared to those who smoked less than 40 c/day.

Information obtained by the interviewees spoke that 30,8% of the diseased persons tried to stop smoking. This percent in the CG members was somewhat higher, being 49,3%.

Majority of diseased persons with LarC had smoking length, ranging from 31 to 45 years (56,2%). In this group considerably great was the number of those who were smoking longer than 45 years (49 or 33,5%), contrary to CG in whom this number was smaller, and was 11 (14,6%) (Table 2).

Univariate analysis in current smokers showed significantly higher risk for the persons to become ill, who were smoking longer than 40 years, compared to those who were smoking less than 40 years (OR=3,73; 95% CI, 2,03-6,84).

When a combination of the number of daily smoked cigarettes and the length of the smoking habit are taking into account, the risk of occurring LarC has been

**Table 2.** Distribution of interviewees according to length of smoking (years)

| Duration of smoking (years) | Investigated group | | Control group | |
|---|---|---|---|---|
| | N | % | N | % |
| ≤ 15 | / | / | 2 | 2.7 |
| 16-30 | 15 | 10.3 | 20 | 26.7 |
| 31-45 | 82 | 56.2 | 42 | 56.0 |
| > 45 | 49 | 33.5 | 11 | 14.6 |
| Total | 146 | 100 | 75 | 100 |

**Table 3.** Habit of cigarette smoking and the risk of laryngeal cancer

| Variable | Cases[1] | Controls[2] | Crude OR[3] | 95% CI[4] |
|---|---|---|---|---|
| *Smoking habit* | | | | |
| Never smokers | 5 | 57 | 1.00 | |
| Current and ex-smokers | 180 | 128 | 16.03** | 6.25-41.12 |
| Never smokers | 5 | 57 | 1.00 | |
| Current smokers | 146 | 75 | 22.19** | 8.53-57.71 |
| *Age when interviewees started to smoke* | | | | |
| After 20 years of age | 25 | 26 | 1.00 | |
| Before 20 years of aage | 121 | 49 | 2.57* | 1.35-4.88 |
| *Number of cigarettes per day (c/day)* | | | | |
| Never smokers | 5 | 57 | 1.00 | |
| Current smokers < 20 c/day | 46 | 50 | 10.49** | 3.87-28.45 |
| Never smokers | 5 | 57 | 1.00 | |
| Current smokers > 20 c/day | 100 | 25 | 45.60** | 16.55-125.68 |
| *Duration of smoking (years)* | | | | |
| ≤ 40 years | 62 | 55 | 1.00 | |
| > 40 years | 84 | 20 | 3.73** | 2.03-6.84 |
| *Number of cigarettes per day (c/day) and duration of smoking (years) (combined)* | | | | |
| ≤ 20 c/day > 20 years | 48 | 49 | 1.00 | |
| > 20 c/day > 20 years | 97 | 23 | 4.31** | 2.35-7.88 |

[1]IG, Investigated group;[2]CG, Control group; [3]OR - Odds Ratio; [4]CI - Confidence Interval; *Significant at p< 0,05; ** Significant at p < 0,01.

four times (95% CI, 2,35-7,88), significantly higher in the interviewees who are smoking longer than 20 years, more than 20 c/day, compared to those, who in the same time period, smoke less than 20 c/day (Table 3).

**Discussion**

According to many estimates, the smoking habit causes about 30% of all cancers, because it represents the most significant risk ractor for occurence of this disease in humans.

Nowadays, it is known that cigarette smoking has been a risk factor for cancer development in several locations: lung, oral cavity, pharynx, larynx, oesophagus, stomach, pancreas, kidneys and urinary bladder (8). The investigation of Mocarska et al. (9) is one of many, in which the role of cigarette smoking has been stressed as the most significant risk factor for occurence of the laryngeal cancer. In the group of 120 patients, the majority were the current smokers. The author stresses the meaning of taking preventive measures to decrease their number.

