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Rewiev

HOW RELIGION IMPACTSBLOOD DONATION

КАКО РЕЛИГИЈАТА ВЛИЈАЕ НА КРВОДАРУВАЊЕТО

Sadula Useini[1], Rada Grubovic [1,2] and Goran Andonov[1]

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Abstract

The possibility of using blood and blood products for medical treatment of patients represents one of the biggest achievements in modern medicine. The main objective of this process is to take blood from healthy persons without any harm to their health and to give this blood to the sick and injured.
The main question in achieving this objective is how to motivate the healthy persons to donate blood. Motivation is a major aspect on which transfusion services paygreat attention. In some countries, these services achieve excellent results, but on the other hand, there are countries where blood donation is on a very low level, which depends on social factors, political issues, economic development, cultural level, religion and some other hidden motivation problems. Understanding the blood to be "vital for life liquid" in the human development created a myth belief and according to that many different religious beliefs have appeared.The aimof this paper wasto present the attitudes of different religions towards blood donation and its influence on it.
Religion as a compilation of spiritual and philosophical views and understanding of the world and life needs has some general points of acceptance towards blood donation. Religious motives and attitudes towards blood donation as a reaction of altruism and humanity can be accepted only within the frames of general understanding and principles of certain religion.

Keywords: religion, blood donation, humanism

Абстракт

Можноста за употреба на крвта и крвните деривати во лечењето на болните представуваат една од најголемите продобивки на современата медицина. Во овој процес основно е да се земе крв од здрав човек, при тоа да не му се наштети и истата безбедно да се трансфундира во циркулацијата на болниот или повредениот човек. Така се поставува прашањето на крводарувањето-убедување на здравите луѓе да даруваат крв. Мотивирањето на крводарителите го претставува основниот аспект на кој службите за трансфузија на крв му придаваат голема важност. Во одредени држави службите за трансфузија постигнуваат одлични резултати, а од друга страна постојат средини каде дарувањето крв е на многу понизок степен, зависно од социјалната средина, политичките уверувања, имотната состојба, културното ниво, верата и другите скриени мотивациски проблеми. Разбирањето на крвта како "неопходна течност за животот" во развојот на човекот створи митски сфаќање на истата, и во однос на тоа постојат повеќе различни религиозни убедувања. Целта на овој труд е да се согледаат ставовите на различните религии во однос на крводарувањето и нивното влијание на истото.
Религијата како збир на духовениот и филозофски поглед и разбирањето на светот и животните потреби има одредени општи толкувања за крводарувањето. Религиозните мотиви на луѓето и нивните ставови кон крводарувањето како израз на возвишен чин на хуманост и несебичност, можат да се согледаат и да бидат прифатени само во рамките на генералното учење и прициите на одредена религија.

Клучни зборови: религија, крводарување, хуманизам

Blood Donation and Religion through History

Prehistoric religion developed through the perception of hunters, fishermen, farmers, collectors of fruits, who gave us a historic view of the world within its early roots in Egypt, Mesopotamia and China [1]. Prehistoric period started 3000 years before Christ was born; there is no written history about that period, no written notes about myths, rituals and believes of the people.
Knowledge about religion is a need of the contemporary culture; you cannot know the man if you do not know his religion [2]. The secrets of death and life are the challenges that have been moving the mankind to start the search of their existence. As far as we can go

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backward in the history, even before the first written documents, there are proofs that religion was a key aspect in human life. Progressive movements in further development of religion (including Hinduism, Judaism, Christianity, Islam), without any doubts, showed that in one moment they depend on each other, which can be seen especially in Judaism, Christianity and Islam, which had good relations and influence in Europe in that time.

In the period of the ancient Romans the understanding of the blood as a therapeutic, rejuvenating and strengthening matter developed. "Anima omnis in sanguine est" is not only a phrase; it is also a sign that old civilization knew the importance of blood and its connection with life. Ancient people used the blood in baths tocure them. Ancient Romansdrank the victim's blood in the gladiators' arenas, and believed that itwould bring them rejuvenation and revitalization. Ancient Greeks in their rituals used the blood of a bull or a goat to pour over the sick man body. Mayas [4] in their sacrificing rituals let the blood flow over the walls of the Temples in order to keep the shine and warmth of the sun, and thus they expected that the blood from one man given to another would keep the glory, the shine and the warmth, but, in fact, it kept the life in their hearts.

Christianity and Donation of Blood

No one can understand mankind if he cannot understand his religion. "Sometimes naive, sometimes sharp-witted empyreal, sometimes cruel, sometimes secret, sometimes full of irresistible sweetness and love, sometimes confirming the world, sometimes denying the world, sometimes opened only to itself, sometimes comprehensive and missionary, religions have woven though man's life since the ancient times."-Ninian Smart [5].

The question of creation of the man, his love to God, his love to kindred, moral purity are some of the most important issues of Christianity. The laws of the Old Testament are not given as something that stops, but as something that leads the man's answer to God; laws and sins can be summarized as great commandments as Jesus said "love your God with your whole being, and love your kindred as you love yourself" [6]. The issue of blood donation can be seen in adirect and clear position tothe roots of Christianity. This issue can generally be seen withinthe frames of humanism, that is, in the core of the Christian religion. As a proof that Christianity has positive examples regarding blood donation, there is a written document in which believers are asked to donate blood in Vatican with the following words: "Christ gave blood for you, so you should give it for the others".

Islam and Donation of Blood

The issue of blood donations in Islamic religion cannot be seen in a direct and close positioning in the roots of Islam-Kuran, Haids. Actually, this issue can be seen only in the frames of general understanding of the creation of people and the humanity that is preached by Islam. In relation to the issue of creation of the man, Islam preaches that all men are created by one man Adem (Adam), actually from his blood, and in this way mankind continues to exist. Kuran's attitude is clear "O people, we made you, really, from one man and one woman and then created tribes and nations to get to know each other". According to this, the act of creation of people is equal no matter of the color of the skin, class, nation or religion. There are many quotations from Kuran "there are no advantages for Arabs, before non-Arabs, the whites before the blacks" that speak about uniqueness of human nature. The biological development is common to all people as well as the biological needs. In relation to other hypotheses about the humanism in Islam, the Islam religion considers the human life as the most precious thing. This comes from Kuran's clear conception, which without doubt doesn't have a parallel saying: "And for this we tell the sons of Israel that anyone who kills one men, is as if he killed all man/people, and the one who saves one man is the same as if he save all man…" [7]. This citation speaks about sons of Israel that were Musa's (Moses) followers. Saving human life in the holiness of Kuran's citation is considered as a religious, human act. All that is aimed at helping, generosity and humanity can be considered as a virtuous act, andis, in fact, confirmation of believing.

Inthe end, most Kuran's citations speak about believing and virtues. Donation of blood is only one of these acts of humanism and altruism.

Other Religions and Sects and Donation of Blood

From a philosophical and sociological point of view no religion is against the act of blood donation as an act of humanism [8]. But, on the contrary of general understanding, there are some religions and sects such as Buddhism, Mormons and Jehovah's Witnesses, which forbid receiving and donation of blood. There isa study about patients who were Jehovah's witnesses [9] and whorefused to receive blood; consequently the mortality rate wasmore than 50%.

Conclusion

Studying the religious beliefsof people, the influence that religion has on community and economic structure [10-13], are precious source of recognition, which

allows finding new ways to motivatepeople for blood donation. Most religions, especially Christianity and Islam, are not against blood donation, but on the contrary, by means of the two main principles of the creation and humanity have a positive attitude towards blood donation. Some sects and religions, such as Jehovah's Witnesses, have a negative attitude towards using blood for therapeutic purposes.

Religion as a compilation of spiritual and philosophical views and understanding of the world and life needs has some general points of acceptance towards blood donation. The religious motives and attitudes towards blood donation as a reaction of altruism and humanity can be accepted only within the frames of general understanding and principles of certain religion.

Preciselypromotion activities for blood donation should be defined in cooperation with the representatives of all religions in our country, bywhich we believe the number of blood donors among believers will significantly increase. New studies should be encouraged to bring new insights in the understanding of the role played by the different dimensions of religiosity in the intention to donate blood, frequency of donations, and to practice altruistic attitudes concerning promotion of health.

Conflict of interest statement. None declared.

References

1. Marko Visic. Zakonici drevne Mesopotamije. *Svetlost*, Sarajevo, 1989.
2. Religijesvijeta-enciklopediskiprirucnik. Graficki zavod Hrvatske (Krscanska sadasnjost), 1991.
3. Trevor Ming. Istorija religije Istoka i Zapada, Srpska knizevna zadruga, Beograd, 2003.
4. Sijakovic B. Miodrag. Mitologija i religija Acteka, *Decija kniga*-Beograd, 1990.
5. Kosidovski Zenon. Bibliske legende. Narodna knjiga-Pogorica & *Miba books*, 2013.
6. BIBLIJA.
7. KURAN – Sura Maida, 32 citat.
8. Simon TL, McCullough J, Snyder EL, *et al*. Rossi's Principles of Transfusion Medicine. 5th edition. Hoboken (NJ): *Wiley-Blackwell*; 2016.
9. Descombes HM. Jehovah's Witnesses and blood transfusions. *Journal of Medical Ethics* 2001; 27: 355.
10. Abohghasemi H, Divkalayi NSH, Seighali F. Contribution of religion to blood donation: Iran experience. *Asian Journal of Transfusion Science* 2011; 5(2): 185-186. doi:10.4103/09736247.83262.
11. Martinez EZ, Almeida RGS, Braz ACG, *et al*. Association between religiousness and blood donation among Brazilian postgraduate students from health-related areas. *Rev Bras Hematol Hemoter* 2014; 36(3): 184-190. doi:10.1016/j.bjhh.2014.03.012
12. Gillum RF, Masters KS. Religiousness and blood donation: findings from a national survey. *J Health Psychol* 2010; 15: 163-172.
13. Otto PE, Bolle F. Multiple facets of altruism and their influence on blood donation. *J Socio-Econ* 2011; 40: 558-563.

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Original article

COMPARISON OF THERAPY WITH BOTULINUM TOXIN A, LOCAL NIFEDIPINE IN COMBINATION WITH LIDOCAINE AND MANUEL ANAL DILATION IN TREATMENT OF BLEEDING OCCURRENCE IN PATIENTS WITH PRIMARY CHRONIC ANAL FISSURES

СПОРЕДБА НА ТЕРАПИЈАТА СО BOTULINUM TOXIN A, ЛОКАЛЕН NIFEDIPINE ВО КОМБИНАЦИЈА СО LIDOCAINE И МАНУЕЛНА АНАЛНА ДИЛАТАЦИЈА ВО ТРЕТМАН НА ПОЈАВАТА НА КРВАВЕЊЕ КАЈ ПАЦИЕНТИ СО ПРИМАРНИ ХРОНИЧНИ АНАЛНИ ФИСУРИ

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Abstract

Introduction. The primary aim of this study wasto compare the success and clinical effect of injection therapy with botulinum toxin A (ITBT) versus manual anal dilatation (MAD) and local nifedipine in combination with lidocaine (LNCL) on the occurrence of bleeding during treatment of patients with primary chronic anal fissures (CAF). Anal fissure is a longitudinal tear of the mucosa of the anal canal extending from the outer anal orifice in the direction of the dentate line of the inner anal wreath. Fissures are divided into primary and secondary, and acute or chronic. Besides pain, which is a crucial symptom in CAF, bright red blood in the stool, hematochezia or so-called minimal rectal bleeding are also possible.

Methods. This controlled retrospective prospective longitudinal study includeda total of 94 patients, divided in 3 groups. The first group of subjects was treated with ITBT A, the second with MAD, and the third group with LNCL. Injection therapy with botulinum toxin A was performed using Clostridium botulinum A toxin-hemagglutination complex dissolved with sterile saline to a solution withfinal concentration of 200 U/ml. Following previous digital palpation of internal anal sphincter (IAS), the solution was applied to both sides of the CAF in the IAS at a total dose of 20U and volume of 0.1 ml. In order to minimize the risk of the occurrence of adverse effects, a variant of the Lord's stretch procedure was applied using a different technique and duration of the MAD. After extraction of the scope, gradually and progressively 3-4 fingers of one hand were introduced into the anal canal, followed by gradual lateral distraction acting on IAS in direction of the position of the 3 and 9 o'clock. The duration of the anal dilatation was 1 minute. Local therapy with nifedipine and lidocaine was conducted usingthe cream composed of nifedipine (0.3%) and lidocaine (1.5%), which had beenapplied by the subjectsthemselves at home, 2 times over 24 hours within 21 days, applying the cream in the anal canal at a depth of about 1 cm and in the perianal region to a total amount of 2.5-3g. All patients from the three groups were followed-up at4 and12 weeks afterwards.

Results. Of the total of 94 patients, 50 were males (53.2%), and 44 females (46.8%), at the median age of 46.6±13.9 years. The first group consisted of 31 patients treated with botulinum toxin A, the second group comprised 33 patients with MAD and the third group of 30 patients wastreated with LNCL. Distribution of patients with and without bleeding during defecation prior to intervention among the three groups, for p=0.0051 was confirmed as statistically significant. The overall significance is due to the significant difference between the first group in relation to the second and third group of patients (p=0.0049, p=0.0008, respectively). The type of conservative therapy had a significant effect on bleeding during defecation, at the first control at week 4(p=0.00012). Patients that still bled,treated with ITBT were significantly less common than patients treated with MAD, and significantly more frequent than patients treated with LNCL (p=0.007). Patients treated with MAD bled significantly more often than patients treated with LNCL (p=0.00003). Patients from the three groups had a significantly different time of bleeding disappearance during defecation, between the 4th and the 12th week (p<0.0001). The average time to stop bleeding was 4.7 ±2.5 weeks in the ITBT group of patients; 8.7±2.05 weeks in the MAD group of patients (the longest time); 4.2±1.5 weeks in patients treated with LNCL (the shortest time).

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Conclusion. Local nifedipine in combination with lidocaine significantly more often and faster than injection therapy with botulinum toxin A and manual anal dilatation reduces and ceases bleeding in patients with primary chronic anal fissures.

Keywords: chronic anal fissure, bleeding, botulinum toxin A, manual anal dilatation, local nifedipine in combination with lidocaine

Абстракт

Вовед. Примарната цел на оваа студија е да се спореди успешноста и клиничкиот ефект на инјекциската терапија со ботулински токсин А (ИТБТ) наспроти локален нифедипин во комбинација со лидокаин (ЛНКЛ) и мануелната анална дилатација (МАД) врз појавата на крвавење при третман на пациенти со примарни хронични анални фисури (ХАФ). Аналната фисура е надолжен расцеп на лигавицата на аналниот канал што се протега од надворешниот анален отвор во правец на назабената линија на внатрешниот анален венец. Фисурите се делат на примарни и секундарни и акутни или хронични. Иако болката е клучен симптом кај ХАФ, покрај неа, можна е и појава на светла црвена крв во столицата, хематохезијата или таканаречено минимално ректално крвавење.
Методи. Оваа контролирана ретроспективна проспективна лонгитудинална студија опфаќа вкупно 94 пациенти, поделени во 3 групи. Првата група на пациенти беше третирана со ИТБТ А, втората со МАД, и третата група со ЛНКЛ. Инјекциската терапија со ботулински токсин А беше изведена со употреба на Clostridium botulinum А токсин-хемаглутинирачки комплекс растворен со стерилен физиолошки раствор до конечна концентрација од 200 U/ml. По претходна дигитална палпација на внатрешниот анален сфинктер (ВАС), растворот беше аплициран на двете страни на ХАФ во ВАС во вкупна доза од 20U во 0,1ml волумен. Со цел да се минимизира ризикот од појава на несакани ефекти, беше искористена варијанта на Лордовата процедура за дилатација со употреба на поинаква техника и различно времетраење на МАД. По екстракција на аноскопот постепено и прогресивно беа воведувани 3-4 прста од едната рака во аналниот канал, по што следуваше постепена латерална дистракција на аналниот сфинктер делувајќи секогаш во насока на положбата на 3 и 9 часот. Времетраењето на аналната дилатација беше скратено на 1 минута. Локалната терапија со нифедипин и лидокаин беше спроведена со користење на крем составен од нифедипин (0,3%) и лидокаин (1,5%), кој беше применуван од самите учесници во домашни услови, 2 пати во текот на 24 часа во рок од 21 ден, со вметнување на кремот во аналниот канал во длабочина од околу 1 см и перианално во вкупна количина од 2,5-3г. Сите пациенти потоа беа следени на две контроли по 4 и 12 недели.
Резултати. Од вкупно 94 пациенти, 50 се мажи (53,2%) и 44 жени (46,8%), на средна возраст од 46,6±13,9 години. Првата група се состоеше од 31 пациент третирани со ботулински токсин А, втората група од 33 пациенти со МАД и трета група од 30 пациенти третирани со ЛНКЛ. Дистрибуцијата на пациенти со и без крвавење за време на дефекација пред интервенцијата меѓу трите групи, за p=0.0051 беше потврдена како статистички значајна. Севкупното значење се должи на значајната разлика помеѓу првата група во однос на втората и третата група на пациенти (p=0.0049, p=0.0008, соодветно). Типот на конзервативната терапија на ХАФ имаше значаен ефект врз крвавењето на првата контрола во 4-та недела (p=0.00012). Пациентите кои сеуште крвавеа и беа третирани со ИТБТ беа значително помалку од пациенти третирани со МАД и значително почесто од пациентите третирани со ЛНКЛ (p=0.007). Пациентите третирани со МАД крвавеа сигнификантно почесто од пациентите третирани со ЛНКЛ (p=0.00003). Пациентите од трите групи имаа сигнификантно различно време на исчезнување на крвавењето, помеѓу 4 и 12 недели (p <0,0001). Времето до стопирање на крвавењето изнесуваше во просек 4,7±2,5 недели кај пациентите во групата со ИТБТ; 8.7±2.05 недели кај пациенти во групата со МАД (најдолго време); 4.2±1.5 недели кај пациенти третирани со ЛНКЛ (најкратко време).
Заклучок. Локалниот нифедипин во комбинација со лидокаин значително почесто и побрзо од инјекциската терапија со ботулински токсин А и мануелната анална дилатација го намалува и анулира крвавењето кај пациенти со примарни хронични анални фисури.

Клучни зборови: хронична анална фисура, крвавење, ботулински токсин А, мануелна анална дилатација, локален нифедипин во комбинација со лидокаин

Introduction

Anal fissure is a longitudinal tear of the mucosa of the anal canal extending from the outer anal orifice in the direction of the dentate line of the inner anal wreath.It is essential to recognize the linear zone of delineation of the anoderm of the anal canal that can usually be identified in its lower half. Anal fissure as a disease was described for the first time by the British surgeon John Percy in 1934 [1]. Fissures are divided into primary and secondary, and acute or chronic. Primary ones are those that do not appear as part of other diseases. Most ofthe anal fissures are primary and appear asa consequence of a local trauma, such as passage of hard

stool, prolonged diarrhea, vaginal delivery, or anal intercoitus. Other anal fissures, which are usually multiple, large and irregular, are found in patients with previous surgical interventions, inflammatory bowel diseases, granulomatous disorders (sarcoidosis and tuberculosis), malignant diseases (planocellular cancer of the anal canal and leukemia) and some sexually transmitted diseases (syphilis, infection with *Chlamydia*, HIV and some others) [2].

There are two definitions that determine more closely the primary chronic anal fissures (CAF): anatomical and temporal. For the latter, there is still no clear, generally accepted consensus on a global scale on how long an anal fissure should persist and the difficulties it causes, in order to be able to say that it is chronic. Thus, according to studies from the older date of the last century, anal fissures were defined as chronic in all cases in which morphological signs and anal pain lasted longer than 4 weeks [3]. More recent data, such as those from the last Guide to the treatment of benign anorectal diseases by the American College of Gastroenterology of 2014, show that this period should last from 8 to 12 weeks [4]. But, according to other authors of the same period, this timeframe should be at least 6 weeks, or even shorter, but with similar episodes in the past [5, 6].

Anatomically, CAF is characterized by changes resulting from the associated presence of local edema and the onset of fibrosis. In other words, they have raised edges, with the so-calledsentinel pile (cutaneous duplication) at the distalend of the fissure and/or hypertrophic anal papilla at the upper end in the anal canal above the fissure. The first is often described by patients as a "painful hemorrhoidal node," and the second one can be seen by the endoscopic retroflection made in the ampulla of the rectum during colonoscopy. The muscle fibers of the IAS can be visible at the bottom of the CAF [3,4].

Chronic anal fissuresmanifest with anal pain, which is often present at rest, and is exacerbated by the act of defecation. The pain worsening with defecation can last for hours, but usually 1-2 hours. This symptom is described as the most troubling by patients. Besides pain, which is a crucial symptom of CAF, bright red blood, hematochezia or so-called minimal rectal bleeding are also possible. Other possible symptoms that occur less frequentlyinclude: pruritus, a feeling of spasm of the anal sphincter and constipation. It has to be keept in mind that anal fissures often interfere with the clinical presentation of the hemorrhoidal disease because both conditions are very common, and they also have common symptoms and signs and are very often present simultaneously. Finally, for these reasons, part of the presentation and the symptoms of anal fissures, especially bleeding and its intensity, may partly be a consequence of the presence of a simultaneous hemorrhoidal disease.

Monitoring patients with CAF is necessary after the diagnosis is confirmedand initiation of therapy in order to have insight, not only in the process of healing, eventual persistence or repeated occurrence, but also in the improvement of symptoms, using scores for measurement of intensity of the pain and bleeding. The common goals of all forms of therapy, both conservative and surgical, are to eliminate the symptoms of pain, bleeding and pruritus, and thereby achieve healing of the fissure with its full epithelization.

The main motive for the conducting of this study stems from the fact that in the majority of other studies published on this subject and related to the treatment of primary chronic anal fissures, the response of pain is mainly investigated and analyzed as a symptom, and much less the response of bleeding during treatment. The primary aim of this studywasto compare the success and clinical effect of injection therapy with botulinum toxin A versus anal dilatation and local nifedipine therapy in combination with lidocaine on the occurrence of bleeding in the treatment of patients with primary chronic anal fissures.

Material and methods

The study was designed as a controlled retrospective prospective longitudinal study conducted at the University Clinic of Gastroenterohepatology in the period from March 2015 to March 2018. The prospective time of conducting the study corresponds with the period required for proper collection and monitoring of the three samples of patients; in other words, until a minimum of 90 patients were analyzed, divided into three groups of at least 30 subjects, treated with ITBT, with MAD and with LNCL, respectively. To fulfillthe goals of the study, the data obtained from the patients themselves, from the personal observations of the researcher and from available medical documentation of the examinations, were used. All patients included in the study wereaged between 18 and 80 years and had objective, clinically and endoscopically confirmed primary CAF. Patients hadpreviously undergonetotal colonoscopywith a mandatory description of the exact position of the fissure and its morphological features. The study exclusively included patients with a minimum duration of anal pain of 6 weeks prior to initiation of treatment. The presence of acute anal fissure, inflammatory bowel disease, active local or systemic malignant disease, tuberculosis or sarcoidosis, perianal fistulas and abscesses, planned or present pregnancy, prior and parallel therapy with oral calcium channel blockers, present local infection, chemotherapy, data for prior local radiotherapy, previous surgery in the area of the anal canal, and the presence of a fourth degree hemorrhoidal disease were used as exclusion criteria.

The study includeda total of 94 patients, treated on outpatient basisor daily hospital. The first group of sub-

jects were treated with botulinum toxin A, the second group with MAD, and the third group with LNCL.One day before the intervention, all patients underwent colon cleansing using a macrogol solution (macrogol = polyethylene glycol 4500) as part of thepreparation. After the intervention all patients were advised to ingest an increased amount of fibers and fluids. None of the patients within the identified groups was applied any other additional form of therapy, nor was transition from one form of therapy to anotherallowed.To complete the physical findings, anoscopy was made after a previously performed external anal examination and anorectal digital examinationin all subjects when they joined the study, after obtaining a written informed consent. Following the administration of one of the three forms of therapy, the intensity of bleeding was measured by applying a visual analog scale (VAS) expressed in 1 to 10 consisting of a flat line divided into equal parts from 0 to 10, wherein one end marked with 0 denotes the absence of symptoms, and the other end marked by 10 marks the strongest possible symptoms. Scores from 1 to 3 indicate the presence of mild bleeding, 4 to 6-moderate bleeding and 7 to 10-intense bleeding. Improving of the bleeding was defined by its decrease or disappearance and was used as a measure of the effectiveness of therapeutic methods. The presence and intensity of bleeding prior to intervention were noted, and the presence or disappearance of bleeding on each of the controls with the exact time passed was observed.

Injection therapy with botulinum toxin A was performed by using Clostridium botulinum A toxin-a hemagglutination complex (Dysport®, Ipsen Biopharm Ltd, Wrexham, United Kingdom) in the form of a powder for injection solution, packaged in vial with a volume of 3 ml containing 500 units (U) botulinum toxin A, previously placed and stored at 2-8ºC, and then dissolved with sterile saline solution in an amount of 2.5 ml, giving a final concentration of 200 U/ml. Afterwards and following previous digital palpation of IAS with the thumb and index finger of the left arm, the solution made in this manner was applied to both sides of the CAF in the IAS at a total dose of 20 U and volume of 0.1 ml. The application was carried out by using 1 ml insulin syringe and a needle of 10 mm, 25 G. The application was carried out after an obligatory initial examination with an anoscope (Hirschmann 65 mm) with the possibility of a detailed inspectionof the anal canal, the location and morphological features of CAF, with the assistance of endoscopy nurse that helps during the entire procedure, and during the application when it is necessary to spread the gluteal region.

Anal dilatation, as a manual technique, was performed in all patients using a local anesthetic-20% lidocaine in the form of a gel. During the intervention, patients were placed in the knee-elbow position. In order to minimize the risk of the occurrence of adverse effects, a variant of the Lord's procedure was applied using a different technique and the different duration of the procedure, as follows: each dilatation was carried out with the initial insertion of the anoscope with the possibility of a detailed overview of CAF. After the extraction of the instrument, 3 to 4 fingers were graduallyand progressively introduced only from one hand into the anal canal, followed by a lateral distraction of the IAS, acting only in the direction towards positions of 3 and 9 o'clock (never cranio-caudally in the direction of 6 and 12 o'clock) (Figure 1). This variant of the technique, from the aspect of the mode of execution and its shortend duration, was applied as a result of the idea that the intensity and extent of the strength by applying fingers only from one hand are much smaller compared to the classical technique using the fingers from both hands, and thus the strength of the two forearms for a period of 3-4 minutes. The duration of each individual MAD was shortened compared to the Lord's technique and lasted for one minute. In cases of hemorrhage from the site of the treated CAF after the intervention, bleeding was controlled using short-term local compression.

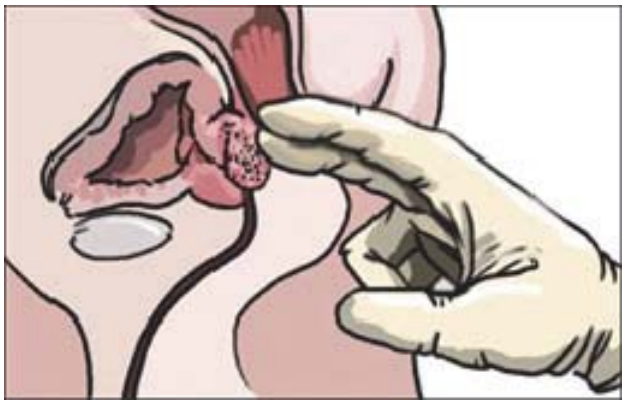

Fig. 1. Manual anal dilatation technique using one arm

Local therapy with nifedipine and lidocaine consisted of the use of a cream composed of nifedipine (0.3%) and lidocaine (1.5%), which had beenapplied by the participants themselves at home, 2 times over 24 hours within 21 days, applying the cream in the anal canal at a depth of about 1 cm and perianally in a total amount of 2.5-3g, that corresponds to a length of about 1 cm of squeezed cream in the endorectal applicator. In order to achieve satisfactory response, all patients were given special attention that a high level of cooperation is necessary, without any attempts to prematurely stop the usage of the cream.

