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СПИСАНИЕ ЗА МЕДИЦИНА, ВЕТЕРИНА И ФАРМАЦИЈА

REVUE MÉDICALE MACÉDONIENNE

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Неговиот труд и стручност,
ММП го стави во рангот меѓу релевантните стручни списанија

НН: ТРАНСФЕРЗАЛА НА НЕПОВТОРЛИВИТЕ

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Проф. д-р Костандина Корнети - Пекевска

Превземено од Вох Медицин Скопје, мај 2010

Проф. д-р Ѓорѓи Зографски, патолог, хистопатолог и цитодијагностичар, го достигна за секого посакуваното врвно ниво, што за нашата средина значи врвен авторитет и вонсериски професионалец. Беше одличен во школувањето, сериозен, темелен и безграничен во надградувањето.

Рационален до крајност, инвентивен во ограничените просторни и кадровски услови. Покрај максималната ангажираност со пункционалните биопсии од пациенти, во клеточно-анализирачките системски студии, во прогностичките анализи добиени со споредбените сознанија од патохистолошките извештаи со клиничка прогноза, учествуваше и во презентација и публицирање на кумулираните извештаи за дејноста на Лабораторијата за ХКЦ, со сопствена електронска база на податоци, поставена и втемелена од 1997 година. Побудуваше нескриен интерес и за онаа, човечката страна на докторот, која ја поседуваше на особен начин и за што често зборуваа неговите пациенти, колеги и студенти. Уште еден од оние што се посветени и работохолично оддадени на професионалните стручни, научни и наставни преокупации и активности.

Во факултетската средина беше најчест корисник на Централната факултетска библиотека. Се информираше за најновите достигнувања од неговата област и 10 години го раководеше Библиотечниот одбор, со високи стандарди, познавања и компетенции за унапредување на библиотечно-информатичкиот сервис на факултетот, со посебен сенс за позитивна атмосфера и стимулирање на активностите. Го одработуваше третиот мандат на главен и одговорен уредник на "Македонски медицински преглед".

Имаа привилегија колегите од онколошката ЛЦПХ и од Катедрата по патологија да ја чувствуваат неговата харизма, дух, интелект, ерудиција и култивираност во заедничката работа, а го прифаќаа и го поддржуваа секој негов аргументиран и образложен предлог. Уживаше безрезервен авторитет меѓу непосредните соработници и колеги. А делуваше тивко и ненаметливо. За сите нас тој беше големина во сенка. Не прифаќаше, не се нудеше ниту се наметнуваше за функции и позиции што ги заслужуваше. Беше почесен претседател и член на републички комисии и тела, како и во управниот одбор на престижната меѓународна хуманитарна организација Сју Рајдер од формирањето.

Проф. Зографски, пред сè, беше семеен човек посветен на семејното огниште. Грижливо ја штитеше и со благороден израз на лицето уживаше во зачуваната приватност на семејната хармонија.

Со него беше престижно да се води дијалог. Претставуваше не воин, туку витез на медицинскиот академски трудбенички ред.

Така, проф. д-р Ѓорѓи Зографски, нашиот омилен Ѓоре, се впиша во трансферзалата на неповторливите.

Костандина Корнети - Пекевска
Превземено од Вох Медици
Скопје, мај 2010

ОБРАЌАЊЕ НА РЕДАКЦИСКИОТ ОДБОР ПО ПОВОД 70 ГОДИНИ МАКЕДОНСКИ МЕДИЦИНСКИ ПРЕГЛЕД

Почитувани колешки и колеги, соработници на Македонскиот медицински преглед,

Го дочекавме големиот јубилеј-70 години постоење на вашето и наше медициинско списание, негувач на пишаниот медицински збор, сведок на бројни напори на нашите лекари своите секојдневни резултати преточени во бројни практични и научни осознавања да стигнат до медицинската јавност во Македонија и надвор од неа.

Зад нас е долга историја и многу спомени, трудови за паметење, автори кои своите професионални животи ги преточуваа во пораките преку ова списание. И навистина секој поглед наназад низ долгите години сведочи за многу високи дострели на плејадата трудови и автори кои придонесоа и ја насликаа историјата на Македонскиот медицински преглед. Сето тоа е причина да бидеме горди на сето она што произлезе од ова списанието, неговите автори, уредници и соработници. Долга и горда историја.

Како и целиот живот во оваа средина во изминатите 70 години, така и медицината и овој горд славеник, минаа низ разни премрежија, тешкотии, непознаници. Имаше бројни светли моменти, но и потешкотии, падови. Разни проблеми кои се закануваа со негово гаснење, со пад на квалитетот и неговото значење. Благодарение на бројни ентузијасти, Македонскиот медицински преглед одолеа на сите проблеми и до ден денес го има клучното значење во публикувањето на македонската медицинска наука, мисла и пишан збор.

Со почит кон историјата и кон сите што го создадоа и негуваа оваа стручно списание, мораме да се вратиме во сегашниот момент и да се проектираме кон идните периоди.

Успеавме да го вратиме ритамот на редовно излегување на ММП и така дојдовме да излезе овој јубилеен број и да го држиме в рака. Се надеваме дека квалитетот на објавените трудови се враќа на повисоко ниво што е исклучително важно на современото небо на стручни и научни списанија насекаде околу нас. Во завршна фаза е подготовката на веб изданието со што ќе се олесни достапноста на оваа периодична публикација. Крајна цел е добивањето на импакт факторот, со што самото стручно списание, а и трудовите објавени во него ќе добијат на значење и вредност. Сакаме да се стимулираат сите позитивни вредности на нашите лекари и преку ММП да ги споделат своите успеси и сознанија. Исто така, е потребно да се даде поголемо меѓународно значење на оваа списание со вклучување наши колеги од странство во уредувачките тела и во неговите содржини. Ова почнува да се остварува и тоа не само со еминентни колеги од регионот, туку и од целиот свет.

Затоа, почитувани колешки и колеги, драги пријатели, да застанеме сите заедно зад оваа стручна публикација, да бидеме негови соработници и почитувачи, да ги споделиме со него и преку него нашите успеси, нашите резултати, нашите вредности и тајни... А тој ќе не награди со тоа што сето тоа ќе го прошири насекаде до каде што допира и се шири. ММП заслужува исто толку колку што и тој ни е потребен на сите нас.

Ве повикуваме да се гордееме и да се сеќаваме на неговите 70 години. Ве повикуваме да направиме нашите поколенија да ги прославуваат следните јубилеи и со гордост да се сеќаваат и да ги паметат научните дострели на македонската медицинска наука и практика која ја проследиле низ страниците на Македонскиот медицински преглед.

Нека ни е честит јубилејниот број и уште мнооооогу, мнооооогу броеви и јубилеи.

Претседател на Македонско лекарско друштво
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ММП

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ММП

Review

MALIGNANT DISEASES IN PREGNANCY - ETHICAL, DIAGNOSTIC AND TREATMENT CHALLENGE

МАЛИГНИ БОЛЕСТИ ВО ТЕК НА БРЕМЕНОСТ - ЕТИЧКИ, ДИЈАГНОСТИЧКИ И ТЕРАПИСКИ ПРЕДИЗВИК

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Abstract

The biological uniqueness of malignant tumors in pregnancy lies in the combination of uncontrolled growth of malignant tumors and controlled growth of fetoplacental complex in the same host. The risk of pregnancy associated with a malignant disease is approximately 0.1% (1 case per 1, 000 deliveries). According to results from one of the largest studies of malignant tumors in pregnancy, which involved almost thousand cases, the most frequent types were breast and cervical cancer, then melanoma, lymphoma and leukemia, gastric and rectal cancer, bone sarcoma and other sarcomas of the soft tissue. According to a Swedish retrospective study on cancer during pregnancy, the incidence rate is 37.4 cases per 100 000 deliveries.

Pregnancy-associated malignant disease brings a range of specific problems, such as: difficulties related to diagnosis and staging of the disease; the risk of performing diagnostic and therapeutic procedures during pregnancy; counseling and treatment plan of pregnant women having malignant disease.

The treatment plan of a pregnant patient with cancer has three possibilities: termination of pregnancy; postponement of treatment to reach fetal viability; treatment during pregnancy.

The management of malignant disease during pregnancy may be highly dangerous or even fatal for the fetus. The treatment includes surgery, radio and chemotherapy.

In malignant diseases during pregnancy, the prognosis is the same as in general population cancers of the same stage, localization and type. Termination of pregnancy is indicated in cases of, either high absorbed fetal radiation dose, or high grade, aggressive or metastatic cancer.

Keywords: malignant diseases, pregnancy, cancer treatment, surgery, radiotherapy, chemotherapy

Абстракт

Биолошката единственост на малигните тумори во бременоста се засновува на комбинацијата на неконтролиран раст на малигните клетки наспроти контролираниот на фето-плацентарниот комплекс, во ист домаќин. Ризикот за малигна болест поврзана со бременост е 0,1% (1 случај на 1000 породувања). Според резултатите на една од најголемите студии за малигни тумори во тек на бременост, која вклучувала околу 1000 случаи, најчести биле карциномот на дојка и цервикалниот карцином, меланомот, лимфомот и леукемијата, карцином на желудник и ректум, саркомот на коски и други саркоми на меките ткива. Според шведската ретроспективна студија за карцином во тек на бременост, инциденцата е 37,4 на 100 000 породувања.

Малигните болести асоцирани со бременост носат низа на специфични проблеми: проблеми поврзани со дијагнозата и стеџингот на болеста; ризик за изведување на дијагностички и тераписки процедури; советување и план за третман на бремената жена.

Планот за треман на бремената пациентка вклучува три можности: завршување на бременоста; одложување на третманот за да се постигне вијабилност на плодот; третман во тек на бременост.

Менаџментот на малигната болест во тек на бременост може да биде многу опасен дури и фатален за плодот. Третманот вклучува операција, хемо и радиотерапија.

Кај малигните заболувања во тек на бременост, прогнозата е слична како и кај другата популација со исти стадиум, локализација и тип на болест. Завршување на бременост се препорачува само кај високо абсорбирана фетална радијациона доза, висок градус, агресивен или метастатски карцином.

Клучни зборови: малигно заболување, бременост, третман на карцином, радио-хемо терапија

Introduction

The biological uniqueness of malignant tumors in pregnancy lies in the combination of uncontrolled growth of malignant tumors and controlled growth of fetoplacental complex in the same host. Thus, the major physiological

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processes for maintaining human kind and major pathological processes when untreated nearly always end up with death, are united in the battle for biological immortality.
Malignant diseases develop, as a rule, in the advanced age. However, due to the increasing number of women who decide to postpone pregnancy, the association between the malignant disease and pregnancy may be expected more frequently. In the period of 1970-2000, the mean age of women who gave birth for the first time was increased by 3.5 years (from 21.4 to 24.9) [1]. Since then, the postponement of the first birth has become an ongoing process. During the last decade, the age at first birth in Europe has increased in average 2 years and today, ranges between 25 and 29 years [2,3].
The incidence of malignant diseases during pregnancy is difficult to calculate due to the lack of central registries for these conditions. The risk of pregnancy associated with a malignant disease is approximately 0.1% (1 case per 1, 000 deliveries) [4]. According to results from one of the largest studies of malignant tumors in pregnancy, that involved almost thousand cases, the most frequent types were breast and cervical cancer, then melanoma, lymphoma and leukemia, gastric and rectal cancer, bone sarcoma and other sarcomas of the soft tissue [5]. It is hard, however, to determine, the total number of pregnancies in the population, and thus the incidence of malignant diseases during pregnancy is expressed per number of deliveries. According to a Swedish retrospective study on cancer during pregnancy, the incidence rate is 37.4 cases per 100 000 deliveries [6].
It is usuallyestablished that the incidence of malignant tumors during pregnancy is similar to the one in the general female population of the same age. To acquire the best reliable data from each country it is necessary to look into the local cancer registry, withdraw the number of patients diagnosed in a year in the age cohort of 15-39 years and thus estimate the co-incidence of malignant tumors in pregnancy. The estimation for Europe is shown in Table 1.

Table 1. Estimation of incidence of cancer during pregnancy for European countries*

Malignant tumor	Cohort 15-39 per 100 000 inhabitants	Incidence in pregnancy per 100 000 pregnancies
Breast	15.88	16
Cervix	10.59	11
Ovary	3.04	3

**Globocan 2008*[6]

Malignant disease in pregnancy is bringing an extreme stress for the pregnant patient, as well as for the doctor. Every oncologist that has treated pregnant patients with malignancy knows the difficulty of bringing decisions and emotional factors involved in them.
Treatment of malignant tumors in pregnancy is very delicate, since a pregnant woman has to choose between her life and the life of her unborn child. Also, the diagnostic and therapeutic procedures must be done with a special care having in mind the conjoined risk for the mother and the child.
Pregnancy-associated malignant disease brings a range of specific problems, such as: difficulties related to diagnosis and staging of the disease; the risk of performing diagnostic and therapeutic procedures during pregnancy; counselling and treatment plan of pregnant women having malignant disease.

Difficulties related to diagnosis and staging of the disease
One of the main problems associated with malignant disease during pregnancy is late diagnosis, generally as a result of several factors: malignant tumors are presented with symptoms that are usually accredited to pregnancy; physical examination may be compromised by the anatomic and physiological changes that occur in pregnancy; during pregnancy, the majority of serum markers (beta HCG, AFP, CEA, CA 125.) are physiologically increased; the optionsfor invasive diagnostic procedures or imaging techniqueare limited.
In pregnant women with cervical, ovarian or breast cancer, clinical investigationsoftencould be untrustworthy. For metastatic liver tumors, in general population as well as in pregnant women (where investigations show physiological increase of alkaline phosphatase values), liver function tests are not useful due to their low sensitivity. Also, examinations in patients with cancer showed presence ofoncofetal antigens, which are normally expressed in developing fetus and abnormally in cancer. One of these oncofetal antigen is alpha-fetoprotein (AFP) which is used for detection of neural tube defects in pregnancy, while in general population increased values of AFP are significantly associated with presence of the germinal cell tumor, hepatocellular cancer or less commonly, with adenocarcinoma. So, differenttiation of fetal and malignant expression of these antigensis rather difficult.
Staging principles during pregnancy remain the same as in non-pregnant patients with adjustment for gestational age, and the used procedures depend on the localization and type of malignant disease.
Some malignant diseases like early Hodgkin's disease or ovarian cancer require laparotomy for staging, which brings a certin risk in pregnancy. During pregnancy, some diagnostic as well as therapeutic procedures are difficult and some are dangerous such as conization which may be associated with greater risk of severe bleeding, spontaneous abortion or premature delivery, compared with general population. On the other hand, fine needle aspiration, core needle or open excisional biopsy as well as minor staging procedures such as bone marrow biopsy, spinal tap or endoscopies are relatively safe to perform during pregnancy [4].
In order to detect local tumor spread or distant metastases, staging requires radiographic imaging techniques. In spite of the common view of radiographic examinations as being risky during pregnancy, majority of them are far below threshold dose for fetal damage (0.05-0.1 Gy (5-10cGy)) [7]. Usually the dose delivered to the fetus in most conventional radiographic and nuclear medicine examinations is less than 0.01 Gy (Table 2), so, if necessary, single X-Ray scan of head and chest may be performed, with a risk for fetal malformations below 1% [8]. If the fetus is directly exposed to several radiations, the radiation level should be measured, and if it is above 5-10 cGy, the risk for fetal malformations is high and abortion should be considered.

Table 2. Estimated average dose to fetus per some radiographic examination

Examination	Average dose (in millirems)*
Dental	0.06
Head	< 0.5
Chest	0.5
Extremities	< 0.5
Mammography	< 10
Upper G.I. series	170
Pelvis	210
Intravenous pyelography	590
Abdominal CT	2000

** adapted from Fenig EZ et al, 2005[9]*

Note: The unit **gray** measures the absorbed dose of radiation (D), absorbed by any material. The unit **sievert** measures the equivalent dose of radiation (H), supposed to have a damaging effect equivalent to the same dose of gamma rays. Both the gray, with symbol Gy and the sievert, with symbol Sv are SI derived units, defined as a unit of energy (joule) per unit of mass (kilogram): 1 Gy=1 Sv=1 J/kg. An older unit for the equivalent dose, is the rem, still often used in the United States. One sievert is equal to 100 rem: 1Sv=100 rema.
Fluoroscopic procedures, CT, usage of radionuclides or bone scans can cause severe damage and should be replaced with MRI [10]. The correlation between the use of CT and radionuclides during pregnancy has been studied in a large population-based cohort study in Ontario [11]. Authors concluded that the possibility of the carcinogenic impact of CT or radionuclide imaging on the fetus cannot be excluded, so whenever possible, MRI and ultrasonography should be used as the most preferable diagnostic procedures during pregnancy.
The data for the impact of PET during pregnancy are limited, but since their estimation showed potential fetal harmful radiation, the procedure should be avoided [12].

Therapeutic interventions during pregnancy

The management of malignant disease during pregnancy may be highly dangerous or even fatal for the fetus and it depends on the: maternal risk correlated with treatment delay for accomplishing fetal viability or higher gestational age; the type of cancer, the localization, the stage, and prognosis; possible adverse effects of fetal exposure to treatment including late occurring complications; premature delivery forempowering timely cancer treatment and associated fetal complications.

Surgical interventions during cancer in pregnancy

The abdominal and pelvic surgeries should be planed in the 2nd trimsetar of pregnancy to preventpremature delivery or fetal loss.
General anesthesia during pregnancy is associated with far more possible complications due to the increased blood volume and stroke volume of the heart, but the medicines used like nitrous oxide or halothane are proved to be safe for the fetus[13].
Laparoscopy is also possible, but only for well-trained laparoscopists, since large uterus is threatened to be harmed by inserted troacars.
On the other hand, extra-abdominal surgery is usually safe with proper postopertive treatment and preventive womb analgesia [14].
Sentinel lymph node mappingin breast cancer patients with an injection of 18.5 MBq Technetium (Tc) with a fetal exposure of 0-0.05 mGy, was shown to be safe during pregnancy. The estimation of fetal exposure in vulvar cancer is about 0.1 mGy, which is also in the safe threshold [15]. However, it is noted that this technique should be used only in a 1-day protocol, with an extensive hydratation to minimize the irradiation effects. Instead of patent blue which is contraindicated during pregnancy for the risk of an anaphylactic reaction, Indocyanine green might be a safe option.

Radiotherapy

The effects of radiotherapy differ according to gestational age of the fetus. In the first 12 weeks of gestation the effect of radiation is teratogenic, and afterwards carcinogenic, with a potential induction of malignancy in the first decade of child's life.
The potential consequences of fetal exposure to radiotherapy are presented in Table 3 [15].

Table 3. Possible effects of radiotherapy on pegnancy

Gestational age	Effect of radiotherapy
From conception to 9/10 days	Lethal effect
From 2-6 weeks	Malformations Growth retardation
From 12-16 weeks	Growth and mental retardation Microcephaly Sterility
From 20/25 weeks to delivery	Malignancies Genetic defects

In the treatment of breast cancer, in early pregnancy, the absorbed fetal dose is 5 cGy, and later, more than 1 Gy. The fetal exposure of therapeutic doses of 5000-6000 cGyis10 cGy in early pregnancy and 200 cGy or more in late pregnancy. Therefore, when deciding whether to start the radiation treatment during pregnancy, it should be considered that 10 cGy absorbed dose is a borderline for fetal malformations, with increasing risk of 50% in fetal absorbed dose of 1 Gy5,[15]. Therefore, radiotherapy should be avoided during pregnancy, except in selected cases with far cancer localization and respected safety measures.