The retrospective study of Alagic-Smailbegovic comprised 156 persons sick of LarC. The greatest percent of them were current cigarette smokers (93%), and only 11 (7%) were non-smokers (10).

Our realized study showed a high percent of current cigarette smokers among the patients with LarC (79%). It was interesting that only 5 persons, representing 2,7% of the patients, did not smoke cigarettes. In the CG, the percent of the current cigarette smokers was twice smaller (40,5%), while there were much more non-smokers in relation of the patient (57 or 30,8%).

In the study of Yun et al. (11), the risk of occurence the LarC has been analyzed in men, older than 30 years. The risk has been calculated concerning the age, body mass index, the frequency of physical activity, alcohol consumption and intake of some food types. The results showed that the current cigarette smokers have 3,01 times significantly greater risk to become ill in relation to the non-smokers [(adjusted relative risk - aRRs) = 3,01; 95% CI, 1,58-5,72].

Similar results were with the meta-analysis, conducted by Gandini et al. According to them, the relative risk for occurence of laryngeal cancer was 8,96 (95% CI, 6,73-12,11), times greater in the current smokers, compared to the non-smokers (12).

Case-control study performed in our country showed that the current and former smokers together had 16,03 (95% CI, 6,25-41,12), times significantly greater risk to become ill from laryngeal cancer, compared to the non-smokers. When only the current smokers were taken into consideration, the risk was even greater, being 22,19 (95% CI, 8,53-57,71).

Case-control study of Yokoyama et al. (13), investigated the occurence of the cancer of oesophagus and oropharyngolarynx. In this study 808 examinees were included, cigarette smokers and alcohol consumers, followed within the period from 1-148 months. In 53 of them, cancer was diagnosed during the period of the follow-up, the oesophagus cancer in 23, and oropharyngolarynx in 30. The results from the analysis showed that the persons smoking more than 30 c/day had 2,52 (95% CI, 1,22-5,22), times greater risk to become ill concerning to those, who smoked 0-19 c/day.

The evidence, according to which the cigarette smoking is the main risk factor for occurence of cancer of the upper aerodigestive tract (oral cavity, pharynx, larynx and oesophagus), has been known for a long time. On the other side, there is a small number of studies which study the effect of consuming small quantitites of tobacco products. One of them was the study realized by Polesel et al. (14). According to the results obtained, the risk for occurence of LarC was significantly greater in smokers, compared to the non-smokers, almost in consuming of 6 cigarettes per day. By this, the harmful effects from smoking was proved and the necessity of the social activation in fight against this habit.

Our investigation showed that the current smokers who were smoking less than 20 c/day had 10,49 (95% CI ,3,87-28,45), while those, who were smoking more than 20 c/day, had 45,6 (95% CI, 16,55-125,67), times significantly higher risk to become ill from laryngeal cancer, compared to the non-smokers.

**Conclusion**

Laryngeal and other tobacco related cancers could not often be treated successfully in the moment of diagnosing. Because of that, the key for reduction of these cancers lies in prevention.

A great number of published studies warn of drastic increase of smoking habit in the young age groups, that could bring to significant increase of the number of LarC patients in future. This threat could be avoided with implementation of strict anti-tobacco rules and health education: prohibition of smoking in educational institution at all levels and prohibition of cigarette sale to juvenile persons.

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# EPIDEMIOLOGIJA NA DAUNOV SINDROM VO REPUBLIKA MAKEDONIJA VO PERIODOT 1995 - 2007 GODINA