After initial treatment with any of the three methods, patients were clinically monitored at intervals of 4 and 12 weeks after the intervention. Regardless of the scheduled controls in this way, in case of a worsening of the symptoms, the patients had the opportunity to consult at any time at their request. The implementation of the study was approved by the Ethics Committee ofthe Medical Faculty in Skopje and wasin accor-

dance with the European Directive 2001/20 / EC of the European Parliament and of the Council from 4th of April, 2001.

Kolmogorov-Smirnov test and Shapiro-Wilki test were used for testing the normality of the distribution. The categories are represented by absolute and relative representation, the quantitative marks-with mean, standard deviation (SD), median and rankings (IQR). For comparison of the three methods of treatment of CAF in relation to the analyzed parameters, parametric (Student's t-test for independent and dependent samples, Wilcoxon Matched paired test, ANOVA repeated measurements) and nonparametric methods (Chi-square test, Mann-Whitney test, Kruskal-Wallis ANOVA test) were used. For the level of significance, the value of $p < 0.05$ was taken; and for the highly significant value of $p < 0.01$.

Results

This section presents the results obtained by treatment and analysis of 94 patients with primary chronic anal fissures. Of the total of 94 patients, 50 were males (53.2%), and 44 females (46.8%), at the median age of 46.6 ± 13.9 years in the range of 20-75 years. Patients weredivided into 3 groups, depending on the type of therapeutic treatment: the first group consisted of 31 patients treated with botulinum toxin A, the second group comprised 33 patients with MAD and the third group of 30 patients treated with LNCL.

Male patients weremore commonly present in the groups treated with MAD and LNCL-54.55% (18) and 56.7% (17) respectively, while female patients were more commonly included in the botulinum toxin group - 51.6% (16). The tested differences in the distribution of males and females among the three groups were statistically non-significant (p=0.79), thus making the groups homogeneous in relation to sex (Table 1 and Table 1a).

Table 1. Descriptive analysis of patientsby gender

Gender	N	Type of intervention group 1 n=31 (%)	group 2 n=33 (%)	group 3 n=30 (%)
Male	50	15 (48.39)	18 (54.55)	17 (56.67)
Female	44	16 (51.61)	15 (45.45)	13 (43.33)

gr. 1: injection therapy with botulinum toxin A; gr. 2: manual anal dilatation; gr. 3: local nifedipine in combination with lidocaine

Table 1a. Tested gender differences

Tested differences– all groups(Chi-square=0.46 p=0.79 ns)		
Type of intervention	**group 2**	**group 3**
group 1	p=0.058 ns	p=0.52 ns
group 2		p=0.86 ns

Table 2. Descriptive analysis of patientsby age

Type of intervention	N	Descriptive Statistics(age) mean ± SD	std err	Min-max	p value
group 1	31	48.55±11.7	2,105	20-73	F=0.0054
group 2	33	48.48±14.1	2,452	21-71	p=0.99 ns
group 3	30	48.83±15.9	2,920	25-75	

gr. 1: injection therapy with botulinum toxin A, gr. 2: manual anal dilatation, gr. 3: local nifedipine in combination with lidocaine

The average age of patients in the three groups was similar and statistically insignificantly different (Table 2). Distribution of patients with and without bleeding during defecation prior to intervention among the three groups, for p=0.0051 was confirmed as statistically significant. The overall significance wasdue to the significant difference between the first group relatively to the second and third group of patients (p=0.0049, p= 0.0008, respectively) (Figure 1 and Table 3).

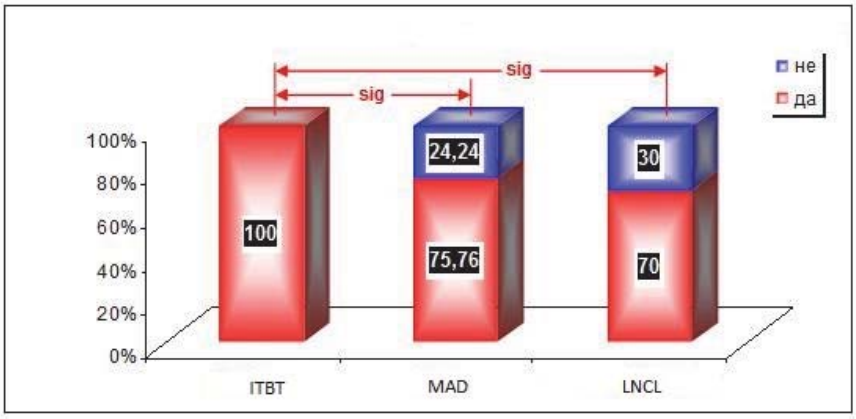

Fig. 1. Bleeding during defecation prior to intervention

Table 3. Tested differences for bleeding during defectation prior to intervention

Tested differences-all groups (Chi-square=10.56 p=0.0051 sig)		
Type of intervention	**group 2**	**group 3**
group 1	p=0.0049 sig	p=0.0008 sig
group 2		p=0.82 ns

gr. 1: injection therapy with botulinum toxin A, gr. 2: manual anal dilatation, gr. 3: local nifedipine in combination with lidocaine

Table 4 shows the distribution of patients from the three groups with respect to their assessment of the intensity of bleeding before the intervention, expressed with the VASscores in absolute values (1-the worst bleeding, 10-the strongest bleeding) (Table 4).

The type of CAF conservative therapy had a significant effect on bleeding during defecation, onthe first control at week 4 (p=0.00012). Patients treated with ITBT were significantly less common than

Table 4. Intensity of bleeding before intervention according to VAS in absolute values

Before intervention				
Intensity of bleeding, absolute values, VASscores − 1-10	**N**	**Type of intervention**		
		group 1 n=31 (%)	**group 2** n=33 (%)	**group 3** n=30 (%)
1	3	1 (3.2)	1 (3.85)	1 (4.76)
2	7	0	4 (15.3)	3 (14.2)
3	14	4 (12.9)	5 (19.2)	5 (23.8)
4	15	8 (25.8)	6 (23)	1 (4.76)
5	15	8 (25.8)	2 (7.69)	5 (23.8)
6	16	7 (22.5)	6 (23)	3 (14.2)
7	5	2 (6.45)	2 (7.69)	1 (4.76)
8	2	0	0	2 (9.52)
10	1	1 (3.23)	0	0

gr. 1: injection therapy with botulinum toxin A, gr. 2: manual anal dilatation, gr. 3: local nifedipine in combination with lidocaine

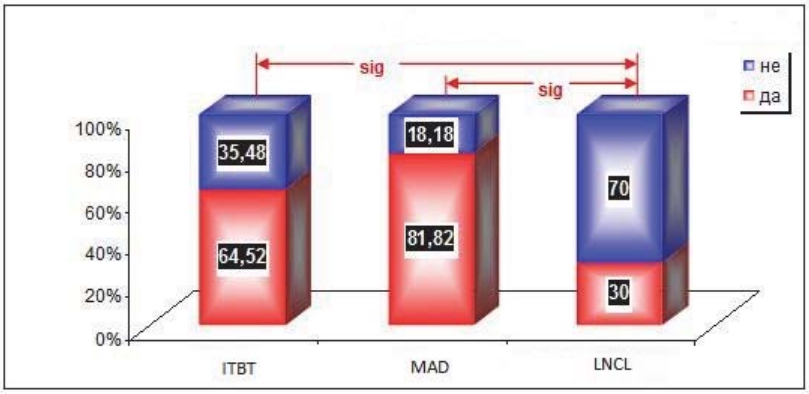

Fig. 2. Presence of bleeding at first control

patients treated withMAD that still bled, and significantly more often than patients treated with LNCL (p =0.007). Patients treated with MAD bled significantly more often than patients treated with LNCL (p= 0.00003) (Figure 2, Table 5).

Table 5. Tested differences on the presence of bleeding at first control

Tested differences– all groups (Chi-square=17.98p=0.00012 sig)		
Type of intervention	**group 2**	**group 3**
group 1	p=0.12 ns	p=0.007 sig
group 2		p=0.00003 sig

gr. 1: injection therapy with botulinum toxin A, gr. 2: manual anal dilatation, gr. 3: local nifedipine in combination with lidocaine

In the period of 4 weeks after the onset of the study, patients in the three groups most commonly found bleeding as poor-69.2% (18); 74.1% (20); 82.35% (14); respectively.

After 12 weeks of intervention, the presence of blood during defecation had 19.35% (6) of patients from the first group; 15.15% (5) of the second group; 10% (3) of patients in the third group. In the period of 12 weeks after the start of the study, during the second control, patients who still bled, rated the bleeding as a mild 100% (19) in the group with ITBT, 100% (27) in the MAD group and 87.5% (7) in the third group of LNAs, and only 1 patient in the last group had a moderate bleeding. No significant difference in bleeding intensity was observed in defecation, and depending

on the type of treatment of CAF for a period of 12 weeks (p = 0.15).

Patients from the three groups had a significantly different time of bleeding disappearance during defecation, between the 4th and 12th weeks (p<0.0001). The average time to stop bleeding was 4.7±2.5 weeks in the ITBT group of patients; 8.7±2.05 weeks in the MAD group ofpatients (the longest time); 4.2±1.5 weeks in patients treated with LNCL (the shortest time). Post-hoc analysis of intergroup comparisons showed that patients treated with manual anal dilatation had a significantly longer duration of bleeding in defecation compared to patients treated with botulinum toxin (p= 0.00012) and compared to patients treated with local nifedipine in combination with lidocaine (p=0.0001) (Figure 3 and Table 6).

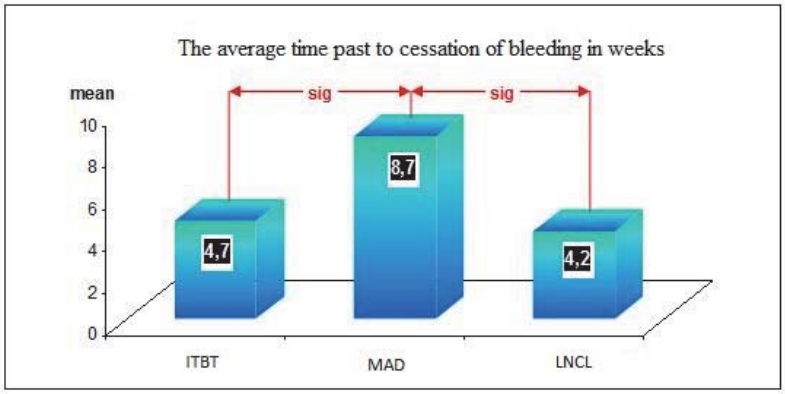

Fig. 3. Average time past to cessation of bleeding atsecond control

Table 6. Tested differences in time past to end of bleeding at second control

Tested differences–all groups F=28.85 p=0.00000 sig Post hoc Tukey test		
Type of intervention	**group 2**	**group 3**
group 1	p=0.00012 sig	p=0.77 ns
group 2		p=0.00012 sig

gr. 1: injection therapy with botulinum toxin A, gr. 2: manual anal dilatation, gr. 3: local nifedipine in combination with lidocaine

Discussion

There is almost no single publication related to the occurrence of CAF in which the term bleeding during and after defecation does not occur. Although it is the second most common symptom in CAF after pain and occurs in a significant proportion of patients, it is still far from being systematically investigated, such as pain. The frequency of its prevalence is usually analyzed, or in other words, the number of patients with CAF in whom there is bleeding before the application of therapy. Thus, Hananel N and Gordon PH found that bleeding wasa common symptom of CAF found in 71.4% of patients (7), but this percentage variedin some other studies and ranged from 74.6% (8) to 84% (9). Our results showed that bleeding during defectation before the intervention had all patients treated with ITBT; 75.8% treated with MAD and 70% treated with LNCL. The distribution of patients with and without bleeding prior to the intervention among the three groups, for p =0.0051 was confirmed as statistically significant due to the significant difference between the first group in relation to the second and third group of patients (p= 0.0049; p=0.0008; respectively). In only a small number of studies, bleeding in CAF has been further elaborated, analyzed and monitored throughout their duration until completion. In support of this statement, is the evidence that in one meta-analysis, which included 34 studies with a total of 1577 patients, a bleeding questionnaire was used in only 10 studies [10]. Furthermore, in the last meta-analysis of Nelson RL. for non-surgical forms of CAF treatment from 2012, out of a total of 77 studies analyzed, only in 2 the term bleeding was present [11]. Inthese two studies, as well as in few other, bleeding wasanalyzed as a measure of outcome for the applied forms of therapy [12,13]. The presence and intensity of bleeding before the intervention, and the presence or interruption of bleeding ateach control with a precisely stated time to the interruption was a measure of outcome in our study. At the first control, after week 4, the type of conservative therapy of CAF had a significant influence on the presence of bleeding during defecation (p=0.00012). Patients treated with ITBT and with MAD were significantly more likely to bleed than patients treated with LNCL for p=0.007 and p=0.00003, respectively.

Patients from the three groups had significantly different past time to the end of bleeding during defecation (p<0.0001). The time to stop bleeding was the shortest (4.2±1.5 weeks) in patients treated with LNCL and significantly less bleeding was registered in the same group of patients (p=0.0015) at 4 weeks after the intervention. No significant difference in bleeding intensity was observed depending on the type of treatment, for a period of 12 weeks (p=0.15). In a study comparing controlled intermittent dilatation and LIS, no statistically significant difference was observed in relation to the occurrence of bleeding [14]. In a double-blind study,

in which patients were divided into two groups, one of which was treated with nifedipine and the other with isosorbide dinitrate, the disappearance of bleeding was 91.4% and 97.1% at 2 and 3 weeks in the nifedipine group compared to 71.4% and 91.4% in the group treated with isosorbide dinitrate, respectively (p=0.149 and p =0.498) [15]. In the study of Guy F. initial bleeding was reported in 84% of patients. There was a statistically significant difference in the bleeding reduction between the group treated with self-dilatation of IAS and the other treated with passive anal dilatation (group A: p=0.0001; group B: p=0.0001) [9]. Other CAF studies mentioning bleeding, report it only as a complication of the invasive forms of therapy rather than as an outcome measure [16]. Thus, in a study describing endoscopic dilatation as a new method for treating CAF, after an average period of 19 months of follow-up no case of bleeding and other complications were reported in 62 patients [17].

Conclusion

Despite the positive effect of all three analyzed forms of treatment on the occurrence of bleeding, treatment with local nifedipine in combination with lidocaine significantly more often and faster than injection therapy with botulinum toxin A and manual anal dilatation reduces bleeding in patients with chronic anal fissures.

Conflict of interest statement. None declared.

References

1. Lockhart-Mummery JP. Diseases of the rectum and colon and their surgical treatment. Toronto: *MacMillan;* 1934.
2. Nelson RL, *et al*. Prevalence of benign anorectal diseases in a randomly selected population. *Dis Colon Rectum* 1995; 38: 341.
3. Goligher JC. Stretching of anal sphincters in treatment of fissure-in-ano. Surgery of the Anus, Rectum & Colon. 3rd Edition. London: *Balliere & Tindall*, 1975.
4. Wald A, *et al*. ACG clinical guideline: management of benign anorectal disorders. *Am J Gastroenterol* 2014; 109: 1141-57. http://dx.doi.org/10.1038/ajg.2014.190.
5. Zaghiyan KN, Fleshner P. Anal fissure. *Clin Colon Rectal Surg* 2011, 24: 22-30. http://dx.doi.org/10.1055/s-0031-1272820.
6. Beaty JS, Shashidharan M. Anal Fissure. *Clin Colon Rectal Surg* 2016; 29: 30-37.
7. Hananel N, Gordon PH. Re-examination of clinical manifestations and response to therapy of fissure-in-ano. *Dis Colon Rectum* 1997; 40(2): 229-233.
8. Vanella S, *et al*. Botulinum toxin for chronic anal fissure after biliopancreatic diversion for morbid obesity. *World J Gastroenterol* 2012; 18(10): 1021-1027.
9. Gaj F, Bivianob I, Candelorob L, Andreuccettic J. Anal self-massage in the treatment of acute anal fissure: a randomized prospective study. *Ann Gastroenterol* 2017; 30 (4): 438-441.
10. Bobkiewicz A, Francuzik W, Krokowicz L, Studniarek A, *et al*. Botulinum Toxin Injection for Treatment of Chronic Anal Fissure: Is There Any Dose-Dependent Efficiency? A Meta-Analysis. *World J Surg* 2016; 40: 3064-3072.
11. Nelson RL, Thomas K, Morgan J, Jones A. Nonsurgical therapy for anal fissure. *Cochrane Database Syst Rev* 2012; 2: CD003431.
12. Eshghi F. The efficacy of L-arginine gel for treatment of chronic anal fissure compared to surgical sphincterotomy. *Journal of Medical Sciences* 2007; 7(3): 481-484.
13. Kenny SE, Irvine T, Driver CP, *et al*. Double blind randomised controlled trial of topical glyceraltrinitrate in anal fissure. *Arch Dis Childhood* 2001; 85: 404-407.
14. Yucel T, *et al*. Comparison of controlled-intermient anal dilatation and lateral internal sphincterotomy in the treatment of chronic anal fissures: a prospective, randomized study. *Int J Surg* 2009; 7(3): 228-231.
15. Khaledifar B, Mahmoudi MYA, etMobasheri M. A Double-Blind Randomized Trial Comparing the Effectiveness and Safety of Nifedipine and Isosorbide Dinitrate in Chronic Anal Fissure. *Malays J Med Sci* 2015; 22(5): 42-49.
16. Nelson RL, Chattopadhyay A, Brooks W, *et al*. Operative procedures for fissure in ano (Review). *Cochrane Database Syst Rev* 2011; 11: CD002199.
17. Perez-Miranda M, *et al*. Endoscopic anal dilatation for fissure-in-ano: a new outpatient treatment modality. *Rev EspEnferm Dig* 1996; 88(4): 265-272.

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ММП

Original article

SINONASAL MICROBIOTA IN PATIENTS WITH CHRONIC RHINOSINUSITIS

СИНОНАЗАЛЕН МИКРОБИОТ КАЈ ПАЦИЕНТИ СО ХРОНИЧЕН РИНОСИНУЗИТИС

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Abstract

Introduction. Chronic rhinosinusitis (CRS) is a common, chronic inflammatory disorder of the nose and paranasal sinuses. Microbiome refers to the genetic potential of the entire cohort of resident microorganisms that inhabit the sinus cavities and function as an organized community. A microbiota is an ecological community of commensal, symbiotic and pathogenic microorganisms. The aim of the study was to investtigate and to compare the microbiome results of middle nasal meatus swab samples and corresponding maxillary sinus aspirate samples, taken from the same patients with chronic rhinosinusitis.

Methods. Twelve patients with clinical and radiological evidence of CRS were included in this study. All patients underwent general anesthesia and functional endoscopic sinus surgery technique as a minimally invasive procedure. Two different types of sampling for investigating the sinonasal microbiome were performed in each patient-the first sample was endoscopically-guided middle nasal meatal swab, while the second sample was maxillary sinus aspirate using a Mucoset with a sterile container, obtained intraoperatively. For the purpose of cultivation of the microorganisms, different types of media were used.

Results. The most frequent species determined in middle nasal meatal swabs were: *Staphylococcus aures* in 5 (20.8%), *Staphylococcus Coagulasae negative* in 4 (16.7%) and *Streptococcus pneumoniae* in 3(12.5) samples. *Staphylococcus Coagulasae Negative* was found in 5 (20.8%) while *Staphylococcus aures* in 4(16.7%) maxillary sinus aspirates as the most permanent species. Fisher's exact test was used for testing the distrubition of isolated microbes in middle nasal meatus and maxillary sinuses, and for p=0.8 the difference was statistically insignificant.

Conclusion. Microbiomes in middle nasal meatus do not differ significantlly from microbial flora in maxillary sinus. Microbiome in middle nasal meatus is highly representative and reflects the microbiome in corres ponding maxillary sinus.

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Keywords: chronic rhinosinusitis, microbiota, middle nasal meatus swab, maxillary sinus aspirate

Апстракт

Вовед. Хроничниот риносинузитис претставува хронично инфламаторно заболување на носот и параназалните синуси. Микробиом го опфаќа целокупниот генетски потенцијал на резидентните микроорганизми кои ги населуваат носната и синусните празнини и функционираат како заедница. Целта на студијата беше да се истражи и спореди микробиомот добиен со брис од средниот носен ходник со микробиомот добиен од аспират на истостраниот максиларен синус кај пациенти со хроничен риносинузитис.

Методи. Во студијата беа вклучени дванаесет пациенти со клинички и радиолошки знаци за хроничен риносинузитис. Во општа ендотрахеална анестезија беше релизирана функционална ендоскопска синусна хирургија како минимално инвазивен метод. Кај секој пациент, во тек на оперативната интервенција беа користени два различни начини на земање примероци за истражување на синоназалниот микробиом-првиот начин беше ендоскопски земен брис од среден носен ходник додека вториот начин беше колекција на аспират од истостраниот максиларен синус со употреба на Mucoset во стерилен контејнер. Земените примероци беа засадувани на различни типови на органски и неоргански подлоги.

Резултати. Најчести бактериски специеси изолирани од брисот од среден носен ходник беа *Staphylococcus aures* во 5(20,8%), *Coagulasa negative staphylococcus* во 4(16,7%) и *Streptococcus pneumoniae* во 3(12,5) примерока. *Coagulasa negative staphylococcus* беше детектиран во 5(20,8%) додека *Staphylococcus aures* во 4(16,7%) примероци на аспирати од максиларен синус како најзастапени бактериски соеви. Fisher-овиот exact test беше користен за испитување на дистрибуцијата на изолираните микроорганизми во средните носни ходници и максиларните синуси,

и за p=0,8 разликата беше прикажана како статистички несигнификантна.
Заклучок. Од резултатите добиени во студијата можеме да заклучиме дека микробиомот во средниот носен ходник не се разликува сигнификантно од микробната флора во максиларниот синус. Наодот добиен од брис од среден носен ходник е високо репрезентативен и го отсликува микробиомот во кореспондентниот максиларен синус.

Клучни зборови: хроничен риносинузитис, микробиом, брис од среден носен ходник, аспират од максиларен синус

Introduction

Chronic rhinosinusitis (CRS) is a common, chronic inflammatory disorder of the nose and paranasal sinuses characterized by two or more symptoms, one of which is either nasal blockage or nasal discharge with or without facial pain and reduction of smell. As the anatomic region responsible for initially filtering the inspired external environment, the warm and moist sinonasal cavities are, not surprisingly, colonized by a high burden of microbes. Multiple host and environmental factors have been implicated in the development of CRS; however, understanding the role of microbes has become increasingly important.
Microbiome refers to the genetic potential of the entire cohort of resident microorganisms (commensal, symbiotic, pathogenic) that inhabit a given niche (*e.g.*, the sinus cavities) and function as an organized community [1]. In recent years, composition of native microbiota of the sinuses has changed considerably. Once presumed to be sterile in the healthy state, the sinuses are now known to harbor a diverse consortium of microorganisms [2,3]. Disruption of indigenous microbiota (dysbiosis) may lead to pathogen overgrowth and enhanced susceptibility to infection, similar to what has been observed in the gastrointestinal tract and lower airways. Resident microbes may also influence the behavior of pathogenic species in a "community as pathogen" model, which further promotes development of CRS [4,5].
Sampling technique is also somewhat variable between studies, making cross-study meta-analyses a challenge. It appears that the anterior nasal cavity microbiome differs from the middle meatus and sphenoethmoid recess [6]. The middle meatus is frequently used as a representative sampling site for the deeper sinuses, given its high agreement in culture comparison studies with the maxillary sinus [7], likely resulting from its position within the anterior ethmoid drainage pathway of the maxillary, anterior ethmoid, and frontal sinuses [8].
The aim of the study was to investigate and to compare the microbiota results of middle nasal meatus swab samples and corresponding (ipsilateral) maxillary sinus aspirate samples, taken from the same patients with chronic rhinosinusitis.

Material and methods

Twelve patients with clinical or radiological evidence of CRS without nasal polyposis, who visited the University Clinic of Otorhinolaryngology (Skopje, Macedonia) for functional endoscopic sinus surgery (FESS), were included in this study. Diagnosis of CRS was made based on the diagnostic criteria of the EPOS guidelines (presence of symptoms, nasal endoscopy and CT findings) [9]. Patients who had taken antibiotics or antifungals in the month prior to surgery were excluded from the study.
All patients underwent general anesthesia and FESS technique as minimally invasive procedure by protecting sinonasal mucosa at the same time widening natural sinus ostia to enable normal mucocialiary clearance and ventilation of sinus cavities. Depending on the extent of the disease, middle meatal antrostomies and/or ethmoidectomies and/or sphenoidotomies were performed.
Two different types of sampling for investigating the sinonasal microbiota were performed in each patient - the first sample was a swab samples, while the second sample was a sinus aspirate. Both types of samples were obtained intraoperatively, swab from the middle nasal meatus and aspirate from the ipsilateral maxillary sinus. Endoscopically-guided swab was passed through a sterile cannula, then into the middle nasal meatus, to protect the swab tip from contamination from other sites especially nasal vestibule. Each swab was placed in a sterile Eppendorf tube. After swabbing, middle meatal antrostomy was done and aspirate sample was taken from the maxillary sinus using a Mucoset with a sterile container and a tube inserted into the sinus cavity through a sterile curved 4 mm aspirator. A total of 24 swab samples were collected from middle nasal meatus of both sides (left and right) and 24 aspirate samples from both maxillary sinuses. All collected samples were transferred within a period of 30 minutes to the Institute of Microbiology and Parasitology, Medical Faculty (Skopje, Macedonia) for microbiological evaluation. A microbiological culture is a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions. For the purpose of breeding the microorganisms, different types of organic and inorganic substrates (medium) were used. The period of incubation lasted 24-48 hours and the results were obtained within 5 days.

Results

The most frequent species determined in middle nasal

meatal swabs were Gram (+) bacteria: *Staphylococcus aures* in 5(20.8%), S*taphylococcus Coagulasae Negative* in 4 (16.7%) and *Streptococcus pneumoniae* in 3(12.5) samples followed by Gram (-): *Haemophilus influenzae, Moraxella catarrhalis* and *Klebsiella pneumoniae* found in 2 (8.3%) samples (Table 1). Neither anaerobes nor fungi were isolated in middle meatus smears.

Table 1. Microbes identified in middle nasal meatus in patients with CRS

Microbes		Number	%
Aerobic bacteria			
Gram (+)	*Staphylococcus aureus*	5	20.8
	Staphylococcus epidermidis	2	8.3
	Staphylococcus Coagulasae Negative	4	16.7
	Streptococcus pneumoniae	3	12.5
Gram (-)	*Klebsiella pneumoniae*	2	8.3
	Haemophilus influenzae	2	8.3
	Moraxella catarrhalis	2	8.3
Total aerobic bacteria		20	83.3
Total microbes		**20**	**83.3**
None		**4**	**16.7**

The distribution of microbes identified in maxillary sinus aspirates is presented in Table 2. Only 3 samples were sterile. *Staphylococcus Coagulasae Negative* was found in 5(20.8%) while *Staphylococcus aures* in 4 (16.7%) aspirates as the most permanent species, followed by *Haemophilus influenzae* and *Streptococcus pneumoniae.* Also, in maxillary sinus aspirates anaerobic bacteria (*Propionibacterium, Peptostreptococcus*) were identified as well as fungi (*Aspergillus*, *Mucor*).