Chemotherapy

In pregnancy, due to changes in gastrointestinal motility, increased plasma volume leading to 50% decreased peak drug concentration in the amniotic fluid as a 3rd pharmacological space thus delaying metabolism and excretion with a potentially increasing toxicity, and increased blood flow and renal clearance, alter the pharmacokinetics of chemotherapy. Most of the cytotoxic agents can pass across the placenta and are teratogenic for the fetus, but they are more rapidly eliminated than the same agents in general population, except methotrexate which is captured in the amniotic fluid longer. Nevertheless, the highest risk for teratogenicity is given by the antimetabolites and

alkylating agents. The malformations frequency in the first trimester ranges from 12.7-17% in single and 25% in poly-chemotherapy, with a general population rate of 1-3% [16],reaching up to 20% at term [17]. Therefore, in the first trimester, chemotherapy should be avoided. In the second (after 16th week when CNS is developed) or third trimester mostcytostatics are safe till the 34th week of pregnancy [18]. The fetal plasma samples of doxorubicin, epirubicin, paclitaxel and docetaxel are below 10%, and for that reason are safe for second and third trimester treatment. However, the risk of premature delivery and intrauterine growth retardation should be kept in mind. The administration of chemotherapy should not go beyond the 34th week of pregnancy, because delivery within two weeks of the last chemotherapy dose may lead to fetal neutropenia. Anti-neoplastic drugs are excreted in the breast milk andtherefore, breast feeding should be avoided [19].
Studies of the long-term effects on fetuses exposed in utero to chemotherapy showed no major neurologic deficits nor psychological changes in the offspring [20,21]. Late potential risk of chemotherapy during pregnancy might be carcinogennesis in offspring, as well as intellectual and reproductive impairment [20].

Prognosis

In malignant diseases during pregnancy, the prognosis is the same as in general population cancers of the same stage, localization and type. Breast and ovarian cancers have worse prognosis if diagnosed during lactation. Some cancers are detected rather late, since pregnancy may conceal the symptoms [22]. Termination of pregnancy is indicated in cases of, either high absorbed fetal radiation dose, or high grade, aggressive or metastatic cancer.

Counselling and treatment plan of pregnant patient with malignant disease

The treatment plan of a pregnant patient with cancer has three possibilities: termination of pregnancy; postponement of treatment to reach fetal viability; treatment during pregnancy.
According to western medicine, a pregnant woman with a malignant disease should be accepted as an autonomous person with the right to choose between several possibilities for treatment in the best interest of herself and her fetus. She should be presented with all risks and opportunities while choosing whether to be treated, to postpone treatment, or to terminate pregnancy. It is highly difficult to choose which is the best offer, and at the same time to minimize the risk both for the mother and for the child. It is very delicate and challenging for the doctor to make the best approach to the family, and to explain all aspects of the disease and treatment options in the language that can be completely understood by the patient and her closest family. Nevertheless, the decision should be made together with the patient and her closest family in her and her baby's best interest.

Conflict of interest statement. None declared.

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Review

PHARMACOGENETICS OF OPIOID THERAPY IN TREATMENT OF POSTOPERATIVE PAIN: A REVIEW

ФАРМАКОГЕНЕТИКА НА ОПИОИДНАТА ТЕРАПИЈА ПРИ ТРЕТМАН НА ПОСТОПЕРАТИВНА БОЛКА: ПРЕГЛЕД

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Abstract

The safe and effective analgesia during and after surgery is an important clinical, social and economic problem. The goal of good analgesia is an individual balancing that patient's pain is reduced to a low level, and side effects are minimized. Data from the literature suggest genetic differences between patients in their ability to metabolize a particular drug. The effect of the drug is determined by interreaction of several genetic polymerphisms that affect the pharmacokinetics and pharmacodynamics of the drug, including differences in potency of binding to receptors and activity of drug enzymes and transportters. There is evidence suggesting that mutations of the mu-opioid receptor gene affects interindividual differences in opioid sensitivity. This review of the literature aims to analyze the current knowledge on the impact of genetic polymorphisms of CYP3A4, ABCB1 C3435, ABCB1 2677 and OPRM1 A118G gene on analgesic effect and side effects of opioids in the treatment of postoperative pain.

Keywords: pharmacogenetics, opioids, postoperative pain, polymorphisms

Апстракт

Безбедната и ефективна аналгезија во тек и по хируршките интервенции претставува важен клинички, социјален и економски проблем. Целта на добрата аналгезија е индивиуално балансирање на аналгетиците со кои кај пациентот болката ќе се сведе на најниско ниво, а несаканите ефекти ќе се минимизираат. Податоците од литературата сугерираат на генетските разлики помеѓу пациентите во нивната способност да метаболизираат одреден лек. Ефектот на лекот е детерминиран од интерреакцијата на неколку генетски полиморфизми кои влијаат врз неговата фармакокинетика и фармакодинамика, вклучувајќи ги разликите во потентноста на врзување за рецепторите како и активноста на метаболизирачките ензими и транспортери. Има докази кои сугерираат дека мутации на mu-опоидниот рецепторен ген влијаат врз интериндивидуалните разлики во опиоидната осетливост. Овој преглед од литературата има за цел да ги анализира досегашните сознанија за влијанието на генетските полиморфизми на CYP3A4, ABCB1 C3435, ABCB1 2677 и OPRM1 A118G генот врз аналгетскиот ефект и несаканите ефекти на опиоидите при третманот на постоперативна болка.

Клучни зборови: фармакогенетика, опиоиди, постоперативна болка, полиморфизми

Introduction

One of the most important objectives during surgery and early postoperative period is pain relief with minimal side effects of analgesics used. The safe and effective analgesia during and after surgery is an important clinical, social and economic problem. This problem is important due to individual differences between patients sensitivity compared to opioid analgesics used. Each year in the US alone more than 30 million children and adults are subjected to painful surgical procedures, while worldwide more than 250 million patients are in need of any type of surgery within one year. Many patients cope with inadequate pain control or side effects of opioids that are commonly used in the early postoperative period. The goal of good analgesia is individual balancing that patient's pain is reduced to

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a low level, and side effects are minimized. There are many variables that determine the degree of pain: age, weight, sex, race, presence of anxiety and type of surgery [1]. New research suggests that the different structure of the genes responsible for a particular part of pharmacokinetics or pharmacodynamics of opioids affect efficacy. Studies done on animals and humans suggest that some metabolizing enzymes and transporters play an important role in opioid metabolism impacting opioid movement in and out of opioid target cells. This process has the power to influence the interindividual differences in the concentration of the opioid in blood and brain [2].

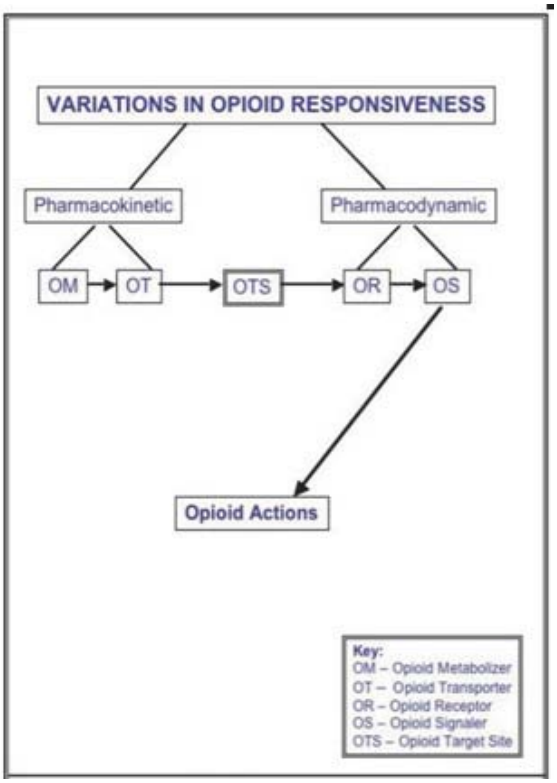

Fig. 1. Variation in opioid responsiveness

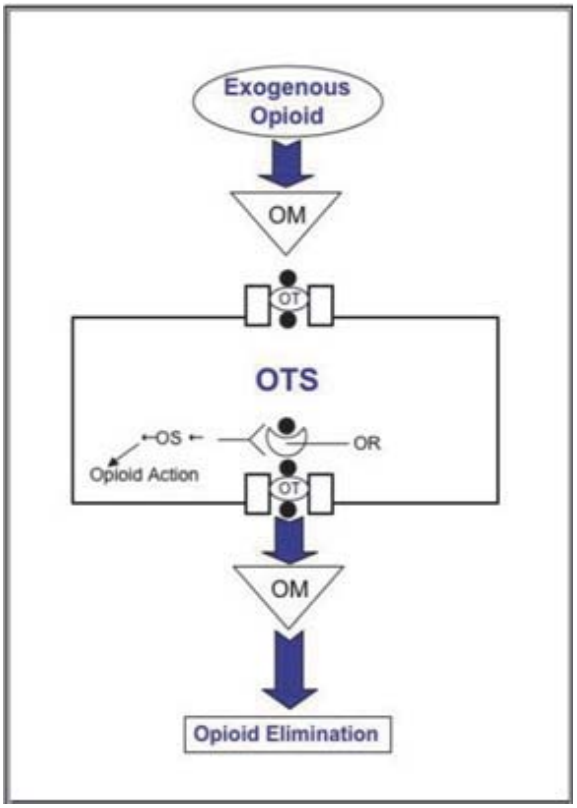

Fig. 2. Schematic of some principle processes potentially contributing to variations in opioid responsiveness
Howard HS. Variations in Opioid Responsiveness. Pain Physician 2008; 11: 237-248

1. Variations in the pharmacokinetics of opioids

1.a. Opioid metabolizers

In the literature genetic differences between patients in their ability to metabolize a particular drug are presented. Opioid analgesics are metabolised mainly by cytochrome P450 system and less than/by?? UDP-glucuronosyltransferases (UGTs), participating in the secondary metabolic processes. Codein drug can be totally ineffective in 10% of the Asian population due to mutation of the enzyme CYP2D6, which is necessary for metabolism of the drug in the active substrate. There are other genetic changes that may affect a particular part of the drug metabolism, and increase or decrease of its efficiency [4]. Genetic variability in the expression or density of opioid receptors, receptor potential or ability to transfer may explain the different sensitivity in patients on drug morphine. Also, variability in the expression of enzymes responsible for the metabolism of opioids may affect differences in individual consumption of opioid analgesics and their toxicity. In the future pharmacogenetic mapping can serve as a predictor of individual dosage [4]. The effect of the drug is determined by interreaction of several genetic polymorphisms that affect the pharmacokinetics and pharmacodynamics of the drug, including differences in potency of binding to recaptors. Together 20-25% of the effects of clinically used drugs are altered by genetic differences in enzymes involved in their metabolism. Enzyme system cytochrome P450 (CYP) is the biggest part because this system is responsible for 80% of the phase I metabolism of the drug. There are four metabolic structures by the enzyme system responsible for the metabolism of drugs: PM-poor metabolizers, IM-intermediate metabolizers, EM-extensive metabolizers and UM-ultrarapid metabolizers [5]. Genetic polymorphisms play an important role in variations of sensitivity to the drug. Patients with genetic enzyme system that belong to the group of PM-poor metabolizers have at least one mutant (inactive alleles) within the gene and may exhibit more side effects with normal dose of the drug, due to reduced metabolism and increased concentration of the drug in the blood. Patients in the group of strong EM-metabolizers, or those wich have two active alleles within the genetic system that encodes enzymes for dissolution

of the drug are expected to have a good response to the drug they take. Clinically relevant CYP3A enzymes, CYP3A4, CYP3A5 and CYP3A7 substrate have similar characteristics and are located predominantly in the liver. CYP3A4 is the largest cytochrome P450 system present in the liver. There is a large interindividual variability in CYP3A4 gene expression [6]. Differences in metabolism of codein between strong and poor metabolizers have influence on the analgesic efficiency in patients who received codein by the PCA pump (patient controlled analgesia). Patients according to genetic analysis of metabolizing enzymes were in the group of poor metabolizers (PM) received a greater concentration of the drug due to inadequate analgesia, unlike patients who were strong metabolizers (EM [7]. The study for efficacy of tramadol in the treatment of postoperative pain, from 241 patients in the group of strong metabolizers and 30 in the group of poor metabolizers, 47% of PM and 22% of the EM were tramadol insensitive; 43% of PM and 22% of EM had a need for additional analgesia [8]. The genetic polymorphism of opioid metabolizing enzymes has great variability and affect the pharmacokinetics of opioid analgesic efficacy and toxicity [9].

1.b. Opioid transporters

Drug transporters are important structural proteins that affect absorption, distribution and elimination of opioids [9]. In the gastrointestinal tract and the liver cells they have the power to affect the bioavailability of opioids imported through the gastrointestinal tract by reducing or increasing the intestinal absorption and biliary elimination [10,11]. The activity of transporters at the blood-brain barrier have the potential to affect the clinical efficacy and safety of opioids. The main action of these drugs is in the brain [9]. Opioid transporter is acting as drug transport out of the target cells. The effectiveness of carriers depends on the crossing of the opioid through biological membranes. There are two large families of opioid transporters integrated in the pharmacokinetics of these drugs, which are ATP binding cassette (ABC)-efflux transporters and solute carrier (SLC)-influx transporters [9]. ABC transporters efflux family contains nearly 50 active polymorphisms divided into seven smaller groups [12]. The most studied so far by ABC transporters is ABCB1 (MDR1, P-glucoprotein (P-gp)), efflux transporter which operates on the capillary endothelial cells of the blood-brain barrier and blood-CSF barrier [12]. Opioid analgesia is delayed in mice with a genetic mutation in the P-glucoprotein. Morphine, methadone, loperamide and fentanyl are P-glucoprotein substrates [13-18]. ABCB1 gene wich is encoding P-gp is highly polymorphic with over 100 identified single polymerphisms. Most genetic polymorphism has been researched in exon 26, it is C3435T, which has a different distribution and frequency of people with different ethnic backgrounds. There are compelling data that the distribution of morhine in the brain, which is transported by P-gp, is dependent on genetic polymorphism of ABCB1 3435. There is a connection between increased concentration of morphine in CSF in patients mutant homozygous compared to 3435 ABCB1 gene [19]. The explanation is that patients who have two mutant alleles of this gene, lack appropriate active efflux transport of opioid substance (P-gp), it increases the concentration of the opioid in the liquor and occur more severe side effects, such as sedation. From this group of genes responsible for P-gp, as the main transporter of opioid drugs in and out of the target cells and gene ABCB1 2677.

Patients with at least one mutant allele in both ABCB1 genes ABCB1 3435 and 2677 have significantly higher concentration of loperamide in plasma, unlike the patients who have two wild-type alleles of these two genes [20]. Mutation of the gene ABCB1 2677 affects the concentration of opioid substance in the blood and adverse effects [21,22]. In his study Campa *et al*, placed the hypothesis that patients who have two wilde type alleles of the gene responsible for opioid transport ABCB1 3435 CC and two mutant alleles of the gene encoding the mu-opioid receptor OPRM1 118 GG will be the least sensitive of opioids (worst responders). On the other hand, patients with two mutant alleles of the gene ABCB1 3435 TT, having ineffective efflux transport of the opioid out of the cell and two wilde type alleles of the gene encoding the opioid receptor OPRM1 118 AA are expected to be most sensitive to opioid therapy (best responders). In a retrospective study of Coller *et al*, the authors concluded that the variability of the ABCB1 gene affects the daily needs of methadone. Patients who had two active alleles of this gene (active efflux transport) were in need of higher daily doses than in patients who had at least one mutant allele of the same gene (inactive efflux transport) [23]. Variability in the degree of pain and the need for opioids was significantly associated with genetic polymorphism, particularly taking into account the combined structure of potential predictors genes responsible for certain parts of the pharmacokinetics and pharmacodynamics of these drugs.

2. Variations in the pharmacodynamics of opioids

2.a. Opioid receptors

Mu, kappa and delta opioid receptors are encoded by OPRM1, OPRK1 OPRD1 and all genes have polymerphism. There is evidence to suggest that mutations of the mu-opioid receptor gene affect interindividual differences in opioid sensitivity [24]. OPRM1 gene can occur in the following allelic presentation AA homozygous with wilde type allele, heterozygous AG with one mutant allele and GG homozygotes with two mutant alleles. It is assumed that patients with two wilde type alleles AA have a strong potential to bind the opioid substance, unlike patients with at least one mutant allele had a

weak affinity and power to bind opioid drug. Single nucleotide polymorphism of 118A>G within the OPRM1 mu-opioid receptor gene resulting in different performance of opioid analgesia, presented in patients with malignant disease homozygous to the mutant allele (GG), who received higher doses of morphine for pain relief, unlike patients with homozygous wild-type alleles (AA) [25]. In a study that evaluates candidate genes for pain A118G of OPRM1 gene is rated eight, scoring system where the highest grade was nine [26].

One of the first experimental study on the possible relationship between genetic polymorphism within the OPRM1 gene and the degree of pain is made in the US in the study of Fillingim *et al*. This study found that there are differences in the level of pain caused by painful stimuli (thermal, mechanical and ischemic) in patients with different profile OPRM1 gene [27]. Another study investigating the correlation between 118A>G polymerphism and genetic required dose of morphine (PCA-patient controlled analgesia) among the patients after knee arthroplasty surgery. Patients with homozygous variant (GG) consumed about 60% higher amount of morphium, unlike patients who were heterozygous or homozygous with wild type allele (AA) for 48 hours postoperatively investigation. Demographic data are not correlate with pain and opioid claim [28]. During the research done among the women after hysterectomy, patients homozygous with mutant alleles (GG) within the 118A>G polymorphism required a 30% higher dose of morphine to control postoperative pain, unlike patients homozygous wild type alleles (AA) in the first 24 hours after abdominal hysterectomy [29]. Significant difference between the degree of pain and 118A>G genotype was found in the study conducted in patients with malignant disease treated with morphine in the first two months of therapy. In the first seven days of starting treatment with morphine, patients with homozygous wild type allele (AA) had weaker pain from baseline, in contrast to patients homozygous mutant type alleles (GG), in which the response to pain-killers on the level of pain was insignificant [30]. The study of Sia *et al*, made in England included 631 female stump operated by caesarean section. The women were divided into 3 groups according to genotype: AA group, Group AG and GG group. All patients were guided in spinal anesthesia (marcaine 0,5% 2 ml + morpine 0,1 mg intrathecal). Postoperatively they were placed on PCA (patient controlled analgesia) and followed 24 hours with the following parameters every 4 hours pain (VAS visual anallog scale), nausea, and pruritus. The conclusion from this study is that A118G polymorphism of human mu-opioid receptor gene have a significant effect on the perception of pain, need for analgesics and the occurrence of nausea as a side effect in patients who received intrathecal morphine and intravenous PCA postoperatively. Patients with AA genotype had the faintest pain, received less morphine intravenously in the postoperative period, and had a higher incidence of nausea and vomiting. Patients from group GG had the strongest pain [31] Study of Fukuda *et al*, made in Japan 2009 in the field of oral surgery, produces results that one of the five most common single nucleotide genetic polymorphisms OPRM1 gene responsible for mu-opioid receptors influence the perception of postoperative pain and the need of fentanyl. This strong opioid is less effective in patients with a G allele of the OPRM1-118G unlike patients with the A allele [32]. The study made in China 174 patients after gynecological interventions analyzes the relation of the degree of pain and the need for fentanyl (PCA) with A118G polymorphism of the mu-opioid receptor gene (OPRM1). Fentanyl consumpation is greater in patients with G allele. OPRM1 genetic analysis may be a predictor of opioid sensitivity and to optimize post-operative pain control. [33]. The impact of genetic polymorphism A118G within the OPRM1 gene on the level of pain was researched in a study in patients with diabetes and diabetic foot. Variations within this gene is closely related to the degree of pain. Patients with mutant type alleles within the 118 OPRM1 had weaker pain than other patients [34]. After the initial assumptions OPRM1 gene that is responsible for binding the drug morphin to opioid receptors, Deb *et al*, explored the association of genetic variations within this gene and addiction to heroin and alcohol use among Indian population. Patients who consumed heroin and alcohol, and had G allele in A118G single genetic polymorphism was associated with a greater dependence, unlike patients with the A allele [35]. Several studies refer to the demographic characteristics of the patients such as age, sex and the type of operation affect the level of pain [36,37]. Differences in the distribution and frequency of alleles within the genetic polymorphism of the OPRM1 is referenced in different ethnic groups [38-40]. Stronger pain and greater need of fentanyl in patients with 118G allele is likely due to changed less binding capability of mu-opioid receptor within the genetic variability of OPRM1 gene in patients with a G allele [41]. Two studies that refer to their investigations found no differences in binding ability of opioids to mu-opioid receptors in different variations of OPRM1 gene [42,43]. So far it is known that the effects of the fentanyl-representing mu-opioid agonist, is variable in different individuals. The exact mechanism of these individual differences are not yet known. Multiple factors affect postoperative pain such as ethnicity, anxiety, and presence of preoperative pain. Opioid receptor is encoded by the mu-opioid recaptor gene (OPRM1), it is the most important candidate for the examination of genetic variation on the level of pain in the field of pharmacogenetics. This receptor is mainly place of action and the endogenous opioids such as enkephalins and endorphins β- and represents a principal place of action of most opioid analgesics [44-46]. Genetic variations influence susceptibility to pain, the likelihood of developing chronic pain and res-

ponse to pharmacotherapy in managing pain [47,48]. Farmacogenetic polymorphism is definitely important role in individual differences of analgesic effects and the emergence of side effects of opioid analgesics. However, genetic factors of the individual response of the analgesic pain treatment. Other factors affecting the treatment of pain include biological differences (ethnic, age and gender), factors of environment (smoking), comorbidities and use of other drugs (potential interreaction) must be considered along with genetic variations, because they summed together affecting the pharmacokinetics and pharmacodynamics of drugs used to control pain. Additional studies are necessary to characterize the combined effect of genetic variation with demographic and clinical variables in order of selection and appropriate opioid dosage to individual patients for suprimiranje middle and severe pain. It requires additional randomized prospective studies to assess the appropriate dosing and make individual drug algorithm based on genotypic information. Incorporating this new knowledge in the standards of anesthesiologists will result in benefits for patients and greater safety. In the near future farmacogenetic approach in the management of pain will provide individualization of the treatment and selection of an appropriate analgesic early treatment in order to provide sustained efficacy with minimal side effects [49].