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**Izvadok**

Daunoviot sindrom pretstavuva arhetipi~no hromozomsko naru{uvawe i e prvata medicinska sostojba koja se manifestira kako hromozomska abnormalnost. Cel na trudot e da se odredi stapkata na incidenca i prevalenca na Daunoviot sindrom vo Republika Makedonija vo periodot 1995 - 2007 godina, kako i da se analiziraat site zaboleni spored pol, vozrast, tip na hromozomskata aberacija, starost na majkata, starost na tatkoto i stepen na mentalna retardacija. Trudot pretstavuva deskriptivna studija, a istara ‘uvaweto e sprovedeno vo Zavodot za mentalno zdravje na deca i mladi pri J.Z.U Zdravstvena dom Skopje. Analizirani se site pacienti so dijagnosticiran Daunov sindrom, so i bez mentalna retardacija, koi se evidentirani vo periodot od 1995-2007 godina. Podatocite se izvadeni od postoe~kite ambulantski dnevnici i registri vo Zavodot za mentalno zdravje na deca i mladi vo Skopje. Vo periodot od 1995 do 2007 godina, vo Zavodot za mentalno zdravje na deca i mladi pri J.Z.U Zdravstvena dom Skopje vo Republika Makedonija, registrirani se vkupno 89 zaboleni so dijagnoza Daunov sindrom, od koi 47(53%) bea ma{ki, a 42(47%) od ‘enski pol. Incidencata e najgolema vo 1995 godina i iznesuva 0,96/100 000 ‘iteli, a najmala vo 2002 godina - 0,045/100 000 ‘iteli. Prevalencata na Daunoviot sindrom vo na{ava zemja vo ispituvaniot period iznesuva 4,38/100 000 ‘iteli. Slobodnata trisomija 21 be{e dijagnosticirana kaj 66(74%) na{i ispitanici.

***Klu~ni zborovi:*** Daunov sindrom, incidenca, prevalenca, rizik-faktor

# EPIDEMIOLOGY OF DOWN'S SYNDROME IN THE REPUBLIC OF MACEDONIA DURING THE PERIOD OF 1995 - 2007

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**Abstract**

Down's syndrome is an archetypal chromosome disorder and it is the first medical condition manifested as a chromosomal abnormality. The aim of this paper was to determine the incidence and prevalence rates of Down's syndrome in the Republic of Macedonia during the period from 1995 to 2007 as well as to analyze all patients by gender, age, type of chromosomal aberration, maternal age, paternal age and degree of mental retardation. This is a descriptive study and the investigation was realized in the Institute of Mental Health of Children and Young Adults at the Health Center in Skopje. The investigation included all patients with Down's syndrome with or without mental retardation who have been registered during the period of 1995-2007. Data were taken from the existing outpatient diaries and registers of the Institute of Mental Health of Children and Young Adults in Skopje. In the period from 1995 to 2007, there were 89 registered patients with Down's syndrome, of whom 47 (53%) were males and 42 (47%) females. The biggest incidence rate was in 1995 – 0.96/100 000 inhabitants, and the smallest in 2002 – 0.045/100 000 inhabitants. Prevalence rate of Down's syndrome in our country during the examined period was 4.38/100 000 inhabitants. Free trisomy 21 was diagnosed in 66 (74%) of our subjects.

***Key words:*** Down's syndrome, incidence, prevalence, risk factor

## Introduction

Down's syndrome is the archetypal chromosome disorder and it is the first medical condition that is manifested as a chromosomal abnormality. It was first reported in 1959 by Lejeune, who proved the association between clinical features of Down's syndrome and extra chromosome 21.

Down' syndrome (DS) was named after Dr. John Langdon Down. In 1866 in his essay this physician described the typical characteristics of DS in a group of children with mental retardation, who looked like people from Mongolia and thus, were called mongoloids (1).

Numerous studies have pointed out the fact that DS characteristics appear as a result of chromosomopathies in which chromosome 21 is involved: free trisomy 21, Robertsonian translocations and mosaicism.

Free trisomy 21 is a trisomy of the pair 21 of chromosomes. It occurs during gametogenesis as a result of non-disjunction of chromosomes during the first or the second meiotic division resulting in gametes with 24 chromosomes instead with 23 chromosomes. In 90% of patients, free trisomy 21 reflects a maternal meiotic error (2, 3).

Robertsonian translocations 14/21 and 21/21 occur as a result of reciprocal translocation between two chromosomal groups. According to literature data, a healthy individual bears the translocated chromosome and he/she transfers it to the next generations (4).