Table 2. Microbes identified in maxillary sinus in patients with CRS

Microbes		Number	%
Aerobic bacteria			
Gram (+)	*Staphylococcus aureus*	4	16.7
	Staphylococcus epidermidis	1	4.2
	Staphylococcus Coagulasae Negative	5	20.8
	Streptococcus pneumoniae	2	8.3
Gram (-)	*Klebsiella pneumonia*	1	4.2
	Haemophilus influenza	3	12.5
	Moraxella catarrhalis	1	4.2
Total aerobic bacteria		17	70.9
Anaerobic bacteria			
Gram (+)	*Propionibacterium*	1	4.2
	Peptostreptococcus	1	4.2
Total anaerobic bacteria		2	8.4
Fungi			
Aspergillus		1	4.2
Mucor		1	4.2
Total fungi		2	8.4
Total microbes		21	**87.5**
None		**3**	**12.5**

The same bacterial species were identified in 14(87.5%) middle nasal meatus swab samples and corresponding (ipsilateral) maxillary sinus aspirate samples, taken from the same patients with chronic rhinosinusitis. *Staphylococcus aureus* and *Staphylococcus Coagulasae Negative* were the most frequent microbes isolated in both types of samples (Table 3).

Table 3. Microbes identified in middle nasal meatus and in correspondent maxillary sinus in same patients with CRS

Microbes - maxillary sinus/ middle nasal meatus	Number
Staphylococcus aureus	4
Staphylococcus epidermidis	1
Staphylococcus Coagulasae Negative	3
Streptococcus pneumonia	2
Klebsiella pneumonia	1
Haemophilus influenza	2
Moraxella cararrhalis	1
Total	**14**
Fisher's exact test p=0.88	

Fisher's exact test was used for testing the distrubition of isolated microbes in middle nasal meatus and maxillary sinuses, and for p=0.8 the difference was statistically insignificant. We can conclude that microbiota in middle nasal meatus do not differ significantlly from microbial flora in maxillary sinuses.

Discussion

Although culture-dependent techniques have been the mainstay of the microbial diagnostics in CRS, it has been estimated that >70% of bacterial species inhabiting body surfaces cannot be successfully cultivated under standard culture conditions because specific microenvironments necessary for bacterial growth may not be reproducible in the laboratory [4]. It is informative that 10-45% of CRS specimens have been reported to yield negative cultures [10,11].

In the study we investigated the microbiota of middle nasal meatus and corresponding maxillary sinus. The most frequent species determined in middle meatal swabs were Gram (+) bacteria: *Staphylococcus aures* in 5(20.8%), *Staphylococcus Coagulasae Negative* in 4(16.7%) and *Streptococcus pneumoniae* in 3(12.5%) samples followed by Gram (-), *Haemophilus influenzae, Moraxella catarrhalis* and *Klebsiella pneumoniae* found in 2 (8.3%) samples. We found that maxillary sinus aspirate samples had higher observed species richness than swab samples. Beside aerobes we identified anaerobic bacteria (*Propionibacterium, Peptostreptococcus*) as well as fungi (*Aspergillus, Mucor*).

Araujo *et al*. in their study that included 114 patients isolated aerobes in 86% of CRS patients, anaerobes were isolated in 8% of CRS patients, and fungi were isolated in 11% of CRS patients; the most frequent microorganisms were *Staphylococcus aureus* (36%),

Staphylococcus Coagulasae Negative (20%), and *Streptococcus pneumoniae* (17%). Middle meatus and maxillary sinus cultures presented the same pathogens in 80% of cases. In healthy individuals, coagulase-negative *Staphylococcus* (56%), *S. aureus* (39%), and *S. pneumoniae* (9%) were the most frequent isolates [12].
Our results suggest also a good correlation between middle nasal meatus swab samples and corresponding maxillary sinus aspirates samples, with no major significant differences in bacterial composition in patients with chronic rhinosinusitis.
Benninger *et al*. described a high rate of concordance, 77% between microbes found in the middle nasal meatus and maxillary sinus [13].
The use of endoscopic middle meatal cultures as a noninvasive method to determine the bacteriology of the maxillary sinus has not accurately been established. Because of this fact Dubbin *et al*. made a literature review that compared cultures obtained by endoscopic middle meatal swabs with those obtained from maxillary sinus aspirates. They analyzed studies published between January 1966 and October 2003 identified in multiple databases, bibliographies, and original articles. Studies were included for analysis if they had compared the results of endoscopic middle meatal cultures to aspirate cultures. The conclusion was that endoscopic middle meatal cultures had a high concordance with maxillary sinus aspirates [7].
The presence of fungi in the sinonasal cavities of patients with and those without CRS has been well established by using both culture-based and molecular detection techniques [14,15]. In the study of Boase *et al.*, fungi were found in only a small proportion of patients with CRS (n=35) and was completely absent in patients without CRS (n=6). Culture, PCR, and fluorescence *in situ* hybridization (FISH) techniques were used, and all three methods exhibited similar sensitivities. Culture was able to detect two of the *Aspergillus* cases, and an additional two patients with *Penicillium chrysogenum* and *Trichosporon*, respectively [16]. In our study *Aspergillus* and *Mucor* were identified only in two aspirate samples from maxillary sinus but fungi were not found in the swab samples from the middle nasal meatus in patients with CRS. However, the study realized by Ponikau *et al.* determined a high incidence of allergic fungal sinusitis in patients with chronic rhinosinusitis. Fungal cultures of nasal secretions were positive in 202 (96%) of 210 consecutive CRS patients [15].
Antibiotics have traditionally been the primary medical treatment for CRS, although evidence to support their efficacy is still lacking. More than 90% of otolaryngologists continue to use prolonged systemic antibiotics as "maximal medical therapy" before surgical intervention [17]. Facing the increasing antibiotic resistance and challenges with new drug development, new treatment modalities are urgently needed [18]. However, since we have a better understanding of the potential role of the sinus microbiome in promoting sinus health, such nonselective medications may disrupt the dynamics of the entire bacterial community. Rather than prescribing antibiotics to eradicate pathogenic bacteria, timely probiotic or prebiotic (nonviable food components that modulate microbiota to benefit the host) supplementation may emerge as a new mode of therapy to competitively inhibit pathogens or to facilitate sinus recolonization with desirable commensals. [19]. Probiotics involve administration of live microbes in sufficient amounts to directly confer beneficial physiologic effects on the host [20]. Systemically, ingestion of probiotics has been reported to enhance production of γ-interferon, and interleukin-2 lymphocyte responses, and to shift the balance of Th cells toward an increased Th1:Th2 ratio [21-23].
Extensive research is required to elucidate the role of the sinus microbiome and the influence of specific microbial species and/or strains on health or disease status. Better delineation of the complex dynamics between resident microbiota and the host immune system is also critical in guiding future medical therapy.

Conclusion

Microbiota in middle nasal meatus do not differ significantlly from microbial flora in maxillary sinus. Microbiota in middle nasal meatus is highly representative and reflects the microorganisms in corresponding maxillary sinus.

Conflict of interest statement. None declared.

References

1. Wilson MT, Hamilos DT. The nasal and sinus microbiome in health and disease. *Curr Allergy Asthma Rep* 2014; 14: 485.
2. Meltzer EO, Hamilos DL, Hadley JA, *et al*. Rhinosinusitis: Establishing definitions for clinical research and patient care. *Otolaryngol Head Neck Surg* 2004; 131: S1-S62.
3. Ramakrishnan VR, Feazel LM, Gitomer SA, *et al*. The microbiome of the middle meatus in healthy adults. *PLoS One* 2013; 8: e85507.
4. Han MK, Huang YJ, Lipuma JJ, *et al*. Significance of the microbiome in obstructive lung disease. *Thorax* 2012; 67: 456-463.
5. Frank DN, Zhu W, Sartor RB, Li E. Investigating the biological and clinical significance of human dysbioses. *Trends Microbiol* 2011; 19: 427-434.
6. Yan M, Pamp SJ, Fukuyama J, *et al*. Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and S. aureus carriage. *Cell Host Microbe* 2013; 14: 631-640.
7. Dubin MG, Ebert CS, Coffey CS, *et al*. Concordance of middle meatal swab and maxillary sinus aspirate in acute and chronic sinusitis; a meta-analysis. *Am J Rhinol* 2005; 19: 462-470.
8. Lund VJ, Stammberger H, Fokkens WJ, *et al*. European position paper on the anatomical terminology of the internal nose and paranasal sinuses. *Rhinol Suppl* 2014; 24: 1-34.

9. Fokkens WJ, Lund VJ, Mullol J, *et al*. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology* 2012; 50: 1-12.
10. Hsu J, Lanza DC, Kennedy DW. Antimicrobial resistance in bacterial chronic sinusitis. *Am J Rhinol* 1998; 12: 243-248.
11. Mantovani K, Rodrigues DdeO, Tamashiro E, *et al*. Comparing different methods used to collect material for a microbiological evaluation of patients with chronic rhinosinusitis. *Braz J Otorhinolaryngo* 2010; l76: 321-325.
12. Araujo E, Palombini BC, Cantarelli V, *et al*. Microbiology of middle meatus in chronic rhinosinusitis. *Am J Rhinol* 2003; 17(1): 9-15.
13. Benninger MS, Anon J, Mabry RL. The medical management of rhinosinusitis. *Otolaryngol Head Neck Surg Suppl* 1997; 127: 7-12.
14. Catten MD, Murr AH, Goldstein JA, *et al*. Detection of fungi in nasal mucosa using polymerase chain reaction. *Laryngoscope* 2001; 111: 399-403.
15. Ponikau JU, Sherris DA, Kern EB, *et al*. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999; 174: 877-884.
16. Boase S, Foreman A, Cleland E, *et al*. The microbiome of chronic rhinosinusitis: Culture, molecular diagnostics, and biofilm detection. *BMC Infect Dis* 2013; 13: 210.
17. Dubin MG, Liu C, Lin SY, Senior BA. American Rhinologic Society member survey on "maximal medical therapy" for chronic rhinosinusitis. *Am J Rhinol* 2007; 21: 483-488.
18. Boucher HW, Talbot GH, Benjamin DK, *et al*. 10 x '20 Progress-development of new drugs active against gram-negative bacilli: An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2013; 56: 1685-1694.
19. Pineiro M, Asp NG, Reid G, *et al*. FAO Technical meeting on prebiotics. *J Clin Gastroenterol* 2008; 42(suppl. 3 pt. 2): S156-S159.
20. Reid G, Sanders ME, Gaskins HR, *et al*. New scientific paradigms for probiotics and prebiotics. *J Clin Gastroenterol* 2003; 37: 105-118.
21. Donnet-Hughes A, Rochat F, Serrant P, *et al*. Modulation of nonspecific mechanisms of defense by lactic acid bacteria: Effective dose. *J Dairy Sci* 1999; 82: 863-869.
22. Solis-Pereyra B, Aattouri N, Lemonnier D. Role of food in the stimulation of cytokine production. *Am J Clin Nutr* 1997; 66: 521S-525S.
23. Wheeler JG, Bogle ML, Shema SJ, *et al*. Impact of dietary yogurt on immune function. *Am J Med Sci* 1997; 3: 120-123.

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Original article

IMPORTANCE OF EXTRAMURAL VASCULAR INVASION IN PREOPERATIVE STAGING OF RECTAL CANCER WITH MRI

ЗНАЧЕЊЕТО НА ЕКСТРАМУРАЛНАТА ВАСКУЛАРНА ИНВАЗИЈА ВО ПРЕДОПЕРАТИВНИОТ СТЕЈЏИНГ НА РЕКТАЛЕН КАРЦИНОМ СО МАГНЕТНА РЕЗОНАНСА

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Abstract

Introduction. Rectal cancer is the third most common malignant disease worldwide with a high mortality rate in developed countries. The prognosis of rectal cancer has been significantly improved over the past decade, and this is mainly due to progress in preoperative staging, which has been reflected in the therapeutic approach, where a significant change was made from simple surgical treatment to multimodal treatment. Although extramural vascular invasion (EMVI) is not included in the classical protocol for preoperative staging, itis a significant prognostic indicator of the recurrence rate.
Methods. The study is a prospective one, and it included 61 hospital patients with previously proven rectal cancer, who had been operated on at the Department of Abdominal Surgery ofthe University Clinic for Surgical Diseases “St. Naum Ohridski” in Skopje, and who underwent a magnetic resonance staging preoperatively.
Results. Comparison of extramural vascular invasion determined with MR preoperatively with pathohistological postoperatively obtained result.
Conclusion. MR as an ideal imaging method in preoperative staging of rectal carcinoma. It is a tool that determines with high accuracy the extramural vascular invasion in patients with rectal cancer.

Keywords: rectal cancer, magnetic resonance, preoperative staging, extramural vascular invasion

Абстракт

Вовед. Ректалниот карцином е трета по честота малигна болест ширум светот со висока стапка на морталитет во развиените земји. Прогнозата на ректален карцином е значајно подобрена во изминатава декада, и ова е главно благодарејќи на напредокот во предоперативниот стејџинг, што рефлектираше во терапискиот пристап, каде што значајна промена е направена од едноставен хируршки третман до мултимодалити третман. Иако не спаѓа во класичниот протокол за предоперативен стејџинг-екстрамуралната васкуларна инвазија (ЕМВИ) е значаен прогностички показател за стапката на рецидивирање.
Методи. Студијата е проспективна, и вклучува 61 болнички пациенти со претходно докажан ректален карцином, кои се оперирани на Абдоминална Хирургија при УК по хируршки болести Св.Наум Охридски-Скопје, и кај кои предоперативно е направен стејџинг со магнетна резонанса.
Резултати. Компарација на Екстра муралната васкуларна инвазија одредена со МР предоперативно со патохистолошки постоперативно добиениот резултат.
Заклучок. МР како идеална имиџинг метода во предоперативен стејџинг на ректален карцином. Алатка која со висока точност ја одредува Екстра муралната васкуларна инвазија ка пациенти со ректален карцином.

Клучни зборови: ректален карцином, магнетна резонанса, предоперативен стејџинг, екстра мурална васкуларна инвазија

Introduction

Rectal cancer is the third most common malignant disease worldwide with a high mortality rate in developed countries. The prognosis of rectal cancer has been significantly improved over the past decade, and this is mainly due to the progress in preoperative staging, which reflected in the therapeutic approach, where a significant change was made from simple surgical treatment to multimodal treatment [1]. This reduced the

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local recurrence rate by 11% and increased the 5-year survival rate by 58% [1,2].
About 50% of colorectal cancer is localized in the rectum. Rectal cancer is defined as a tumor whose upper margin is measured with a rigid rectoscopy at 16cm or less of the anocutaneous line. The highest percentage ofrectal cancer belongs to adenocarcinoma (98%). The remaining rectal tumors are relatively less common, carcinoid (0.1%), lymphomas around 1% and gastrointestinal stromal tumors (GIST) less than 1% [2].
Mesorectal fascia is a significant anatomic indicator for the diagnostic evaluation of the local tumor spread. Fascia is a connective tissue that surrounds the rectum and mesorectal fat, including lymph nodules and lymph vessels to the pelvic floor, and is actually a natural barrier to tumor spread [3,4].
Surgical treatment of rectal cancer is a challenge to achieve a balance between minimizing the risk of local recurrence and preserving the anorectal and genitourinary function. Total mesorectal excision (TME is the removal of the tumor, rectum and surrounding mesorectal fat [4-6].
Today, TME is a surgical choice for treatment of rectal cancer. The introduction of this surgical technique has reduced the rate of mortality from rectal cancer from 16% to 9%.
TME involves resection of the tumor margins. TME is a mesorectal compartment that includes the rectum, surrounding mesorectal fat weaving, surrounding lymph nodules and mesorectal fascia [6].
The next advancement in the treatment of rectal cancer is a transition from adjuvant to neoadjuvant chemoradiotherapy, which has resulted in an increase in the percentage of five years of survival and a decrease in the recurrence rate, has decreased the percentage of multivisceral and extensive resections in the surgical treatment of the rectal cancer [7-9].
The goal of neoadjuvant therapy is to reduce the size and stage of advanced rectal cancer, to minimize the risk of distant metastases and to allow less extensive surgical therapy and, preferably, a sphincter preservative technique [8].
It is a question whether a patient with a rectal cancer is a candidate for TME alone or a preoperative chemoradiotherapy followed by TME. Preoperative staging with MR may be the answer to this question because it is the most important tool in the staging of rectal cancer [9].
The magnetic resonance imaging method plays a crucial role in the preoperative staging of the rectal cancer. MR is a modality of choice for rectal cancer staging, which assists the surgeon in achieving negative resection margins.In fact, MR assists the surgeon in planning the type of surgical treatment, and helps predict the response to treatment and disease detection [10,11].
Surgical treatment with negative resection margins (no tumor presence within 1mm of resection margins, seen on histopathology) is the only standard for treatment.
Positive postoperative margins often result in relapse of the tumor, the possibility of the disease being incurable, poor quality of life, and a recurrent rate of 5-year-survival. The initial preoperative staging is aimed at selecting patients requiring chemoradiotherapy.
In patients with rectal cancer, local relapse is difficult to treat; it can cause a variety of symptoms, and most often has a fatal outcome [12-14].

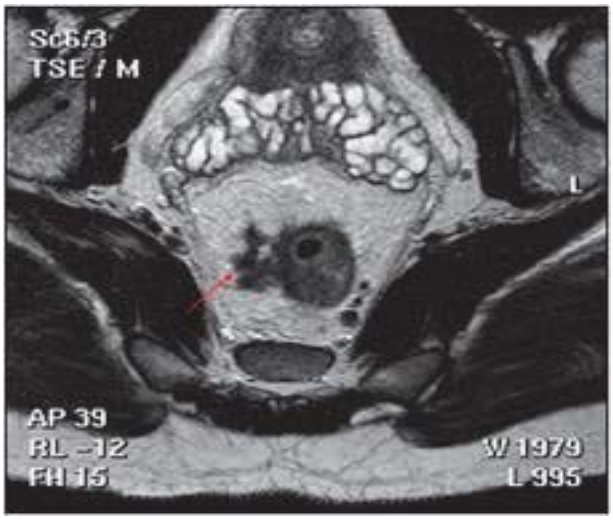

Fig. 1. Example of positive EMVI in rectal cancer

Preoperative staging of rectal carcinoma with MR allows patients, usually in T1, T2 and T3a stage, to benefit from only TME without preoperative neoadjuvant therapy, in contrast tothose patients with extrarectal spread that might benefit from a preoperative radio chemotherapy, in order to reduce the tumor [15,17-19].
Extramural vascular invasion (EMVI) is a significant prognostic indicator for the eventual recurrence of rectal cancer after surgery. EMI is a direct invasion of the blood vessels, usually veins from the tumor itself. This occurs at the macroscopic level and can be detected by MR. It is also a significant prognostic indicator,

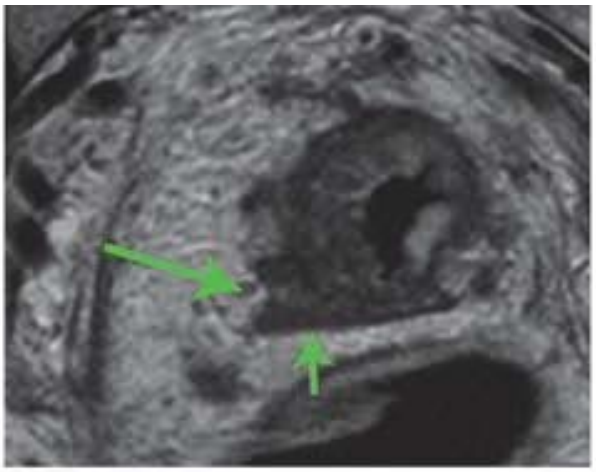
Fig. 2. Positive EMVI in rectal cancer

a predictor of hematogenous tumor spread. MR is a highly specific and moderately sensitive tool in the detection of EMVI [18-20]. EMVI pozitiv is defined as the presence of tumor cells in the blood vessels in mesorectal fat. Its manifestation is a local advanced tumor that penetrates deeper into the mesorectum and is used as a marker for a lower rate of 5-year-survival and local recurrence [21,22].
Earlier EMVI was detected on the histopathological sample of the operative specimen. Lately, EMVI has been detected in MR before and after neoadjuvant chemoradiotherapy. MR has the ability to determine this parameter *in vivo* [22,23].
Extramural venous invasion (EMVI) plays a role in preoperative risk stratification and influences the choice of possible preoperative neoadjuvant treatment. EMVI responds to chemoradiotherapy by making fibrosis of blood vessels that can be detected on MR. The regression of EMVI as a result of neoadjuvant treatment can be a measurable indicator and can be used as a biomarker to evaluate the effect of treatment [24].

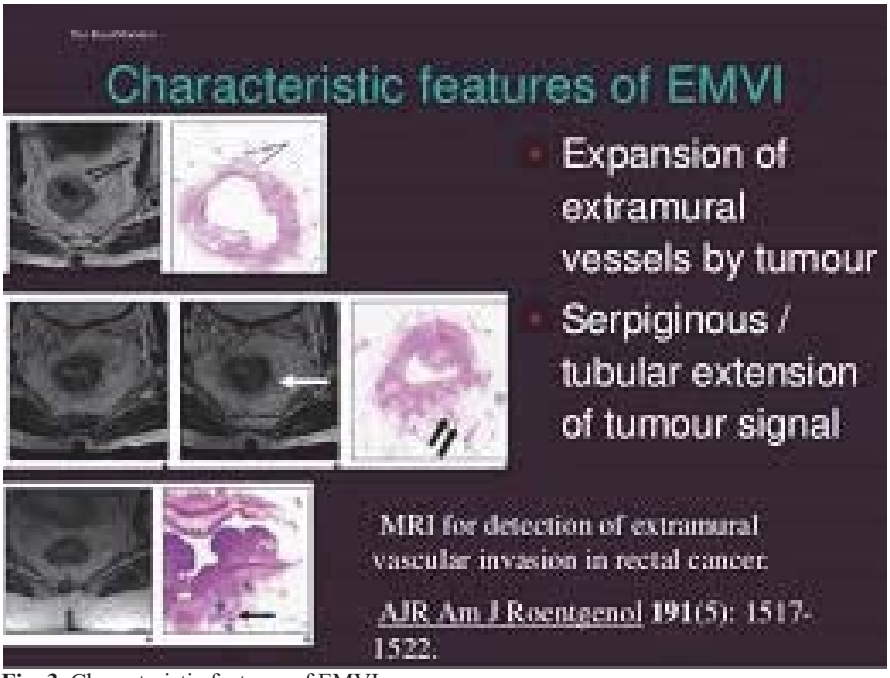

Fig. 3. Characteristic features of EMVI

Material and methods

This was aprospective study, which included 61 hospital patients with previously proven rectal cancer, who wereoperated on at the Department of Abdominal Surgery of the University Clinic of Surgical Diseases "St. Naum Ohridski" in Skopje.

- Inclusion criteria for participation in the study were: patients in whom rectal cancer had been proven previously by colonoscopyand were scheduled for surgery.
- Patients excluded from the study werethose who had a body weight higherthan 120 kg, patients who had implanted metal parts and patients who couldnot undergothe examination due to claustrophobia.

The following parameters were considered: sex, age, family history, localization of the tumor in (low, middle, high) rectum, and T and N stages determined by MR. A CT waspreoperatively made to determine the possible existence of distant MS deposits.
A correlation was then made with the results obtained from the histopathological finding.

MRI should determine the following:

- Localization of the tumor-whether it wasa high or low rectal tumor, its size, as well as the way of its growth.
- T staging - T1, T2, T3 and T4
- The distance of the tumor to the mesorectal fascia
- Tumoral growth or the existence of lymph nodes up to 1mm from the margin of resection
- The presence of lymph nodes mesorectally
- The presence of extramural vascular invasion (EMVI positive or negative).

Recent studies performed on 3T MR have shownno significant difference in the differentiation of the T2 stage and early T3 stage. The latest views in the literature show that both 1.5 T and 3 T are equally useful in the TMN staging.The MR protocol includes the SAG T2 pulse sequence that starts the scan [13,14].
The cranial boundary of the scanning field is the level of the vertebrae L5, and the caudal is below the anal duct. This pulse sequence provides a longitudinal cross-section of the tumor giving an insight into its length and the way of its growth. This pulse sequence

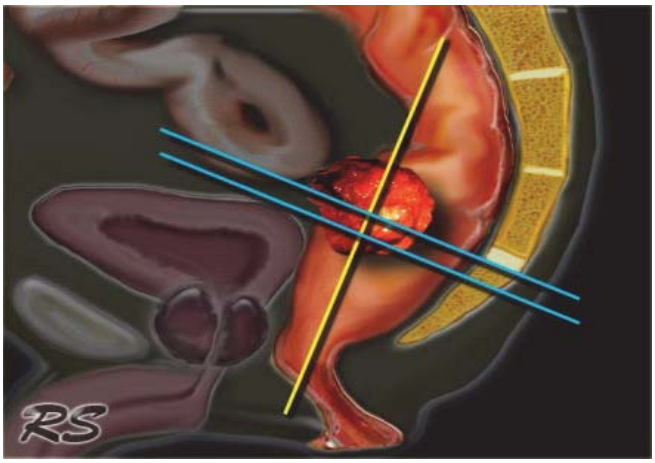

Fig. 4. Scanning of rectal cancer with MRI

measures the distance from anorectal blend to the lower edge of the tumor to determine the localization of the tumor in the rectum [22-24].
Based on the sagittal pulse sequence, the axial pulse sequences (AX T1, T2, DWI) have to be perpendicular to the axis of the tumor to avoid the partial volume effect.Coronal shots are planned perpendicular to axial and distally localized tumors parallel to the anal canal [19,20].

Discussion

This study shows that MR is a tool with high accuracy for prediction of tumor recidives depending on positive or negative EMVI preoperatively.

Fig. 5. Correlation of EMVI MRI with pathohistology

The comparison of EMVI detected preoperatively with MR and postoperative pathohistological results give us the right to regard MR as the gold standard in preoperative staging of rectal carcinoma [27,28]
Figure 5 shows correlation of EMVI MR with EMVI pathohistology shows that 27 patients have EMVI MR positive correlation with the results from pathohistology it have been 30 patients with EMVI positive. MR EMVI negative has been detected within 34 patients correlating to the results from pathohistology there have been 31 patients with EMVI negative.

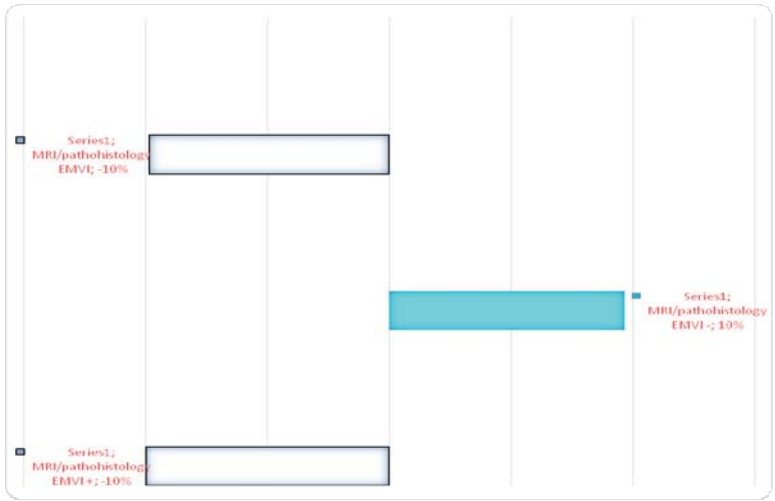

Fig. 6. Percentage of correlation of EMVI MRI with pathohistology

Figure 6 shows that difference between EMVI MR and EMVI pathohistology was not more than 10%, which was in agreement with the results from the scientific literature.