Conflict of interest statement. None declared.

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Original article

METHODS FOR VASCULAR CONTROL IN LIVER RESECTIONS DUE TO COLORECTAL METASTASES - IMPACT ON RESIDUAL PARENCHYMA

МЕТОДИ ЗА ВАСКУЛАРНА КОНТРОЛА ПРИ РЕСЕКЦИИ НА ЦРН ДРОБ ПОРАДИ КОЛОРЕКТАЛНИ МЕТАСТАЗИ - ВЛИЈАНИЕ ВРЗ РЕЗИДУАЛНИОТ ПАРЕНХИМ

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Abstract

Introduction. Massive blood loss while performing resections of the liver continues to be a serious problem with potentially lethal outcome. Therefore in the last 2-3 decades there has been a significant developement of techniques for vascular control during liver resections.
Methods. In the period from 01.01.2006 to 31.12.2015 in KOCPH UMBAL "Aleksnadrovska" a total of 239 patients with colorectal liver metastases underwent surgery of whom: 179 patients were radicaly operated on and 57 patients were subjected to Pringle maneuver. Using the statistical software SPSS-19 we analyzed various factors that may affect the early postoperative results.
Results. In resections of colorectal liver metastases there was a significant difference in the postoperative functional parameters (AST, ALT), which correlated with the degree of liver damage, in patients with Pringle and without Pringle maneuver 265.32 vs. 448 (p=0.001), and 300.53 vs. 481.91 (p=0.002),-respectively. There was no significant difference in the postoperative results in comparisson of resections <15 minutes, performed without Pringle and with Pringle maneuver. The blood loss is another factor that affects the postoperative complications (p = 0.048), and it was lowest in the Pringle group <15 min.
Conclusion. Pringle maneuver is a simple and effecttive method for vascular control. As a result of its use we can observe the damage of the residual liver volume from the continuous ischemia to the reperfusion period. Thus, in liver resections, due to colorectal metastasis, vascular control strategy should be individual and corresponding to the extent of the procedure and associated diseases of the liver-fatty liver, cirrhosis, chronic hepatitis and others.

Keywords: colorectal liver metastases, liver resection, vascular occlusion of hepato-duodenal ligament, ischemia, residual parenchyma.

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Апстракт

Вовед. Масивната загуба на крв при извршување ресекции на црн дроб продолжува да биде сериозен проблем со потенцијално летален исход. Затоа, во последните две-три децении, значително се развиваат техниките за васкуларна контрола при ресекции на црниот дроб.
Методи. Во периодот од 01.1.2006 г. до 31.12.2015 г. во КОЦПХ УМБАЛ (КОСРН UMBAL) "Александровска" се оперирани 239 пациенти со колоректални метастази на црниот дроб, радикално се оперирани 179 пациенти, а кај 57 пациенти е направен Pringle maneuver. Со статистички софтвер SPSS-19 беа анализирани различни фактори коишто може да влијаат на раните постоперативни резултати.
Резултати. При ресекции на црниот дроб поради колоректални метастази постои сигнификантна разлика на постоперативните функционални параметри (AST, ALT), коишто корелираат со степенот на оштетување на црниот дроб, кај пациенти без Pringle и со Pringle-соодветно 265,32, наспроти 448 (p=0,001) 300,53, наспроти 481,91 (p=0,002). Нема значајна разлика на постоперативните вредности при споредба на ресекции без Pringle и со Pringle под 15 мин. Загубата на крв е друг фактор којшто влијае на постоперативните компликации (p=0,048), таа е најниска во групата со Pringle <15 мин.
Заклучок. Pringle maneuver претставува прост и ефективен метод за васкуларна контрола. Како резултат од неговата употреба може да се набљудува оштетување на резидуалниот волумен на црниот дроб од продолжителната исхемија и периодот на реперфузија. Затоа, при ресекции на црниот дроб, поради колоректални метастази, стратегијата за васкуларна контрола треба да

биде индивидуална и да кореспондира со екстензитетот на процедурата и со асоцираните болести на црниот дроб-стеатоза, цироза, хроничен хепатит и др.

Клучни зборови: Колоректални метастази на црниот дроб, црнодробна ресекција, клемување на хепатодуоденалниот лигамент, исхемија, резидуален паренхим

Intoduction

The significant blood loss in liver resections and the perioperative blood transfusion are associated with an increased rate of postoperative complications and mortality [1-3]. Recent studies have even shown that perioperative blood transusion increases the risk of recurrence in patients with colorectal liver metastases. [2,3] Therefore, in recent decades a number of different techniques for vascular control during liver resection are presented and described I the literature. Methods for occlusion of liver blood vessels are effective in reducing the blood loss during transection of the liver [1,3]. Their use on the other hand is associated with a potentially damaging influence to residual parenchyma due to the effect of continuous ischemia-reperfusion period [3].

Materials and methods

In the period from 01.01.2006 to 31.12.2015 in KOCPH UMBAL "Aleksnadrovska" 239 patients with colorectal liver metastases underwent surgery, of whom: 179 patients were radicaly operated on and 57 patients were subjected to Pringle maneuver. Using the statistical software SPSS-19 we analyzed various factors that may affect the early postoperative results.

Results

The study include 239 patients with colorectal liver metastases: radical intervention was done in 179 while in the remaining 60 palliative intervention was performed or only biopsy was taken-55 patients. The type of intervention applied depended significantly on the type of liver metastases ($p<0.001$). Metachronous metastases significantly more than synchronous were treated with radical intervention (93.33% vs. 56.3%). Palliatively treated or biopsied were only 6.67% metachronous and 43.7% synchronous metastases.

Tabela 1. Distribution of the types of radical interventions in synchronous and metachronous liver metastases

Type of liver resection	Liver metastases		*p-value*
	synchronus	**metachronous**	
Radical	67(56.3%)	112(93.33%)	[a] <0.001
palliative/biopsy	52(43.7%)	8(6.67%)	
Total	119	120	

[a] (Chi-square test)

In 57(23.8%) patients during liver resection Pringle maneuver was used, with an average duration of 16.37±8.3 minutes. The shortest duration of the Pringle maneuver lasted for 5 minutes, the maximum duration was 60 minutes.

The group of patients in whom the Pringle maneuver was used were divided in terms of duration of less than 15 minutes and more than 15 minutes. In 33(18.43%) patients this vascular procedure was lasted up to 15 minutes, with an average duration of 12.06±2.7 minutes. In 24(13.4%) patients the Pringle maneuver was used for more than 15 minutes, with an average duration of 22.3±9.7 minutes (Figure 1).

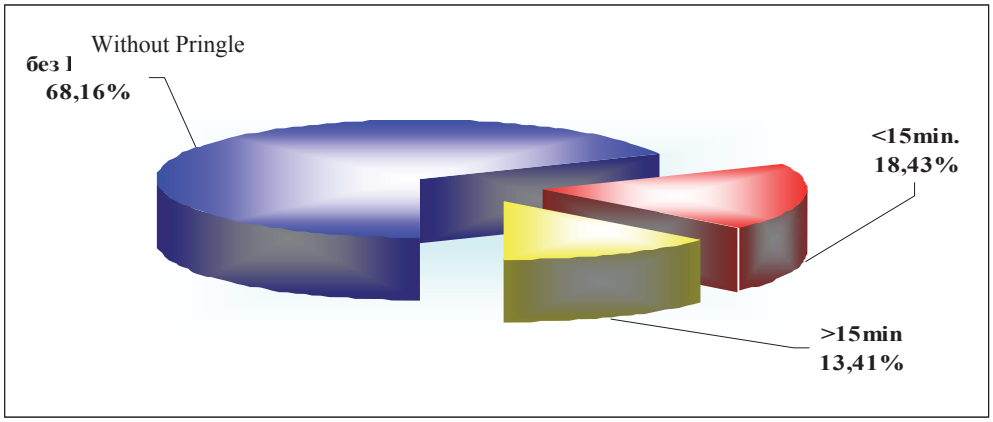

Fig. 1. Liver resections with and without Pringle maneuver

Table 2. Distribution of liver resection with and without Pringle maneuver and time of it

Types of resections of the liver	Pringle maneuver		
	without Pringle N=122	Pringle <15min N = 33	Pringle >15 min N = 24
atypical resections	49	7	1
resections of 2 segments	12	10	2
resections of 3 segments	11	5	2
resections of more than 3 segments	6	1	3
left lobectomy	11	4	0
left hemihepatectomy	2	1	1
right hemihepatectomy	3	1	8
metastasectomy	19	1	0
resections + another procedure	9	3	7

Table 2 shows the grouping of surgical interventions regarding the use of the Pringle maneuver. As it can be seen, this intervention was more frequently used in patients who underwent right hemihepatectomy (9/12) and in combined radical operations (10/19). The Pringle maneuver was used in 34.17% of large-extent operations, and in 27.12% of small-extent operations. Differences in the distribution of patients with and without Pringle maneuver, in terms of the extent of the intervention according to the number of resected liver segments showed no statistical significance (p=0.34) (Figure 2).

Tabela 3. Comparison of blood loss volume and the length of applications of Pringle maneuver with patients operated without clamping technique

Pringle maneuver	The operation extent according to the number of segments resected liver		*p-value*
	major resections	minor resections	
no	79(65.83)	43(72.88)	0.34
yes	41(34.17)	16(27.12)	

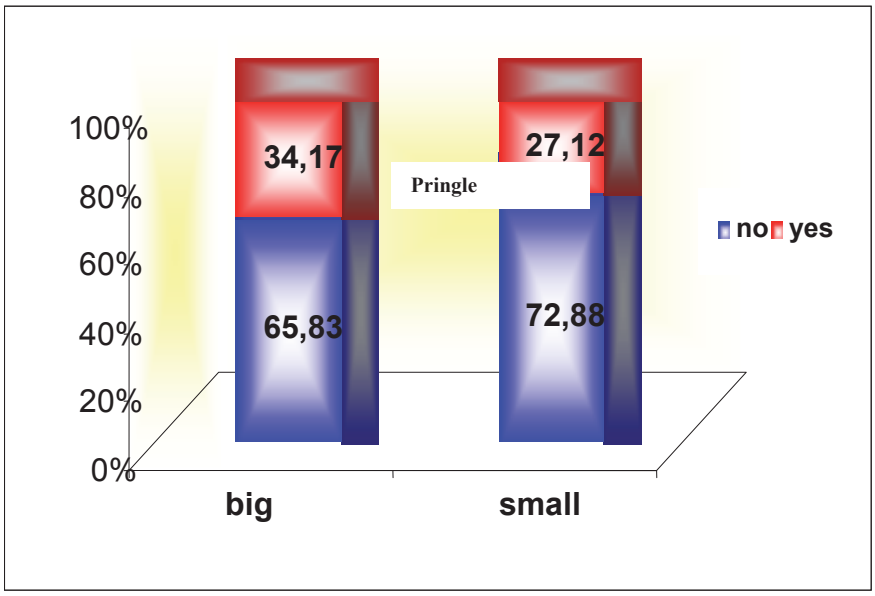

Fig. 2. Extent of surgical intervention according to the number of resected liver segments

There were more complications in patients with synchronous metastases compared to matachronous (6.72% vs 4.17%); with unilateral localized metastases compared to bilateral (7.75% vs 2.73%), and patients with advanced age and associated liver disease such as cirrhosis , steatosis, chronic hepatitis and others.

Blood loss is another factor that affects the postoperative morbidity and mortality. When comparing patients with observed complications postoperatively with patients without complications we noticed that the first group had a

Table 4. Difference in values of AST and ALT pre- and postoperatively depending on the application and duration of Pringle maneuver

	Pringle	Average value	Statistical deviation	Average statistical error	P
Blood loss	without Pringle	488.10	170.509	18.604	0.972
	Pringle >15 min	486.96	123.599	25.772	
Blood loss	without Pringle	488.10	170.509	18.604	0.000
	Pringle <15 min	319.35	55.793	10.021	
Blood loss	Pringle >15 min	486.96	123.599	25.772	0.000
	Pringle <15 min	319.35	55.793	10.021	

significantly higher blood loss-537.50 ml compared to 441.67 ml (p=0.048). For this reason we analyzed the amount of blood loss when we divided patients into groups with and without Pringle maneuver compared to the duration of blood vascular occlusion-under and over 15 minutes. The lowest blood loss was observed in the group of patients with Pringle <15 min (Table 4).
The extent of damage of residual parenchyma postoperatively is closely monitored through the measurement of serum transaminases and their comparison before and after surgery (Table 5). Raised values are usually a result of surgical trauma and ischemic injury of the liver due to techniques of vascular control. The analysis of patients with resections of colorectal liver metastases showed no significant difference in postoperative functional status in patients without Pringle and with Pringle maneuver. The difference between the preoperative and postoperative AST values in patients without Pringle and with Pringle maneuver was 265.32 vs. 448 (p=0.001), and ALT-300.53 vs. 481.91, respectively (p=0.002). There was no significant difference of postoperative ALT values comparing resections without Pringle and Pringle maneuver with duration less then 15 min.

Table 5. Difference in values of AST and ALT pre- and postoperatively depending on the application and duration of Pringle maneuver

	Pringle	Average value	Statistical deviation	Average statistical error	*P*
Difference in pre- and postoperative values of AST	without Pringle	265.32	258.467	28.201	0.001
	with Pringle	448.00	322.816	43.930	
Difference in pre- and postoperative values of ALT	without Pringle	300.53	240.603	26.252	0.002
	with Pringle	481.91	371.606	50.569	
Difference in pre- and postoperative values of AST	without Pringle	265.32	258.467	28.201	0.006
	with Pringle >15 min	464.74	296.907	61.909	
Difference in pre- and postoperative values of ALT	without Pringle	300.53	240.603	26.252	0.009
	with Pringle >15 min	518.09	348.598	72.688	
Difference in pre- and postoperative values of ALT	without Pringle	300.53	240.603	26.252	0.081
	with Pringle <15 min	455.06	391.278	70.276	

Discussion

Intraoperative bleeding and perioperative blood transfusion are assotiated with increase in postoperative morbidity and mortality [4]. Also, blood transfusions increase the recurrent rate in patients treated with liver resection due to malignancy [2,5-7]. Pringle first described the efficacy of vascular occlusion of hepatoduodenal ligament in patients with liver damage in 1908 [8]. His maneuver it has been used routinely in the practice and it is very easy method for vascular control of the afferent blood flow to the liver [1,3]. Nevertheless, the Pringle maneuver poses no risk to the general hemodynamic damage of the liver and bowel congestion, especially in patients with chronic liver disease [3]. Using the Pringle maneuver in duration less than 15 min. we found no statistically significant increase in the impairment of the residual parenchyma, which was demonstrated by the ghange of postoperative transaminase levels. At the same time, blood loss in this group of patients was the lowest, presenting this method to be sufficiently effective. This is also associated with the experience of the surgeon and the ability to perform transection of the liver in a shorter period. Patients who require a prolonged period for the Pringle maneuver pose a problem. Belghiti *et al*. [5] reported that intraoperative blood loss during parenchymal transection was higher, in intermitentniot PM 230 ml vs 530ml, the intraoperative transfusion is higher 28% vs 32%, but there are better results in terms of tolerance and stabilization of hepatic function of healthy and sick liver compared to continuous PM. Petrowsky *et al*. [9] compared the ischemic preconditioning preparation (PM-10 min. 30-reperfusion) with CPM and IPM in large hepatectomy observing less blood loss during liver transection (146 vs. 250 ml) and shorter transection time (40.4 vs 50.6 min). Makuuchi *et al*. [10] proposed hemihepatic pedicular occlusion technique to reduce the level of visceral stasis and general hepatic ischemia. In a randomized tria Fu *et al.* [11] compared the hemihepatic occlusion of the liver with CPM and IPM and found that the operating time was shorter in the Pringle group; the three groups were different in terms of intraoperative blood loss and postoperative mortality, but the Pringle group had a significantly more severe ischemia-reperfusion injury of the liver, a greater number of complications and longer intrahospital period. Hemihepatic occlu-

sion is particularly useful in patients with liver cirrhosis and peripheral lesions due to lower ischemia- reperfusion injury of the liver [11]. Heneay *at al.* [12] were the first who described the total hepatic vascular exclusion (THVE). It combines the control of vascular inflow and outflow and vascular occlusion of the lower and upper part of v. cava inferior. Chen *et al.* [13] modified the technique of THVE and proved the difference between PM and modified THVE, in terms of intraoperative blood loss and transfusion (750 ml vs. 350 ml and 46.5% vs 13.3%), but no significant difference in terms of postoperative functional stabilization of liver enzymes AST, ALT, bilirubin and morbidity rate (29.3% vs. 31.6%). Selective hepatic vascular exclusion (SHVE) limits the branches with vascular occlusion with extraparenchymal control of hepatic veins, but without interrupting the caval flow. Thus, this method is not associated with haemodynamic and biochemical deficiencies of THVE [14,15]. Selective vascular exclusion compared to PM had less intraoperative blood loss and transfusion (420 ml vs. 880 ml), but there was no significant difference in terms of postoperative morbidity (49% vs. 52%) [16]. Man *et al.* [17] showed that resection with vascular occlusion compared to resection without vascular occlusion resulted in significantly less blood transecion surface (12 vs. 22 ml/cm^2), after a short time of transection (2 vs. 2.8 min/cm^2) and less post-operative complications (26% vs. 30%). These results are similar to other showing no significant difference in intraoperative blood loss, percentage of hemotransfusion and between postoperative morbidity and no difference mortality. [18]

Conclusion

The Pringle maneuver is a simple and effective method for vascular control. It helps in monitor the damage to the residual volume of the liver from the continuous ischemia and reperfusion period. Therefore, in liver resections due to colorectal metastasis, vascular control strategy should be individual and corresponding to the extent of the procedure and associated diseases of the liver- fatty liver, cirrhosis, chronic hepatitis and others.

Conflict of interest statement. None declared.