Mosaicism or mixoploidy is a phenomenon when two or several cells are with different chromosomal complement due to non-disjunction, but they have origin from the same zygote (4). If non-disjunction happens earlier, then the percentage of trisomic cells is bigger and has obvious phenotypic manifestations of DS. If non-disjunction happens later in embryogenesis, trisomic cells are with a smaller percentage and phenotypic characteristics of the syndrome are poorly expressed (5, 6). Mosaicism is found in 2-4% of children with DS (4).

The possibility of creation of one extra 21 chromosome increases dramatically with advancing maternal age. It has been established that older mothers participate with 25% in giving birth to a child with DS, although only 9% of all pregnancies account for giving birth at older age (7). Although maternal age effect is known to be an important factor in Down's syndrome, in 20-25% of the cases the carrier of the mutation is the father (8, 9).

**Aim**

1. Determination of incidence and prevalence rate of Down's syndrome in the Republic of Macedonia between 1995 and 2007.
2. Registration and analysis of all patients with Down's syndrome in the Republic of Macedonia during the period of 1995-2007 according to gender, age, type of chromosomal aberration, maternal age, paternal age and degree of mental retardation.

**Material and Methods**

This is a descriptive study. The investigation was conducted in the Institute of Mental Health of Children and Young Adults at the Health Center in Skopje. All patients with diagnosed Down's syndrome, with or without mental retardation, registered in the period from 1995 to 2007 were analyzed. Data were taken from the existing outpatient diaries and registers at the Institute of Mental Health of Children and Young Adults in Skopje. Descriptive epidemiological method was applied. Analysis of the structure of the data obtained was done by relations, proportions and rates.

**Results**

During the period from 1995 to 2007, a total of 89 patients with Down's syndrome were registered in the Institute of Mental Health of Children and Young Adults at the Health Center in Skopje. The highest incidence was recorded in 1995 (0.96/100 000 inhabitants) and the smallest in 2002 (0.045/100 000 inhabitants), indicating a decreasing tendency. Down's syndrome prevalence rate in our country in the analyzed period was 4.38/100 000 inhabitants.

Of the total number of patients with Down's syndrome (89), 47 (53%) were males and 42 (47%) females. Seventy-eight (87.6%) patients were at the age of 10 years, 8 (9%) were up to 20 years and 3 (3.4%) were older than 21 years.

Free trisomy 21 was the most common chromosomal aberration in our patients and it was diagnosed in 66 (74%) of them. Down's syndrome due to Robertsonian translocations was registered in 4 (4.5%) patients and due to mosaicism in 19 (21.5%) patients.

**Table 1.** Incidence and prevalence rate of Down's syndrome in R. Macedonia during the period of 1995-2007

| Year | Number of citizensin R. Macedonia | Number of patients with Down's syndrome in R. Macedonia | Incidence rate / 100.00 |
|---|---|---|---|
| 1995 | 1.974.800 | 19 | 0,96 |
| 1996 | 1.991.398 | 7 | 0,35 |
| 1997 | 2.002.340 | 4 | 0,35 |
| 1998 | 2.012.705 | 4 | 0,19 |
| 1999 | 2.021.578 | 6 | 0,29 |
| 2000 | 2.031.112 | 11 | 0,54 |
| 2001 | 2.038.651 | 9 | 0,44 |
| 2002 | 2.023.654 | 1 | 0,045 |
| 2003 | 2.029.892 | 5 | 0,24 |
| 2004 | 2.035.196 | 4 | 0,19 |
| 2005 | 2.041.231 | 9 | 0,44 |
| 2006 | 2.078429 | 6 | 0,28 |
| 2007 | 2.083181 | 4 | 0,19 |
| Total | Average2.028.681 | 89 | Periodic prevalence 4,38 |