Although EMVI is not included in the classical protocol for staging of rectal cancer preoperatively, its significance as a prognostic indicator of tumor relapse is indisputable.

MR is a standard procedure in the diagnosis of rectal cancer in developed countries. In addition, there are no exact criteria for performing this imaging technique in our country, which are essential for determining the preoperative stage of the disease, isolation of patients who are candidates for neoadjuvant therapy and multimodal treatment with additional performance of lower rectal UC [29,30].

All thesecould lead to a reduction in the number of extensive surgeries, and increase of the number of sphincter-preserving surgical procedures. This implies completion of MRI procedure, supplemented with lower rectal US (for better differentiation between T1 and T2 stages), which would improve the diagnostics and radicality of the overall treatment in rectal cancer [31].

MR isan ideal imaging method for preoperative staging for a local or advanced stage of rectal cancer. MR allows evaluation of extramural spread, determines the mesorectal involvement and involves the margins of resection [32,33].

Conflict of interest statement. None declared.

References

1. Kang H, O'Connell JB, Leonardi MJ, *et al*. Rare tumors of the colon and rectum: a national review. *Int J Colorectal Dis* 2007; 22: 183-189. [Crossref] [Medline] [Google Scholar]
2. Adam IJ, Mohamdee MO, Martin IG, *et al*. Role of circumferential margin involvement in the local recurrence of rectal cancer. *Lancet* 1994; 344: 707-711. [Crossref] [Medline] [Google Scholar]
3. Kelly SB, Mills SJ, Bradburn DM, *et al*. Northern Region Colorectal Cancer Audit Group. Effect of the circumferential re-section margin on survival following rectal cancer surgery. *Br J Surg* 2011; 98: 573-581. [Crossref] [Medline] [Google Scholar]
4. Heald RJ, Moran BJ, Ryall RD, *et al*. Rectal cancer: the Basingstoke experience of total mesorectal excision, 1978-1997. *Arch Surg* 1998; 133: 894-899. [Crossref] [Medline] [Google Scholar]
5. Kosinski L, Habr-Gama A, Ludwig K, Perez R. Shifting concepts in rectal cancer management: a review of contemporary primary rectal cancer treatment strategies. *CA Cancer J Clin* 2012; 62:173-202. [Crossref] [Medline] [Google Scholar]
6. Bipat S, Glas AS, Slors FJ, *et al*. Rectal cancer: local staging and assessment of lymph node involvement with endoluminal US, CT, and MR imaging-a meta-analysis. *Radiology* 2004; 232: 773-783. [Crossref] [Medline] [Google Scholar]
7. Beets-Tan RG, Lambregts DM, Maas M, *et al*. Magnetic resonance imaging for the clinical management of rectal cancer patients: recommendations from the 2012 European Society of Gastrointestinal and Abdominal Radiology (ESGAR) consensus meeting. *Eur Radiol* 2013; 23: 2522-2531. [Crossref] [Medline] [Google Scholar]
8. Fernandez-Esparrach G, Ayuso-Colella JR, Sendino O, *et al*. EUS and magnetic resonance imaging in the staging of rectal cancer: a prospective and comparative study. *Gastrointest Endosc* 2011; 74: 347-354 [Crossref] [Medline] [Google Scholar]
9. Intven M, Reerink O, Philippens ME. Diffusion-weighted MRI in locally advanced rectal cancer: pathological response prediction after neo-adjuvant radiochemotherapy. *Strahlenther Onkol* 2013; 189: 117-122. [Crossref] [Medline] [Google Scholar]
10. Jung SH, Heo SH, Kim JW, *et al*. Predicting response to neoadjuvant chemoradiation therapy in locally advanced rectal cancer: diffusion-weighted 3 Tesla MR imaging. *J Magn Reson Imaging* 2012; 35: 110-116. [Crossref] [Medline] [Google Scholar]
11. Barbaro B, Vitale R, Valentini V, *et al*. Diffusion-weighted magnetic resonance imaging in monitoring rectal cancer response to neoadjuvant chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2012; 83: 594-599. [Crossref] [Medline] [Google Scholar]
12. Lambregts DM, Vandecaveye V, Barbaro B, *et al*. Diffusion-weighted MRI for selection of complete responders after chemoradiation for locally advanced rectal cancer: a multicenter study. *Ann Surg Oncol* 2011; 18: 2224-2231. [Crossref] [Medline] [Google Scholar]
13. Kim SH, Lee JM, Hong SH, *et al*. Locally advanced rectal cancer: added value of diffusion-weighted MR imaging in the evaluation of tumor response to neoadjuvant chemo- and radiation therapy. *Radiology* 2009; 253: 116-125. [Crossref] [Medline] [Google Scholar]
14. DeVries AF, Piringer G, Kremser C, *et al*. Pre-treatment evaluation of microcirculation by dynamic contrast-enhanced magnetic resonance imaging predicts survival in primary rectal cancer patients. *Int J Radiat Oncol Biol Phys* 2014; 90: 1161-1167. [Crossref] [Medline] [Google Scholar]
15. Lim JS, Kim D, Baek SE, *et al*. Perfusion MRI for the prediction of treatment response after preoperative chemoradiotherapy in locally advanced rectal cancer. *Eur Radiol* 2012; 22: 1693-1700. [Crossref] [Medline] [Google Scholar]
16. Oberholzer K, Menig M, Pohlmann A, *et al*. Rectal cancer: assessment of response to neoadjuvant chemoradiation by dynamic contrast-enhanced MRI. *J Magn Reson Imaging* 2013; 38: 119-126. [Crossref] [Medline] [Google Scholar]
17. Intven M, Reerink O, Philippens ME. Dynamic contrast enhanced MR imaging for rectal cancer response assessment after neo-adjuvant chemoradiation. *J Magn Reson Imaging* 2014 Aug 14. [Epub ahead of print] [Google Scholar]
18. Kim SH, Lee JM, Gupta SN, Han JK, Choi BI. Dynamic contrast-enhanced MRI to evaluate the therapeutic response to neoadjuvant chemoradiation therapy in locally advanced rectal cancer. *J Magn Reson Imaging* 2014; 40:730-737. [Crossref] [Medline] [Google Scholar]
19. Gollub MJ, Gultekin DH, Akin O, *et al*. Dynamic contrast enhanced-MRI for the detection of pathological complete response to neoadjuvant chemotherapy for locally advanced rectal cancer. *Eur Radiol* 2012; 22:821-831. [Crossref] [Medline] [Google Scholar]
20. Ozis SE, Soydal C, Akyol C, *et al*. The role of ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography in the primary staging of rectal cancer. *World J Surg Oncol* 2014; 12: 26. [Crossref] [Medline] [Google Scholar]
21. Maas M, Lambregts DM, Lahaye MJ, *et al*. T-staging of rectal cancer: accuracy of 3.0 Tesla MRI compared with 1.5 Tesla. *Abdom Imaging* 2012; 37:475-481. [Crossref] [Medline] [Google Scholar]

22. Donmez FY, Tunaci M, Yekeler E, Balik E, Tunaci A, Acunas G. Effect of using endorectal coil in pre-operative staging of rectal carcinomas by pelvic MR imaging. *Eur J Radiol* 2008; 67: 139-145. [Crossref] [Medline] [Google Scholar]
23. Slater A, Halligan S, Taylor SA, Marshall M. Distance between the rectal wall and mesorectal fascia measured by MRI: effect of rectal distension and implications for preoperative prediction of a tumour-free circumferential resection margin. *Clin Radiol* 2006; 61: 65-70. [Crossref] [Medline] [Google Scholar]
24. Furey E, Jhaveri KS. Magnetic resonance imaging in rectal cancer. *Magn Reson Imaging Clin N Am* 2014; 22: 165-190, v–vi. [Crossref] [Medline] [Google Scholar]
25. Suzuki C, Torkzad MR, Tanaka S, *et al*. The importance of rectal cancer MRI protocols on interpretation accuracy. *World J Surg Oncol* 2008; 6: 89. [Crossref] [Medline] [Google Scholar]
26. Mir N, Sohaib SA, Collins D, Koh DM. Fusion of high b-value diffusion-weighted and T2-weighted MR images improves identification of lymph nodes in the pelvis. *J Med Imaging Radiat Oncol* 2010; 54: 358-364. [Crossref] [Medline] [Google Scholar]
27. Cong GN, Qin MW, You H, *et al*. Diffusion weighted imaging combined with magnetic resonance conventional sequences for the diagnosis of rectal cancer [in Chinese]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 2009; 31: 200-205. [Medline] [Google Scholar]
28. Alberda WJ, Dassen HP, Dwarkasing RS, *et al*. Prediction of tumor stage and lymph node involvement with dynamic contrast-enhanced MRI after chemoradiotherapy for locally advanced rectal cancer. *Int J Colorectal Dis* 2013; 28: 573-580. [Crossref] [Medline] [Google Scholar]
29. Heijnen LA, Lambregts DM, Martens MH, *et al*. Performance of gadofosveset-enhanced MRI for staging rectal cancer nodes: can the initial promising results be reproduced? *Eur Radiol* 2014; 24: 371-379. [Crossref] [Medline] [Google Scholar]
30. Rudisch A, Kremser C, Judmaier W, *et al*. Dynamic contrast-enhanced magnetic resonance imaging: a non-invasive method to evaluate significant differences between malignant and normal tissue. *Eur J Radiol* 2005; 53: 514-519. [Crossref] [Medline] [Google Scholar]
31. Tamakawa M, Kawaai Y, Shirase R, *et al*. Gadolinium-enhanced dynamic magnetic resonance imaging with endorectal coil for local staging of rectal cancer. *Jpn J Radiol* 2010; 28: 290.298. [Crossref] [Medline] [Google Scholar]
32. Yao WW, Zhang H, Ding B, *et al*. Rectal cancer: 3D dynamic contrast-enhanced MRI-correlation with microvascular density and clinicopathological features. *Radiol Med (Torino)* 2011; 116: 366-374. [Crossref] [Medline] [Google Scholar]
33. Lollert A, Junginger T, Schimanski CC, *et al*. Rectal cancer: dynamic contrast-enhanced MRI correlates with lymph node status and epidermal growth factor receptor expression. *J Magn Reson Imaging* 2014; 39: 1436-1442. [Crossref] [Medline] [Google Scholar]

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ММП

Original article

DISTRIBUTION OF HPV TYPES AMONG PATIENTS WITH POSITIVE HISTOLOGICAL FINDINGS FOR CERVICAL PRECANCEROUS LESIONS AND INVASIVE CANCER OF THE UTERINE CERVIX

ДИСТРИБУЦИЈА НА ХПВ ТИПОВИТЕ МЕЃУ ПАЦИЕНТКИ СО ПОЗИТИВНИ ХИСТОЛОШКИ НАОДИ ЗА ЦЕРВИКАЛНИ ПРЕКАНЦЕРОЗНИ ЛЕЗИИ И ИНВАЗИВЕН ЦЕРВИКАЛЕН КАРЦИНОМ

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Abstract

Introduction. The aim of this study was to provide basic data on the prevalence of different HPV types among the female population in R. Macedonia with histologically proven cervical intraepithelial neoplasia or invasive cervical cancer, in order to better plan the vaccination program and screening for cervical cancer.
Methods. This study retrospectively statistically (using Statistica SPSS for Windows) analyzed histological findings positive for cervical intraepithelial neoplasia or invasive cervical cancer and positive HPV genotyping results of564 patients who came to the University Clinic for Gynecology and Obstetrics due to an abnormal Pap smear test during the last year (2017).
Results. HPV isolation showed the presence of human papillomavirus in78% of the total of 564 subjects. The prevalence of HPV in LSIL, HSIL, invasive squamocellular carcinoma of the uterine cervix and adenocarcinoma of the uterine cervix was 69.2%, 87.2%, 97.6% and 71.4%, respectively. HR HPV types were isolated in 75%of LSIL and 96% of HSIL. Predominantly isolated were HPV types 16, 18, 35, 31, 33, 58, 6, 11 and 40. HPV type 16 wasthe most commonisolated genotype among all patients with 48.4%, 30.1% and 19.6% in HSIL, invasive carcinoma and LSIL, respecttively. HPV type 18 had the highest rate in patients with invasive adenocarcinoma of the cervix (30.1%).
Conclusion. Human papillomavirus types 16, 18, 35, 31, 33, 58, 6, 11 and 40were the predominant high risk types in patients with invasive cervical cancer and its precursors in the Republic of Macedonia.

Keywords: HPV types, SIL, cervical cancer

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Апстракт

Вовед. Целта на оваа студија беше обезбедување на базични податоци за преваленцата на различните ХПВ типови меѓу женската популација од Р. Македонија со хистолошки докажана цервикална интраепителна неоплазија или цервикален карцином, со цел подобро планирање на програмата за вакцинација и на скринингот за цервикален карцином.
Методи. Оваа студија опфати статистичка ретроспективна анализа на хистолошките наоди позитивни за цервикална интраепителијална неоплазија (ЦИН) или инвазивен цервикален карцином и позитивен резултат од ХПВ генотипизација кај 564 пациентки кои се јавиле на Универзитетската Клиника за гинекологија и акушерство за хистолошка верификација на абнормален цитолошки резултат во текна 2017 година. Статистичката обработка беше изведена во SPSS /Windows.
Резултати. ХПВ анализата покажа позитивитет кај 78 % од вкупно 564 пациентки. ХПВ преваленцата кај групите LSIL, HSIL, инвазивен цервикален планоцелуларен и аденокарцином изнесуваа 69.2%, 87.2%, 97.6% и 71.4%,соодветно. Високоризичните ХПВ типови беа изолирани кај 75% од LSIL и 96% од HSIL. Предоминантно изолирани ХПВ типови беа 16, 18, 35, 31, 33, 58, 6, 11 и 40. ХПВ тип 16 беше најчесто изолиран меѓу сите ХПВ типови со 48.4%, 30.1% и 19.6% кај HSIL, инвазивните цервикални карциноми и LSIL, соодветно. ХПВ тип 18, втор по честота покажа највисока стапка меѓу цервикалните аденокарциноми-30.1%.
Заклучок. Хуман папилома вирусите тип 16, 18, 35, 31, 33, 58, 6, 11 и 40 се веројатно најпревалентните типови меѓу популацијата во Република Македонија.

Клучни зборови: ХПВ типови, SIL, цервикален карцином

Introduction

Cervical cancer is the third most prevalent malignant tumor in women and the most common gynaecological malignancy. Approximately 500,000 new cases and 250,000 deaths from cervical carcinoma are registered each year, and 80% of them are in underdeveloped and developing countries [1]. Cervical intraepithelial neoplasia (CIN) is a one-way pathophysiological process that has two different outcomes. The first is the spontaneous regression of cervical lesions after their occurrence, while the second outcome is the potential occurrence of cancer, that is, if the changes are not timely screened or adequately treated in their early stages, they can develop into invasive cervical cancer. Persistent infection with a high-risk human papilloma virus (HPV) is the main etiological cause of cervical precancerous lesions and invasive cervical cancer [2]. The prevalence of this infection worldwide varies from 2 to 44%. The isolation of HPV types has shownthat there are about 100 different HPV types that differ in each other according to the tropism towards the epithelium of the skin and the epithelium of the mucosal membranes. Clinical HPV types are classified as high risk and low risk based on their oncogenicity. Low-risk types may be associated with a low-grade SIL, but are almost never isolated in invasive carcinoma. At least 15 HPV genotypes have been identified as high risk (HR), among which HPV 16 hasshown to be the most strongly linked with cervical cancer, followed by HPV 18 [3,4]. World literature describes differences in the geographical distribution and prevalence of individual HPV types in different countries and even in different regions of a country [5,6]. Furthermore, the prophylactic effect of HPV vaccination depends largely on the type of the virus. Therefore, for better planning of HPV vaccination programs and screening programs for cervical cancer, basic data on the prevalence and distribution of individual HPV genotypes in the female population in Republic of Macedonia are needed.

Materials and methods

The study was designed as a retrospective analytical study and was carried out at the University Clinic for Gynecology and Obstetrics in Skopje, Macedonia.
This retrospective study included data from 564 patients who came to the University Clinic for Gynecology and Obstetrics in Skopje, in the period from January 2017 until December 2017, for histological verification due to an abnormal cytological finding. In all patients, a cervical smear was also taken during the intervention for HPV isolation. All patients were consent to the above diagnostic procedures. The analysis excluded pregnant patients, followed by patients with histopathological analysis who did not show cervical cellular intraepithelial abnormalities or invasive cervical cancer, patients who did not receive HPV typing data or who did not clearly have HPV isolation and genotyping during the histopathological verification of cytological abnormalities. The bioptic material was histopathologically evaluated at the University Clinic of Oncology and Radiotherapy, in Skopje, Macedonia. HPV DNA analysis was done at the University Clinic for Gynecology and Obstetrics through standardized protocols of HPV DNA isolation, screening and genotyping according to the procedure recommended by the manufacturer ACCU-Prep Genomic DNA Extraction Kit of the Bioneer manufacturer**;** QIA amp DNA Mini Kit; Seeplex HPV 4A ACE Screening, Seegene; High + Low Papilomastrip, by Operon. The statistical analysis of the collected data was made with the software package SSPS for Windows.

Results

Distribution of histological findings is presented in Table 1. Of the total of 564 patients, the distribution of group findings was as follows: HPV cervicitis: 146 patients, (25.7%); CIN I: 177 patients (31.3%), moderate grade dysplasia (CIN II): 65 patients, (11.52%), severe dysplasia (CIN III), 68 patients (12.07%); carcinoma in situ (CIS): 48 patients (8.5%). Invasive cervical carcinomas were histologically confirmed in 60 patients (10.2%) and we divided them according to the histological type of the cancer: invasive squamocellular carcinoma of the uterine cervix: 43(7.61%), invasive adenocarcinomaof the uterine cervix: 17(3.01%).
In 438 patients -78% of the total of 564 subjects, HPV isolation showed the presence of human papillomavirus.

Table 1. Distribution of histological findings

Bethesda	Richart	n	%
Low-grade SIL	HPV	146	26.02%
	CIN I	177	31.55%
High-grade SIL	CIN II	65	11.58%
	CIN III	68	12.12%
	Invasive planocellularCa	43	7.66%
	In situ carcinoma	48	8.55%
	Invasive adenocarcinoma of uterine cervix	17	3.01%
	Total:	564	100.00%

Negative findings were obtained in 123 patients (26.9%). Of all isolated HPV types, 100 patients had only low-risk HPV types (22.8%), while in 378 patients, 86.30% were high-risk HPV types. In the classification of low and high risk, high-risk patients were considered patients who had at least one high-risk HPV type regardless of possible co-infection with another low risk. The diagnoses HPV cervicitis and CIN 1 were grouped as LSIL, CIN 2, CIN 3 and in-situ carcinoma as HSIL, while squamocellular and adenocarcinomas were classified as invasive cervical carcinomas (ICC).

Table 2. Distribution of low-risk and high-risk HPV types

HPV genotype	Total isolated	Isolated in LSIL	Isolated in HSIL	Isolated in ICC
Low risk	100	47	13	2
High risk	378	178	145	50
Total	438	225	158	52

Table 2 shows the distribution of HPV types (low and high risk) in relation to histological diagnoses. Genotyping (Table 3) showed the presence of 23 different low and high-risk HPV types. Of the totalof 23 isolated HPV genotypes, the HPV types that were predominantly isolated in our study were: high-risk (16, 18, 35, 33, 31, 58), and low-risk (6.11 and 40).

Table 3. Distribution of HPV genotypes in relation to histological diagnosis

HPV type	HPV	LSIL- CIN1	HSIL CIN2	HSIL CIN 3	Ca in situ	ISC	IAC
16	11(8.6%)	14(11.0%)	17(13.3%)	33(25.6%)	29(22.8%)	20(15.8%)	3(2.3%)
18	8(12.6%)	1(1.6%)	5(7.9%)	14(22.2%)	6(9.5%)	10(15.8%)	19(30.1%)
35	9(16.1%)	23(40.2%)	7(11.8%)	3(5.3%)	5(8.9%)	10(16.7%)	1(2.1%)
31	5(12%)	15(36%)	11(26%)	4(9%)	3(7%)	4(9%)	0(0%)
11	20(48.7%)	13(31.7%)	2(4.8%)	3(7.3%)	1(2.4%)	2(4.8%)	0(0%)
33	3(8%)	21(55%)	4(11%)	5(13%)	2(5.2%)	3(8%)	0(0%)
40	17(41%)	9(26.4%)	2(5.8%)	1(2.9%)	4(4.3%)	1(2.9%)	1(2.9%)
58	10(31.2%)	8(25%)	5(15.6%)	2(6.25%)	4(4.3%)	2(6.25%)	0(0%)
6	11(42.3%)	11(42.3%)	2(7.6%)	0(0%)	1(3.8%)	0(0%)	1(3.8%)

*ISC – Invasive squamocellular cervical carcinoma IAC- invasive adenocarcinoma of the uterine cervix

Discussion

The HPV prevalence in our study showed a high rate of positive HPV isolation among all subjects (78%). This high rate is probably due to the fact that the study was conductedin a tertiary medical institution.The prevalence of HPV in LSIL, HSIL, invasive squamocellular carcinoma of the uterine cervix and adenocarcinoma of the uterine cervix diagnostic groups was69.2%, 87.2%, 97.6% and 71.4%, respectively, which is approximately equal to worldwide studies [7] in which the prevalence is reported to be 99. 7% in positive cytological or histological findings. Our results showed that the prevalence of HPV in general and high-risk HPV increases with the severity of the cervical lesion, indicating that HPV infection plays a key role in the development of cervical cancer and its precursors. In this study, an increase in the prevalence of HPV type 16 infection with the severity of the cervical lesion was detected, with 48.4% in high-grade lesions (CIN2, CIN3, *insitu* carcinoma), followed by the rate of 30.1% in patients with invasive cervical cancer, and the lowest in low-grade lesions, 19.6%. In addition, HPV type 16 wasthe most common isolated genotype among all patients. This data corresponds with those found in the literature [8,9]. Although the pathogenesis of individual HPV genotypes remains unclear, HPV type 16 may be considered the underlying risk factor forthe development of squamous cervical lesions and invasive cervical cancer. Hence, these patients should be subjected to regular control, especially those with a high risk HPV infection that is theoretically on the step of transition from a high-grade lesion to invasive cervical cancer. HPV type 18 wasthe second most frequently encountered type among patients in our study, with the highest rate in patients with invasive adenocarcinoma of the uterine cervix (30.1%) compared to LSIL (14.2%). Safaeian*et al*. [10] also reported a sub-presentation of cervical precancerous lesions caused by HPV type 18 compared to cervical carcinomas attributed to this HPV type. One explanation of this phenomenon may be that HPV type 18, in contrast to other high risk types, has a higher potential to cause cervical cancer, and therefore cervical lesions may develop faster than others caused by the rest of the HPV types. The occult pathology of HPV 18 requires further research. In our study, HPV 18 was the most widespread genotype in patients with cervical adenocarcinoma. This is in line with several studies focusing on cervical adenocarcinoma. The worldwide study of HPV18 is highly associated with the onset of cervical adenocarcinoma [11]. The rough distribution of low and high-risk HPV types in the groups showed that 86.3% of all patients had a high-risk HPV type. The rate of positive HPV types of high-risk strains increases as the degree of cervical change increases, from about 75% in LSIL to 96% in

invasive cervical carcinoma. In general, HPV type 16 and 18 are the two most prevalent genotypes worldwide, and are incriminated for approximately 50% and 20% of cervical carcinomas, respectively. In contrast, lately, non-HPV 16/18 types of cervical cancer and precancerous lesions have proven geographical variations in prevalence [29-31]. Our study found that among non-HPV 16/18 high-strain strains, namely HPV 31, 33, 35, 58,are the most commonly reported in low-grade lesions. At the same time, LSIL have higher rate of low-risk HPV types: HPV type 6, 11 and 40 (see Table 3). Such data on the epidemiological status of HPV differences from region to region, as well as within a country can significantly contribute to the design of appropriate vaccination strategies. Of the total of 23 isolated HPV genotypes, the HPV types that were predominantly isolated in our study were: high-risk (16, 18, 35, 31, 33, 73 and 58), and low-risk (6.11 and 40). This result corresponds to the so far publishedfindings that pay attention to the fact that high-risk HPV types are detected more often with higher-level lesions.

Conclusion

This study provides only a basic description of the distribution of HPV genotypes among patients diagnosed with cytological changes in the Republic of Macedonia. Human papillomavirus types 16, 18, 53, 58, 33 and 31 are perhaps the predominant high risk types in patients with cervical cancer and its precursors in the Republic of Macedonia. A more extensive controlled study of a larger population is needed for a better analysis of the problem and an adequate evaluation of the effectiveness of prophylactic HPV vaccination and eventual screening programs.

Conflict of interest statement. None declared.

References

1. Bayu H, Berhe Y, Mulat A, Alemu A. Cervical Cancer Screening Service Uptake and Associated Factors among Age Eligible Women in Mekelle Zone, Northern Ethiopia, 2015: A Community Based Study Using Health Belief Model. *PLoS One* 2016; 11: e0149908.
2. Munoz N, Bosch FX, de Sanjose S, *et al*. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003; 348: 518-527.
3. Thomsen LT, Frederiksen K, Munk C, *et al*. Long-term risk of cervical intraepithelial neoplasia grade 3 or worse according to high-risk human papillomavirus genotype and semi-quantitative viral load among 33,288 women with normal cervical cytology. *Int J Cancer* 2015; 137: 193-203.
4. Ferris DG, Cox JT, O'Connor DM. The biology and significance of human papillomavirus infection.On Haefner HK, Krumhoiz BA, Massad LS (eds): Modern colposcopy, Dubuque, IA. *Kendall/Hunt Publishing* 2004; 89.
5. Clifford GM, Smith JS, Plummer M, *et al*. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2003; 88(1): 63-73.
6. Wei H, Wang N, Zhang Y, *et al*. Distribution of various types of low-risk human papillomavirus according to cervical cytology and histology in northern Chinese women. *Int J GynaecolObstet* 2014; 126: 28-32.
7. Walboomers JM, Jacobs MV, Manos MM, *et al*. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999; 189(1): 12-19.
8. Zhao Y, Lin H, Shen D, *et al*. Distribution of HPV genotypes in uterine cervical lesions in Yanbian, northern China. *PatholInt* 2008; 58: 643-647.
9. Hong D, Ye F, Chen H, *et al*. Distribution of human papillomavirus genotypes in the patients with cervical carcinoma and its precursors in Zhejiang Province, China. *Int J Gynecol Cancer* 2008; 18(1): 104-109.
10. Safaeian M, Schiffman M, Gage J, *et al*. Detection of precancerous cervical lesions is differential by human papillomavirus type. *Cancer Res* 2009; 69(8): 3262-3266.
11. Berrington de Gonzalez A, Green J. Comparison of risk factors for invasive squamous cell carcinoma and adenocarcinoma of the cervix: collaborative reanalysis of individual data on 8097 women with squamous cell carcinoma and 1374 women with adenocarcinoma from 12 epidemiological studies. *Int J Cancer* 2007; 120(4): 885-891.