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Original article

SEASONAL INFLUENZA- FACTORS ASSOCIATED WITH A SEVERE CLINICAL FORM OF THE ILLNESS

СЕЗОНСКА ИНФЛУЕНЦА-ФАКТОРИ АСОЦИРАНИ СО ТЕШКА КЛИНИЧКА ФОРМА

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Abstract

Introduction. The risk factors associated with the progression of a severe clinical form of seasonal influenza are of a particular importance in developing a current and accurate decision in terms of treatment options.
Aim. The aimof the study was to identify the specific factors associated with a severe form of seasonal influenza.
Method. The studywas conducted as a prospective, group comparison at the University Clinic for Infectious Diseases in Skopje, Macedonia, during the period of January 01, 2012, until January 01, 2015. This study analyzed 122 adult patients, who were clinically-confirmed to be infected with seasonal influenza by laboratory analyses and other necessary tests. These patients were grouped into two categories: patients with a mild form of seasonal influenza, and patients with a severe form of seasonal influenza. Furthermore, the demographic, clinical, and biochemical results obtained were analyzed. The variables in the univariable analysis which were significantly associated with a severe form of seasonal influenza were included in the multivariable logistic regression analysis in order to extract and determine the independent predicttors of a severe form of seasonal influenza.
Results. The multivariable analysis yielded cardiovascular diseases (p=0.01), dyspnea (p=0.001), tachypnea >20 respiration/ minute (p=0.005), values of LDH greater than 618 U/L (p=0.048) and SAPS 2score (p=0.031) as independent variables which predict the severity of the illness. The area under the ROC curve [0.826 (95% CI)] suggests that the probability of a severe form of influenza was82.6%. The global accuracy for this model to predict a severe form of influenza was 81.1%, with the sensitivity being 88.5%, and the specificity 72.9%.
Conclusion. Cardiovascular diseases, dyspnea, tachypnea, elevated levels of LDH and SAPS 2 score are independent predictive indicators for severe influenza. Early identification of these indicators will allow implementation of adequate medical intervention which will in turn reduce mortality rates.

Keywords: seasonal influenza, severe clinical form, predictors

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Апстракт

Вовед. Од посебно значење се ризик факторите за развој на тешка клиничка форма на сезонска инфлуенца за донесување навремена и правилна одлука за спроведување соодветен третман.
Цел на оваа студија е идентификување на факторите, асоцирани со тешка инфлуенца.
Методи. Истражувањето е проспективно, групно, споредбено и е изведено на Универзитетската клиника за инфективни болести во Скопје во период од 1 јануари 2012 година до 1 јануари 2015 година. Анализирани се 122 возрасни пациенти соклинички и лабораториски потврдена сезонска инфлуенца, кои понатаму се поделени на група пациенти со лесна и со тешка форма наинфлуенца. Анализирани се демографски, клинички и биохемиски податоци. Варијаблите од универијантната анализа, сигнификантно асоцирани со тежината на болеста, беа вклучени во мултиваријантната логистичка регресиска анализа, за да се детерминираат независните предиктори за тешка форма на инфлуенца.
Резултати. Мултиваријантната анализа ги издвои кардиоваскуларните болести (p=0.01), диспнеја (p= 0.001), тахипнеја >20респирации/минута (p=0.005), вредности на ЛДХ поголеми од 618 U/L (p=0.048) и САПС 2 скорот (p=0.031), како независни показатели кои на приемот ја предвидуваат тежината на болеста. Површината под ROC кривата изнесува 0.826 (95% CI), што сугерира веројатност за тешка форма на инфлуенца од 82.6 проценти. Глобалната точност на овој модел за предвидувањетешка форма на инфлуенца изнесува 81.1%, сензитивноста е 88.5%, а специфичноста 72.9%.

Заклучок. Кардиоваскуларните заболувања, диспнеа, тахипнеа, покачените вредности на ЛДХ и САПС 2, речиси се независни предиктори за тешка инфлуенца. Раната идентификација на овие показателите ќе овозможи имплементација на адекватни медицински постапки и ќе придонесе за намалување на морталитетот.

Клучни зборови: сезонска инфлуенца, тешка клиничка форма, предиктори.

Introduction

Seasonal influenza is an acute, viral, respiratory disease whichis causedby the influenza viruses A (H1N1), A (H3N2) and B. It is clinically presented in a range from mild to severe complicated disease with exacerbation of other underlying conditions, severe pneumonia, multiorgan failure and invasive bacterial co-infection [1]. Annual seasonal epidemics affect 5-10% of adult population and 20-30% of children. During seasonal epidemics from 3 to 5 million severe cases and about 250.000-500.000 lethal cases are registered worldwide [2,3]. Until now there has been nolaboratory test which has served as a potential marker for identification of patients with a high risk of developing severe clinical form of influenza and lethal outcome [4]. It is known that adult patients and patients with different comorbid conditions such as diabetes mellitus, chronic cardiovascular diseases, pulmonary diseases and immunosuppressive conditions are at a higher risk of developing severe clinical course of the disease and lethal outcome [5-9]. Pneumonia is the main reason for the progression in the severe form of the illness and lethal outcome [10, 11]. Delayed antiviral treatment, severe hypoxemia and multisystem organ failure are the most commonly-referred leading risk factors for thesevere form of the illness [12,13]. The largest number of studies has evaluated isolated risk factors leading to lethal outcome and only a few of them have been focused on the complete palette of predictors for development a severe form of the disease and lethal outcome [14-17]. From the clinical practice point of view, the recognition of the risk factors and predictors for progression of thesevere form of the disease and lethal outcome of influenza is of particular importance in bringing timely and exact decision for hospitalization, treatment or undertaking special measures for intensive monitoring of these patients.

Severe influenza is defined by signs ofrespiratory weakness (dyspnea, tachypnea, hypoxia, cyanosis), that is, partial pressure of arterial oxygen (PaO2<70mmHg; <9.0kPa) and/or need of mechanical ventilation or signs of ARDS (PaO2/FiO2≤200), intensive care, severe complications, exacerbation of the existing chronic disease [18-20].

The **aim** of the study was to identify the specific factors associated with a severe form of seasonalinfluenza.

Material and methods

The study was designed in accordance with the ethics principles of the Declaration of Helsinki for patients and their rights, and was approved by the Ethics Committee of the Medical Faculty of Ss Cyril and Methodius University in Skopje.

The study was a clinical, prospective, group comparison and it was conductedat the University Clinic for Infectious Diseases and Febrile Conditions in a three-year-period (1.1.2012-1.1.2015).

A total of 122 patients with clinically and laboratory confirmed influenza were analyzed. The patients were divided into two groups:

Group 1 contained 35 patients with a mild form of influenza,

Group2 contained 87 patients with severe influenza.

All of the patients with clinical and laboratory confirmed influenza and aged ≥16 years were included in the study. Patients were excluded if they died in the first 24 hours of their inclusion in the study.

On admission of patients, the following parameters were noted: demographic characteristics, clinical symptoms and signs, laboratorybiochemical characteristics, and evaluation of SAPS 2 score.

For determining the presence of the influenza virus nasopharyngeal smear was used. In the Laboratory of virology and molecular diagnosis at the Institute for Public Health from the previously isolated RNA (ribonucleic acid) real time RT-PCR (reverse transcripttase/ion-polymerase chain reaction in real time) was performed on the apparatus IQ (BioRad) for detection of matrix gene of influenza A and influenza B. The samples positive to influenza A were subtyped by the same method, by RT-PCR, with a specific set of primers for highly conserved regions of H1, H3 and H1 pdm (pandemic).

In order to have an objective judgment for the severity of the illness and prediction of mortality, SAPS 2 score (Simplified Acute Physiology Score II) was evaluated in the first 24 hours.The score consists of a scale of different parametars suchas: age, temperature, pulse, urine output, urea, leukocytes, potassium, sodium, bicarbonates, bilirubin, Glasgow coma scale, AIDS, hematological malignancies and metastatic carcinoma. For example, SAPS 2 score value of 0.43 shows that there is 43% risk of lethal outcome [21].

The data were statistically analyzed with the program SPSS for Windows 13.0, using relevant statistical methodologies. Distribution of frequencies (absolute and relative incidence) was used for qualitative parameters. Descriptive methods such as mean, median and mode were used for mean and typical values of data as well as measures of declination, standard deviation and standard error. For testing the significance of the differentce between certain analyzed factors parametric tests (t-test for independent samples, Analysis of Variance) were used and non-parametric tests for independent samples

(Mann-Whitney U test, Chi-square test, Fisher-exact test). Regarding determination of prognostic factors of death in patients with influenza the method of multivariate analysis was used (Logistic Binary Regression), by which the relation of probability of exposure (OR) was determined as an approximate value of the real risk (RR). Statistical precision (OR) was obtained by calculation of the confidence intervals (CI) about the estimated values. The value of $p<0.05$ was considered to be statistically significant, and the value of $p<0.01$ highly significant.

Results

The study included 122 patients (87 with severe vs. 35 mild form) of clinically and laboratory confirmed influenza from whom12 (9.83%) died, all suffering from a severe form of the disease. Virus type A was detected in 116(95.08%) patientsand the remaining6 (4.92%) patients had virus type B. Subtype of influenza Avirus was detected in only 72(62.07%) patients. Subtype H3N2 was detectedin 13(48.15%) patients with mild influenza andin 29(64.44%) with a severe form of influenza. Subtype H1N1 was detected in 14(51.85%) patients with mild and 16(35.56%) with severe influenza.

Table 1 shows general characteristics of patients noted on admission. Males were dominant in both groups (65.71% and64.37%; p=ns). Patients with severe influenza were older than those with a mild form of the disease (54.8±17.3 vs. 47.1±14.8 years; p=0.023). The majority of patients in both groups consisted of urban patients (90.98%).

It is also importantthat patients who suffered from a severe form of the disease asked for medical treatment with delay in comparison to patients who suffered from mild influenza. About 50% of patients with severe influenza sought medical treatment after 5 days or even later.

Table 1. General characteristics of patients regarding severity of illness

Variable	Total n = 122	Influenza Mild n = 35	Severe n = 87	p value
Type of influenza virus [n (%)]				
A	116(95.08)	35(100)	81(93.1)	[c]0.18
B	6(4.92)	0	6(6.9)	
Gender [n (%)]				
Female	43(35.25)	12(34.29)	31(35.63)	
Male	79(64.75)	23(65.71)	56(64.37)	[a]0.889
Age (mean±SD)				
	52.6±16.9	47.1±14.8	54.8±17.3	[b]0.023*
Place of living [n (%)]				
City	111(90.98)	33(94.29)	78(89.66)	[a]0.42
Village	11(9.02)	2(5.71)	9(10.34)	
Days prior to admission (medianIQR)				
	median 2(4-7)	median 3(1-4)	5(3-7)	[d]0.00006**
Use of osaltamivir prior to admission [n (%)]				
no	112(91.8)	34(97.14)	78(89.66)	[c]0.28
yes	10(8.2)	1(2.86)	9(10.34)	
Comorbidities [n (%)]				
no	43(35.25)	21(60)	22(25.29)	[a]0.000008**
yes	79(64.75)	14(40)	65(74.71)	
Cardiovascular diseases [n (%)]				
no	71(58.2)	27(77.14)	44(50.57)	[a]0.007**
yes	51(41.8)	8(22.86)	43(49.43)	
Pneumonia [n (%)]				
no	24(19.67)	13(37.14)	11(12.64)	[a] 0.002**
yes	98(80.33)	22(62.86)	76(87.36)	

[a]p (Chi-square test) [c]p (Fisher exact test) [d] (Mann-Whitney U test)[b]p(Student's t-test)
*p<0.05 **p<0.01

Only 10 patients used oseltamivir before admission and almost all had severe influenza. Of these, only 5 began treatment in the first 48 hours of the illness. In 79 (64.75%) patients comorbid conditions were noted, which were significantly more commonin those with a severe form of the disease (74.71% vs. 40%; p=0.000008). Chronic cardiovascular diseases were the most common comorbid conditions and were observedin 51(41.8%) patients, of whom 8(22.86%) had mild and 43(49.43%) had a severe form of the disease; (p= 0.007). All of the other comorbid conditions remained insignificantly associated with the severity of the disease. Chronic pulmonary disease was found in15 (12.3%) patients; 2 (5.71%) had mild influenza and 13(14.94%) had severe influenza (p= 0.23); 19(15.57%) patients had endocrine disease, of whom 2(5.71%) were categorized in themild and 17(19.54%) in thesevere (p=0.057) influenza; hematological disease were insignificantly more frequent in patients with severe influenza (6.9% vs. 2.86%; p=0,67). Accompanying neurological, renal, immunological and

liver diseaseswerenoted only in those patients with severe influenza (11.49%, 5.57%, 1.15% and 1.15%, respectively). There was only one patient with a malignant disease (suffering from mild influenza), 4 pregnant patients (2 with severe and 2 with mild), and 3 obese patients (1 had mild and 2 had severe form of influenza).

Pneumonia was present in 98(80.33%) patients, and was foundsignificantly more frequently in patients with severe influenza [76(87.36%) vs.22(62.86%; p=0.002)] (Table 1). The analysis conducted to demonstrate association between the symptoms and severity of the illness showed that the severe form of influenza wasmore frequently present along with rhinorrhea (20.69% vs.2.86%; p=0.014), dyspnea (65.52% vs.25.71%; p=0.00007), cyanosis (present only in those with severe influenza (24.14%; p= 0.0014), pulse>80/min(89.66% vs. 60%; p=0.0015), respiration>20/min (80.46% vs. 48.57% p=0.0004) (Table 2).

Table 2. Symptoms and vital signsregarding severity of illness

Variable	Influenza			p value
	Total n = 122	Mild n = 35	Severe n = 87	
Rhinorrhea [n (%)]				
no	103(84.43)	34(97.14)	69(79.31)	[a]0.014*
yes	19(15.57)	1(2.86)	18(20.69)	
Sore throat [n (%)]				
no	103(84.43)	25(71.43)	78(89.66)	[a]0.012*
yes	19(15.57)	10(28.57)	9(10.34)	
Dyspnea [n (%)]				
no	56(45.9)	26(74.29)	30(34.48)	[a]0.00007**
yes	66(54.1)	9(25.71)	57(65.52)	
Cyanosis [n (%)]				
no	101(82.79)	35(100)	66(75.86)	[a]0.0014**
yes	21(17.21)	0	21(24.14)	
Temperature>37.8° [n (%)]				
on admission	89(72.95)	28(80)	61(70.11)	[a]0.27
Pulse/min. >80 [n (%)]				
on admission	99(81.15)	21(60)	78(89.66)	[a]0.0015**
Respiration/min>20 [n (%)]				
on admission	87(71.31)	17(48.57)	70(80.46)	[a]0.0004**

[a]p (Chi-square test) [b]p(Student's t-test) [c]p (Fisher exact test) *p<0.05 **p<0.01

Table 3 demonstrates laboratory and biochemical parameters presented upon patient admission.The following parameters weresignificantly more frequently registered in patients with thesevere form of the disease: anemia with hematocrit level<35% (26.44 vs.8.57%; p=0.001),

Table 3. Laboratory-biochemical parameters and SAPS 2score regarding severity of illness

Variable on admission	Influenza			p value
	Total n = 122	Mild n = 35	Severe n = 87	
Leukocytes x 10_9/L > 9				
	50(40.98)	7(20)	43(49.43)	[a]0.0028**
Neutrophils (%)				
<0.54	12(9.84)	3(8.57)	9(10.34)	[a]0.04*
>0.66	98(80.33)	24(68.57)	74(85.06)	[c]0.005**
Hematocrit (%) <35				
	26(21.31)	3(8.57)	23(26.44)	[a]0.001**
Glucosemmol/L >6.3				
	68(55.74)	13(37.14)	55(63.22)	[a]0.0087**
Ureammol/L >8.3				
	31(25.41)	4(11.43)	27(31.03)	[a]0.024*
CPK **U/L>170**				
	50(40.98)	8(22.86)	42(48.28)	[a]0.0098**
LDH U/L>618				
	67(54.92)	11(31.43)	56(64.37)	[a]0.00094**
Albuming/L <35				
	69(56.56)	9(25.71)	60(68.97)	[a]0.000013**
SAPS 2 score (mean±SD) median (IQR)				
	32.3±28 med 22(16-37)	22.1±22.3 med 18 (13-21)	36.4±29.1 med 26 (17-42)	[d]0.0002**

[a]p (Chi-square test) [c]p (Fisher exact test) *p<0.05 **p<0.01

leukocytes level >9 x10$_9$/L(49.43% vs. 20%; p=0.0028), neutrophils level <54% and >66%; (p=0.04 and p=0.005), glucose values>6.3 mmol/L(63.22% vs. 37.14%; p= 0.0087), urea values>8.3 mmol/L (31.03%vs 11.43%; p=0.024), creatine phosphokinase-CPK (48.28% vs. 22.86%; p=0.0098), lactate dehydrogenase-LDH (64.37% vs.31.435; p=0.00094), albumin level<35 g/L (68.97% vs. 25.71%; p=0.000013).

The value of SAPS 2 score showed a significant association with the severity of the disease. It was significantly lower in patients with severe influenza (p=0.0002).

The variables which in the univariateanalysis were significantly associated with the severity of the disease were included in the multivariatelogistic regression analysis so that the independent predictors of severe influenza could be determined.

The results of this analysis showedthe following variables to beindependent predictors for severe influenza: cardiovascular diseases (p=0.01), dyspnea (p=0.001), respiration more than 20 perminute (p=0.005), value of LDH greater than 618 U/L (p=0.048), and SAPS 2 score (p=0.031).

Patients with influenza and a cardiovascular disease had 2.964 times greater chance of developing severe influenza in comparison to those without a history of such a disease (OR=2.964 95% CI 1.382-6.370), patients with dyspnea 3.056 times (OR=3.056 95% CI 1.87-7.2), and respiration more than 20 per minute, 2.17 times (OR=2.17 95% CI 1.529-6.187) greater chance.

Patients with LDH values greater than 618 U/Lhad 1.706 times greater chance of developing severe influenza in comparison to those with normal LDH levels (OR= 1.706 95% CI 1.014-3.224). Increase in SAPS 2 score for one score increases the chance for severe influenza- 8.7% (OR=1.87 95% CI 1.23-2.98) (Table 4).

Table 4. Multivariate logistic regression analysis for prediction of severe form of influenza

Variable	Adjusted OR 95% CI for OR	p value
Cardiovascular	2.964 (1.382-6.370)	0.01*
Dyspnea	3.056 (1.87-7.2)	0.001**
Respirations>20	2.17 (1.529-6.187)	0.005**
LDH > 618 U/L	1.706 (1.014-3.224)	0.047*
SAPS 2score	1.87 (1.23-2.98)	0.031*

ROCanalysis showed that cardiovascular diseases, dyspnea, tachypnea, LDH values greater than 618 U/L, and SAPS 2 score in combination acted as good indicators in distinguishing between severe and mild influenza. The area under the ROCcurve, AUC, was 0.826, with 95% confidence intervalof 0.738-0.914, which suggests that a combination of these predictors yields a probability for developing severe influenza at a rate of 82.6%.

Global precision of this predictive model to foresee a severe form of influenza was 81.1%, with a sensitivity of 88.5%, and specificity of 72.9% (Figure 1).

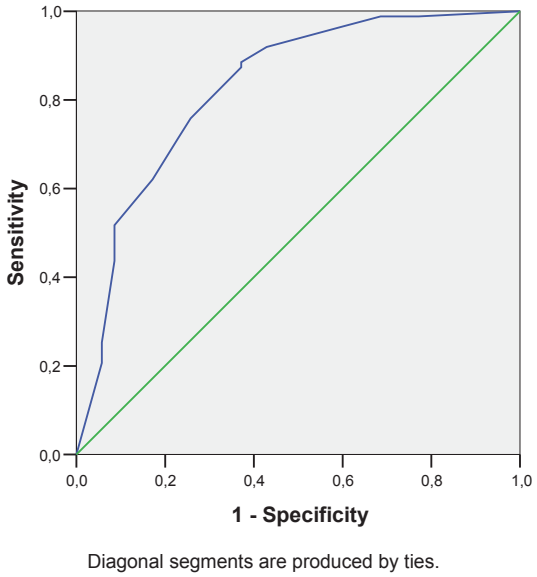

Fig. 1. ROC curve for the influence of cardiovascular diseases, dyspnea, tachypnea, LDH and SAPS score in prediction of severe form of influenza

Discussion

Determining the severity of the illness at the very admission has a significant role in early detection of seriously affected patients, allowing for prompt and suitable medical intervention and administration of proper treatment in order to ultimately improve the outcome of the illness. So far, a large number of studies have been published presenting the factors associated with lethal outcomes, but only a few studies have focused on determining the factors associated with a severe clinical form of influenza [7,22-25]. Our study has shown that routinely analyzed demographic, clinical and biochemical parameters inpatients with seasonal influenza can be used to predict the severity and the outcome of the illness. In terms of gender, males were predominant in both groups (65.71% and 64.37%), a result similar to most studies identifyingmale gender as a risk factor for influenza [26]. In a study conducted in Canada, out of 29 lethal outcomes 27.6% were males, whereas 72.4% females, which is a completely different result compared to the previously mentioned one [27].