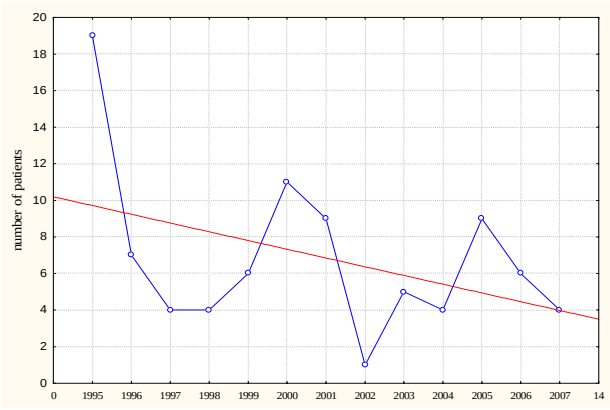


**Fig. 1.** Tendency of Down's syndrome in R. Macedonia during the period of 1995-2007

amniocentesis has been introduced for early diagnosis of this syndrome. In addition to the maternal age as a major risk factor, the most common indications for amniocentesis are: number of spontaneous abortions, positive family history, abnormalities in the development of some member of the family if there is a child with Down's syndrome, maternal/paternal diseases, increased drug consumption, radiation, mother's exposure to viral infections.

Free trisomy 21, which is the most common chromosomal aberration, was diagnosed in 66 (74%) of our patients. This percentage is smaller in comparison with its percentage in the world (90%). Mosaicism, on the other hand, was found in 21.5% of our patients, which is significantly higher compared to that found in the world (2-4%) (4).

**Table 2.** Distribution of patients by gender and age

| age / years | male | female | total |
|---|---|---|---|
| 0 - 5 | 24 | 22 | 46 |
| 6 - 10 | 16 | 16 | 32 |
| 11 - 15 | 4 | 1 | 5 |
| 16 - 20 | 2 | 1 | 3 |
| 20 < | 1 | 2 | 3 |
| total | 47 | 42 | 89 |

Mental retardation of different degree was registered in 73 (82%) patients, of whom 41 (56%) were males and 32 (44%) females. Of these, 27 (30%) patients had mild, 34 (38%) moderate and 12 (14%) severe form of mental retardation. In the remaining number of patients with Down's syndrome - 16 (18%), a psychophysical development delay was observed.

Distribution by parental age showed that the largest number of infants with Down's syndrome was born when their parents were at the age between 25 and 35 years. There was a large number of parents older than 35 years, and the smallest number was of those under the age of 25.

The possibility of creation of one extra 21 chromosome increases dramatically with advancing maternal age. The incidence of a 30-year-old mother to give birth to a DS infant is 1 per 1000, of a 35-year-old mother is 1 per 400, whereas of a 49-year-old mother is 1 per 12. The incidence increases proportionally as the mother's age advances (7, 8). In young mothers, Down's syndrome in their children is mainly due to chromosomal translocations (9).

In Serbia it was found that 70% of mothers who have DS children were younger than 35 years. Maternal age in this study has shown bimodality, meaning that the

**Table 3.** Distribution of patients by parental age

| age / years | mather | father |
|---|---|---|
| < 25 | 22 | 9 |
| 25 - 35 | 36 | 44 |
| > 35 | 31 | 36 |
| total | 89 | 89 |

**Discussion**

Prevalence rate of Down's syndrome in our country during the period from 1995 to 2007 was 4.38/100 000 inhabitants. Incidence rate of newborns with Down's syndrome is with a decreasing tendency since for older mothers as a high risk group a prenatal screening –

curve of dependence of DS on maternal age had two peaks. The first one was between 20 and 24 years and the second between 40 and 44 years when majority of DS children were born (10).

One of the latest theories about the onset of Down's syndrome is based on the impaired

microcirculation of the ovarian follicle. It occurs as a result of lack of oxygen and excess of carbon dioxide and milk acid that lead to chromosome damage. This hypothesis explains why mothers of all ages can give birth to a child with Down's syndrome. As reported in the literature, in 20-25% of DS children the carrier of chromosomal aberration was the father (4, 9).