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Original article

EARLY WOUND INFECTIONS FOLLOWING REMOVAL OF POSITIONING SCREW FROM THE DISTAL TIBIO-FIBULAR SYNDESMOSIS

РАНИ ИНФЕКЦИИ НА РАНАТА ПО ВАДЕЊЕ НА ПОЗИЦИОНИОТ ШРАФ ОД ДИСТАЛНАТА ТИБИО-ФИБУЛАРНА СИНДЕЗМОЗА

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Abstract

Introduction. The routine removal of the positioning screw from the syndesmosisin a period of 8-12 weeks from the index surgery is under debate.The aim of the present study wasto examine the incidence ofearly surgical wound infection after removal of the positioning screw.
Methods. Atotal of 114 patients that had undergone a screw removal from the distal tibio-fibular syndesmosis in theperiod between January 2011 and June 2016 were examined. No antibiotic prophylaxis was given during the procedure. The patients' follow-up was one week, two weeks, one month and three months following the surgery. The occurrence of an infection was statistically examined in correlation with the sex, age, body mass index, diabetes, smoking and the American Society of Anaesthesiology score.
Results. An infection of the surgical wound following removal of the distal tibio-fibular syndesmosis screw was registered in 8 patients (7%). Five of them had *S. aureus* isolated from their surgical wound, one had *Pseudomonas aeruginosa*and one had *Enterococcus faecalis*. One patient had a negative microbiological finding. One patient needed hospitalization, parenteral antibiotic therapy and a surgical treatment of the wound. Statistically significant risk factors were: diabetes, body mass index, and smoking.
Conclusion. Our results support prophylactic use of antibiotics during the removal of the positioning screw from the distal tibio-fibular syndesmosis.

Keywords: syndesmosis, ankle, screw, extraction, infection

Апстракт

Вовед. Рутинското вадење на позициониот шраф за фиксација на синдезмозата 8-12 седмици по иницијалната операција е предмет на дебати. Целта на оваа студија е да се испита инциденцата на рана инфекција на раната по вадење на позициониот шраф од синдезмозата.
Методи. Во студијата учествуваа вкупно 114 пациенти лекувани во периодот јануари 2012-јуни 2016 година. При вадењето на позициониот шраф не беше давана антибиотска профилакса. Следењето се спроведуваше една и две седмици, како и еден и три месеци по операцијата. Појавата на инфекција статистички се испитуваше во корелација со полот, возраста, Индексот на телесна маса, дијабетот, пушењето и Скорот на Американската асоцијација за анестезиологија.
Резултати. Инфекција на хируршката рана беше регистрирана кај 8 испитаници (7%). Кај петмина беше изолиран *S. Aureus*, а кај по еден *Pseudomonas aeruginosa*и *Enterococcus faecalis*. Кај еден испитаник микробиолошкиот наод беше неативен. Кај еден испитаник беше потребна хоспитализација, парентерална антибиотска терапија и хируршки третман на раната. Статистички сигнификантни ризик фактори беа дијабет, Индекс на телесна маса и пушење.
Заклучок. Резултатите од ова истражување ја поддржуваат профилактичката употреба на антибиотици при вадење на позициониот шраф од синдезмозата.

Клучни зборови: синдезмоза, скочен зглоб, шраф, вадење, инфекција

Introduction

The ankle fractures are the most common injury of the lower extremity [1], and their incidence has increased in the past decades [2]. Simultaneously, they are the most common injury of a weight-bearing joint. The articular surface have specific morphology, and the distal tibio-fibular syndesmosis has a very important role in the biomechanics of the joint; thus the challenge during thereduction of the joint, which makes the absolute stability and the healing of the ligaments that form the

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distal tibio-fibular syndesmosis is difficult to be achieved [3]. The ankle fractures are considered to be accompanied by a disruption of the syndesmosis in 15-23% of the cases [4]. The disruption of the distal tibio-fibular syndesmosis completely disturbs the delicate biomechanics of the joint [5-7]. Syndesmotic reconstruction is widely regarded as the cornerstone in the treatment of these injuries [8-10]. Even though transfixation with the positioning screw is still a "gold standard" [11], there are opposing opinions about the technical details of the procedure itself. There is no consensus regardingthe number and calibre of the screws, the level at which they should be placed, the number of cortices that should be engaged, and the duration of the transfixion of the syndesmosis. In the past few years, several cadaveric biomechanical studies confirmed the importance of the syndesmosis during the movements of the ankle, so the question for the duration of the syndesmotic transfixion has been raised [12-14]. On the other hand, the results from many studies have questioned the need for removal of the positioning screws in terms of the functional outcome following this injury, stating that the routine screw removal has no effect when it comes to achieving pre-injury activity level [15-17]. In the meantime, these authors believe that another surgery to remove the screws increases the risk of postoperative complications, which may affect the patient's overall satisfaction from the treatment.

We were motivated to analyze the occurrence of infection of the surgical wound following removal of the positioning screws, which is another element in the scientific discussion focused on the need of removal of the positioning screws.

Materials and methods

A prospective study was conducted at the University Clinic of Traumatology at the Medical Faculty in Skopje, in the period from January 2011 to June 2016. The study was focused on patients with ankle fracture, who had their positioning screw removed 8-12 weeks after the initial surgery.The need for removal of the positioning screw was determined by the surgeon. Patients who had a serious wound infection after the initial surgery, another fracture which was surgically treated, visceral or cranio-cerebral injury which was surgically treated and was acquired during the incident that also caused ankle fracture, patients who had an ASA score ≥3 during the initial procedure and patients who were on corticosteroid therapy, were excluded from the study.

The procedure of screw removal was performed by infiltrating a local anesthetic at the place of the planned incision, making a skin incision no longer than one centimeter, identifying and removing ofthe positioning screw. The wound was closed with prolene suture and a sterile dressing was applied. A prophylactic antibiotic was not given. The follow-up of the wound was on the first postoperative day, seven days, fourteen days, one month and three months after the screw removal. Following the wound check on the first postoperative day, the dressing was changed and remained in place till the next wound check (a week following surgery) when the stitch was removed. The dressing was applied again and the patient was advised to remove it the next morning. The patients were discharged from the hospital during the first postoperative day. The presence of an infection was diagnosed based on the generally accepted recommendations by the Centre for Disease Control and Prevention (Table 1) [18].

Table 1. Criteria for wound infection diagnosis

Presence of at least one of the following factors:	
	purulent discharge
	positive microbiological finding from a sample taken aseptically
	opening of the wound by the surgeon
Presence of one of the signs/symptoms:	
	pain
	swelling
	redness
	high temperature at the site of the wound
	the surgeon believes there is a wound infection

A swab was taken from the wound of the patients that had positive findings, and an oral antibiotic treatment was ordinated depending on the microbiological results. The patients that needed another hospitalization, a wound revision and parenteral antibiotic treatment, were considered to have a serious wound infection. It was considered that the patient did not have an early wound infection related to the positioning screws removal, if there were no signs of an infection during all four check-ups.

Other factors that were registered, besides the status of the surgical wound, were the demographic characteristics of the patients, the mechanism of the injury, the BMI (Body Mass Index) [19], the American Society of Anesthesiology (ASA) score [20], diabetes and smoking. All data wereenteredin an electronic data base (Microsoft Excel, Microsoft, Redmond, Washington), and after finishing the study, the data were transferred to SPSS (SPSS for Windows 22.0, Chicago IL). The qualitative variables were described as absolute and relative numbers, and the quantitative variables were described as an average value and standard deviation. The t-test for

independent samples and the Fisher'sexact two-tailed test were used for variable analysis.

Results

Enrolment in the study, demographics, type of fracture

In the stated period, 563 patients underwent an ankle fracture surgery at the University Clinic of Traumatology. Among them, 186 (32.9%) had a syndesmotic fixation, and 131(69%) had their positioning screw/screws removed within 8-12 weeks from the initial procedure. The decision for the removal was made by the surgeon. Ofthese 131 patients, 114(87%) were involved in the study (6 patients did not want to participate, and 11 did not satisfy the inclusion and exclusion criteria). The demographics of the patientsare shown in Table 2.

Table 2. Demographic characteristics of patients

Sex	Number (%)	Mean (age)	SD (age)	SE (age)	Min	Max
Men	73 (64%)	49.6	14.2	1.66	21	75
Women	41 (36%)	54.3	13.3	2.07	23	78
Total	114 (100%)	51.3	14	1.31		

SD-standard deviation; SE-standard error; Min-minimal value (age); Max-maximal value (age)

The participants were mostly male, and the average age of acquiring the ankle fracture was higher among women.The average screw removal period after the initial procedure was 81.3±14.7 days.The analysis of the type of the fracture related to age and sex of the patients is shown in Figure 1.

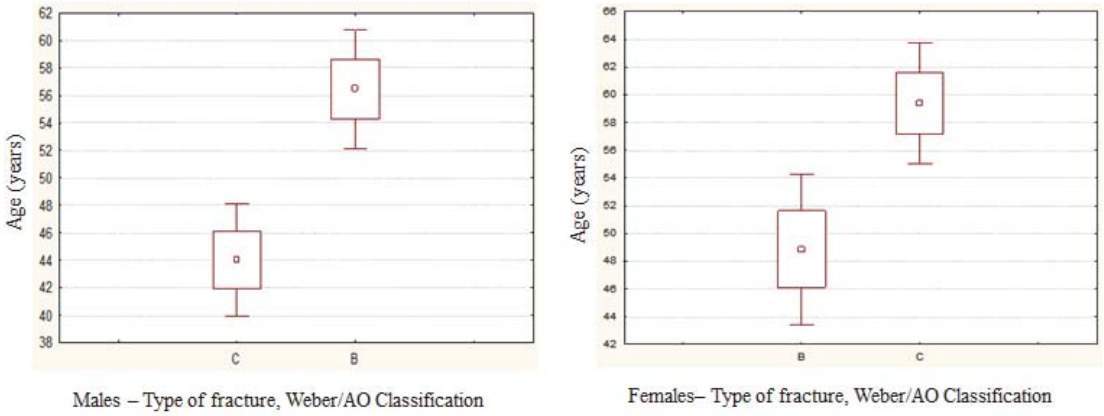

Fig. 1. Analysis of type of fracture by age and gender

Wound infection, causes and treatment

Early wound infection was diagnosed in eight patients (7%) during the control check-up, which was implemented with the previously described methodology. In four (50% of those in whom the infection was diagnosed) patients, the infection was diagnosed one week after the surgery and in three (37.5% of those in whom the infection was diagnosed) patients it was diagnosed during the control check-up two weeks after the performed procedure. In one (12.5% of those in whom infection was diagnosed) patient, the infection was diagnosed one month after the surgery. The eight patients, in which the infection was diagnosed, were represented as group B, and the rest of 106 patients were represented as group A. The microbiological finding was positive in seven (87.5% of group B) patients. In fiveof them, *Staphylococcus aureus*was isolated, in one-*Enterococcus faecalis* and one of the patientshad *Pseudomonas aeruginosa.* The patient who was infected with *Pseudomonas aeruginosa*had the wound opened by the surgeon and parenteral antibiotic therapy was administered. The antiobiogram of this patient showed Imipenem, Meropenem, Cefazidin and Colistin sensitivity. In this case, Cefazidin was administrated parenterally for 7 days, which resulted inreduced local inflammation and the patient was treated with dressingsfor the next 2 weeks. Patients with *Staphylococcus aureus* and the one with *Enterococcus faecalis*were given oral antibiotic therapy withamoxicillin-clavulanic acid for 7-10 days. The symptoms and clinical signssubsided without any further complications. There were no signs of spreading of the infection in the deeper structures nor did any other complication occur in the osteosynthetic material. The patient with the negative microbiological finding was administrated amoxicillin-clavulanic acid for 7 days, afterwhich the patient was symptom-free.

Risk factors for surgical infections

Besides the characteristics stated previously, we calculated BMI, checked the presence of diabetes, asked patients about their habit of smoking cigarettes and calculated the ASA score.

As shown in Table 3, we found no statistical association between the infection and the ASA score (Fisher's exact test: p=1.00). Also, we found that both smokers and non-smokers equally got infection (Fisher's exact test: p=0.448).

There was a significant difference between the two groups related to the level of BMI in favor of higher value in the group with infection (t-test=4.7; df=112; p<0.001) (Table 3). Our analysis showed significant association between the infection and the presence of diabetes (Fisher's exact test: p=0.025) (Table 3).

Table 3. Results of the analysis of risk factors

Parameter		group A	group B
ASA score	score 1	71 e. (66.9)	5 e. (62.5)
Fisher exact, two tailed p=1.00	score 2	35 e. (33.02)	3 e. (37.5)
Body Mass Index	mean value	25.68	29.75
t-value-4.77, df=112, p<0.001			
Diabetes	No DM	82 e. (77.36)	3 e. (37.5)
Fisher exact, two tailed p=0,025	DM	24 e. (22.64)	5 e. (62.5)
Smoking	smoker	36 e. (33.9)	4 e. (50)
Fisher exact, two tailed p=0,448	non-smoker	70 e. (66.1)	4 e. (50)

e-examinees; df-degree of freedom; the numbers in the parentheses show the percentage of group A and group B

Discussion

The results of the present studyshowedthat the wound infection occurred in 7% of the patients after the positioning screw was removed from the syndesmosis. The authors have a strong opinion that it is a significant percentage, especially having in mind thetechnical simplicity of the positioning screw removal procedure. In addition, the study of Bonneville *et al.*, which included 1617 examinees, reported significantly less cases of wound infection after skeletal trauma procedures for osteosynthesis and arthroplasty [21]. Similar results were presented by Astagneau *et al.*; wound infection occurred in 1.5% following surgery for skeletal trauma [22]. On the other hand, the study of Andersen found wound infection in 5% of patients following positioning screw removal from the syndesmosis [23%]. Yet, another study reported up to 9% following the procedure discussed [24]! Theoretically, it is hard to address the cause of the high rate of wound infection following this simple procedure; however, it is common for our study and the other studies describing this complication that no antibiotic prophylaxis was given during the procedure.

The microbiological findings showed that up to 62.5% of patients had *Staphylococcus aureus*. Most of the studies that have analyzed this issuehave stated that the number one cause of surgical wound infection in skeletal surgery is the abovementioned microorganism [25,26].

It is not surprising that smoking, diabetes and high BMI were significantly more present in patients with wound infection [25,27-30]. However; we found intriguing that the study of Andersen did not confirm the significance of the smoking and BMI in the occurrence of the wound infection followingthis procedure, although the study group was quite similar to ours [23]. It is also very important to be stated that BMI in this study wassignificantly higher compared to the Andersen's study. Nonetheless, itmay be noteworthy that the mean BMI in the present study was much higher compared to the study of Andersen.

Even though the present study was not focused on the need of syndesmotic screw removal, the high rate of wound infections we found, gave us a reason to briefly address the subject abovementioned. The removal of the positioning screw is the second surgicalprocedure that patients experience and it comes with its own risks, which can lead to prolonged hospital stay and higher health expenses. Huber's cadaveric study focused on the syndesmosis movement the restrictions in the joint movement in presence of positioning screw that wentthrough the tibia and fibula [31]. Similar anatomical and radiological studies have discussedthe same issue [32-34]. However, the clinical significance of the prolonged fixation of the syndesmosis is still uncertain. Most of the clinical studies that have discussed this issue failed to prove the impact of the prolonged fixation on the functional outcome [35-39]. At least two studies demonstrated the best functional results in patients in whom the positioning screw was found to be broken [40,41].

Having in mind all these notions, the high percentage of postoperative infection following removal of the positioning screw should be regarded asyet another risk factor while deciding whether or not the syndesmotic screw should be removed.

The limitations of the present study wereits small number of patientsthat weretreated in a single institution, the lack of a control group and the strict inclusion/exclusion criteria. Also, we did not analyze the functional result with regard to the occurrence of wound infection and costs of the treatment. On the other hand, the risk factors did give useful informationthough this procedure is recognized by many surgeons as a minor one, it might be the cause of serious complications and therefore they should be modified before the procedure is undertaken. Complications following partial or total implant removal have already been re-

ported. Namely, the study of Sanderson (1992) described an infection following implant removal in 15% of patients [42]. The authors of the present study believe that prophylactic antibiotic use will reduce the rate of wound infection following syndesmotic screw removal.

Conclusion

The need of routine syndesmotic screw removal remains controversial. Our results demonstrated a high percentage of wound infection following the procedure. The postoperative wound infection carries risks of spreading in the deeper structures and it may lead to preterm implant removal, which will renderuncertain functional result. The routine use of the antibiotic prophylaxis while performing this procedure might reduce the rate of wound infections. Our results do not support routine syndesmotic screw removal.

Conflict of interest statement. None declared.

References

1. Jensen S, Andersen B, Mencke S, Nielsen P. Epidemiology of ankle fractures: a prospective population-based study of 212 cases in Aalborg, Denmark. *Acta Orthop Scand* 1998; 69(1): 48-50.
2. van Staa T, Dennison M, Leufkens H, Cooper C. Epidemiology of fractures in England and Wales. *Bone* 2001; 29(6): 517-522.
3. van Heest TJ, Lafferty pM. Injuries to the Ankle Syndesmosis: current concepts review. *J Bone Joint Surg Am* 2014; 96-A(7): 603-613.
4. Kemler E, van de Port I, Valkenberg H, *et al*. Ankle injuries in the Netherlands: trends over 10-25 years. *Scand j Med Sci Sports* 2015; 25(3): 331-337.
5. Hermans JJ, Beumer A, de Jong TAW, Kleinrensink Gj. Anatomy of the distal tibiofibular syndesmosis in adults: a pictorial essay with a multimodality approach. *J Anat* 2010; 217: 633-645.
6. Ramsey P, Hamilton W. Changes in tibiotalar area of contact caused by lateral talar shift. *J Bone Joint Surg Am* 1976; 58(3): 356-357.
7. Tile M. Fractures of the ankle. In Schatzker M, Tile J. The rationale of operative fracture care. NewYork: NY: *Springer-Verlag* 2005; 580-581.
8. Schepers T, van Zuuren WJ, van den Bekerom MPJ, *et al*. The management of acute distal tibio-fibular syndesmotic injuries: Results of a nationwide survey. *Injury Int J Care Injured* 2012; 43(10): 1718-1723.
9. Heim D, Schmidlin V, Ziviello O. Do type B malleolar fracctures need a positioning screw? *Injury Int J Care Injured* 2002; 33(8): 729-734.
10. Scolaro JA, Marecek G, Barei DP. Management of Syndesmotic Disruption in Ankle Fractures: A Critical Analysis Review. *J Bone Joint Surg Reviews* 2014; 2(12): 1-8.
11. Schepers T, Dingemans SA, Rammelt S. Recent developments in the treatment of acute syndesmotic. *Fuß & Sprunggelenk - FussSprungg* 2016; 14(2): 66-78.
12. Egol KA, Pahk B, Walsh M, *et al*. Outcome after Unstable Ankle Fracture: Effect of Syndesmotic Stabilization. *J Otrhop Trauma* 2010; 24(1): 2-11.
13. Weening B, Bhandari M. Predictors of Functional Outcome Following Transsyndesmotic Screw Fixation of Ankle Fractures. *J Orthop Trauma* 2005; 19(2): 102-108.
14. Miller AN, Paul O, Boraniah S, *et al*. Functional Outcomes After Syndesmotic Screw Fixation and Removal. *J Orthop Trauma* 2010; 24(1): 12-16.
15. Kukreti S, Faraj A, Miles JNV. Does position of the syndesmotic screw affect functional and radiological outcome in ankle fractures? *Injury Int Care Injured* 2005; 36(9): 1121-1124.
16. Veen EJD, Zuurmond RG. Mid-term results of ankle fractures with and without syndesmotic rupture. *Foot and Ankle Surgery* 2015; 21(1): 30-36.
17. van den Bekerom MPJ, Raven EEJ. Current Concepts Review: Operative Techniques for Stabilizing the Distal Tibiofibular Syndesmosis. *Foot and Ankle International* 2007; 28(12): 1302-1308.
18. Centers for Disease Control and Prevention. [Online].; 1999 [cited 2011 May 02]. Available from: HYPERLINK "https://www.cdc.gov/hicpac/SSI/table1-SSI.html"https://www.cdc.gov/hicpac/SSI/table1-SSI.html.
19. Centers for Disease Control and Prevention. [Online]. [cited 2015 May 15]. Available from: HYPERLINK "http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/english_bmi_calculator/bmi_calculator.html"http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/english_bmi_calculator/bmi_calculator.html.
20. ANZ Journal of Surgery. [Online]. Available from: HYPERLINK"http://www.anzjsurg.com/view/0/ASAscore. html"http://www.anzjsurg.com/view/0/ASAscore.html.
21. Bonnevialle P, Bonnomet F, Philippe R, *et al*. Early sirgical site infection in adult appendicular skeleton trauma surgery: A multicenter prospective series. *Orthop Traumatol Surg Res* 2012; 98: 684-689.
22. Astagneau P, Rioux C, Golliot F, Brucker G. Morbidity and mortality associated with surgical site infections: Results from the 1997-1999 INCISO surveillance. *I Hosp Infect* 2001; 48: 267-274.
23. Andersen M, Frihagen F, Madsen J, Figved W. High complication rate after syndesmotic screw removal. *Injury Int J Care injured* 2015; 46(11): 2283-2287.
24. Schepers T, Van Lieshout E, de Vries M, Van der Elst M. Complications of syndesmotic screw removal. *Foot Ankle Int* 2011; 32(11): 1014-1014.
25. Emori T, Gaynes R. An overview of nosocomial infections, including the role of microbiology laboratory. *Clin Microbiol Rev* 1993; 6: 428-442.
26. Weiglet J, Lipsky B, Tabak Y, *et al*. Surgical site infections: causative patogens and associated outcomes. *Am J Infect Control* 2010; 38: 112-120.
27. Anderson D, Kaye K, Classen D. Strategies to prevent surgical site infections in acute care hospitals. *Infect Control Hosp Epidemiol* 2008; 29(1): S51-S61.
28. Dronge A, Perkal M, Kancir S, *et al*. Long-term glycemic control and postoperative infectious complications. *Arch Surg* 2006; 128: 375-380.
29. Nasell H, Ottosson C, Tornqvist H, *et al*. The impact of the smoking on complications after operatively treated ankle fractures-a follow-up study of 906 patinets. *J Orthop trauma* 2011; 25: 748-755.
30. Costigan W, Thordarson D, Debnath U. Operative management of ankle fractures in patients with diabetes melitus. *Foot Ankle Int* 2007; 28: 32-37.
31. Huber T, Schmoelz W, Bolderl A. Motion of the fibula relative to the tibia and its alterations with syndesmosis screws: acadaver study. *Foot Ancle Surg* 2012; 18(3): 847-856.

32. McKeon K, Wright R, Johnson J, *et al.* Vascular Anatomy of the Tibiofibular Syndesmosis. *J Bone Joint Surg* 2012; 94(10): 931-938.
33. Dikos G, Heisler J, Choplin R, Weber T. Normal Tibiofibular relationships at the Syndesmosis on Axial CT Imaging. *J Orthop Trauma* 2012; 26(7): 433-438.
34. Grenier S, Benoit B, Rouleau D, *et al*. ATFP: Anteroposterior Tibiofibular Ratio, A New Reliable Measure to Assess Syndesmotic Reduction. *J Orthop Trauma* 2013; 27(4): 207-211.
35. Schepers T, van der Linden H, van Lieshout E, *et al*. Technical aspects of the syndesmotic screw and their efect on functional outcom following acute distal tibiofibular syndemosis injury. *Injury Int J Care Injured* 2014; 45(4): 775-779.
36. van der Bekerom M, Hogervorst M, Bolhuis H, van Dijk C. Operative aspects of the syndesmotic screw: Review of current concepts. *Injury Int J Care Injured* 2008; 39(4): 491-498.
37. Bell D, Wong M. Syndesmotic screw fixation in Weber C ankle injuries-should the screw be removed before weight bearing? *Injury Int J Care Injured* 2006; 37(9): 891-898.
38. Schepers T. To retain or remove the syndesmotic screw: review of literature. *Arch Orthop Trauma Surg* 2011; 131(7): 879-883.
39. Manjoo A, Sanders D, Tieszer C, MacLeod M. Functional and Radiographic Results of Patients with Syndesmotic Screw Fixation: Implications for Screw Removal. *J Orthop Trauma* 2010; 24(1): 2-6.
40. Hamid N, Loeffler B, Braddy W, *et al*. Outcome after fixation of ancle fractures with an injury to the syndesmosis. *J Bone Joints Surg [Br]* 2009; 91-B(8): 1069-1073.
41. Kaftandziev I, Spasov M, Trpeski S, *et al*. Fate of the syndesmotic screw-Search for a prudent solution. *Injury Int Care Injured* 2016; 46S: S125-S129.
42. Sanderson P, Ryan W, Turner P. Complications of metalwork removal. *Injury* 1992; 23(1): 29-30.

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Original article

КОРЕЛАЦИЈА НА ВРЕМЕТО ОД ПОЧЕТОК НА СИМПТОМИ ДО ЛАПАРОСКОПСКАТА ОПЕРАЦИЈА И НЕЈЗИНИОТ ИСХОД КАЈ ПАЦИЕНТКИ СО АДНЕКСАЛНА ТОРЗИЈА: ПЕТ ГОДИШНО ИСКУСТВО

CORRELATION OF TIME FROM BEGINNING OF SYMPTOMS TO LAPAROSCOPICOPERATION AND ITS EFFECT IN PATIENTS WITH ADNEXAL TORSION: FIVE-YEAR-EXPERIENCE

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Abstract

The aim of this study was to present the correlation between the time from the beginning of symptoms to laparoscopic operation and the effect of the operation in patients with adnexal torsion.

Methods. In a retrospective study, we analyzed the results from medical documentation of 31 patients with adnexal torsion operated laparoscopically in the Department for Urgent Gynecology (University Clinic for Gynecology and Obstetrics in Skopje, R. Macedonia) in the period of five years (January 2013-July 2018).
We analyzed the used diagnostic tests, operative protocols and histopathology reports**.**

Results. The mean age of patients was 27.03±10.05 years (range 14-53 years). Adnexal torsion on the right side *vs.* adnexal torsion on the left side was presented in 17 patients (54.84%) *vs.* 14(45.16%), without significant difference in presentation. The average time from the beginning of symptoms to laparoscopic operation was 16.39±10.25 hours. In 22 patients (70.97%), the time from the beginning of symptoms to operation was ≤ 24 hours. In all 9 patients with the time from the beginning of symptoms to operation >24 hours adnexectomy was done. Statistical analysis showed a very strong positive correlation between the time from the beginning of symptoms to laparoscopic operation and adnexectomy.

Conclusions. It is very important to react quickly in cases with adnexal torsion. In patients with the time from the beginning of symptoms to operation shorter than 24 hours conservative laparoscopic operation should be done. Radical laparoscopic treatment (oophorectomy or adnexectomy) should be done only in cases with extreme damage of adnexa.

Keywords: adnexal torsion, laparoscopy

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Абстракт

Цел на студијата е да се презентира корелацијата помеѓу времето од почеток на симптомите до лапароскопската операција и нејзиниот ефет кај пациентки со аднексалнаторзија.

Методи. Во ретроспективна студија, ние ги анализиравме резултатите од медицинската документација на 31 пациентка со аднексална торзија оперирани лапароскопски на Одделот за Ургентна гинекологија (Универзитетска клиника за гинекологија и акушерство во Скопје, Р Македонија) во период од пет години (Јануари 2013-Јули 2018).

Резултати. Просечната возраст на пациентките беше 27,03±10,05 години (14-53).Аднексална торзија на десната страна наспрема аднексална торзија на левата страна беше присутна кај 17 пациентки (54,84%) наспрема 14(45,16%), без сигнификатно значајна разлика во презентацијата. Средното време од почеток на симптомите до лапароскопската операција изнесуваше 16,39±10,25. Кај 22 пациентки (70,97%) времето од почеток на симптомите до операцијата беше ≤24 часа. Кај сите 9 пациентки со време од почеток на симптомите до операцијата >24 часа беше направена аднексектомија. Статистичката анализа покажа силно позитивна корелација помеѓу времето од почеток на симптомите до лапароскопската операција и аднексектомија.