Age as theanalyzed demographic data in our study has had a significant influence on the illness outcome. The average age of the patients in the group with severe influenza was 54.8±17.3 and it was significantly higher than the average age of the patients in the group with mild influenza 47.1±14.8 (p=0.023). It is this fact as well as data from a large number of studies that point out adult patients as a vulnerable group, with a risk to progress into a severe form of the illness and/or/death. Thus, certain preventive and therapeutic measures in primary and secondary health protection may have significant influence on the outcome [28,29]. Yet, age in our multivariate analysis was not set apart to be an in-

dependent predictor for a severe form of influenza. Furthermore, the patients' place of residence did not influence on the outcome, although majority ofpatients [111 (90.98%)] came from an urban environment.

The use of neuraminidase inhibitors for 48 hours since the start of the symptoms according to many studies lowers the risk of progression into a severe form or death ofpatients with influenza. In addition, the World Health Organization (WHO) recommends early treatment with oseltamivir even insuspicious cases of influenza, since the delayed medical treatment increases the mortality rate [17,26]. In our study 112(91.8%) patients did not use oseltamivir before admission, whereas only 10(8.2%) used this drugand almost all of them belong to the group with severe influenza. Only 5 of these patients started using therapy in the recommended 48 hours since the onsetof the symptoms. There wasno statistically significant difference in terms of the illness severity between those patients who did use and those who did not use oseltamivir before admission (p=0.28). The answer to this can probably be found in two significant issues. The first refers to the small group of patients included in this study, who used oseltamivir; this fact is at the same time a limitation of this study. The second issueis that some forms of viruses are resistant to oseltamivir [29].

The mortality rate of hospitalized patients with severe influenza in our study was 9.83%. The mortality rate percentage differs between the published studies which certainly depends on different conditions and criteria under which patients are being analyzed, as well as on the criteria for admission to intensive care [15-17]. Thus, in the study conducted in China, out of 60 patients with a severe form of influenza 44% were treated in an intensive care, and the death rate was 14,7% [30].

Several studies suggest that a high body temperature is one of the most frequent symptoms and initial presentation in more than 80% of the cases with influenza [26,31]. In our study 110(90.16%) patients had body temperature higher than 37.8 degrees, with almost equal representation in both groups. The other patients did not have elevated body temperature since they received antipyretic therapy on initial clinical presentation, but it did not exclude influenza. In terms of clinical symptoms and signs of the illness in our study the following statistically significant clinical parameters, which indicated severe influenza onadmission, had been affirmed: dyspnea, cyanosis, with registered cyanotic patients only in the group with severe influenza, tachycardia and tachypnea. In the study of Damak H. *et al.* From 2011, in which 32 adult patients with severe influenza were analyzed, dyspnea and tachypnea had beenobservedas significant factors for a severe form of the illness (88% against 36%), which is in agreementwithour findings (65.52% against 80.46%). Cyanosis had been presenting 8% of patients unlike our study where on admission 24.4% of patients were cyanotic. The difference is most likely due to the difference in the number of severely affected patients and stricter criteria [32].

With regard to laboratory biochemical parameters, our study has pointed out the following indicators for severe influenza: hematocrit <35%, leucocytesabove $9x10_9$/L, neutrophilsunder 54% and above 66%, urea values >8.3 mmol/L, creatine phosphokinase-CPK, lactate dehydrogenase-LDHand albumin level <35 g/L, but multivariate analysis has shown only LDH as an independent variable. The study conducted in Shanghai, China in 2009, whichanalyzed 68 patients, the following factors were shown to be significant for severe influenza and death outcome: lymphopenia, total number of leukocytes, increased level of lactate dehydrogenase, low albumin levels and C-reactive protein. The multivariate model for severe outcome disclosed only LDH and CRP as independent variables [30]. Some studies stress the role of CPK and neutrophils, However, in studies analyzing children CRP is not indicated as a risk factor for severe clinical form of the illness. This is probably due to the specific immunological response of children and the difference in time of CRP synthesizing, which takes 6-12 hours, unlike neutrophilswhich have been verified as significant in the multivariate analysis, most likely because of their faster synthesizing [33]. The increase of urea levelsin our study has been a significant factor for severe influenza; however, multivariate analysis it did not show significance. In our study lactate dehydrogenaseof all laboratorybiochemical parameters has shown to be the most significant factor using the multivariate model. In the literature intensive rhabdomyolysis has been presented as a complication of influenza, which leads to increase of CPK and LDH. This is a result of myositis and is the cause of intensive myalgiaand muscular weakness in patients. The mechanism of intensive rhabdomyolysisis not yet enough elucidated, but it is assumed that it is direct virus invasion of the muscle structure or an immunological response [32-35].

The severity of the illness on admission was evaluated through SAPS2 score and in our study it was set apart as significant in the multivariate analysis. There was an established correlation between the illness severity and the measured valuables of SAPS2 score which correlates with majority of studies that have analyzed these predictive scores [21,36].

Conclusion

This study has defined a prognosis model of 4 independent variables: dyspnea, tachypnea, increased LDH levelsand SAPSscore which predict the illness severity at the very admission with global accuracy of 81.1%.

Early detection of the indicators of the illness severity in patients with seasonal influenza will allow implementation of appropriate medical intervention which will in turn decrease mortality rates.

Conflict of interest statement. None declared.

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Original article

EVALUATION OF PANFUNGAL MARKER (1,3)-β-D-GLUCANIN DIAGNOSIS OF INVASIVE INFECTIONS WITH *CANDIDA* SPECIES

ЕВАЛУАЦИЈА НА ПАНФУНГАЛНИОТ МАРКЕР (1,3)-B-D-ГЛИКАН ЗА ДИЈАГНОЗА НА ИНВАЗИВНИ ИНФЕКЦИИ СО *CANDIDA* SPECIES

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Apstract

Introduction. Although blood culture is considered a gold standard in diagnosis of invasive infections, it is still not reliable and fast enough for diagnosis of candidemia. Determination of serum (1,3)-β-D-glucan is a highly sensitive and specific test for invasive mycosis, and could probably be of benefit to patients with high risk for invasive infections with *Candida* species.
Aim. The aim of this study was to prospectively evaluate the diagnostic performance of serum (1.3)-β-D-glucan BDG (Fungitell) assay, in comparison with blood culture, for diagnosis of invasive infections with *Candida* species.
Methods. Blood and sera from 120 patients divided in 4 groups, hospitalized at the University clinics in Skopje, during a 2-year period, were investigated for invasive *Candida* infections. Blood was examined with conventional methods (automated BacT/Alert system, Gram stain and culture on fungal media). Identification of *Candida* species was performed with VITEK-2 system. Serum (1,3)-β-D-glucan was determined by means of Fungitell assay.
Results. Positive blood culture was registered in 23.33%, 43.33%, 23.8% and in 3.33% of samples only in groups I, II, III and IV, respectively. Positive findings with (1,3)-β-D-glucan Fungitell assay at the same time with blood culture were detected in 83.33%, 76.67%, 30% and 26.67% in groups I, II, III and IV, respectively. The average concentration of BDG was highest in group I, followed by group II, group IV and group III.
Conclusion. Our results suggest that a positive (1,3)-β-D-glucan assay could be a superior test in addition to the blood culture for diagnosis of candidemia and highlights the value of this test as a diagnostic adjunct in the serodiagnosis of an invasive candidiasis.

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Keywords: *Candida*, candidemia, blood culture, (1,3)-β-D-glucan, serodiagnosis

Апстракт

Вовед. Хемокултурата, иако се смета за златен стандард во етиолошката дијагноза на инвазивните инфекции, сè уште не е сигурен и брз метод за дијагноза на кандидемија. Одредувањето на серумскиот (1,3)-β-Д-гликан е високо сензитивен и специфичен тест за инвазивни микози, кој може да биде од голема корист за лицата со висок ризик за инвазивни инфекции со *Candida* species.
Цел. Целта на оваа студија епроспективно евалуирање на дијагностичкиот потенцијал на серумскиот (1,3)-β-Д-гликан, во споредба со хемокултурата, за дијагноза на инвазивни инфекции со *Candida* species.
Методи. Крв и серум од 120 пациенти класифицирани во четиригрупи, хоспитализирани на универзитетските клиники во Скопје, во период од две години, беа испитувани за инвазивни инфекции со *Candida* species. Крвта беше обработувана со конвенционални методи (автоматизиран БацТ/Алерт систем за хемокултури, боење по Грам и култура на фунгални подлоги). Идентификацијата на *Candida* species се изведуваше со ВИТЕК-2 системот. Серумскиот (1,3)-β-Д-гликан се одредуваше со Фунгителл тестот.
Резултати. Позитивна хемокултура беше консеквентно регистрирана во 23.33%, 43.33%, 23.8% и 3.33% примероци во групите I, II, III и IV. Позитивен наод на (1,3)-β-Д-гликан истовремено со хемокултурата, Фунгителл тестот детектираше кај 83.33%, 76.67%, 30% и 26.67% во групите I, II, III и IV, консеквентно. Просечната концентрација на БДГ беше највисока во групата I, следена од групите II, IV и III.

Заклучок. Нашите резултати укажуваат дека позитивен (1,3)-β-Д-гликан може да биде супериорен тест, покрај хемокултурата за дијагноза на кандидемија, и ја истакнува користа од овој тест како дијагностичка поддршка во серодијагнозата на инвазивната кандидијаза.

Клучни зборови: *Candida*, кандидемија, хемокултура, (1,3)-β-Д-гликан, серодијагноза

Introduction

The incidence of invasive fungal infections (IFI) caused by *Candida* species has dramatically increased over the last decades, despite the availability of effective treatments, and is directly associated with increased morbidity and mortality [1], especially among the growing population of immunocompromised patients or patients receiving critical care in intensive care units (ICUs) [2]. The most frequent predisposing factors for development of invasive fungal infections are prolonged stay in ICUs, broad spectrum antimicrobial agents, prolonged use of corticosteroids, chemotherapy and radiotherapy, prematurity, intravascular catheters and parenteral nutrition, immunosuppression and disruption of mucous membranes, and HIV/AIDS are among [1]. *Candida* species are ranked on the 4th position as agents of nosocomial septicemia in many studies across USA, and cause approximately 9-12% of all septicemias [3] and on the 6th or 7th position as causes of nosocomial septicemia in many European studies [4]. To achieve a favorable prognosis of these deadly infections, an early initiation of an antifungal treatment is necessary. It relies on a timely and accurate diagnosis, which in turn is still challenging. In the absence of specific signs and symptoms, there is a need to evolve an appropriate diagnostic approach. Histopathologic demonstration of organisms in tissue specimens or growth of fungal agents in culture media is still the "gold standard" method, but obtaining such specimens may be difficult, and conventional microbiological methods' results (blood culture) are relatively insensitive, since they are positive in less than 50% of all invasive *Candida* infections, are time-consuming and not generally accessible in all laboratories [5]. Therefore, diagnosis of invasive fungal infections remains challenging, due to a limited choice of sensitive early diagnostic markers. As a consequence of the difficulties with diagnosis, a significant effort has gone into developing non-culture-based diagnostic techniques for detecting invasive candidiasis [6]. Recently, particular emphasis has been placed on the detection of fungal markers within biological samples. Among possible culture-independent serum markers, *Candida* mannoproteins (13), and (1,3)-β-D-glucan (BDG) offered some promise [7,8].

(1,3)-β-D-glucan (BDG) is a panfungal marker that is a component of the cell wall (cell wall polysaccharide) found in many pathogenic fungi, including *Candida* species, which can be present early in the blood and fluids from patients suffering from IFI. Serum β-D-glucan concentrations show a constant rise before clinical and microbiological evidence of infections, then decrease, and eventually become negative if patients respond to antifungal therapy. Conversely, patients not responding do not show a decrease or show a continuous rise. The Fungitell test (Associates of Cape Cod) is a chromogenic kinetic test that was approved in 2003 by the U.S. Food and Drug Administration for the presumptive diagnosis of IFI [9]. It may allow earlier diagnosis of IFI, which is otherwise feasible with traditional methods.
The aim of this study was to prospectively evaluate the diagnostic performance of serum (1,3)-β-D-glucan BDG (Fungitell), in comparison with blood culture, for diagnosis of invasive infections with *Candida* species.

Study design

A prospective diagnostic study was performed at the Institute of Microbiology and Parasitology, Medical Faculty, Skopje, during a 2-year period, from March 2012 until May 2014.

Material and methods

This study analyzed primarily sterile specimens (blood and serum) from 120 patients classified in 4 different groups according to the clinical diagnosis and risk factors for development of invasive candidiasis (group I (n=30)-patients with primary immune deficiency, group II (n=30)-patients with prolonged stay in ICU receiving broad spectrum antibacterial treatment, group III (n= 30)-patients with mucosal candidiasis and group IV (n=30)-cystic fibrosis patients). Invasive fungal infection was defined according to the revised definitions by the EORTC/MSG (European organization for research and treatment of cancer/mycoses study group) consensus group, with the necessary modification that BDG was not included in the microbiological criteria [10]. Blood culture was performed with automated BacT/Alert system (*bioMerieux*, France), Gram stain and culture on selective Sabouraud and CALBmedium (Oxoid). Identification of *Candida* species was performed with automated VITEK-2 system (*bioMérieux,* France) [11]. Serological (1,3)-β-D-glucan detection was performed with Fungitell assay (Associates of Cape Cod) (9). Briefly, total of 5 µl of serum were briefly pretreated with 20 µl alkaline reagent solution (0.125 M KOH/0.6 M KCl) for 10 min at 37°C and then 100 µl reconstituted Fungitell reagent was added to the sample placed into triplicate wells of a 96-well microtiter plate.The reaction was incubated for 40 min at 37°C and the optical density was measured at 405/490 nm with a spectro-

photometer. The mean rate of optical density change was determined for each well, and the BG concentration was determined by comparison to a standard curve. Interpretation of BG values was as follows: <60 pg/ml, negative; 60 to 79 pg/ml, indeterminate; ≥80 pg/ml, positive. The test results of the BDG assay were not available for the clinician for decision making (BG results were not used for the management or classification of IFI). Proven and probable IFI were considered to be true-positive cases for analysis. Patients with possible IFI were considered to have true-negative cases.

Results

According to the gender structure of the participants in our study, men were more frequently identified in groups I, III and IV (60%, 56.67%, 53.33%, respectively), and women in the group II (56.67%). The average age of patients with primary immune deficiency was 39.13± 21.8 years, 65.63±29.8 of patients in the second group, 51.91±18.4 of the third group of patients and 16.23± 6.4 years of the fourth group of patients (Table 1).

Table 1. Characteristics of patients according to age and gender

Variable	Groups of patients				
	group I n=30 (%)	**group II n=30 (%)**	**group III n=30 (%)**	**group IV n=30 (%)**	**Total n=120 (%)**
Gender					
women	12(40%)	17(56.67%)	13(43.33%)	14(46.67%)	56(46.7%)
men	18(60%)	13(43.33%)	17(56.67%)	16(53.33%)	64(53.33%)
Age					
years, mean±SD	39.13±21.8	65.63±29.8	51.91±18.4	16.23±6.4	43.0±24.1
min-max	3-68	21-83	18-93	7-30	3-82
[a]*p = 0.6*					

p (Chi-square test)

Distribution of the examined participants in all groups, according to clinical diagnosis with EORTC/MSG criteria, showed that a proven fungal infection was most frequently registered in patients with prolonged ICU stay receiving a broad spectrum antibiotic treatment (56.67%), followed by patients with primary immune deficiency (33.33%) (Table 2). Differences in distribution of proven, probable and possible fungal infection were statistically significant between group I versus groups III and IV, and between group II versus groups III and IV.

Table 2. Characteristics of patients according to EORTC/MSG criteria classification

Variable	Groups of patients				
	group I n=30 (%)	**group II n=30 (%)**	**group III n=30 (%)**	**group IV n=30 (%)**	**Total n=120 (%)**
Classification					
proven	10(33.33%)	17(56.67%)	0	1(3.33%)	28(23.33%)
probable	12(40%)	11(36.67%)	6(20%)	8(26.67%)	37(30.83%)
possible	8(26.67%)	2(6.67%)	24(80%)	21(70%)	55(45.83%)
	Chi-square: 54.08[a]*p*< 0.001				
	I vs II ns		II vs III *p*< 0.001		III vs IV ns
	I vs III *p*< 0.001		II vs IV *p*< 0.001		
	I vs IV *p*< 0.0009*				

Regarding the distribution of host factors, 53.33% of patients in group I had hematological malignancy, 30% had AIDS; 10% presented with hypogamaglobulinemia and 3.33% each had chronic granulomatous disease or CARD9 deficit. In group II, host factors distribution was: neonatal sepsis (16.66%), abdominal surgery (13.33%), solid organ cancer (13.33%), diabetes mellitus (13.33%), neonatal meningitis (10%), sepsis in pediatric ICU (6.66%), urosepsis (6.66%), renal failure with hemodialysis (6.66%), endocarditis (6.66%), pancreatitis with diabetes mellitus (3.33%), and burns (3.33%). In group III, 56.66% of patients had esophageal candidiasis with comorbidities like COPD, asthma, obesity, diabetes mellitus, alcoholism, ulcus disease and cancer, and 43.33% ICU patients with diabetes mellitus as comorbidity, presented with signs and symptoms of candiduria. In group IV, CF patients with acute exacerbations were analyzed.

The blood culture was positive in 23.33% patients in group I, 43.33% in group II, 23.08% in group III and one patient in group IV. The statistical analysis confirmed that positive blood culture was a significantly less frequent finding in patients with cystic fibrosis, compared to both groups of patients with primary immune deficiency (p=0.023), and patients with prolonged ICU stay and antibiotic treatment (p=0.00025). The most frequent isolates from blood culture in group I were *C. tropicalis* and *C. krusei*, followed by *C. albicans* and *C. parapsilosis.* In group II, *C. albicans* and *C. pelliculosa* were equally presented, followed by *C. parapsilosis* and *C. Glabrata* (Table 3). Positive findings with (1-3)-β-D-glucan Fungitell BDG assay at the same time with

Table 3. Yeast species in blood culture

Variable	Groups of patients				
	group I n=30 (%)	group II n=30 (%)	group III n=30 (%)	group IV n=30 (%)	Total n=120 (%)
Blood culture					
negative	23(76.67%)	17(56.67%)	10(76.92%)	29(96.67%)	79(76.7%)
positive	7(23.33%)	13(43.33%)	3(23.08%)	1(3.33%)	24(23.3%)
	Chi-square: 13.4 p = 0.0038**				
	I vs IV p= 0.023*				
	II vs IV p= 0.00025**				
C.parapsilosis	1	2	0	0	3
C.tropicalis	3	1	0	0	4
C.albicans	1	4	1	1	7
C.krusei	2	0	0	0	2
C.pelliculosa	0	4	0	0	4
C.glabrata	0	2	2	0	4

p (Chi-square test) *p < 0.05 **p< 0.01

Table 4. Characteristics of patients according to positivity of (1-3)-β-D-glucan test

Variable	Groups of patients				
	group I n=30 (%)	group II n=30 (%)	group III n=30 (%)	group IV n=30 (%)	Total n=120 (%)
(1-3)-β-D-glucan test					
negative	5(16.67%)	5(16.67%)	21 (70%)	19(63.33%)	50 (41.66%)
positive	25(83.33%)	23 (76.67%)	9 (30%)	8 (26.67%)	65 (54.16%)
intermediate	0	2 (6.67%)	0	3 (10%)	5 (4.16%)
	[a]p<0.001				
	I vs III [a]p=0.00003**			II вс III [a]p=0.000066**	
	I vs IV [a]p=0.00004**		II вс IV [a]p=0.00009**		

[a]p (Chi-square test) **p<0.01

blood culture were detected in 25/30 (83.33%) patients of group I, 23/30 (76.67%) of group II, 9/30 (30%) of group III, and 8/30 (26.67%) patients of group IV. Described differences were confirmed as statistically significant between group I and group III (p=0.00003), and between group I and group IV (p=0.00004), and also between group II and group III (p=0.000066), and between group II and group IV (p=0.00009) (Table 4).