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Ovie instrukcii se vo soglasnost so "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Site podatoci se dostapni na veb stranata www.icmje.org.).

Rakopisite }e bidat objaveni samo pod uslov ako nieden materijal, nitu negov del, tabela ili sli~no ne se i nema da bidat pe~ateni i dadeni nikade za objavuvawe pred nivnoto pe~atewe vo Acta Morphologica.Ovie pravila ne va' at za apstrakti ili slu~ai (case report ) prezentirani na nau~ni kongresi.

Izdava~ite }e gi razgleduvaat prifatenite i neprifatenite trudovi. Avtorite treba da objasnat kako nivnata rabota se razlikuva od ve}e postoe~kite nivni recenzirani trudovi no vo plik vo koj se ispra}a trudot.

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Rakopisot treba da gi potvrdi instrukciite vo Uniform Requirements 5th edition , New Engl J Med 1997;336 (4 ): 309-315. Trudot mora da sodr ' i ne pove}e od 5000 zbora . Na plikot vo koj se ispra}a se potpi{uvaat site avtori i ostavaat podatoci (po{.faks, tel. Broj, e- mail), dostapni za ponatamo{no kontaktirawe. Sekoj avtor mora da potpi{e izjava deka toj ili taa }e gi ispolni kriteriumite na Uniform Requirements.

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*Trud:*

1. Greenblatt DJ, Abernethy DR,Shader Jr RI.Pharmacocinetic aspects of drug therapy in the elderly ( commentary ). Ther drug Monit 1986; 8 ( 6 ): 249 - 255.

*Kniga:*

2. Mitchell JR, Horning MG ( Eds ). Drug metabolism and drug toxicity. New York; Raven Press, 1984:1 – 25.

*Poglavie vo kniga:*

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*Kniga vo serija:*

4. Usdin E,Asberg M,Bertilisson L (Eds). Frontiers in biochemical and pharmacological research in depression.New York; Raven Press,1984. (Advances in biochemical psychopharmacology; vol 39).

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Nestandarni karakteristiki (gr~ki bukvi, matemati~ki simboli i dr.) treba da se {ifriraat vo kontekst na tekstot. Ve molime napravete lista na koristewe na {ifrite.

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Manuscript must contain no more than 5000 words. A cover letter signed by all authors should identify the person (post address, telephone number, and e-mail address) responsible for negotiations. Each author must sign a statement attesting that he or she fulfills the authorship criteria of the Uniform Requirements. Each author must significantly contribute to the submitted work.

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3. Kutt H, Pippenberg CE et al. Plasma clearance of nor-methsuximide in a uremic patient. 223-226. In: Levy RH, Pitlick WH, Meijer J (Eds). Metabolism of antiepileptic drugs. New York; Raven Press, 1984.

**Book in a series:**

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**Internet:**

5.http://www.med.monash.edu.au/medical

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A structured abstract should be provided on a separate page with no more than 250 words, presenting essential data in five paragraphs introduced by separate headings in following order: Objectives, Background, Methods, Results, Conclusion. Complete sentences should be used. All data in the structured abstract must be present also in the submitted text or tables. Three to five key words should be added. Terms from Index Medicus should be used.

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| | | | |
|---|---|---|---|
| A a | A a | N n | N n |
| B b | B b | W w | Nj nj |
| V v | V v | O o | O o |
| G g | G g | P p | P p |
| D d | D d | R r | R r |
| \ \| | G g | S s | S s |
| E e | E e | T t | T t |
| @ ‘ | Zh zh | ] } | K k |
| Z z | Z z | U u | U u |
| Y y | Dz dz | F f | F f |
| I i | I I | H h | Kh kh |
| J j | J j | C c | Ts ts |
| K k | K k | ^ ~ | Ch ch |
| L l | L l | X x | Dzh dzh |
| Q q | Lj Lj | [ { | Sh sh |
| M m | M m | | |

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