Заклучоци. Многу е важно да се реагира брзо во случаевите со аднексална торквација. Кај пациентки со време од почеток на симптомите до операцијата пократко од 24 часа треба да се направи конзервативна лапароскопска операција. Радикален лапароскопски третман (оофоректомија или аднексектомија) треба да се применат само кај случаеви со екстремно оштетување на аднексата.

Клучни зборови: аднексалнаторквација,

лапароскопија

Introduction

Adnexal torsion is a condition when ovary with or without fallopian tube rotates along its vascular pedicle, leading to partial or complete occlusion of the blood supply. Torsion of the uterine adnexa accounts for approximately 2.7% to 7.4% of all gynecological emergencies and is also a rare but important cause of acute abdominal pain in gynecology [1].

Diagnosis can be difficult and is mainly based on clinical symptoms, gynecological examination, transvaginal or transabdominal ultrasound examination and imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). The most common symptoms in clinical presentation are: acute lower abdominal pain, nausea, vomiting and fever.

On gynecological examination adnexal tenderness or palpable adnexal mass have been found in most of the cases. On transvaginal ultrasound examination adnexal torsion is frequently described as unilateral ovarian enlargement and edema with peripherally arranged follicles or as a solid mass with hypoechoic and hyperechoic areas as a result of hemorrhage and necrosis. Also, ovarian cysts or paraovarian cysts are a common finding in twisted ovaries. The size of the cysts is usually moderate (around 5 cm) and the cysts tend to become hemorrhagic as a result of venous congestion of ovaries. The tube may also be involved in torquation and can be filled with hemorrhagic fluid. There is often hemorrhagic fluid in the pouch of Douglas [2,3]. On color Doppler examination low peripheral vascularity and absence of blood flow are usually found. The pedicle that is twisted may be seen as a ”whirlpool“ sign [4].

CT and MRI are useful in diagnosis of adnexal torsion with findings as ovarian enlargement, tube thickening and fluid in the pouch of Douglas. CT may confirm and help to exclude other nongynecologic and extrapelvic diseases [5]. MRI is useful and safe in diagnosis of adnexal torsion in the second and third trimester of pregnancy because in these patients ovaries and appendix are more difficult to be visualized by ultrasound [6].

Blood is usually taken for detection of infection, anemia or inflammation in all patients in emergency departments with acute abdominal pain. The easiest marker for examination is C-reactive protein that is raised in the presence of inflammation. Also, the white cell count is raised in about 50% of patients with adnexal torsion. But, these markers are not routinely used in the diagnosis of adnexal torsion because of low sensitivity and specificity. Recently, several other pro-inflammatory markers such as interleukin-6 and tumor necrosis factor-α have been used in diagnosis of adnexal torsion [7].

In patients with ovarian torsion obstruction of venous and lymphatic drainage are present at the beginning (Figure 1).

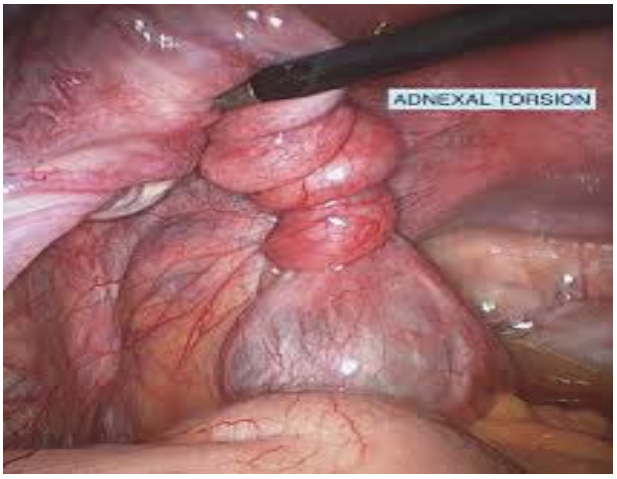

Fig. 1. Adnexal torsion (our material)

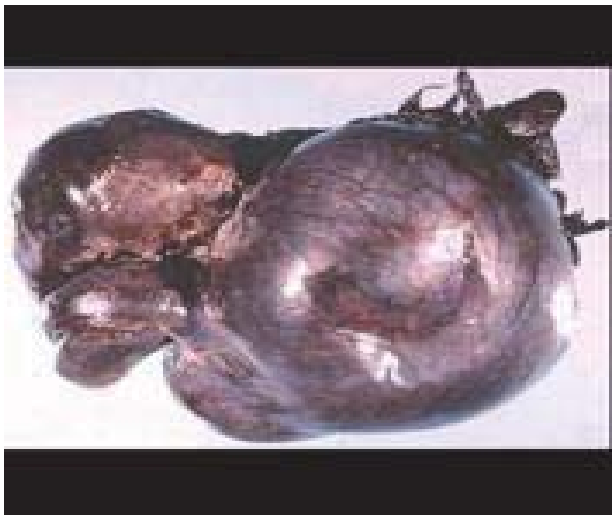

Fig. 2. Necrosis of the ovary as a result of adnexal torsion (our material)

Over time, the arterial supply of the ovary becomes compromised with infarction and necrosis of the ovary (Figure 2).

There are several types of adnexal torsion (torsion of ovary, torsion of tube, torsion of adnexa).

If diagnosis of adnexal torsion is made before infarction, the ovary can be detorsed and normal blood flow restored. In these cases conservative treatment can be used (extirpation of cyst, partial resection of ovary). In cases with infarction of ovary, adnexectomy must be done. On histopatological examination in such cases ovarian necrosis is found.

Ovarian salvage depends on early diagnosis and surgical management.

In the past, adnexal torsion was treated by salpingo-oophorectomy (laparotomy) without untwisting the adnexa to avoid potential thromboembolism from ovarian vein thrombosis.

Recently, a review of the literature has shown that the risk of pulmonary embolism after adnexal torsion was 0.2% and was not increased when the adnexa was untwisted [8].
In recent years laparoscopic surgery is the procedure of choice for treatment of patients with adnexal torsion, because detorsion and ovarian cyst extirpation can be made laparoscopically.
During laparoscopy exploration of the whole abdominal cavity must be done. The twisted ovary must be carefully untwisted with non-traumatic instruments. This is followed by inspection of ovarian surface, tubal condition, presence of ovarian cysts, degree of ischemia and necrosis. In cases with restoration of ovarian color and vascularization extirpation of cyst or partial resection of ovary is made. Oophoropexy in cases with long ovarian pedicle is done for prevention of recurrence. In patients with ovarian necrosis as the result of adnexal torsion, adnexectomy must be done.

Aim of the study

This retrospective study was undertaken in order to evaluate the correlation of the time from the beginning of the symptoms of adnexal torsion to laparoscopic operation and its effect on adnexa in patients operated on at the University Clinic for Gynecology and Obstetrics in Skopje, R. Macedonia over a five-year period (Department for urgent gynecology).

Materials and methods

We evaluated the results from laparoscopic treatment of 31 patients with adnexal torsion hospitalized in the Department for Urgent Gynecology at the University Clinic for Gynecology and Obstetrics in Skopje, R. Macedonia over a 5-year period (from January 2013 to July 2018). Diagnosis for adnexal torsion was made by clinical examination, ultrasound examination with color Doppler, but was confirmed during laparoscopy. All patients signed a full informed consent before operation.
For ultrasound examination GE Medical System Voluson E-8 ultrasound machine with a RIC 5-9-D transvaginal transducer (3.7-9.3 MHz) or RAB 4-8-D transabdominal transducer (4-8,5 MHz) was used. Laparoscopy was made in all patients by using the Storz equipment.
During laparoscopy for adnexal torsion, firstly detorsion was made, followed by a 15 min waiting period for recovery of adnexa. During that time the operative field was continuously irrigated with saline solution and we observed for any sign of reperfusion manifested as a slight change in the color of adnexa. In cases with recovery of adnexa conservative surgery was done. If reperfusion did not occur, then the ovary, tube, or both were resected.
Statistical analysis was made by using SPSS software package, version 20.0 for OS Windows. Quantitative data were described by using mean and mediana with standard deviation. Percentage difference was presented with Difference test. Correlation between variables was made by using Spearman's Rank Order Correlation. The value of $p<0.05$ was considered statistically significant.

Results

In the period of five years, at the Department for Urgent Gynecology of the University Clinic for Gynecology and Obstetrics in Skopje, R. Macedonia, 31 patients with adnexal torsion treated with laparoscopic operation were evaluated.
The mean age of patients was 27.03±10.05 years [95% CI (23.52-30.74)], with range 14-53 years.
According to nationality, there were 18(58.06%) Macedonians, 11(35.48%) Albanians, and 2 patients were from other nationality (6.45%). Only one patient was pregnant in 14 weeks of gestation and successfully terminated pregnancy at time. On ultrasound examination ovarian cysts were found in 21 cases (67.74%), paraovarian cysts in three cases (9.68%), and ovarian enlargement and edema were found in 6 cases (19.35%).In one case (3.23%) PCOS of affected adnexa was found.
Conditions associated with adnexal torsion are presented in Table 1.

Table 1. Conditions associated with adnexal torsion

Condition	No. of patients	Percentage (%)
Ovarian cysts (functional and organic)	21	67.74
Paraovarian cysts	3	9.68
Ovarian enlargement/edema	6	19.35
PCOS	1	3.23
Total	31	100

All 31 patients were operated laparoscopically. All of them had unilateral adnexal torsion. The torsion was more common on the right side (54.84%, n=17) than on the left side (45.16%, n=14), without a significant difference (Difference test: Difference 9.68% [95% CI (-14.45-32.31)]; Chi-square=0.572; df=1 p=0.4496).
The average time from the beginning of the symptoms to laparoscopic operation was 16.39±10.25 hours [95% CI (12.96-19.68)] with minimal versus maximum time of 4 *vs*. 36 hours. In 22 patients (70.97%) the time from the beginning of the symptoms to operation was ≤24 hours. In all 9 patients with the time from the beginning of the symptoms to laparoscopic operation >24 hours adnexectomy was done (Table 2).

Table 2. Analysis of effect of time from beginning ofsymptoms to laparoscopic operation on type of laparoscopic operation

Effect of operation	Time from beginning ofsymptoms to laparoscopic operation		Total
	≤24 hours	>24 hours	
adnexectomy	2 (9.09%)	9 (100%)	11 (35.48%)
resectioovarii	7 (31.82%)	0 (0%)	7 (22.58%)
other operation	13 (59.09%)	0 (0%)	13 (41.94%)
Total	22 (70.97%)	9 (100%)	31 (100%)

Statistical analysis showed a significant linear very strong positive correlation between the time from the beginning of the symptoms to laparoscopic operation and adnexectomy as the result of the operation (Spearman rank Order Correlation: R=0.8415; p<0.05) (Figure 3).

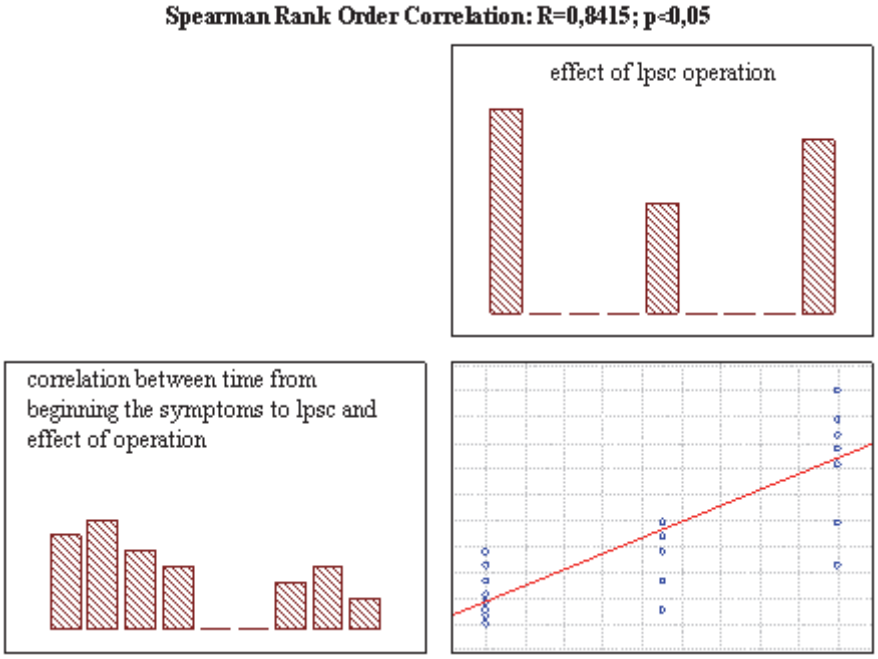

Fig. 3. Correlation between time from beginning ofsymptoms to laparoscopic operation and type of laparoscopic operation

Conservative laparoscopic operation was made in 20 cases (64.52%), while adnexectomy was made in 11 cases (35.48%) because of hemorrhagic necrosis of ovaries. In one patient with PCOS, detorsion with ovarian drilling and ovariopexy were performed. In other cases detorsion with cyst extirpation and ovarian resection or salpingectomy were made. These results are presented in Table 3.

Table 3. Operation performed for adnexal torsion (n=31)

Operation	No.	(%)
Detorsion and cyst enucleation	12	38.71
Detorsion and ovarian drilling	1	3.23
Detorsion and ovarian resection	7	22.58
Detorsion and adnexectomy	11	35.48
Total	31	100

Histopathological examination confirmed simple serous cystadenoma in one case, benign cystic teratoma in one case, dermoid cyst in one case, functional benign ovarian cysts in 18 cases, paraovarian cysts in three cases, and infarctus with necrosis of adnexa in 7 cases. No cases of malignancy were found in our study.

Postoperatively thromboprophylaxis was given to all patients.

None of the patients had serious intraoperative and postoperative complications, such as thromboembolic events. The average hospital stay was 2.5 days (range 1-3 days). After one month ultrasound examination revealed restored ovarian function in the form of follicular development and normal blood flow on color Doppler in all cases treated with conservative laparoscopic operation.

Discussion

Adnexal torsion is a rare condition that usually occurs during the reproductive period of life in women. Historically, the treatment of choice for patients with adnexal torsion was laparotomy with salpingo-oophorectomy. But, over the last years laparoscopy has been

used more often in the treatment of adnexal torsion because of its safety, reliability and preserving fertility. The most common predisposing factors for adnexal torsion are: cases with previous adnexal torsion, induction of ovulation, long tubes, ovarian and paraovarian cysts, endometriosis and pelvic inflammatory disease [9]. In our study, the most common predisposing factors were ovarian and paraovarian cysts in 24 patients (77.4%), which correspond with literature reports [10]. The mechanism of adnexal torsion is not known, but there are several theories such as: presence of long tubes, sudden valsalva maneuver, pelvic congestion, long utero-ovarian ligament etc. Diagnosis can be difficult, especially in cases with intermittent adnexal torsion. Transvaginal and transabdominal ultrasonography associated with power and color Doppler can be used for diagnosis but with limitation because these investigations cannot quantify the vascularization of all ovarian tissues [11]. In our study diagnosis of adnexal torsion was based on clinical signs for acute lower abdominal pain with nausea and vomiting, transvaginal or transabdominal ultrasonography that showed ovarian enlargement with ovarian or paraovarian cysts and reduction or absence in arterial flow on color Doppler examination.

But, definitive diagnosis was made during laparoscopic operation. In all cases, we were able to make laparoscopic surgery since there was no suspicion for malignancy.

The type of laparoscopic surgery is determined by many factors, including age, desire to preserve fertility, ovarian pathology. It is generally agreed that women with normal or mildly ischemic adnexa can be treated by conservative operation (detorsion of adnexa followed by treatment of the etiology). In cases with severe adnexal ischemic or necrotic lesions majority of authors recommend radical treatment-oophorectomy or adnexectomy, procedures performed in our study [12]. There are several studies withpediatric cases of adnexal torsion which give greater support to conservative approach than to surgical management of adnexal torsion with detorsion with or without oophoropexy [13-15]. It is very important to react quickly in cases with adnexal torsion. In a study of Anders *et al*. from 2005 the mean time from initial examination to operation was 11 hours for salvaged ovaries (range1-23 hours) and 21 hours for nonsalvaged ovaries (range 2-71 hours) [16]. In our study,in 22 patients (70.97%) the time from the beginning of the symptoms to operation was ≤24 hours. In all 9 patients (29.03%) the time from the beginning of the symptoms to laparoscopic operation >24 hours adnexectomy was done.

Parelkar*et al*. in a study with 13 ovarian torsions in children managed to treat all of them by detorsion [17]. In the study of Fady*et al*. oophoropexy was made in 46.8% of cases, especially in cases with recurrent torsion. In our study we made ovarian fixation in three cases (9.68%).Tandulwadkar*et al.* managed to conserve 77.2% of ovaries with adnexal torsion [18]. In a retrospective study, Tsafrir*et al.* reported 80% success in ovarian conservation [19]. In our study we made conservative laparoscopic operation in 70% of cases [20].

Conclusions

Laparoscopy is an effective and safe surgical method for diagnosis and treatment of patients with adnexal torsion. Conservative laparoscopy is treatment of choice for women in the reproductive age desiring fertility. Prognosis is excellent in patients with early diagnosis and urgent treatment. According to the results obtained inour study, the most important factor for type of laparoscopic operation wasthe time from the beginning of the symptom of adnexal torsion to operation.

In patients with the time from the beginningof the symptoms to operation shorter than 24 hours conservative operation should be done. In cases with the time from the beginning of the symptoms to operation longer than 24 hours because of extreme damage of the adnexa, adnexectomy should bedone.

Conflict of interest statement. None declared.

References

1. Damigos E, Johns J, Ross J. An update on the diagnosis and management of ovarian torsion. *The Obstetrician-Gynaecologist* 2012; 14: 229-236.
2. Shadinger LL, Andreotti RF, Kurian RL. Preoperative sonographic and clinical characteristics as predictors of ovarian torsion. *J Ultrasound Med* 2008; 27: 7-13.
3. Smorgick N, Maymon R, Mendelovic S, *et al*. Torsion of normal adnexa in postmenarcheal women: can ultrasound indicate an ischemic process? *Ultrasound ObstetGynec* 2008; 31: 338-341.
4. Vijayaraghavan SB. Sonographic whirlpool sign in ovarian torsion. *J Ultrasound Med* 2004; 23: 1643-1649.
5. Swenson DW, Lourenco AP, Beaudoin FL, *et al*. Ovarian torsion: case-control study comparing the sensitivity and specificity of ultrasonography and computed tomography for diagnosis in the emergency department. *Eur J Radiol* 2014; 83: 733-738.
6. Eskandar O, Eckford S, Watkinson T. Safety of diagnostic imaging in pregnancy. Part2: magnetic resonance imaging, ultrasound scanning and Doppler assessment. *ObstetGynecol* 2010; 12: 171-177.
7. Cohen SB, Stockheim D, Eidman DS, *et al*. The accuracy od serum interleukin-6 and tumour necrosis factor as markers for ovarian torsion. *Hum Reprod* 2001; 16: 2195-2197.
8. McGovern PG, Koenigsberg R, Little AB. Adnexal torsion and pulmonary embolism: case report and review of the literature. *ObstetGynecolSurv* 1999; 54(9): 601-608.
9. Alkatout I, Mettler L, Anlauf M, *et al*. Management of adnexal torsion by laparoscopic approach. *Gynecological Surgery* 2012; 9 (4): 405-409.
10. Argenta PA, Yeagley TJ, Otto G, *et al*. Torsion of the uterine adnexa. Pathologic correlations and current management trends. *J Reprod Med* 2000; 45(10): 831-836.

11. Khatri MA. Laparoscopic management of adnexal torsion. *Sri Lanka Journal of Obstetrics and Gynaecology* 2011; 33: 56-59.
12. Fady M, Shawky M. Adnexal torsion: Management controversy: A case series. *Middle East Fertility Society Journal* 2017; 22(2): 156-159.
13. Breech LL, Hillard PJ. Adnexal torsion in paediatric and adolescent girls. *CurrOpinObstetGynecol* 2005; 17: 483-489.
14. Crouch M, Gyampoh B, Cutner AS, *et al*. Ovarian torsion: to pex or not to pex? *J PaediatrAdolescGynecol* 2003; 16: 381-384.
15. Fuchs N, Smorgick N, Tovbin Y, *et al*. Oophoropexy to prevent adnexal torsion: how, when and for whom? *J Minim Invasive Gynecol* 2010; 17: 205-208.
16. Anders JF, Powell E.C. Urgency of evaluation and outcome of acute ovarian torsion in pediatric patients. *Arch PediatrAdolesc Med* 2005; 159(6): 532-535.
17. Parelkar SV, Mundada D, Sanghvi BV, *et al*. Should the ovary always be conserved in torsion? A tertiary care institute experience. *J PediatrSurg* 2014; 49(3): 465-468.
18. Tandulwadkar S, Shah A, Agarwal B. Detorsion and conservative therapy for twisted adnexa: Our experience. *J GynecolEndoscSurg* 2009; 1(1): 21.
19. Tsafrir Z, Hasson J, Levin I, *et al*. Adnexal torsion: cystectomy and ovarian fixation are equally important in preventing recurrence. *Eur J ObstetGynecolReprodBiol* 2012; 162(2): 203-205.
20. Georgievska J, Tofoski Gl, Daneva A, *et al*. Our experience in laparoscopic management of adnexal torsion. XIX National congress of infertility and reproductive health with international participation, Borovetz 2018. Book of abstracts. (Available on: www.basrh.org).

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Original article

DURATION OF INTERFERON BETA TREATMENT IN MULTIPLE SCLEROSIS IS NOT ASSOCIATED WITH HIGH BINDING ANTIBODY TITERS

ВРЕМЕТРАЕЊЕТО НА ТЕРАПИЈАТА КАЈ МУЛТИПЛА СКЛЕРОЗА СО ИНТЕРФЕРОН БЕТА НЕ ВЛИЈАЕ НА РАЗВОЈ НА ВИСОК ТИТАР НА ВРЗУВАЧКИ АНТИИНТЕРФЕРОНСКИ АНТИТЕЛА

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Abstract

Interferon beta (IFNB) is first line of treatment in patients with multiple sclerosis. Efficacy is well established in many clinical trials and real world efficacy studies. Interferon beta is a protein in the group of biosimilars. It is very similar to biological proteins and is well tolerated in the human body. As protein, interferon is a highly immunogenic and the body develops binding antinterferon antibodies (BAB). A subset from BAB areneutralizing antibodies (NAB), which reduce interferon efficacy and clinical response.

The aim of our study was to determine whether duration of treatment is correlated with high BAB titers.

We included 132 subjects with multiple sclerosis treated with IFNB. From 132 subjects, 71(53.8%) were BAB positive and 61(46.2%) were BAB negative. Subjects were divided in 3 groups depending on the IFNB formulation. In the Betaferon group mean treatment time was 62.6 months, in the Rebif group mean treatment time was 79.9 months and in the Avonex group it was 32 months, which wassignificantly lower treatment time compared to other two groups (P<0.05). In the Avonex group 22% were BAB positive, in the Betaferon group 23.7% were BAB positive and in the Rebif group 8% were BAB positive. Patients treated with Rebif had significantly lower risk for BAB development compared to Betaferon and Avonex groups (P<0.01). In our study, we did not find a significant correlation between duration of treatment and high titer of BAB antibody.

Keywords: multiple sclerosis, binding antibodies, neutralizing antibodies, immunogenicity, interferon beta

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Апстракт

Интерферон бета е прва линија на терапија кај мултипла склероза. Ефикасноста е добро позната и е докажана во многубројни клинички студии и студии од реална пракса. Интерферонот бета е протеин кој е сличен на хуманите протеини и лесно се толерира од организмот. Интерферонот како протеинска структура има високо ниво на имуногеност и организмот развива антиинтерферонски врзувачки антитела (BAB). Дел од BAB антителата се трансформираат во неутрализирачки антитела (NAB) кои ја намалуваат ефикасноста на интерферонот.

Целта на нашата студија беше асоцијацијата на времетраењето со висината на титарот на BAB антителата.

Во студијата беа вклучени 132 пациенти со мултипла склероза третирани со интерферон. Од вкупно 132 пациенти 71(53.8%) беа позитивни за BAB, а 61 (46.2%) беа негативнина BAB. Пациентите беа поделени во 3 групи во зависностод формулацијата на лекот. Кај групата третирана со Бетаферон просечното времетраење на терапија е 62,6 месеци, кај групата третирана со Ребиф просечно времетраење на терапијатабеше 79.9 месеци. Кај групата со Авонекс просечното времетраење на терапијата е 32 месеци, што во споредба со другите групи е статистички значителна p<0.05.

Кај групата третирана со Авонекс 22% беа позитивни за BAB, кај групата третирани со Бетаферон 23,7% беа позитивни за BAB. Кај групата третирана со Ребиф позитивни за BAB беа 8% од испитаниците што е статистички значајно во споредба со Авонекс и Бетаферон p<0.05. Шансите за развој на висок титар на антитела кај пациентите третирани со Авонекс и Бетаферон е значително поголема во однос на групата третирана со Ребиф p< 0,01. Во однос на времетраењето и висината на БАБ антителата статистичката обработка не покажа зна-

чителна корелација. Во нашата студија времетраењето на терапијата не влијае на висината на БАБ антителата.

Клучни зборови: мултипла склероза, имуногеност, интерферон бета, врзувачки антитела, неутрализирачки антитела

Introduction

Interferon beta (IFNB) is the first line treatment for multiple sclerosis (MS). Its efficacy is well established in clinical trials and real world studies [1]. Three formulation of IFNB are widely available.One type is known as IFNb-1b (Betaferon) and two types asIFNb-1a (Rebif) and (Avonex), which differ inexcipients, route of administration and dosage [2]. IFNB is a protein structure in the group of biosimilars and is well tolerated by the organism [3,4]. Despite regular use and similar structure to the body proteins, it is a foreign molecule to the organism and a very largenumber of patients develop antibodies [5,6]. Neutralizing antibodies (NAB) are biologically relevant as they bind to the effective receptors on the IFNB and block the function and reduce its efficacy. In patients with high NAB a biological response of the IFNB is lower and it can affect efficacy of the treatment and disease progression [7,8]. NAB are subset of binding antibodies (BAB) which are formed in almost every patient within 6 months of interferon treatment [9]. BAB can bind to interferon on different sites and it is considered not biologically relevant [10]. New studies show that high titers of BAB has big probability of high titters of NAB and questions the importance of NAB testing which is more complicated and expensive procedure [11]. Almost 50% of patients with high titters of BAB eventually will have biological relevant NAB. HLA genotype Class II molecules areconsidered to be arisk factor [12]. Development of BAB differs in different formulation of interferon. Rate for BAB development is different, some patients will develop high titers in the first year of treatment, and others will have low titters of BAB after many years.

Aim

This study aimed to find out thecorrelation between time of treatment and titer of BAB in Macedonian patients treated with IFNB therapy for multiple sclerosis.

Material and methods

Patients diagnosed with MS and treated with IFNB at least 2 months were included in the study. All of them were treated at the University Clinic of Neurology, Skopje Macedonia. In total 132 patients were enrolled and treated with IFNB in the period of 2013-2017. Blood samples from cubital vein were collected for antibody testing. Sera was isolated and put in freezer on -20 degrees until the test.
All samples were tested for BAB titers with direct ELISA method using BULHMAN kit for interferon antibodies for clinical testing. Units of titers are defined as Bulhman Titer Unit (BTU). A positive result as instructed in the kit for our cohort was >50 BTU.
Duration of treatment in the groups and BTU titers were analyzed using ANOVA method and if there was astatistical significance a post-hoc Tukey test was used.