In the statistical analysis, intermediate results were not included.

In Table 5 the concentrations of the (1-3)-β-D-glucan marker (BDG) are presented. At the same time with blood culture, statistically significantly higher concentration of BDG was obtained in group I compared to group III (p=0.000008) and group IV (p=0.000008), and, statistically significantly higher concentration of

Table 5. Descriptive statistics BDG concentration (pg/ml)

	Descriptive statistics BDG concentration (pg/ml)			
	mean ± SD	min-max	median (IQR)	*p*-value
group I	184.77 ± 106.9	33 - 447	177 (117 – 268)	H=44.4 [d]p<0.001
group II	164.43 ± 98.9	38 - 378	148 (88 – 218)	I vs III[c]p=0.000008**
group III	59.87 ± 27.5	34 - 124	46 (41 – 88)	I vs IV[c]p=0.000008**
group IV	61.17 ± 27.3	25 - 133	47 (41 – 85)	II vs III[c]p=0.0000029** II vs IV[c]p=0.0000028**

[c]p (Mann0Whitney U test) [d]p (Kruskal-Wallis test)

BDG was obtained in group II compared to group III (p=0.0000029) and group IV (p=0.0000028). The average concentration of BDG was highest in group I (184.77±106.9 pg/ml), followed by group II (164.43± 98.9 pg/ml), group IV (61.17±27.3 pg/ml), and group III (59.87±27.5 pg/ml). The median concentration of BDG was 177 pg/ml (range117-268), 148 pg/ml (range 88-218), 46 pg/ml (range 41-88), and 47 pg/ml (range 41-85) in all four groups, respectively.

Positive BDG test in group I was registered in 25 (83.33%) patients' sera. The concentration of BDG in the positive sera was in the range 82-447 pg/ml. The average concentration of BDG was 214±91.9 pg/ml. Sensitivity, specificity, PPV, NPVof BDG were100%, 62.5%, 88%, 100%, respectively (Table 6).

Positive BDG test in group II was registered in 23 (76.67%) patients' sera. The concentration of BDG in the positive sera was in the range 88-378 pg/ml, with an

Table 6. Comparative diagnostic performances of blood culture and (1-3)-β-D-glucan test in group I

Method	Se(%)	Sp(%)	PPV(%)	NPV(%)
Blood culture	31.82	100	100	34.78
β-D-glucan test	100	62.5	88	100

Table 7. Comparative diagnostic performances of blood culture and (1-3)-β-D-glucan test in group II

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Blood culture	46.43	100	100	11.76
β-D-glucan test	88.46	100	100	40

average concentration of 197.74±88.6 pg/ml. Sensitivity, specificity, PPV, NPV of BDG were 88.46%, 100%, 100%, 40%, respectively (Table 7).

In group III positive BDG test was registered in 9 (30%) patients' sera. The concentration of BDG in the positive sera was in the range 88-124 pg/ml, with an average concentration of 99.22±12.8 pg/ml. Sensitivity, specificity, PPV, NPV of BDG were 100%, 87.5%, 66.67%, 100%, respectively (Table 8).

In group IV positive BDG test was registered in 8 (26.67%) patients' sera. The concentration of BDG in the positive sera was in the range85-133 pg/ml, with an average concentration of 99.25±16.5 pg/ml (Table 9).

Table 8. Comparative diagnostic performances of blood culture and (1-3)-β-D-glucan test in group III

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Blood culture	0	72.73	0	80
β-D-glucan test	100	87.5	66.67	100

Table 9. Comparative diagnostic performances of blood culture and (1-3)-β-D-glucan test in group IV

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Blood culture				
β-D-glucan test	100	100	100	100

This test did not identify false negative or false positive results in group IV; all 8 cases of CF with positive BDG assay were in the group of probable/proven IFIs according to EORTC/MSG criteria, and all negative BDG cases were classified as possible infections.

Discussion

Early diagnosis of invasive *Candida* infections is crucial in order to initiate antifungal agents early. Delay in the administration of appropriate antifungal treatment increases mortality from invasive candidiasis. Unfortunately, clinical and radiological signs are often unspecific and conventional culture methods are not sensitive enough [6]. To overcome many obstacles during laboratory work, different tests have been developed, which have been evaluated for diagnosis of invasive candidiasis. In this study we have evaluated the performance of a new (1,3)-β-D-glucan (BDG) detection system (Fungitell) as a diagnostic adjunct for invasive fungal infections. Different studies have evaluated clinical performance of the (1,3)-β-D-glucan assay with focus on specific target populations, like hematological [8], pediatric patients [12], or ICU [13]. It has also been proposed as an early biomarker of invasive fungal infections and is included in diagnostic criteria of invasive fungal infections in the 2008 revised IFI diagnosis criteria of European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) [10]. In order to investigate the diagnostic potential of the BDG test to enhance diagnosis of invasive *Candida* infections, we have examined sera from 120 patients that were divided into 4 groups according to the clinical diagnosis and EORTC/MSG criteria, for presence of elevated concentrations of (1,3)-β-D-glucan and compared them with blood cultures. According to EORTC/MSG criteria, a proven fungal infection was most frequently registered in patients with prolonged ICU stay receiving antibiotic treatment with broad spectrum (group II) (56.67%), followed by patients with primary immune deficiency (group I) (33.33%). Differences in distribution of proven, probable and possible fungal infection were statistically significant between group I versus groups III and IV, and especially between group II versus groups III and IV. This is in consistency with the work of Montagna *et al*. [14] where most of the cases with invasive fungal infection tend to occur in older patients (more than 60 years), as was the average age in our group of patients with prolonged stay in ICU setting (65.63±29.8) that presented the highest rate of proven infections with *Candida* species. This can be attributed to the increased incidence of invasive mycoses with advanced age which most often is associated with impaired immunity. In our study 33.32 % of invasive fun-

gal infections in group II were in the NICU (neonatal intensive care unit) and PICU setting (pediatric intensive care unit). Of all the patients in this group, 56.67% had proven and 36.67% had probable fungal infections. Positive blood cultures were identified in 24 patients in all groups. Most episodes of candidemia developed in group I, and were caused by *C. tropicalis* and *C. krusei*, followed by *C. albicans*, *C. parapsilosis* and *C. tropicalis*. In group II, *C. albicans* and *C. pelliculosa* were equally presented, followed by *C. parapsilosis* and *C. glabrata.* In the third group, 3 cases of candidemia were confirmed with positive blood culture (one patient with *C. albicans*, *C. glabrata* in 2 patients). In our CF patients, only one patient had positive blood culture with *C. albicans*. Although *C. albicans* is still considered the most frequent etiological agent of candidemia, in the recent few decades, a significant increase of candidemia caused by non-*albicans Candida* species [15] has been registered, which are usually less susceptible to antifungal drugs. Our study confirms high prevalence of non-*albicans Candida* species candidemia, as has been demonstrated in many reports from around the world [16,17].

The Fungitell assay for detection of (1,3)-β-D-glucan confirmed elevated concentrations of BDG in: group I-25/30 (83.33%), group II-23/30 (76.67%), group III-9/30 (30%), and group IV-8/30 (26.67%). In group I, this test detected 10/10 (100%) of proven and 12/12 (100 %) of probable cases of IFIs and 3/6 (37.5%) in possible infections. In group II, the test detected 13/17 (76.47%) of proven and 10/11 (90.91%) of probable cases of IFIs. In group III, 6/6 (100%) of probable and 3/24 (100%) of possible infections were detected by the assay. In group IV, the Fungitell assay was positive in 8/30 (26.67%) of proven/probable infections. BDG-positive results have been obtained by the Fungitell assay for most of the cases with candidemia caused by different *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*). Interestingly, the BDG values in two cases of proven infection (candidaemia caused by *C. Pelliculosa* in a neonate and one adult case of candidemia with *C. albicans*) did not reach any of these cut-offs and the test remained negative. However, the BDG detection was not performed at the same time with the blood sample as the one that was *Candida* culture positive, although the sample used was collected at the same time.

In this study the sensitivity of the BDG test was 100%, 88.46%, 100%, 100%, in groups I, II, III and IV respectively, compared to sensitivities of 77.6% (18), 86.7% [19], and 100% [20] in other reports for diagnosis of candidemia. Many studies have reported wide discrepancies on sensitivity (40%-100%) and specificity (45%-99%) of the assay [21-23], probably due to the heterogeneity of their designs, and probably because they have used different cut-off values (10 to 120 pg/ml) for a positive result. In a recent study, Pickering *et al.* evaluated 39 sera samples from 15 patients with blood culture positive yeast infections using a cut-off value of 80 pg/ml (19). Thirty (77%) samples were positive for BDG (range 84 to 1359 pg/ml), and 13 of the 15 patients had at least one specimen positive. In a recent multi-center study of 107 patients with proven candidiasis, 81% had a positive result for BDG at a cut-off of 60 pg/ml and 78% had positive results at a cut-off of 80 pg/ml (18). Other studies have also reported low BDG sensitivity in different IFI and various patient populations. Koo *et al*. (24) and Ostrosky-Zeichner *et al*. (18), who both analyzed the performance of the BDG assay, regardless of the category of patients or type of IFI, observed an average sensitivity of 64%. Koo *et al*. (24) reported even lower BDG sensitivity in patients receiving a hematopoietic stem cell transplant or patients with febrile neutropenia (43% and 38%, respectively).

Specificity of the BDG test in our study was 62.5%, 100%, 87.5%, 100%; PPV was 88%, 100%, 66.67%, 100%, and NPV was 100%, 40%, 100% and 100% in groups I, II, III and IV, respectively. Specificity in group I was considerably lower than that reported by Pazos *et al*. [20] for adult patients with hematological cancer (89.6%). On the other hand, Digby *et al*. have reported positive BDG results in patients hospitalized in ICU with proven bacterial infections [26]. Since these investigators used very low (>20 pg/ml) cut-off values for a positive BDG marker, rather than the recommended cut-off (≥80 pg/ml), it is impossible to accurately interpret their results. In this study population, BDG demonstrated high sensitivity, but poor specificity. But, the high negative predictive value in our study (100%) and other studies as well (>95%) [26] show that their primary value, based on a negative result, is to exclude the presence of IFI instead.

False-positive BDG results have been previously described in many studies. Prattes *et al*. found that unmodified cellulose containing membranes increased the serum BDG levels. False-positive BDG results have also been confirmed after mucosal damage from chemotherapy or radiotherapy, which could allow BDG from dietary sources or from *Candida* colonizing the gastrointestinal tract to enter the bloodstream; as well after receipt of antibiotics from fungal sources [22]. False-positive BDG results are possible after treatment with immunoglobulin or antitumoral polysaccharides such as lentinan and schizophyllan [27], which can elevate BDG levels in the absence of IFI. In our study we also had patients on hemodialysis, but we had no relevant details on the background of the treatment, so we could not be certain if some of the elevated BDG levels actually demonstrated false positive results (data not shown). False-positive BDG results have also been described during some bacterial infections [19]. In our study we had few patients who presented with clinical signs of sepsis, with negative blood culture for fungi, but positive blood culture for bacteria (data not shown). There is a high probability that these were polymicrobial

infections that were also described in many studies and could be an additional reason for false positive results of the BDG test [28]. For all these reasons, BDG assay must be used and interpreted with a great caution. This phenolmenon should be carefully evaluated in further studies, where probably a serial monitoring of sera could clarify more precisely these dilemmas about false-positive results.

To summarize, our findings suggest that a positive (1,3)-β-D-glucan (BDG) assay could be a superior test in addition to the blood culture for diagnosis of candidemia and highlights the value of the BDG assay as a diagnostic adjunct in the serodiagnosis of an invasive candidiasis. This test may also be useful in the evaluation of patients at high risk of IFI. In clinical practice, proper use of this test would require knowledge of its performances and features, particularly for the factors associated with a false-positive test result.

Conflict of interest statement. None declared.

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Original article

INFLUENCE OF TRAUMA, SPORT ACTIVITY AND BODY MASS INDEX ON KNEE LESIONS EVALUATED BY MAGNETIC RESONANCE

VLIJANIETO NA TRAUMATA, SPORTSKATA AKTIVNOST I INDEKSOT NA TELESNA MASA NA LEZIITE NA KOLENOTO EVALUIRANI SO MAGNETNA REZONANCA

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Abstract

Introduction. The aim of the study was to analyze effusion, bone edema, joint/articular cartilage, menisci and ligaments in correlation with pain intensity, sport activity and BMI.
Methods. In our prospective study, 261 knee MRIs of patients with acute knee trauma wereanalyzed, who had a negative x-ray of the knee for fracture, and pain lasting for 1 month despite conservative therapy.
Results. Gender distribution: 65.1% male and 34.87% female patients. Majority of subjects had body mass index from 18.5-24.9 (41.76%) The presence of an edema in the medial condyle of the femur was detected in 12.64% of patients. The most common lesion was lesion on the cartilage of the medial condyle on the femur (33.72%). We found lesions of the ACL in 40% of the cases. All patients with a combined trauma to the anterior cruciate ligament and the posterior horn of the medial meniscus had also a bone edema type 1 and type 2.
Conclusion. Age and body weight do not have an impact on the cause of trauma and the type and grade of the knee lesions. Athletes and not athletes have signifycantly different causes of trauma to the knee. Patients with lesions in the posterior horn of the medial meniscus grade 3 had significantly more bone edemas (p=0.013). Localization of the pain is not linked to the cause of injury, but it depends on the developed lesions on the knee.

Keywords: MRI, knee, trauma

Апстракт

Вовед. Цел на студијата еда се евалуира поврзаноста на лезиите на колено видени со магнетна резонанаца, при акутна траума на коленото во однос на интензитетот на болка, спортската активност како и инедксот на телесна маса.
Материјал и методи. Во нашата студијата беа анализирани 261 магнетни резонанци на колено кај пациенти со траума на коленото со негативен радиограм на колено за фрактура и болак која перзистира последниот месец и покрај спроведената терапија.
Резултати. Од 261 пацнент 65,13% беа мажи а 34,87% жени. Најзастапен индекс на телесна маса од 41,76% во групата од 18,5-24,9. Едем на медијалниот кондил на фемур беше присатен кај 12,64% пацинети. Најчеста лезија беше на дрскавицата на медијалниот кондил на фемур 33,72%. Лезија на предниот вкрстен лигамен беше присутна кај 40% од случаите. Коскениот едем тип 1 и тип 2 беше присутен кај комбинираните лезии на предниот вкрстен лигамент и задниот рог од медијалниот менискус.
Заклучок. Возраста и индексот на телесна маса не покажаа сигнификантна поврзаност со траумата и степенот на лезија. Спортистите и не спортистите имаат сигнификантно различен начин на траума. Пациентите со лезија на задниот рог на медијалниот менискус гр. 3 имаат сигнификантно почесто едем (p=0.013). Локализацијата на болката не е поврзана со начинот на траума, таа зависи од настанатите промени во коленото.

Клучни зборови: МРИ, колено, траума

Introduction

The knee joint as a synovial joint is often exposed to trauma which leads to pain in the knee. Magnetic resonance as a noninvasive technique is a method of choice to detect the cause of the pain. Bone edema as an indirect sign often gives us a direction in depicting the lesion, such as lesion of the ligament, meniscal lesion or lesions of the cartilage [1-4]. The structures of the knee which are covered with cartilage or other structures

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are not available for arthroscopic evaluation; before the era of MRI, such lesions were detected only clinically. The aim of this study was to evaluate the correlation between the MRI of the knee and trauma, intensity of pain, sport activity as well as body mass index (BMI) [5-9].

Materials and methods

Our study included 261 patientswhomet our inclusion criteria for participation in this study.
Inclusion criteria-patients with knee trauma,at age from 19-50 years, with negative x-ray of the knee, persistent pain despite conservative treatment.
Exclusion criteria-disagreement to participate in the study, old trauma, surgery of the knee, sickle cell anemia, rheumatoid arthritis or psoriasis.
All patients signed an informnt consent for participation in the study. The study involvesone year prospective analysis ofmagnetic resonance imaging of the knee using an MRI of 1.5 T. The data wasobtainedusing a questionnnaire regarding the injury filled in by the patients [10-13]. The gradation of pain wasdivided into three degrees: 0-(1-3), 1-(4-6) and 2-(7-10).The cause of trauma was evaluated as: during a fall-0, bending of the knee-1, sports injury-2.
The occurrence of pain was graded as: spontaneous pain-0, pain that appearedwith movement-1, pain that appeared when ascending stairs-11, pain when descending stairs-12, locking of the knee-13.
The body mass index is general and will be measured using a table. SPSS Windows 17.0 was used for statistical analysis of the results (χ^2 test $p<0.001$, Fischer's test $p<0.005$).

Results

In our study 261 knee MRIs of patients with acute knee trauma were analyzed, who had a negative x-ray of the knee for fracture, and pain lasting for 1 month despite conservative therapy.
Gender distribution was: 170 (65.13%) male patients and 91 (34.87%) female patients.
Subjects were aged 19-50 years; the average age was 33.77±10.2 years. We analyzed knee joint trauma within three age groups: subjects from 19-29 years, 30-39 years and 40-50 year. In the age group 40-50 years 35.25% of patients had acute trauma to the knee joint [14].
The majority of subjects had a body mass index (BMI) from 18.5-24.9 (41.76%), followed by 37.93% of subjects with an index of 25-29.9, 18.39% of subjects with a BMI greater than 30, and 1,91% with a BMI lower than 18.5. Table 1 shows the results of the relationship between the demographic characteristics of patients with trauma to the knee. The cause of injury in the larger number of subjects was bending (40.23%) [4,9]. The distribution of the type of pain demonstrated that pain with movement was most commonly encountered (72.41%). In terms of localization of the pain, the majority of subjects reported the medial part of the knee (47.13%). 42.14% of subjects had a weak pain, i.e. on a scale of 1 to 10 they answered between 1 and 3, while 16.09% of subjects reported a strong pain, grading it between 7 and 10.

Table 1. Demographic characteristics of patients with trauma to the knee

Variable	
Gender	*n(%)*
Male	170 (65.13)
Female	91(34.87)
Age groups	*n(%)*
19-29	101 (38.7)
30-39	68(26.05)
40-50	92(35.25)
Mean±SD(33.77±10.2)min– max (19–50)	
Height	*n(%)*
160-170	81(31.03)
171-181	102(39.08)
182-201	78(29.88)
Weight	*n(%)*
55-70	68(26.05)
71-86	111(42.53)
87-102	82(31.42)
BMI	*n(%)*
<18.5	5(1.91)
18.5-24.9	109 (41.76)
25-29.9	99(37.93)
>30	48(18.39)

Effusion in the knee was present in 89 (34.1%) of subjects. Bone edema can only be analyzed using a MRI of the knee and its gradation of type 1 and type 2 can help in the prognosis of the evolution of changes [2]. Edema in the medial condyle of the femur was detected in 33 (12.64%) patients, on the lateral condyle of the femur in 42 (16.09%) patients, edema on the medial segment of the tibia plateau in 14 (5.36%) patients, edema on the lateral segment of the tibia plateau was diagnosed in 16 (6.13%) patients. MRI findings showed that edema on the diaphysis of the femur and tibia was present in 21(8.05%) and 7(2.68%) of the analysed cases, respectively. In 30(11.49%) patients with internal distortions of the knee joint, MRI findings showed edema on the medial facet of the patella, and in 16(6.12%) patients an edema was present in the lateral facet on the patella [15-18].
Type 2 edemas were the most common MRI finding on the medial facet on the patella, detected in 20 (7.66%) subjects, followed by the lateral condyle of the femur in 19(7.28%) subjects, medial condyle of the femur in 16 (6.13%) subjects, lateral facet of the patella in 6 (2.29%) subjects, medial segment of the tibia plateau in 5 (1.91%) subjects, lateral segment of the tibia plateau in 2(0.77%) subjects and in one subject (0.38%) the MRI finding showed an edema on the diaphysis of the tibia.

In acute knee trauma, we often see lesions of the cartilage. Gradation of lesions on the cartilage of the knee is conducted according to ICRS classification [19-23].

Table 2. Distribution of a bone edema in knee joint

Variable	
MCF – medial condyle of the femur	*n(%)*
None	228(87.36)
Type 1	17(6.51)
Type 2	16(6.13)
LCF – lateral condyle of the femur	*n(%)*
None	219(83.91)
Type 1	23(8.81)
Type 2	19(7.28)
MET- medial seg. of the tibia plateau	*n(%)*
None	247(94.64)
Type 1	9(3.45)
Type 2	5(1.91)
LET- lateral seg. of the tibia plateau	*n(%)*
None	245(93.87)
Type 1	14(5.36)
Type 2	2(0.77)
DF- femur diaphysis	*n(%)*
None	240(91.95)
Type 1	21(8.05)
DT- tibia diaphysis	*n(%)*
None	254(97.32)
Type 1	6(2.3)
Type 2	1(0.38)
MFP – medial facet of the patella	*n(%)*
None	231(88.51)
Type 1	10(3.83)
Type 2	20(7.66)
LFP – lateral facet of the patella	*n(%)*
None	245(93.87)
Type 1	10(3.83)
Type 2	6(2.29)

Table 3 shows findings from knee MRI regarding detection of lesions on the joint cartilage.

In our study the most common lesion was a lesion on the cartilage of the medial condyle on the femur found in 88(33.72%) patients, on the lateral condyle of the femur in 53 (20.31%), a lesion on the medial aspect of the tibia plateau in 26(9.96%) and lesions on the lateral aspect of the tibia plateau in 7(2.68%) patients [11]. A lesion of the cartilage on the medial facet of patella was detected in 77(2.68%) patients, a lesion on the cartilage of the lateral facet of the patella in 68 (26.05%) patients. With regard to the type of lesion of the articular cartilage, an edema was most frequently diagnosed on the lateral facet of the patella (8.81%), only one patient with acute trauma to the knee had an edema on the cartilage of the medial segment of the tibia plateau.

A ribbed articular cartilage was the most common type of change on the medial and lateral facets of the patella (8.81%).

We found chondral defects of the medial condyle of the femur in 4 subjects, of the lateral condyle of the femur in 3 subjects, on the medial facet of the patella in one subject, while 2 patients had chondral defects on the articular cartilage on the lateral facet of the patella.

Generally, the most common change in the articular cartilage of the knee diagnosed with MRI was a carilage defect <50% of cartilage depth, i.e. 48 (18.39%) patientshad this type of finding on the medial condyle of the femur, 2(9.96%) on the lateral condyle of the femur, 17(6.51%) patientson the medial segment of the tibia plateau, 4(1.53%) on the lateral segment of the tibia plateau, 31(11.88%) on the medial facet of the patella and 17(6.51%) subjects had a defect on the articular cartilage less than 50% of the lateral facet of the patella.

Table 3. Distribution of the lesions on the joint cartilage

Variable	
R –MCF- medialcondyleofthe femur	*n(%)*
None	173(66.28)
Edema	11(4.21)
Ribbed	10(3.83)
h<50%	48(18.39)
h> 50%	15(5.75)
Chondraldefect	4(1.53)
R –LCF –lateralcondyleof the femur	*n(%)*
None	208(79.69)
Edema	14(5.36)
Ribbed	7(2.68)
h<50%	26(9.96)
h> 50%	3(1.15)
Chondraldefect	3(1.15)
R-MET-medialsegmentofthetibia plateau	*n(%)*
None	235(90.04)
Edema	1(0.38)
Ribbed	3(1.15)
H<50%	17(6.51)
h> 50%	5(1.92)
R-LET-lateralsegmentof the tibia plateau	*n(%)*
None	254(97.32)
Edema	2(0.77)
Ribbed	1(0.38)
H<50%	4(1.53)
R –MFP–medialfacetonthe patella	*n(%)*
None	184(70.49)
Edema	8(3.06)
Ribbed	23(8.81)
h<50%	31(11.88)
h> 50%	14(5.36)
Chondraldefect	1(0.38)
R –LFP-lateralfacetonthepatella	*n(%)*
None	193(73.95)
Edema	23(8.81)
Ribbed	23(8.81)
h<50%	17(6.51)
h> 50%	3(1.15)
Chondraldefect	2(0.77)

Besides cartilage lesions of the knee during trauma the menisci are exposed as well [16,22].

In our study, the MRI findings showed a lesion on the anterior horn on the medial meniscus in 34(13.03%) subjects, a lesion on the posterior horn on the medial meniscus in 175(67.05%) subjects, a lesion on the ante-

rior horn of the lateral meniscus in 36(13.79%) subjectsand a lesion on the posterior horn of the lateral meniscus in 58(22.22%) subjects.

Regarding the type of meniscal lesion detected with magnetic resonance, the results showed that the posterior horn on the medial meniscus wasthe most common predilection area for all three types of lesions, i.e. in 22.99% of patients with trauma to the knee MRI findings showed type 1 lesions, i.e.mucoid degeneration, in 13.03% a type 2 lesion, lesion which runs from the capsule horizontally or to one articular surface, and in a high percentage of patients (31.03%) the lesion was of type 3 [12].

The most common injury with lesions on the knee is encountered in the injury of the anterior cruciate ligament (ACL).

Lesions on the ligaments can be accompanied with an edema and thickening, partial lesion and complete lesion. MRI findings of a lesion on the ACL were present in around 40% of subjects, i.e. 106(40.61%), of which 57 (21.84%) had a tear in this ligament [2,3].

The remaining three ligaments were affected more rarely, 17(6.51%) subjects had an injury to the posterior cruciate ligament, 11(4.22%) had a MRI finding for a trauma to the medial collateral ligament and 7(2.68%) had an injury to the lateral collateral ligament of the knee (Table 4).

Table 4. Distribution of lesions of the ligaments of the knee

Variable	
ACL Anterior cruciate ligament	*n(%)*
None	155(59.39)
Edema	12(4.59)
Partial	37(14.18)
Tear	57(21.84)
PCL Posterior cruciate ligament	*n(%)*
None	244(93.49)
Edema	5(1.91)
Partial	12(4.6)
MCL Medial collateral ligament	*n(%)*
None	250(95.78)
Edema	4(1.53)
Partial	4(1.53)
Tear	3(1.15)
LCL Lateral collateral ligament	*n(%)*
None	254 (97.32)
Edema	3(1.15)
Partial	3(1.15)
Tear	1(0.38)

Medial and lateral retinaculum of the knee were affected in 15(5.75%) and 5(1.92%) subjects, respectively. Amongst the traumas of the medial retinaculum, the most common MRI findingwasapartial lesion (3.45%).

In 3(1.15%) cases with trauma to the knee, the magnetic resonance showed edema localized in the area of the attachment of the ligament to the patella. Traumas to the knee rarely occur as isolated events, they are usually complex and occupy more structures. With regard to the complexity of the trauma to the knee, the results showed that in 119(45.59%) cases trauma was isolated, the remaining 142(54.41%) cases showed complex injuries [15].

In the group of patients without bone edemas, 86 (37.72%) had a trauma to the ACL, in the group with type 1 edema, 10 (58.82%) had a trauma to the ACL, while in the group with type 2 edema this percent was 62.5%. Patients with lesions on the ACL had a significantly more frequent occurrence of bone edema (p= 0.043) [2,3]. In the group of subjects without bone edemas 65(28.519%) had injuries to the posterior horn of the medial meniscus grade 3, in the group with type 1 edema (35.29%) had injuries to the posterior horn of the medial meniscus grade 3, while in the group with type 2 edema this percent was 62.5%. The statistical analysis showed that patients with lesions on the posterior horn of the medial meniscus grade 3 presented with bone edemas significantly more frequently (p=0.013). All patients with a combined trauma to the anterior cruciate ligament and the posterior horn of the medial meniscus also had a bone edema type 1 and type 2 [24,25] (Table 5).

Patients aged 40 to 50 years more frequently exhibited traumas to the knee as a result of a fall or bend (36.96%, 45.65% respectively) when compared to the remaining two age groups; patients in the age group 30-39 exhibited traumas to the knee more frequently as a result of a fall (41.18%) when compared to subjectsin the youngest age group, while subjectsin the age group 19 to 29 exhibited sports injuries to the knee (35.64%) more frequently than subjectsin the older age groups [4,9]. More female than male subjects gave anamnestic data of a fall (36.30% vs 30%) and bending (47.25% vs 36.47%), whereas more male subjects declaredsports injury as the cause of injury (33.53% vs 16.48%) [5]. In the groups of subjectswith body mass index lower than 18.5, from 18.5 to 24.9 and from 25 to 29.9 the primary cause of trauma to the knee was bending (80%, 41.28% and 39.39%, respectively), whereas a fall was the most common cause of trauma to the knee in the group with the highest body mass index (41.67%) [5].

Table 5. Correlation between lesions of the ACL, medial meniscus and bone edema

Lesion of the ACL and medial meniscus	Bone edema		
	None	**Type 1**	**Type 2**
No	49 (211.49%)	0	0
Yes	179 (78.51%)	17 (100%)	16 (100%)

Discussion

In our study the age of subjects had an insignificant influence over the cause of trauma to the knee (p=0.078). In subjectsaged 40-50 years trauma to the knee was more often as a result of a fall or bending (36.96%, 46.56%, respectively) than in the other two age groups; in subjectsaged 30-39 knee traumas were more common due to a fall (41.18%) when compared to the youngest age group; in subjects aged 19-29 we found a higher percentage of knee traumas to be a result of a sports injury (35.64%) when compared to older subjects. However, these results did not exhibit statistical significance [4,9].
Cause of trauma to the knee significantly depended on gender (p=0.013). Female patients more frequently than male gave amnestic data of a fall (36.30% vs 30%) and bending (47.25% vs 36.47%), whereas male patients more frequently than female declaredsports as a cause of trauma (33.53% vs 16.48%) [5].
Body weight and height, as well as body mass index did not prove to be statistically significant characteristics of subjects when determining the manner in which trauma to the knee occurred (p=0.065, p=0.49 and p=0.38, respectively). In the groups of subjectswith body mass index lower than 18.5, from 18.5 to 24.9 and from 25 to 29.9 the primary cause of trauma to the knee was bending (80%, 41.28% and 39.39%, respectively), whereas falling was the most common cause of trauma in the group ofpatients with the highest body mass index (41.67%). The results from the study showed that the type of pain was significantly different within the three causes of trauma to the knee (p=0.004).
In 73.34%of patients with a spontaneous pain the trauma was due to a fall, in 13.33% the spontaneous pain was a result of bending and sport activities. Patients who suffered a knee trauma as a result of bending more commonly experiencedpain while moving and ascending stairs. The percent of subjects experiencing pain while moving was 28.57% in the group with a fall, 40.47% in the group with bending of the knee, 30.69% in the group with a sports injury, whereas the percent of subjects experiencing pain while ascending stairs was 34.09% in the group with a fall, 47.73% in the group with bending of the knee and 18.18% in the group with a sports injury. All three subjects experiencing pain while descending stairs suffered a trauma to the knee due to a fall, while one patient complained about a locking of the knee as a result of a fall, 5 as a result of bending and 4 as a result of a sport activity [13,14].
The cause of trauma did not play a significant role in the localization of the pain (p=0.26). Lateral and medial localization of the pain was most common in subjectsafter a fall (29.41%, 39.02%, respectively), whereas anterior and posterior localization of pain was experienced primarily in subjects who hada trauma due to a bending of the knee (48.72%, 46.15%, respectively). The difference between the three groups of patients with different causes of trauma was statistically insignificant in relation to the intensity of pain (p=0.38). In the groups with weak as well as strong pain, the most common cause of trauma was bending of the knee (46.35% and 42.86%, respectively), whereas patients whoreported a moderate pain most commonly suffered from a traumatic event as a result of a fall (35.78%). The differences that existed in the described distribution wereinsufficient in proving statistical significance. In the group consisting of athletes, 18.68% sustained the trauma as a result of a fall, 13.19% as a result of a bending of the knee and 68.13% with sport trauma. In the group of subjects who were not athletes, 39.41% sustained the injury as a result of a fall, 53.71% as a result of a bending of the knee and only 5.88% while participating in sports activities. These results also proved to be statistically significant, since athletes and non-athletes differed statistically in the manner in which they sustained a trauma to the knee (p<0.001) [8,10,13,14]. Fluid in the knee was detected in 34.83% of patients whoreported a fall as the cause of trauma, 31.46% of patients who reported a bending in the knee and 33.71% of subjects with sports injury. Findings of fluid in the knee did not significantly depend on the cause of trauma (p=0.094).
Bone edema seen in MRI of the traumatized knee most usually goes with lesions of the cartilage, meniscus and anterior cruciate ligament [4,5] where a lesion on the anterior cruciate ligament occurred in 80% of cases with lesion on the meniscus, cartilage and edema [20,23,24].
In our study with regard to the complexity of the trauma to the knee, the results showed that in 119 (45.59%) of subjects the trauma was isolated, in the remaining 142 (54.41%) subjects the trauma was complex.

Conclusion

Age and body weight do not have an impact on the cause of trauma and the type and grade of the lesions of the knee. Females tend to injure the knee more frequently as a result of a fall whereas males as a result of a bending of the knee. Males are more prone to sports traumas. Localization of the pain is not linked to the cause of injury; it depends on the developed lesion on the knee. The intensity of pain does not depend on the cause of injury;it is subjective to the category and the pain threshold is different in each patient. Athletes and subjects who are not athletes have significantly different causes of trauma to the knee. Bending of the knee is most common in athletes, whereas in non-athletes it is the fall. Findings of knee fluids do not significantly depend on the cause of injury. The statistical analysis confirmed that patients with lesions in the posterior horn of the medial meniscus grade 3 had significantly more bone edemas (p=0.013). All patients with a combined trauma to the anterior cruciate ligament and the posterior horn of the medial meniscus had a bone edema type 1 and type 2. Bone edema should always

be looked forduring a MRI analysis of the knee as it can lead to finding the lesion.

Conflict of interest statement. None declared.

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Original article

INFLUENCE OF RADIATION THERAPY ON GLOMERULAR FILTRATION RATE AFTER TREATING PELVIC MALIGNANCY

ВЛИЈАНИЕ НА ЗРАЧНАТА ТЕРАПИЈА ВРЗ ГЛОМЕРУЛАРНАТА ФИЛТРАЦИСКА РАТА ПО ЛЕКУВАЊЕ НА КАРЛИЧНИОТ МАЛИГНИТЕТ

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Abstract

Introduction. Pelvic malignancy (cervical, rectal and endometrial carcinoma) is a very common and deadly disease. Adjuvant therapy consists of regimens that include both concurrent chemotherapy/radiotherapy (RT) and adjuvant chemotherapy. The aim of the study was to evaluate and analyze renal function through the changes in GFR (Glomerular Filtration Rate), using 3D conformal radiotherapy (3DCRT) techniques in the treatment of patients with pelvic malignancy.
Methods. This study was conducted at the Clinical Center of Kosovo, Oncology Department. Several variables were evaluated in 75 patients: sex, age, type of primary malignancy, median tumor dose (TD) evidence over 50 and above 50 Gray (Gy). Time of the appearance of toxicity was followed by GFR changes during3- and 6-month follow-up period. Patients with pretreatment genitourinary morbidity (PGUM) were excluded from the study.
Results. Our cohort consisted of 75 patients with pelvic malignancy, of whom 53(70.7%) were female and 22(29.3%) male. The average age of the patients included in the study was 57.5±11.2 years. Thirty (40.0%) of the 75 patients had rectal carcinoma, 28 (37.3%) cervical cancer (CC) and 17 (22.7%) endometrial carcinoma. The average value of GFR in the beginning was 71.7± 23.1ml/min, it was 75.6±25.6ml/min three months after beginning of therapy and 79.1±25.9 ml/min six months after therapy. The test of comparison showed a signifycant statistical difference between the values of GFR at the baseline of treatment vs three months after therapy ($P<0.05$), baseline of therapy vs six months after therapy ($P<0.001$), while there was no difference between GFR values three months and six months after therapy.
Conclusion. Overall, the kidney function improved at 3 and 6 months in majority of patients.

Keywords: renal function, glomerular filtration rate, pelvic malignancy, radiation therapy

Апстракт

Вовед. Карличниот малигнитет (цервикален, ректален и ендометријален карцином) е доста често и смртоносно заболување. Адјувантната терапија се состои од третмани кои вклучуваат истовремена хемо/радио-терапија или само хемотерапија. Целта на студијата е да се еваулира раната и касната токсичност на генито-уринарниот тракт како и промените во бубрежната функција со бубрежната функција со следење на гломеруларната филтрациска рата (ГФР) кај пациенти со карличен малигнитет третирани со тридимензионална Conformal Радио техника (3ДЦРТ).
Методи. Студијата е изведена во Одделот за онкологија при Клиничкиот центар на Косово. Испитувани беа 75 пациенти со следење на неколку параметри: пол, возраст, вид на примарен малигнитет, средна туморска доза до и над 50 Grey. Во период од три и шест месеци преку промените во ГФР следена е појавата на токсичност од третманот. Исклучувачки критериум: пациенти со пре генито-уринарен морбидитет пред започнување со третманот.
Резултати. Во испитуваната популација од 75 пациенти со пелвичен малигнитет, 53(70.7%) беа жени и 22(29.3%) мажи, со просечната возраст од 57.5±11.2 год. Од пациентите 30(40%) беа со ректален карцином, 28(37.3%) цервикален карцином и 17(22.7%) ендометријален карцином. На почеток на третманот ГФР беше 71.7± 23.1mmol/L, додека по 3 и 6 месеци од третманот ГФР беше 75.6±25.6mmol/L, односно 79.1±25.9 mmol/L. Резултатите покажаа статистички сигнификантна разлика во однос на ГФР на почеток на третманот vs ГФР на третиот и шестиот месец од третманот, но не и во однос на ГФР од 3 vs 6 месеци во тек на третманот.
Заклучок. Студијата покажа дека постои подобрување на функцијата на бубрезите три и шест месеци од почеток по третманот.

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Клучни зборови: бубрежна функција, Гломеруларна филтрациска стапка, карличен малигнитет, нефротоксичност, зрачна терапија

Introduction

Pelvic malignancy (cervical, rectal and endometrial carcinoma) is a very common and deadly disease. Kidney disease frequently complicates malignancy and its treatment. The spectrum of disease in this setting includes acute renal injury (AKI), chronic renal failure, and tubular disorders. Fortunately, these complications are often preventable or reversible with prompt diagnosis and treatment [1]. Rectal cancer is the third cause of cancer death in men and the second cause of cancer death in women. Higher incidence of this carcinoma is found in Europe, USA and Australia, while the lowest is in Africa and Central and South Asia.
Treatment is done depending on the stage of colorectal carcinoma. Adjuvant therapy for rectal cancer consists of regimens that include both concurrent chemotherapy/ RT and adjuvant chemotherapy. A total of approximately 6 months of perioperative treatment is preferred.
Radiation doses: 45-50 Gy in 25-28 fractions to the pelvis. For resectable cancers, after 45 Gy a tumor bed boost with a 2-cm margin of 5.4 Gy in 3 fractions could be considered for preoperativeradiation and 5.4-9.0 Gy in 3-5 fractions for postoperative radiation. Small bowel dose should be limited to 45 Gy. A number of randomized trials have evaluated the effectiveness of the addition of chemotherapy to radiation administered either preoperatively following clinical evaluation/staging (eg, T3-4 by endoscopic ultrasound) or postoperatively following pathologic staging of rectal cancer as pT3 and/or N1-2 [2]. Preoperative chemo RT also has the potential to increase rates of pathologic complete response and sphincter preservation [3].
Cervical cancer is considered one of the most frequent malignant diseases that occurs in females. According to statistical data, cervical cancer occurs in 8-30 new cases in 100,000 females within a year, depending on the region and state. Treatment of cervical cancer is stratified by stage as delineated in the NCCN Guideline. External Beam Radiation Therapy (EBRT) is done by the use of CT-based treatment planning and conformal blocking is considered the standard of care for EBRT [4].
Endometrial cancer is among the most common forms of cancer in women, depending on certain countries, in Albanian territories, for many reasons is somewhat rare. The most affected age group is 60-69 years. The risk of suffering from endometrial cancer increases with age, about2/3 of the endometrial cancer is notified after menopause, and about 1/3 before menopause [5].
Treatment of endometrial carcinoma depends on stage, radical treatment with operation or definitive pelvic radiotherapy [6].
To help select a patient population who may benefit from adjuvant RT, the GOG 99 and PORTEC trials defined risk factors for women at high-intermediate risk (HIR) for recurrence [7,8].
The aim of the study was to evaluate and analyze renal function through the changes in Glomerular Filtration Rate (GFR), using 3D conformal radiotherapy (3DCRT) techniques in the treatment of patients with pelvic malignancy.