Results

In our study we found that 71 of the patients were BAB positive with titers over 50 BTU and 61 patients were BAB negative with titers under 50 BTU. Mean treatment duration in the group treated with Avonex was

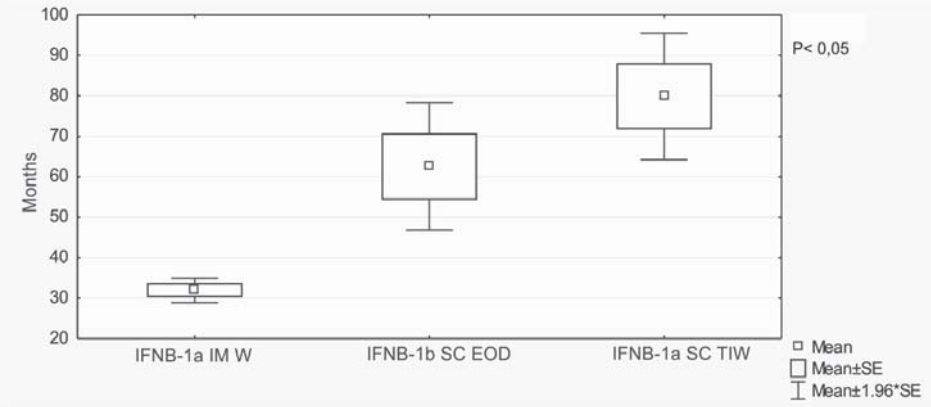

Fig. 1. Duration of IFNB treatment in months
IFNB-interferon beta; IFNB-1a IM W-Avonex intramuscular once weekly; IFNB-1b SC EOD-Betaferon intramuscular every other day; IFNB-1a SC TIW-Rebif subcutaneous three times a week

32 months in the range of 14.2 to 80.2 months. In the group treated with Betaferon mean treatment time was

62.6 months in the range of 10.2 to 158.3 months and in the group treated with Rebif mean treatment time was 79.9 months in the range of 2.03 to 170.4 months. There was astatistical significance in the duration of treatment among interferon groups. The group treated with Avonex had statistically lower mean duration treatment of 32 months compared with Betaferon and Rebif group (62.6 months and 79.9 months, respectively), ($p<0.05$) (Figure 1).

In all 132 patients BAB titers were measured and divided in 3 different treatment interferon groups. In the group treated with Avonex mean BAB titer was402, 1 BTU ranging from 0 to 5526.1 BTU. Mean titer in the Betaferon group was394.0 BTUranging from 11.7-1489.4 BTU and mean titer in the Rebif group was 82.9 ranging from 0 to 823.6 BTU (Figure 2).

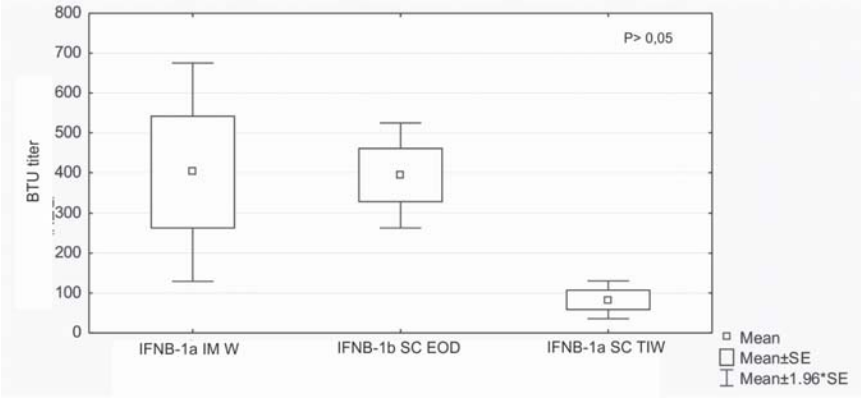

Fig. 2. Mean BTU titer in three IFNB groups
IFNB-interferon beta; IFNB-1a IM W-Avonex intramuscular once weekly; IFNB-1b SC EOD-Betaferon intramuscular every other day; IFNB-1a SC TIW-Rebif subcutaneous three times a week

Mean BAB titer among three different interferon treated drugs wasnot statistically significant (P> 0.05). We founda statistically significant correlation between BAB titer and interferon formulation. Rebif hadby 0.3 times less risk in development of high BAB titers compared to Avonex and by 0.1 less risk compared to Betaferon (Table 1).

In the three IFNBgroups we found no statistically significant correlation between BAB titers and duration of treatment. There was alow negative correlation (Figure 3).

Table 1. Univariate logistic regression analysis

Interferon	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Rebif			17.452	2	.000			
Avonex	-1.149	.446	6.643	1	.010	.317	.132	.759
Betaferon	-2.222	.535	17.259	1	.000	.108	.038	.309
Constant	.934	.356	6.894	1	.009	2.545		

Reference category Rebif

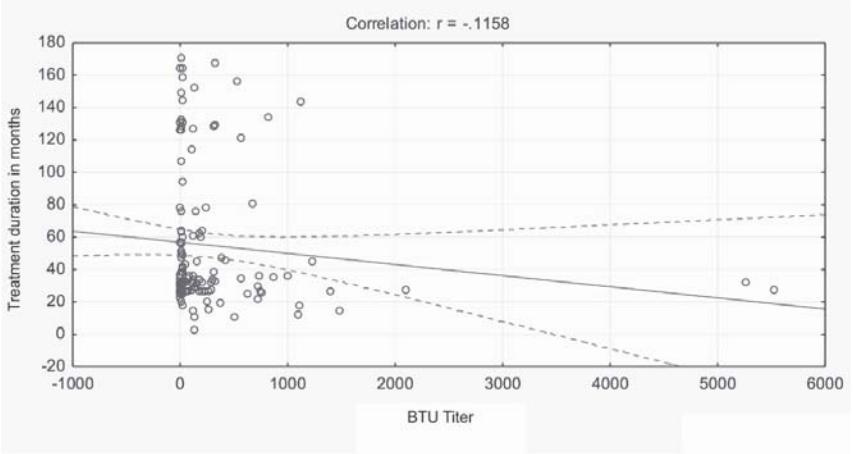

Fig. 3. Correlation between duration and BTU titer

Discussion

Immunogenicity of the IFNB is well known and has beendemonstrated in many studies. In this study we investigated whether duration of treatment wouldaffect high titer of BAB antibody against interferon. We have shownthat in our group of patients treated with IFNB, 71(53.8%) subjects were positive for BAB and 61 subjects (46.2%) were negative for BAB. In our group treated with interferon beta 1a administered subcutaneously a lower risk for antibody development was found compared to interferon beta 1b given every other day subcutaneously and interferon beta 1a given intramuscularly once weekly. In the Danish group, 97% of patients treated with IFNB-1b every other day longer than 12 month were positive for BAB compared to 33% of BAB positive patients treated with IFNB-1a given once weekly and 89% of BAB positive patients in the group treated with IFNB-1a three times weekly (13). In the PRISM study the results were similar to those obtained in the Danish group (14). Our group of patients had different characteristics compared to the Danish group and PRISM study. We have demonstrated that IFNB-1b given subcutaneously every other day and IFNB-1a given intramuscularly once a weekyielded lower incidence of BAB positivity, 22% and 23.7%, respectively. Only 8% were BAB positive in our group treated with IFNB-1a given subcutaneously three times weekly. A similar study conducted on Danish population two year later showed that IFNB-1a given intramuscularlyresulted in ahigh titer of BAB compared to IFNB-1b given subcutaneously. In the same study the subjects treated with IFNB-1a given subcutaneously had equally distributed BAB titers among low and high titers [11].

Our groups weredifferent compared to the groups examined in theabove mentioned studies. Our aim was to show the presence of antibodies above 50 BTU as a positive result for BAB and a correlation with the time of treatment. A Bulgarian study performedin 2017 showed similar results to ours regarding positivity for BAB. They used a similar method to measure BAB using Bulhman kit. In their group they showed that 45.8 of subjects were BAB positive [15]. Another aspect was duration of treatment and BAB development. We wanted to investigate if duration of treatment would give high BAB titers. In our group of patients, there was no significant correlation between time of treatment and high titter of BAB. It seems that time of treatment does not influence high BAB titer development.

High titer of BAB will produce a subset of neutralizing antibodies (NAB) which will neutralize the effect of interferon and its clinical efficacy [16,17]. Measurement of NAB is not standardized and very expensive, as opposed tomeasurement of BAB, which is simple and not expensive [18]. BAB can be used to screen patients at risk for lower therapy effect. The differrences between BAB positivity in our study and other studies are due to different kits and methods used along the years [19].

However, a standardized approach is not yet established. There are different kits and measurements units which can be misleading.

The question remains why some patients are prone todeveloping high titer of BAB compared to others. Genetics can influence different immune response to IFNB, especially HLA class II allele [12,20,21].

Conclusion

Our study did not show a significant correlation between BAB titer and time of treatment. It is difficultto determinewhich patient will develop high titer of BAB in order to predict therapy response. Our study showed BAB positivity relatively similar to other studies.

Conflict of interest statement. None declared.

References

1. Kappos L, Freedman MS, Polman CH, *et al*. Articles Long-term eff ect of early treatment with interferon beta-1b after a fi rst clinical event suggestive of multiple sclerosis: 5-year active treatment extension of the phase 3 BENEFIT trial. *Lancet Neurol* 2009; 8: 987-997.
2. Comi G, Filippi M, Barkhof F, *et al*. Effect of early interferon treatment on conversion to definite multiple sclerosis: a randomised study. *Lancet* 2001; 357(9268): 1576-1582.
3. Cook SD, Quinless JR, Jotkowitz A, Beaton P. Neutralizing Antibody Study Group the NAS. Serum IFN neutralizing antibodies and neopterin levels in a cross-section of MS patients. *Neurology* 2001; 57(6): 1080-1084.
4. Fox E, Green B, Markowitz C, *et al*. The effect of scheduled antibody testing on treatment patterns in interferon-treated patients with multiple sclerosis. *BMC Neurol* 2014; 14(1): 73.
5. Paolicelli D, D'Onghia M, Pellegrini F, *et al*. The impact of neutralizing antibodies on the risk of disease worsening in interferon β–treated relapsing multiple sclerosis: a 5 year post-marketing study. *J Neurol* 2013; 260(6): 1562-1568.
6. Polman CH, Bertolotto A, Deisenhammer F, *et al*. Recommendations for clinical use of data on neutralising antibodies to interferon-beta therapy in multiple sclerosis. *Lancet Neurol* 2010; 9(7): 740-750.
7. Sato DK, Nakashima I, Fukazawa T, *et al*. Neutralizing Antibodies Are Associated with a Reduction of Interferon-β Efficacy during the Treatment of Japanese Multiple Sclerosis Patients. 2012; 85-92.
8. Goodin DS, Hartung H-P, O'Connor P, *et al*. Neutralizing antibodies to interferon beta-1b multiple sclerosis: a clinico-radiographic paradox in the BEYOND trial. *Mult Scler J* 2012; 18(2): 181-195.
9. Sominanda A, Rot U, Suoniemi M, *et al*. Interferon beta preparations for the treatment of multiple sclerosis patients differ in neutralizing antibody seroprevalence and immunogenicity. *Mult Scler J* 2007; 13(2): 208-214.
10. Gneiss C, Reindl M, Berger T, *et al*. Epitope Specificity

of Neutralizing Antibodies Against IFN-β. *J Interf Cytokine Res* 2004; 24(5): 283-290.
11. Jensen PEH, Sellebjerg F, Søndergaard HB, Sørensen PS. Correlation between anti-interferon-β binding and neutralizing antibodies in interferon-β-treated multiple sclerosis patients. *Eur J Neurol* 2012; 19(10): 1311-1317.
12. Fink K, Link J, Lundkvist M, *et al*. HLA Genes and Interferon-beta Preparations Influence Risk of Developing Neutralizing Anti-Drug Antibodies in Multiple Sclerosis (P1.213). *Neurology* 2014; 82(10_Supplement): P1. 213.
13. Ross C, Clemmesen KM, Svenson M, *et al*. Immunogenicity of Interferon-in Multiple Sclerosis Patients : Influence of Preparation, Dosage, Dose Frequency, and Route of Administration. 2000; 706-712.
14. PRISMS Study Group and the University of British Columbia MS/MRI Analysis Group., PRISMS Study Group and the University of British Columbia MS/MRI Analysis Group. TP (Prevention of R and D by I-β-1a S in MSS, Group the U of BCMA. PRISMS-4: Long-term efficacy of interferon-beta-1a in relapsing MS. *Neurology* 2001; 56(12): 1628-1636.
15. Ivanova S, Skrobanska R, Kolyovska V, Milanov I. Do Binding Antibodies Predict Neutralizing Antibody Development in Multiple Sclerosis Patients? 2018.
16. Deisenhammer F, Schellekens H, Bertolotto A. Measurement of neutralizing antibodies to interferon beta in patients with multiple sclerosis. *J Neurol* 2004; 251(S2): II31- II39.
17. Kaya D, Tufan S, Özakbaş S, *et al*. Importance of neutralizing antibody positivity in Tur-kish multiple sclerosis patients. *Health (Irvine Calif)* 2013; 05(11): 1917-1923.
18. Sorensen PS, Ross C, Clemmesen KM, *et al*. Clinical importance of neutralising antibodies against interferon beta in patients with relapsing-remitting multiple sclerosis. *Lancet* 2003; 362: 1184-1191.
19. Creeke PI, Farrell R a. Clinical testing for neutralizing antibodies to interferon-β in multiple sclerosis. *Ther Adv Neurol Disord* 2013; 6: 3-17.
20. Barbosa MDFS, Vielmetter J, Chu S, *et al*. Clinical link between MHC class II haplotype and interferon-beta (IFN-β) immunogenicity. *Clin Immunol* 2006; 118(1): 42-50.
21. Núñez C, Cénit MC, Alvarez-Lafuente R, *et al*. HLA alleles as biomarkers of high-titre neutralising antibodies to interferon-β therapy in multiple sclerosis. *J Med Genet* 2014; 51(6): 395-400.

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Case report

ТЕНКОЦРЕВНА БАКТЕРИСКА ПРОЛИФЕРАЦИЈА КАЈ ПАЦИЕНТ СО СИНДРОМ НА СЛЕПА ВИЈУГА

SMALL INTESTINAL BACTERIAL OVERGROWTH IN PATIENT WITH BLIND-LOOP SYNDROME

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Abstract

Small intestinal bacterial overgrowth (SIBO) is a condition in which the small bowel is colonized by excessive aerobic and anaerobic microbes that are normally present in the colon. A 78-year-old patient with previous Bilroth II resection due to bleeding peptic ulcer was hospitalized because of diarrhea, fatigue, weight lost and bilateral edemas. Laboratory tests showed mild normocytic anemia and hypoalbuminemia. Microbiology stool tests were negative. Upper GI endoscopy showed gastroduodenal anastomosis with two fistulous openings with necrotic surface. The histopathology report from the biopsies taken from the region showed only presence of unspecific inflammation. Initially, the patient was treated with probiotics, nutritive support, fresh frozen plasma, and human albumin but with no remarkable improvement. The treatment with Rifaximin 1200 mg/day led to significant improvement, but because of the anatomic abnormality, the patient was referred to surgery after all. Most of the cases with bacterial overgrowth are successfully treated with antibiotics. However, when the conservative treatment fails and when SIBO is associated with some anatomic abnormality, a surgical treatment may be necessary.

Keywords: small intestinal bacterial overgrowth (SIBO), Bilroth II resection, peptic ulcer, gastrointestinal selective antibiotic

Апстракт

Синдромот на тенкоцревна бактериска пролиферација (СТБП) претставува состојба на колонизација/пролиферација на аеробни и анаеробни бактерии во тенкото црево, кои вообичаено се присутни во дебелото црево. Пациент на 78 годишна возраст со претходна гастрична ресекција по Bilroth II заради крваречки пептичен улкус, беше хоспитализиран поради дијареа, замор, редукција во телесна тежина, билатерални едеми. Лабораториските тестови покажаа присуство на умерена нормоцитна анемија и хипоалбуминемија. Микробиолошките тестови на столицата беа негативни. Горнодигестивната ендоскопија со наод за гастро-дуодено анастомоза со два фистулозни отвори и некротична површина. Хистопатолошкиот наод од биопсиите земени од некротичната површина е во прилог на неспецифично воспаление. Првично пациентот беше третиран со симптоматска терапија (пробиотици, нутритивни суплементи, свежо смрзната плазма, хумани албумини), но без значително пододбрување во клиничката слика. Потоа се отпочна третман со гастроинтестинален селективен антибиотик (Tbl. Rifaximin 1200mg/ден) што доведе до значително подобрување во клиничката слика, но поради анатомската абнормалност како причина за овој синдром, пациентот сепак беше упатен на дигестивен хирург за понатамошен третман. Повеќето случаи на бактериска пролиферација успешно се третираат со антибиотици, меѓутоа кога конзервативниот третман е неуспешен и кога СТБП е асоциран со анатомска абнормалност, хирушкиот третман може да биде неопходен.

Клучни зборови: синдромот на тенкоцревна бактериска пролиферација СТБП, Bilroth II ресекција, пептичен улкус, гастроинтестинален селективен антибиотик

Introduction

Small intestinal bacterial overgrowth (SIBO) is a condition of colonization of the colon flora (aerobes, anaerobes, enterobacteria) in the upper part of the small intestine. SIBO is a result of disruption of the antibacterial mechanisms which are divided into several groups: factors which disrupt the intestinal motility, anatomic causes, gastric hypochlorhydria, metabolic and system diseases [1]. Clinically it is manifested by abdominal pain, diarrhea, weakness, fatigue leading to weight loss,

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malnutrition, nutritive deficiency, malabsorption with appropriate complications following it [1,2,3]. Diagnostic gold standard for this clinical entity is microbial cultivation of jejunal aspirates as an invasive method [2, 3]. The only reliable non-invasive test, nowadays used as much as possible, is the hydrogen and methane exhale test [4,5]. SIBO therapy is individualized and complex, or more accurately it is addressed towards the causes and complications of the disease. It includes treatment of basic causes, nutritive support, and treatment of bacteria overgrowth with cyclic gastrointestinal antibiotics [6-8]. The prognosis usually depends on the cause, i.e. the underlying disease associated with this syndrome.

Case report

A 78-year-old patient was admitted because of loose/ liquid stools, weakness, fatigue, weight loss (about 22 kg for the last 9 months), bilateral edemas. He was examined by a cardiologist, a nephrologist and an infectiologist. Cardiac and nephrotic nature of the edemas were excluded, as well as the infective nature of the diarrhea. A series of examinations were performed, such as upper and lower GI endoscopy, abdominal contrast-enhanced CT scan, but all reports were inconclusive. The patient has arterial hypertension and dilatative cardiomyopathy and was also surgically treated due to bleeding peptic ulcer. He is a former smoker; alcohol consumes occasionally in social quantities; with no food or drug allergies.

From the physical status, the patient was afebrile, conscious, oriented, accessible, skin and visible mucous membranes with pale color, underdeveloped nourishment and muscle build, nonpalpable lymph nodes, and impression of being moderately to severely ill. Blood pressure 100/60 mmHg, pulse 80/min, with no organomegaly but with pitting pretibial bilateral edemas. Laboratory tests with presence of mild normocytic anemia and increased inflammation markers. Degradation products were in normal range as the transaminase activity, cholesterol level and electrolyte and lipid status. The dominating factor was deviation in the protein status manifested with hypoproteinemia (total proteins 47 g/dL) and hypoalbuminemia (albumins 22 g/dL).

Abdominal ultrasound in meanns of meteorismus with no visible changes in the parenchymal organs.

Upper GI endoscopy showed gastroenteric anastomosis with two (fistulous) openings (Bilroth II resection) with necrotic surface in the region. The histopathology analysis of the biopsies taken from the openings, as well as from the distal part of the duodenum, showed a normal mucosa. The histopathology report from the gastroduodenal anastomosis showed an unspecific inflammation of the gastric mucosa. In order to confirm the surgical procedure and according to the endoscopy report for presence of fistulous openings, an indication for duodenoscopy under X-rays was established.

The duodenoscopy confirmed the presence of gastroduodenal anastomosis with two bowel loops, where the distal (efferent) one was clearly identified to have normal mucosa. The proximal (afferent) loop was with substenotic lumen due to ulcerative lesions (Figure 1 and 2). Contrast was applied in both loops but no fistulous opening was identified, with a clear finding for Bilroth II resection and gastro-entero-anastomosis.

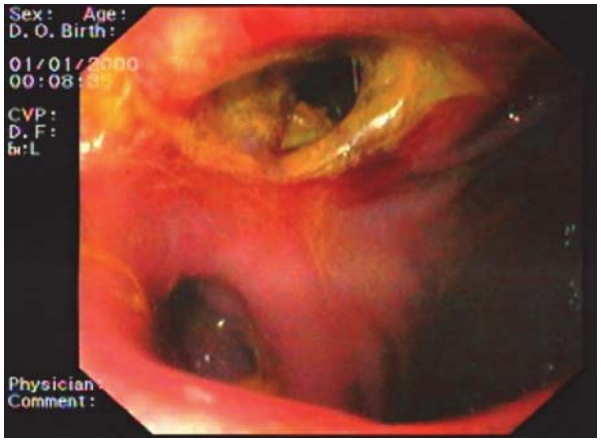

Fig. 1. Duodenoscopy-gastroduodenal anastomosis with two bowel loops, where the distal one was clearly identified and with normal mucosa. The proximal loop was with substenotic lumen due to present ulcerative lesions

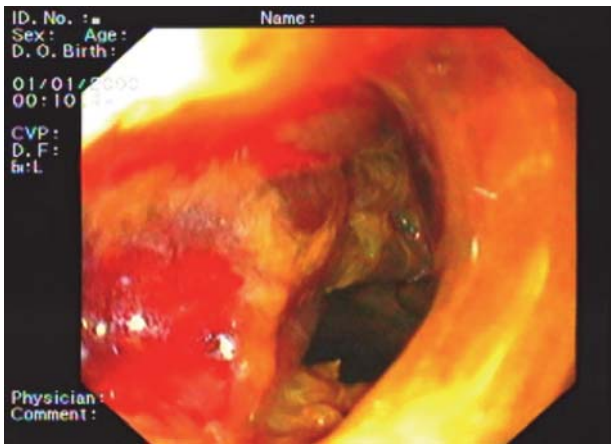

Fig. 2. Proximal loop-substenotic lumen due to present ulcerative lesions with necrotic cell surface. From this region the biopsies were taken

During the hospitalization, the patient was treated with probiotics, nutritive support, fresh frozen plasma and human albumin, but with no significant improvement, i.e. without corresponding stool normalization or withdrawal of the edemas. After the examinations, decision was made to start a therapy with antibiotic (Rifaximin 1200 mg/day), which led to notable clinical improvement manifested with edema withdrawal as well as normalization and consistency of the stool.

Discussion

The patient was presented with a condition after Billroth II resection of the stomach due to ulcerative lesion, with a laboratory report for hypoproteinemia (hypoalbuminemia and hypoglobulinemia), manifested with bilateral edemas and diarrhea. A series of examinations were performed, such as several gastroscopies, colonoscopy with biopsies, CT of the abdomen and MR enterography, all of which were normal. An infective cause for the chronic diarrhea syndrome was excluded, as were the renal and cardiological etiology of the pretibial edemas. The ultrasound examination of the abdomen was inconclusive. Initially, an upper GI endoscopy was performed with an idea for a celiac disease biopsies. But on gastroscopy, a gastroduodenal anastomosis was confirmed. Necrosis was seen in this region and biopsies were taken for histopathology analysis. Biopsies were also taken from the mucosa of the distal (efferent) loop. The histopathology report was normal. The proximal (afferent) loop necrosis was definitely not peptic, as it was noticed in the previous gastroscopies and the patient was also on proton pump inhibitor therapy for a long period. The histopathology report from the small intestinal biopsies showed only presence of unspecific inflammation. Duodenoscopy under X-ray control was obtained, with application of contrast through the substenotic lumen of the proximal loop which was covered with ulcerations (necrosis). This confirmed that the contrast can not run spontaneously through the substenosis. Bearing in mind the general condition and the reports, it was more than obvious that this is a case of blind loop syndrome as a cause for bacterial overgrowth and in this particular case was the cause for maldigestion syndrome with all present forms of nutritive deficiency, which at the same time explained the unspecific inflammation, both at the level of the small intestine and at the level of the colon [10]. In this sense the patient, among the rest was also treated with non-absorbable antibiotic (Rifaximin 1200 mg/day) in a course of 10 days, that led to significant improvement with reduction of the number of stools (from 13 to 3 per day), symptom withdrawal (i.e. edema), and improvement of the laboratory parameters with tendency towards normalization [9]. Nevertheless, in cases with anatomical (postoperative) cause this is not a permanent solution. Accordingly, the patient was additionally sent to an abdominal surgeon for a surgical procedure [11].

Conclusion

This is a case of a bacterial proliferation syndrome with an anatomic etiology, i.e. a condition after Billroth II antrectomy and resulting blind loop formation that leads to stasis of the bowel fluid flow. This is a suitable ground for bacterial overgrowth (so-called blind loop syndrome). Histopathologically it is characterized by the presence of unspecific inflammatory infiltrate. Clinical finding includes diarrhea, weight loss, protein malabsorption, hypoproteinemia i.e. hypoalbuminemia with resulting edema syndrome (presence of pretibial edemas of the lower limbs) and megaloblastic anemia due to iron and vitamin B12 malabsorption. The patient was correspondingly treated according to the symptoms. Gastrointestinal selective antibiotic was also introduced at a corresponding dosage (Rifaximine 1200 mg/24h), with a notable improvement. Surgery as a definite choice of treatment of the anatomic etiology was indicated.

Conflict of interest statement. None declared.

References

1. Vanderhoof JA, Young RJ. Etiology and pathogenesis of bacterial overgrowth. Clinical manifestations and diagnosis of bacterial overgrowth. Treatment of bacterial overgrowth. UpToDate online, vol 18.1; *Wellesley* 2010. Available from: http://www.uptodate.com.
2. Khoshini R, Dai SC, Lezcano S, Pimentel M. A systematic review of diagnostic tests for small intestinal bacterial overgrowth. *Dig Dis Sci* 2008; 53: 1443-1454. [PubMed]
3. Gasbarrini A, Lauritano EC, Gabrielli M, *et al*. Small intestinal bacterial overgrowth: diagnosis and treatment. *Dig Dis* 2007; 25: 237-240. [PubMed]
4. Lupascu A, Gabrielli M, Lauritano EC, *et al*. Hydrogen glucose breath test to detect small intestinal bacterial overgrowth: a prevalence case-control study in irritable bowel syndrome. *Aliment Pharmacol Ther* 2005; 22: 1157-1160. [PubMed]
5. Kerlin P, Wong L. Breath hydrogen testing in bacterial overgrowth of the small intestine. *Gastroenterology* 1988; 95: 982-988. [PubMed]
6. Siebecker A. Small intestine bacterial overgrowth: dietary treatments. http://www.siboinfo.com/diet.html.
7. Di Stefano M, Malservisi S, Veneto G, *et al*. Rifaximin versus chlortetracycline in the short-term treatment of small intestinal bacterial overgrowth. *Aliment Pharmacol Ther* 2000; 14: 551-556. [PubMed]
8. Di Stefano M, Miceli E, Missanelli A, *et al*. Absorbable vs. non-absorbable antibiotics in the treatment of small intestine bacterial overgrowth in patients with blind-loop syndrome. *Aliment Pharmacol Ther*. 2005; 21: 985-992. [PubMed]
9. Pimentel M, Park S, Mirocha J, *et al*. The effect of a nonabsorbed oral antibiotic (rifaximin) on the symptoms of the irritable bowel syndrome: a randomized trial. *Ann Intern Med* 2006; 145: 557-563.
10. Machado JD, Campos CS, Lopes Dah Silva C, *et al*. Intestinal bacterial overgrowth after Roux-en-Y gastric bypass. *Obes Surg* 2008; 18: 139-143. [PubMed]
11. Lakhani SV, Shah HN, Alexander K, *et al*. Small intestinal bacterial overgrowth and thiamine deficiency after Roux-en-Y gastric bypass surgery in obese patients. *Nutr Res* 2008; 28: 293-298. [PubMed]

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Case report

LIPOSUCTION OF A GIANT LIPOMA

ЛИПОСУКЦИЈА НА ГИГАНТСКИ ЛИПОМ

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Abstract

Lipomas are the most frequent benign tumors originating from fatty tissue. They are usually subcutaneously located disfiguring body contour that results in cosmetic discomfort. Therefore, main indication for their removal is of cosmetic reason. Historically, open surgical ablation is a mainstay of their treatment. Recently, new treatment modalities have been employed in order to achieve scarless removal and decreased postoperative morbidity. Of all, liposuction has gained popularity with an increasing rate of utilization due to its simple application, safety, high patient compliance, but mostly because of small incision/scar and eventual total removal. Subcutaneous lipomas are the most common indication for non-cosmetic application of liposuction.
In this report, we present a case of a giant subcutaneous corporallipomathat was successfully removed with liposuction. Preoperative investigations, operative techniques as well as intraoperative findings are shown. There has been no lipomarecurrence in the follow-up periodof 40+ months meaning that a total tumor removal has been achieved. Appropriatepreoperative diagnostic and evaluation are essential when selecting lipomas that can be treated in this way.

Keywords: lipoma, liposuction, lipectomy

Апстракт

Липомите се најчестите бенигни тумори со потекло од масното ткиво. Обично се поткожно лоцирани и ја менуваат телесната контура што води до козметски дискомфорт. Тоа претставува и најчестата индикација за нивно отстранување. Историски, отворената хируршка аблација е метода на избор за нивен третман. Сепак, за да се отстрани липомот без или со мали лузни, а воедно да се намали постоперативниот морбидитет, во поново време се воведоа нови техники за лекување. Од сите тие, заради лесната апликација, високата комплијанса, безбедноста како и радикалноста со употреба на мала лузна, липосукцијата како метода има постојан пораст на употреба и популарна. Поткожните липоми се најчестата индикација за не-козметска апликација на липосукцијата.
Во трудот презентираме случај на гигантски поткожен липомна телото кој што успешно е отстранет со липосукција. Прикажани се предоперативните испитувања, оперативната техника и интраоперативниот тек. Постоперативно, во период на следење од повеќе од 40 месеци, нема присуство на рецидив и постигната е радикалност. Соодветната предоперативна дијагностика и евалуација е круциелна при селекција на липомите кои може да се третираат на овој начин.

Клучни зборови: липом, липосукција, липектомија

Introduction

Lipomas are the most frequent benign tumors in humans, arising from adipose tissue. The incidence is 1-2.1/ 1000 [1].They are usually subdermally located, mainly on the trunk and extremities, and can be easily palpated and inspected. Typical appearanceis soft, clearly localized and mobile solitary lesions in patients in their 40-ties and 50-ties. They grow slowly and progressively without pain. However, lipomas can develop at any age, at any region, at different organs and can have deeper and atypical localizations. They can vary in dimensions; if larger than 10cm in diameter they are referred as giant lipomas [1,2].
Histologically, there are many forms of lipomas according to WHO classification of soft tissue tumors [3]. Out of all, conventional (simple, common) lipoma is by far the most frequent, and when clinicians discuss about lipoma, they generally refer to the conventional type [4]. It is composed ofmature fat cells-adipocytes without atypia, butmorphologicallydifferent from normal fat cells. The cells are arranged in lobules divided with fibro-vascular septa or trabecules. Envelopingfine capsule around the tumor is usually present.
History and physical examination are usually sufficient for establishing the diagnosis. Furthermore, basic

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investigations are consisted of ultrasound imaging with linear probe and fine needle aspiration biopsy as to conclude the diagnosis [5,6]. In doubtful cases, additional imaging can be used, as atypical lipoma and low grade liposarcoma can present clinically quite similar to lipoma [7]. Although rare, lipoma can undergo malignant transformation.In these cases, MRI is a diagnostic tool of choice.

Main indications for operation include cosmetic discomfort, body disfigurement, pain, increased growth or cancer phobia [8-10]. Open surgical extirpation is a method of choicefor treatment, but it bares the risk of larger incisions, longer postoperative morbidity and eventual postoperative complications [1,2,4]. Seeking less scarring and less postoperative morbidity,new modalities have been employed lately. Liposuction, as one of them, raised popularity since its introduction in mid-1970s as a minimal invasive approach with high patients' compliance and high satisfaction rate, being a safe and effective method as well. In literature, there are many reports for liposuction-assisted lipectomyin cases of small (<4cm), [10] medium (4-10cm) [9,11-13] and giant lipomas [14-16]. The main concern is about the probable higher recurrence rate noted in some studies [14], but not in other [11-13].

This paper presents a case of successful removal of conventional lipoma with liposuction and postoperative follow up of 40+ months.

Case report

We present a case of 60-year-old female patient, with a corporal conventional lipoma treated with liposuction. Fifteen years ago, the patient noticed a small subcutaneous lump on the left lumbo-dorsalregion that continued to persist and grew painlessly over the years. Beside body disfiguration, no other symptoms were present. Tumor growth accelerated recently which urged the patient to ask for treatment. Impairment with garments and esthetic mutilation was obvious. Clinically examined, tumor was mobile, subcutaneous, circumscripted, soft and painless, 20x14cm gross (Figure 1). Ultrasound imaging with linear probe showed well- encapsulated soft tissue tumor suprafascially located. Fine needle biopsy result wasI^{st} classification group. These data were in agreement with the clinical evaluation, assuming benign tumor of lipomatous origin. Usual blood count was done preoperatively. Single shot wide spectrum antibiotic was given intravenously 60 minutes before operation that was planned in local anesthesia with mild sedation.

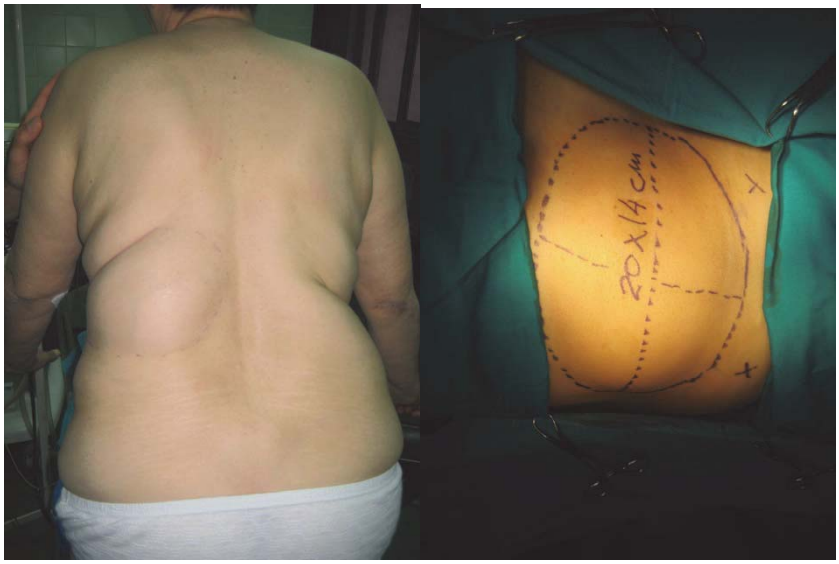

Fig.1. Preoperative photography and markings

Stub incision (0.5cm, with blade No. 11) was planned few centimeters from the tumor margin. After application of local anesthesia (2ml 1% lidocaine, 0.01% adrenaline), incision was made and infiltrating cannula was introduced in the tumorous tissue. Tumescent infiltrating technique was used: about 200 ml of Klein's solution (0.1% lidocaine and 1:1 million adrenalin in 1000 ml 0.9% saline solution) was infiltrated under pressure in the lipoma with a blunt 3mm cannula. End-stage infiltration wasskin anemization and orange peel aspect. Liposuction followed after a waiting period of 20-25 minutes. It was conducted using a 30cm long, blunt Mercedes ǿ3mm cannula (Byron®) and negative pressure manually made with 60 cc syringe that fittedthe cannula.Manually made syringe vacuum wassufficient for liposuctionof thelipoma (Figure 2). Liposuction was finished when smoothness of the overlying skin was reached and/or bloody aspirate predominated. Aspirate was filtrated using gauze and hard partwas sent asa pathohistologicalsample (Figure 3). Finally, incision was closed with one resorptivesubcutaneous suture and compressive dressing was applied. The patient was

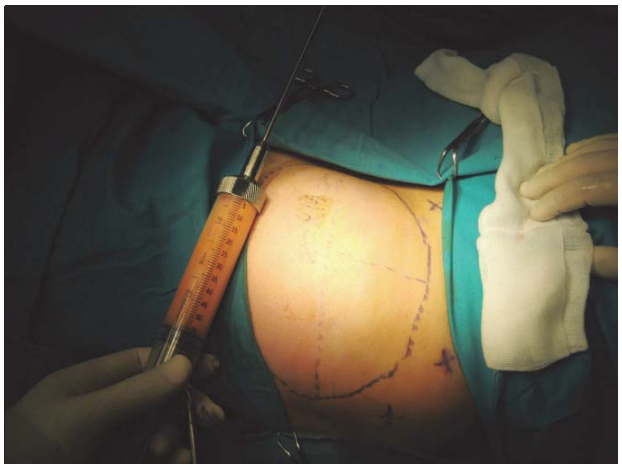
Fig. 2. Liposuction with syringe made vacuum

discharged from hospital the same day. Check-up followed in 3 days. Wearing compressive garment for 3 weeks was advised.

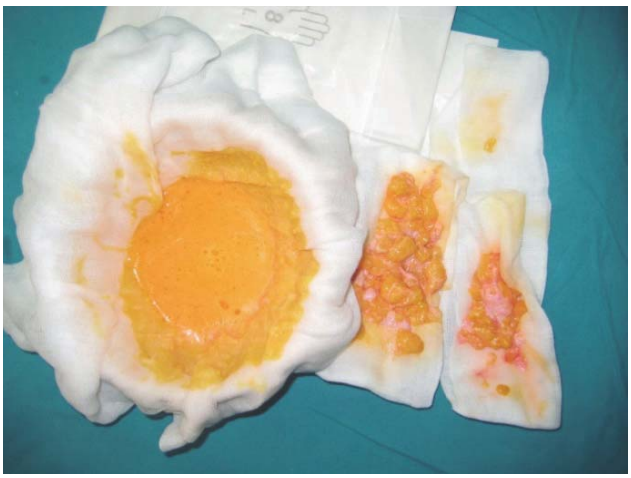
Fig. 3. Filtrated aspirate for PH sampling

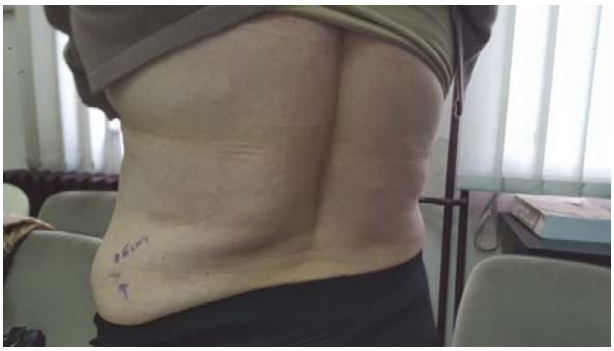
Fig. 4. One year post-op result

On the check-up, bruising and swelling were obvious, but they resolved in 2 weeks. The presenting mild pain at the beginning diminished very fast as well. Lipoma was removed totally. Pathohistological result was lipomatous benign lesion-lipoma. After one year, great results can be seen (Figure 4): no signs of recurrence, good skin alignment and small incisional scar of 0.6 cm lengththat faded over the period. The same findings were noted on the 40th month of the follow-up period. The patient was very satisfied with the outcome.

Discussion

In order to treat lipoma with liposuction, accurate diagnosis prior to surgery is mandatory. Liposuction of misdiagnosed liposarcoma is a very unpleasant scenario that can lead to medico-legal consequences.Having in mind the typical clinical presentation, diagnosing lipoma is usually not difficult [4]. However,the risk of development of sarcoma is greater whenthe tumor is fast growing, painful and larger than 10 cm and/or has atypical or deeper location [17]. In these cases, imaging techniques such as ultrasonography, CT and MRI as well as fine needle/core biopsygive us useful additional data [5-8]. Magnetic resonance is highly sensitive in detection of well-differentiated liposarcomas and highly specificin diagnosis of common lipomas [7]. Furthermore, fine needle aspiration biopsy is a method of high sensitivityindifferentiationof benign and malignant soft tissue tumors [6]. Moreover, the lipoaspirate from liposuctedlipoma should be sent for pathologic examination. Cell integrity is not damagedin the aspirate and accurate pathology sampling can be done accordingly [18]. Hereby, utilizing these diagnostic tools, misdiagnosis ofliposarcomais almost impossible. Diagnostic pathway of our patient comprised anamnesis, clinical examination, ultrasonography, fine needle biopsy and microscopic examinations of the lipoaspirate.
Rubenstein *et al.,* were first that treatedlipoma with liposuctionin 1985 [19]. Nowadays, lipoma is the most frequent indication of non-cosmetic liposuction [20]. Among others, advantages are smaller scar, less pain, good cost-effectiveness, quicker operation, better final aesthetic result, low complication rate [20]. Smaller lipomas in areas where scar is to be avoided also can be removed [10]. As reported, difficulties in removing giant lipomas with liposuction alone are possible [14]. In our case, we did not encounter any technical problem; the cannula we used was long enough to reach tumor margins. Difficulties might arise when a shorter cannula is applied and here, an extra counter incision is a reasonable solution.
Self-resolving early sequels as bruising, ecchymosis, edema, contour dimpling and light pain are concomitant to liposuction. Infection is a rare scenario. Liposuction is a safe procedure when adhering to guidelines [21]. On the other side, open surgery bears the risk of hematomas, infections, seroma formation, dehiscence, larger scars, and indentations. Questionable ability in achieving radical removal and probable higher recurrence rate has been proposed as a main drawback for liposuction. Rubisteinreported difficulties when removing the fibrous capsule with liposuction [19]. Raemdonck *et al*., reporteda higher recurrence risk in giant lipomas treated with liposuction compared to ablative surgery

[14]. Other case reports of giant lipomas showed no recurrences [15,16]. Recent larger studies have reported the same result [10-13]. Al-basty proposed capsule extraction with forceps in order to prevent recurrences and succeeded in not having recurrences in a long-term follow-up [12]. Additional studies showed also excellent results using the proposed technique [11,13]. It seems that extracting hard residual tissue leads to radical removal of the lipomatous tumor. In our case, it was possible to destroy the capsule mechanically and then liposucted. This is a case of non-fibrotic lipoma. Otherwise, using forceps is a reasonable solution.
The risk of recurrence in classical lipectomy is about 2% and the number of reported liposuctedlipomas in the literature is not sufficient to estimate that risk of 2% or higher [13]. Until conduction of larger comparative studies, statements concerning a higher recurrence risk in liposuction are based on small series or are observational.

Conclusion

Liposuction is a relatively novel method for lipoma treatment with excellent results that are cosmetically superior to open surgery. It is indicated in suprafascial/ subcutaneous, lipomatous masses, uni- or multilateral with a size larger than 5 cm. Priorto liposuction of any lipoma, preoperative diagnosis is obligatory. Lipoaspirated tissue can be accurately assessed as a pathology sample.The main drawback for its larger use among surgeons is higher recurrences risk that is not well-scientifically concluded. Therefore, larger randomized prospective studies are needed.

Conflict of interest statement. None declared.

References

1. Hodl S. Regionale und spezielle Erkrankungen des Fettgewebes. In: Braun-Falco O, Plewig G, Wolff HH, Burgdorf WHC, Landthaler M. Dermatologie und Venerologie. 5. Auflage. *SpringerVerlag Heidelberg* 2005; 1021-1023.
2. Shiffman AM. Lipomatous Disorders: Classification and Review of the Literature. *J CosmSurg Med* 2005; 5(3): 26-36.
3. Fletcher CDM, Unni KK, Mertens F. Pathology and Genetics of Tumours of Soft Tissue and Bone. *World Health Organization Classification of Tumours* 2002.
4. Bancroft LW, Kransdorf MJ, Peterson JJ, *et al*. Benign fatty tumors: classification, clinical course, imaging appearance, and treatment. *SkeletalRadiol* 2006; 35: 719-733.
5. Rahmani G, McCarthy P, Bergin D. The diagnostic accuracy of ultrasonography for soft tisselipomas: a systematic review. *ActaRadiologica Open* 2017; 6(6): 1-6.
6. Iyer VK. Cytology of soft tissue tumors: Benign soft tissue tumors including reactive, nonneoplastic lesions. *J Cytol* 2008; 25: 81-86.
7. Gaskin CM, Helms CA. Lipomas, Lipoma Variants, and Well-Differentiated Liposarcomas (Atypical Lipomas): Results of MRI Evaluations of 126 Consecutive Fatty Masses. *AJR* 2004; 182: 733-739.
8. Dalal KM, Antonescu CR, Singer S. Diagnosis and management of lipomatous tumors. *J SurgOncol* 2008; 97: 298-313.
9. Pinski KS, Roenigk HH Jr. Liposuction of lipomas. *DermatolClin* 1990; 8(3): 483-492.
10. Wilhelmi BJ, Blackwell SJ, Mancoll JS, Phillips LG. Another indication for liposuction: small facial lipomas. *Plast Reconstr Surg* 1999; 103(7): 1864-1867.
11. Choi CW, Kim BJ, Moon SE, *et al*. Treatment of lipomas assisted with tumescent liposuction. *JEADV* 2007; 21: 243-246.
12. Al-basti HA, El-Khatib HA. The use of suction-assisted surgical extraction of moderate and large lipomas: long-term follow-up. 2002; 26(2): 114-117.
13. Copeland-Halperin LR, Pimpinella V, Copeland M. Combined liposuction and excision of lipomas:long-term evaluation of a large sample of patients. *PlastSurgInt* 2015; Available athttp://dx.doi.org/10.1155/2015/625396 Accessed 12.12.2018
14. Raemdonck D, De Mey A, Goldsehmidt D. The treatmentof giant lipomas. *Acta Chir Belg* 1992; 92: 213-217.
15. Nichter LS, Gupta BR. Liposuction of giant lipoma. *Ann PlastSurg* 1990; 24: 362.
16. Medina CR, Schneider S, Mitra BS, *et al*. Giant submental lipoma: Case report and review ofthe literature. *Can J Plast Surg* 2007; 15(4): 219-222.
17. Johnson CJD, Pynsent PB, Grimer RJ. Clinical features of soft tissue sarcomas. *Annals of the Royal College of Surgeons of England* 2001; 83(3): 203-205.
18. Shiffman MA, Mirrafati S. Fat transfer techniques: the effect of harvest and transfermethods on adipocyte viability and review of the literature. *Dermatol Surg* 2001; 27(9): 819-826.
19. Rubenstein R, Roenigk H, Garden JM, *et al*. Liposuction for lipomas. *J DermatolSurgOncol* 1985; 11(11): 1070-1074.
20. Coleman WP 3rd. Noncosmetic applications of liposuction. *J Dermatol Surg Oncol* 1998; 14(10): 1085-1090.
21. Housman TS, Lawrence N, Mellen BG, *et al***.** The safety ofliposuction: results of a national survey. *Dermatol Surg* 2002; 28: 971-978.

УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

"Македонски медицински преглед" (ММП) е стручно списание на Македонското лекарско друштво, првенствено наменето на лекарите од општа практика, специјалистите од одделните медицински дисциплини и истражувачите во областа на базичните медицински и други сродни науки.

Списанието ги има следниве рубрики и категории на трудови:

1. **Изворни трудови**
2. **Соопштувања за клинички и лабораториски искуства**
3. **Прикази на случаи**
4. **Од практика за практика**
5. **Едукативни статии**
6. **Вариае (**писма од редакцијата, општествена хроника**,** прикази на книги, извештаи од конгреси, симпозиуми и други стручни собири, рубриката ,,Во сеќавање,, и др).

Изворните трудови имаат белези на научни трудови, додека трудовите категоризирани во рубриките 2-5 имаат белези на стручни трудови.
Во ММП се објавуваат трудови на членовите на МЛД или на членови на други стручни здруженија. Авторите се одговорни за почитувањето на етичките начела при медицинските истражувања, а изнесените ставови, изведени од анализата на сопствените резултати, не се нужно и ставови на Редакцијата на ММП.
Редакцијата ги испраќа ракописите на стручна рецензија; рецензентот (ите) и Редакцијата ја определуваат дефинитивната категоризација на ракописот кој е прифатен за печатење. Редакцијата го задржува правото ракописите да ги печати според рецензираниот приоритет.
Упатството за соработниците на ММП е во согласност со Ванкуверските правила за изедначени барања за ракописите кои се праќаат до биомедицинските списанија.

1. ТЕКСТ НА РАКОПИСОТ

Сите ракописи се испраќаат во електронска форма на електронската адреса (е-маил) на МЛД-ММП, со двоен проред и најмногу 28 редови на страница. Трудот се поднесува на англиски јазик латиничен фонт Times New Roman големина 12 и апстракт на македонски јазик. Лево, горе и долу треба да се остави слободна маргина од најмалку 3 см, а десно од 2,5 см.. Редниот број на страниците се пишува во десниот горен агол.
Ракописот на трудот треба да е придружен со писмо на првиот автор, со изјава дека истиот текст не е веќе објавен или поднесен/прифатен за печатење во друго списание или стручна публикација и со потврда дека ракописот е прегледан и одобрен од сите коавтори, односно со придружна декларација за евентуален конфликт на интереси со некој од авторите.

Насловната страна треба да има: наслов на македонски и англиски, имиња и презимиња на авторите, како и институциите на кои им припаѓаат, имињата на авторите и насловот на установата се поврзуваат со арапски бројки; автор за кореспондеција со сите детали (тел. е-маил); категорија на трудот; краток наслов (до 65 карактери заедно со празниот простор); како и информација за придонесот за трудот на секој коавтор (идеја, дизајн, собирање на податоци, статистистичка обработка, пишување на трудот).

Насловот треба концизно да ја изрази содржината на трудот. Се препорачува да се избегнува употреба на кратенки во насловот.

Изворните трудови и **соопштувањата** го имаат следниов формален редослед: насловна страна, извадок на македонски јазик (вовед, методи, резултати, заклучок) со клучни зборови, извадок на македонски јазик со клучни зборови, вовед, материјал и методи, резултати, дискусија и

заклучоци, литература и прилози (табели, графици и слики) и легенди за прилозите во еден фајл.

Приказите на случаи треба да содржат вовед, детален приказ на случајот, дискусија со заклучок и литература со прилози.

Извадокот на македонски јазик треба да содржи најмногу 250 зборови и да биде структуриран со сите битни чинители изнесени во трудот: вовед со целта на трудот, методот, резултати (со нумерички податоци) и заклучоци. Заедно со извадокот, треба да се достават и до 5 клучни, индексни зборови.

Извадокот на англиски јазик мора да е со содржина идентична со содржината на извадокот на македонски јазик. Клучните зборови треба да се во согласност со MeSH (Medical Sibject Headings) листата на Index Medicus.

Воведот треба да претставува краток и јасен приказ на испитуваниот проблем и целите на истражувањето, со наведување на етичкиот комитет односно институцијата која го одобрила испитувањето (клиничка студија која се работи според принципите на Хелсиншката декларација за пациентите и нивните права).

Методите треба да бидат точно назначени, за да се овозможи повторување на прикажаното истражување. Особено е важно да се прецизираат критериумите за селекција на опсервираните случаи, воведените модификации на веќе познатите методи, како и идентификација на употребените лекови според генеричното име, дозите и начинот на администрација.

Резултатите треба да се прикажат јасно, по логичен редослед. Резултатите се изнесуваат во стандардните СИ единици. Во текстот треба да се назначи оптималното место каде ќе се вметнат табелите и илустрациите, за да се избегне непотребното повторување на изнесените податоци. Значајноста на резултатите треба да се обработи статистички, со детален опис на употребените статистички методи на крајот на делот *методи*.

Дискусијата треба да ги истакне импликациите од добиените резултати, споредени со постојните сознанија за испитуваниот проблем.

Заклучоците треба да не бидат подолги од 150 зборови.

2. ПРИЛОЗИ

Како прилог-документација на трудовите предложени за печатење, може да се достават до 5 прилога (табели, фигури,/слики - илустрации).

Табелите се доставуваат на крајот на трудот во истиот фајл. Секоја табела треба да има свој наслов и реден број кој ја поврзува со текстот. Хоризонтални и вертикални линии на табелата не се дозволени; ознаките на колоните во табелата се пишуваат скратено или со симбол, а нивното објаснување се пишува на дното на табелата, во вид на легенда.

Илустрациите се доставуваат со реден број како слика во црно-бела техника, а секоја слика треба да е придружена со легенда (опис).

Микрофотографиите може да содржат посебни ознаки во вид на стрелки или симболи. Покрај описот на сликата, мора да се наведе и зголемувањето и видот на боењето на препаратот (ако тоа веќе не е направено во секцијата *материјал и методи*).

Сите ознаки на фотографиите мора да бидат доволно големи, за да може јасно да се распознаат и по смалувањето во печатницата, при нивното вклучување во печатената страница на списанието.

3. ЛИТЕРАТУРА

Цитираната литература се пишува на крајот на трудот по заклучоците, со редни броеви според редоследот на појавувањето на цитатот на текстот на трудот ставени во средни загради и без простор меѓу нив (ако се последователни треба да се поврзани со цртичка, на пр. [3-6]).
Литературата се цитира на следниов начин (кратенките за насловите на списанијата треба да се според листата прифатени vo Index Medicus):

а) ***статија во списание*** (се наведуваат сите автори, ако ги има до 4 или помалку; ако ги има повеќе од 4 се наведуваат првите 3 автори и се додава: *и сор*.) Neglia JP Meadows AT, Robison LL *et al*. Second neoplasms after acute lymphoblastic leukemia in childhood. N Engl J Med 1991; 325:1330-6.

б) заеднички автор

GIVIO (Interdisciplinary group for cancer care evaluation). Reducing diagnostic delay in breast cancer. Possible therapeutic implications. *Cancer* 1986; 58: 1756-61.

в) ***без автор*** **-** анонимно. Breast screening: new evidence. (*Editoriall Lancet* 1984; i :1217-8).

г) ***поглавје во книга или монографија***

Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. Vo: Sodeman WA Jr, Sodeman WA, Ed. Pathogenic physiology: mechanisms of disease. Philadelphia; W B Saunders, 1974: 457-72.

Првите отпечатоци на трудовите им се праќаат на авторите за корекција: авторите се должни коригираниот отпечаток да и го вратат на Редакцијата на ММП во рок од 2 дена.

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Известување за членовите на МЛД

Сите што сакаат и натаму да го добиваат списанието треба да ја имаат уплатено членаринатa за 2019 година во висина од 600 денари и за тоа да ја информираат стручната служба на Македонско лекарско друштво, писмено или преку телефон.

Детални информации можете да добиете на телефонот на Друштвото 02 3 162 557.

Известување за рецензентите за ММП

Во склад со правилникот на УКИМ рецензентите што навремено и одговорно ќе ја одработат рецензијата ќе добијат 0.4 бода кои се собираат за унапредување во академските звања. Бодовите можат да се добијат и ретроградно преку побарување во МЛД - 3162 557.