Materials and methods

This is a prospective follow-up study conducted at the Institute of Oncology in Prishtina, Kosovo.
Inclusion criteria: pelvic malignancy (cervical, endometrial and rectal). Exclusion criteria: patients with PGUM (pretreatmentgenitourinarymorbidity).
Our cohort consisted of 75 patients with pelvic malignancy treated with adjuvant radiotherapy. The radiation therapy technique and doses were strictly defined and identical for all regimens. Patients were treated by whole pelvic RT with different site of pelvic malignancy following the International Commission on Radiation Units and Measurements (ICRU) No. 50 recommendations [1].
The clinical target volume (CTV) was defined as pelvic lymph nodes and primary tumor region and was/were? contoured on individual axial CT slices. The lymph node regions were determined by encompassing the blood vessels with a 2 cm margin and based upon primary tumor site. The planning target volume (PTV) has been expanded the CTV by 1 cm. The dose was prescribed so as to encompass at least 95% of the PTV, ranging from a total dose of 45 Gy or 50 Gy, administrated in 1.8 -2 Gy per fraction delivered in 25 to 30 daily fractions. Treatment planning was generated using the Xio software for 3D RT. Dose volume restrictions used for pelvic OARs were described.
In the 3D CRT, whole pelvic irradiation was delivered by anterior-posterior and posterior-anterior parallel ports or a four-field box technique utilizing x-ray energies of 15 Mega Volt (MV). Plans were based on pelvic isocentric conformal fields with energy of 15-MV and patients were treated with a Simens linear accelerator, equipped with 80-leaf multileaf collimator. The pelvic field extended from the upper margin of L5 to the midportion of the obturator foramen or the lowest level where diseasehas been, with a 3 cm margin and laterally 1.5 to 2 cm beyond the lateral margins of the bony pelvic wall (at least 7 cm from the midline). For lateral fields, the anterior border was the pubic symphysis and the posterior border was the space between S2 and S3. The fields were modified to include areas of known tumor.
Measurements for renal function before treatment were: GFR (Cockroft-Gault), urological echo and urine sediment.
CreatClear = Sex*((140-Age)/(SerumCreat))*(Weight/72)

At 3 and 6 months urea, creatinine, GFR (Cockroft-Gault) were analyzed.

Statistical analysis-Testing of qualitative data was done with X2-test, quantitative data that did not have a normal distribution with the Kruskal-Wallis test and Mann-Whitney test. Testing of quantitative data that had normal distribution was done with One Way ANOVA and T-test. Verification of tests was made with 99.7% confidence level (P<0.01) and the reliability of 95% (P<0.05). Data processing was done with the SPSS statistical package.

The data obtained are presented in tables. The following statistical parameters were calculated: index structure, arithmetic mean, standard deviation, minimum and maximum values.

Results

The study included 75 patients with pelvic tumors, of whom 53 or 70.7% were female and 22 or 29.3% male (Table 1). The average age of the patients included in the study was 57.5±11.2 years (range 33-77 years). The average age of the female patients was 56.1±11.1 years (range 33-77years), and of the male patients 60.8±11.1 years (range 37-74 years) (Table 1).

Table 1. Patients included in the study by group and gender

Age (years)		Sex F		Sex M		Total	
		N	%	N	%	N	%
30-39		5	9.4	2	9.1	7	9.3
40-49		9	17.0	1	4.5	10	13.3
50-59		17	32.1	5	22.7	22	29.3
60-69		15	28.3	10	45.5	25	33.3
70+		7	13.2	4	18.2	11	14.7
In total	**N**	**53**	**100.0**	**22**	**100.0**	**75**	**100.0**
	%	**70.7**	**-**	**29.3**	**-**	**100.0**	**-**

Table 2. Patients included in the study based on diagnosis and gender

Diagnosis	Sex F		Sex M		Total	
	N	%	N	%	N	%
Endometrial Cancer	17	32.1	-	-	17	22.7
Rectal Cancer	8	15.1	22	100.0	30	40.0
Cervical cancer	28	52.8	-	-	28	37.3
Total	**53**	**100.0**	**22**	**100.0**	**75**	**100.0**

Of the 75 patients included in the study 30 or 40.0% had rectal carcinoma, 28 or 37.3% had cervical cancer (CC) and 17 or 22.7% had endometrial carcinoma. All male patients had rectal carcinoma (Table 2).

The average value of GFR in the beginning was 71.7± 23.1ml/min, it was 75.6±25.6ml/min three months after baseline of the therapy and 79.1±25.9 ml/min six months after therapy. ANOVA showed an important significant statistical difference between the average value of GFR after therapy (F=10.58, P<0.0001).

Table 3. GFR at the beginning, after three and six months of therapy

	GFR Baseline	GFR After three months	GFR After six months
N	75	75	75
Mean±SD	71.7±23.1	75.6±25.6	79.1±25.9
Repeated measures ANOVA	F = 10.58, P<0.0001		
Tukey-Kramer Multiple Comparisons test	Initially vs. three months later, P<0.05 Initially vs. six months later, P<0.001 Three months vs. Six months, P>0.05		

Tukey-Kramer Multiple Comparisons test showed a significantly statistical difference between GFR values at the beginning vs. three months after using the therapy (P<0.05), baseline of treatment vs. six months after therapy (P<0.001) while there was no difference between GFR values three months vs. six months later (Table 3).

The average value of GFR at the baseline of the therapy in patients with endometrial cancer was 72.7±23.0 ml/min, it was 63.0±22.5 ml/min in patientswith rectal cancer and 80.5±20.8.ml/min in those with CC (Table 4).

The average value of GFR three months after therapy in patients with endometrial cancer was 71.4±27.5 μmol/L, it was 70.1±25.8 in patients with rectal cancer and

83.9±22.7 in those with CC. One Way ANOVA showed borderline significant statistical difference between mean values of GFR according to the type of disease (F= 2.48, P=0.09). Tukey-Multiple Comparisons showed no important significant statistical difference between GFR mean values at the baginningin patients with endometrial cancer vs. rectal cancer (P>0.05), endometrial cancer vs. cervical cancer (P>0.05) and no difference between values of rectal cancer vs.CC (P>0.05), (Table 5).

Table 4. GFR at the beginningin patients according to their diagnosis

GFR Baseline	Diagnosis Endometrial carcinoma	Rectal carcinoma	Cervical cancer (CC)
N	17	30	28
Mean±SD	72.7±23.0	63.0±22.5	80.5±20.8
Dunn's Multiple Comparisons test	Ca endometri vs. Ca recti, P>0.05 Ca endometri vs. Ca cervicis uteri, P>0.05 Ca recti vs. Ca cer. uteri, P<0.01		

Table 5. GFR three months after baseline of therapy in patients according to their diagnosis

GFR after three months	Diagnosis Endometrial cancer	Rectal cancer	Cervical cancer (CC)
N	17	30	28
Mean±SD	71.4±27.5	70.1±25.8	83.9±22.7
One Way ANOVA	F = 2.48, P=0.09		
Tukey-Kramer Multiple Comparisons test	Endometrial Ca vs. Rectal Ca, P>0.05 Endometrial Ca vs. Cervical Ca, P>0.05 Rectal Ca vs. Cervical Ca, P>0.05		

Table 6. GFR six months after the treatmantin patients according to their diagnosis

GFR after six months	Diagnosis Endometrial cancer	Rectal cancer	Cervical cancer (CC)
N	17	30	28
Mean±SD	72.3±29.7	74.6±27.1	88.1±19.7
One Way ANOVA	F = 2.85, P=0.06		
Tukey - Kramer Multiple Comparisons test	Endometrial Ca vs. Rectal Ca, P>0.05 Endometrial Ca vs. Cervical Ca, P>0.05 Rectal Ca vs. Cervical Ca, P>0.05		

Table 7. GFR at the baseline of therapy, after three and six months in patients with rectal cancer

	GFR Rectal Cancer Baseline	After three months	After six months
N	30	30	30
Mean±SD	63.0±22.5	70.1±25.8	74.6±27.1
Repeated Measures ANOVA	F = 8.50, P=0.0006		
Tukey - Kramer Multiple Comparisons test	Initially vs. three months later, P<0.05 Initially vs. six months later, P<0.001 Three months vs. Six months, P>0.05		

The average value of GFR three months after baseline of the therapy in patients with endometrial cancer was 72.3±29.7, it was 74.6±27.1 in patientswith rectal cancer and 88.1±19.7 in those with CC. One Way ANOVA showed borderline significant statistical difference between mean values of GFR according to the type of disease (F=2.85, P=0.06). Tukey-Multiple Comparisons showed no important significant statistical difference between mean values of GFR at the beginning in patients with endometrial cancer vs. rectal cancer (P>0.05), endometrial cancer vs. cervical cancer (P>0.05) and no difference between values of rectal cancer vs. cervical cancer (P>0.05), (Table 6).

ANOVA test showed an important significant statistical difference between the average value of GFR after therapy in patients with rectal cancer (F=8.50, P<0.001). Tukey-Kramer Multiple Comparisons test showed a significant statistical difference between the GFR values at the beginning vs three months after therapy (P<0.05), baseline of therapy vs six months after therapy (P <0.001), while no difference between GFR values three months and six months after (Table 7).

Table 8. GFR at the beginning of therapy, after three and six months in patients with endometrial cancer

	GFR endometrial cancer Initially	After three months	After six months
N	17	17	17
Mean	72.7	71.4	72.3
SD	23.0	27.5	29.7
Repeated measures ANOVA	F = 0.08, P=0.921		

ANOVA showed an important significant statistical difference between the average value of GFR after applying the therapy in patients with endometrial cancer (F=0.08, P>0.05), (Table 8).

Table 9. GFR at the beginning of therapy, after three and six months in patients with cervical cancer

	GFR cervical cancer Initially	After three months	After six months
N	28	28	28
Mean	80.5	83.9	88.1
SD	20.8	22.7	19.7
Nonparametric repeated measures ANOVA	Fr = 13.07, P=0.0015		
Dunn's Multiple Comparisons test	Initially vs. three months later, P>0.05 Initially vs. six months later, P<0.001 Three months vs. six months P>0.05		

Nonparametric repeated measures ANOVA (Friedman test) showed an important significant statistical difference between the average values of GFR after therapy in patients diagnosed with cervical cancer (FR=13.07, P<0.001). Dunn's Multiple Comparisons test showed

an important significant statistical difference between GFR values at the beginning vs.six months after therapy (P<0.001), (Table 9).

Table 10. GFR at the beginning of therapy by gender

GFR initially	Sex		Total
	F	M	
N	53	22	75
Mean	76.6	59.9	71.7
SD	20.6	24.9	23.1
Unpaired T-test	T=3.01, P=0.003		

The average value of GFR at the beginning of therapy in female patients was 76.6 (SD±20.6), while in male 59.9 (SD±24.9).

Unpaired T-test showed an important significant statistical difference between the average value of GFR by gender (T=3.01, P=0.003), (Table 10).

Discussion

Renal failure in cancer patients is often multifactorial, but it is still clinically useful to consider causes as prerenal, intrinsic, and postrenal. Not surprising, prerenal failure is common. The spectrum of intrinsic renal disease in this patient population is broad [2].

According to the new KDIGO (guidelines for chronic kidney disease (CKD) progression) there are 5 stages: 90-120 ml/min-I, 60-90 ml/min-II, 30-60-III, 15-30-IV, <15 ml/min-V for dialysis treatment [9]. The corrected GFR is approximately 8% lower in women than in men, and declines with age at an annual rate of 1 mL/min/1.73m2 from the age of 40.

Creatinine clearance in the beginning has been to the largest number of patients in the second level of CKD (60-90 ml/min). Males after the first 3 months have increased borderline of significance for 7 ml unlike women where it is 2.5ml/min.

Reason of this can be different number of radiation fractions while treating cervical and endometrial cancer vs rectal cancer. Another reason can be anatomical pelvic difference between sexes according to the field or radiotherapy treatment.

The average value of GFR three months after beginning of the therapyin our female patients was 79.1±23.7, while in males it was 67.2±28.4.

Unpaired T-test showed no important significant statistical difference between the average value of GFR by gender (T=1.86, P=0.066).

The average value of GFR six months after therapy in female patients was 81.5±24.0, whilein males it was 73.4±29.8.

Unpaired T-test showed no important significant statistical difference between the average value of GFR by gender (T=1.239, P=0.219).

Martens *et al*. retrospectively reviewed predictors of urinary retention in 207 BT patients. In their model, pre-implant peak flow rate and prostate volume were statistically significant predictors of post-implant urinary retention. They estimated that for every one-unit increase in peak flow rate the odds of catheterization decreased by 6% [10].

Radiation therapy and colorectal cancer-Urinary AEs have not been properly evaluated in the setting of RT for colorectal cancer. The only trial that describes urinary AEs mentions "bladder problems" in 2-4% [10]. Given the close proximity of the bladder as well as its blood and nerve supply to rectum, there is likely to be an additive effect of RT and surgery causing increased risk of bladder dysfunction, although the lower doses of RT used for colorectal cancer may mitigate this effect. Early diagnosis and treatment of renal failure is vital, both to improve renal outcomes and to ensure that patients are best prepared for further oncologic treatment [2].

Conclusion

The aim of this study was achieved managing sides effects that have been accrued during treatments at proper time. Overall, the kidney function was improved at three and 6 months in the majority of patients. Treated group from rectal carcinoma in the beginning had lower GFR rate, which had significant improvement after three and six months in comparison to other groups. It can beconcluded that incurred significant impairment of renal function, registered at 3 and 6 months after treatment, compared with beginning of therapy, after is stabilized without further deterioration in the period from 3 to 6 months.

Conflict of interest statement. None declared.

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Original article

INSULIN RESISTANCE IN PATIENTS WITH CHRONIC HEPATITIS C

ИНСУЛИНСКА РЕЗИСТЕНЦИЈА КАЈ ПАЦИЕНТИ СО ХРОНИЧЕН ХЕПАТИТИС Ц

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Abstract

Introduction. Insulin resistance is the most common extrahepatic manifestation associated with hepatitis C virus, which leads to developing more pronounced fibrosis and liver steatosis. The aim of the study was to assess the prevalence of insulin resistance in non-diabetic, treatment naive patients with chronic hepatitis C and to analyze the relation of insulin resistance with genotype, viral load, gender, age, laboratory parameters, inflammatory and fibrotic changes in the liver, body mass index (BMI) and the presence of steatosis.
Methods. In this cross sectional study, 224 patients with hepatitis C viral infection were included. The patients were divided into two groups. The first group was with no insulin resistance and the second one with present insulin resistance. They were compared in terms of genotype, viral load, gender, age, inflammatory and fibrotic changes in the liver, BMI and liver steatosis.
Results. Insulin resistance was present in 45.5% of patients. The following factors were associated with insulin resistance: age (p=0.0022), inflammatory and fibrotic changes in the liver (p=0.001, p=0.006, respectively), steatosis (p=0.015) and transaminase activities (for AST, p=0,002, for ALT, p=0.001).
Conclusion. In the Republic of Macedonia, a high percent of 45.5% among non-diabetic and treatment naïve patients with chronic viral hepatitis C, had insulin resistance. Insulin resistance was more prevalent in older patients, in those with more pronounced inflammatory and fibrotic changes in the liver, in patients with steatosis and in those with higher transaminase activity.

Keywords: chronic hepatitis C, insulin resistance, steatosis, inflammation, fibrosis, body mass index

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Апстракт

Вовед. Инсулинската резистенција претставува најчеста екстрахепатична манифестација, асоцирана со вирусот хепатитис Ц, која е асоцирана и со развој на поизразена фиброза и стеатоза на црниот дроб. Цел на студијата е процена на застапеноста на инсулинската резистенција кај пациенти со хроничен хепатитис Ц, кои не се дијабетичари и кои досега не се лекувани, како и анализа на асоцираноста на инсулинската резистенција со генотипот, виремијата, полот, возраста, лабораториските параметри, инфламаторните и фибротични промени на црниот дроб, индекс на телесна маса и присуството на стеатоза.
Методи. во оваа студија на пресек се вклучени 224 пациенти со вирусна инфекција хепатитис Ц. Пациентите се поделени во група без инсулинска резистенција и во група со присутна инсулинска резистенција, кои потоа се споредувани во однос генотип, виремија, пол, возраст, инфламаторни и фиброзни промени во црниот дроб, индекс на телесна маса и стеатоза на црн дроб.
Резултати. Инсулинска резистенција е присутна кај 45.5% од пациентите. Фактори кои се асоцирани со инсулинска резистенција се возраста (p=0.0022), воспалителните и фиброзни промени во црниот дроб (p=0.001, p=0.006, соодветно), стеатозата (p=0.015) и трансаминазната активност (за АСТ p=0,002, за АЛТ p=0.001).
Заклучок. Постои висок процент на присуство на инсулинската резистенција од 45.5% меѓу пациентите со хроничен вирусен хепатитис Ц во Република Македонија, кои не се дијабетичари и кои досега не се лекувани. Инсулинската резистенција е позастапена кај постари пациенти, кај оние со поизразени инфламаторни и фибротични промени во црниот дроб, како и кај пациентите со присутна стеатоза и со зголемена трансаминазна активност.

Клучни зборови: хроничен хепатитис Ц, инсулинска резистенција, стеатоза, инфламација, фиброза, индекс на телесна маса

Introduction

Chronic hepatitis C virus infection is widespread throughout the world, affecting approximately 2.8% of the population, or about 185 million people worldwide are infected with the disease [1].
Impaired glucose metabolism is often seen in patients with chronic hepatitis C (CHC), so diabetes mellitus type 2 (DM) is the most common extrahepatic manifestation associated with hepatitis C virus (HCV) [2-3]. This HCV-diabetes association is due to insulin resistance (IR). Basically, IR is a pathological condition in which cells, especially those of adipose tissue, the muscles and the liver do not respond appropriately to insulin secreted by the pancreas. Thus, glucose cannot be absorbed from the circulation, which increases its level in the blood and stimulates the pancreas to secrete larger amounts of insulin in order to reduce serum glucose. Insulin resistance, pre-diabetes and finally, type 2 diabetes mellitus (DM) as known pro-atherogenic conditions are risk factors for developing atherosclerosis and cardiovascular events in this group of patients [4]. Unlike chronic hepatitis B, impaired glucose metabolism is often found in patients with hepatitis C virus infection. IR is also associated with the development of pronounced fibrosis and liver steatosis [5-9]. Mechanisms for HCV induced IR are several, such as the role of tumor necrosis factor-alpha (TNF-α) and the direct effects of HCV core protein in inhibiting insulin signaling pathway [10-13]. The presence of IR leads to a lower rate of sustained virological response (SVR) [14]. The reason for this negative association is not completely known, but some possible mechanisms have been mentioned: HCV core protein by stimulating the suppressor of cytokine signaling-3 (SOCS-3), which is a negative regulator of interferon-α (IFN-α) signaling; obesity by modulating INF signaling pathway, as well as increasing the lipid droplets in hepatocytes or resulting in poor lymphatic circulation [15]. Achieved SVR has a positive impact on the IR reduction [14]. Long duration of infection associated with metabolic abnormalities is the main reason for development of more advanced forms of liver damage. All these result in cirrhosis, requiring liver transplantation [16]. Hepatocellular carcinoma (HCC) is often associated with this type of infection, but also IR is associated with the development of HCC in patients with chronic HCV infection [17-18].
The primary goal of the study was to assess the prevalence of insulin resistance expressed by fasting plasma glucose (FPG), fasting level of the insulin in the blood and Homeostasis Model Assessment of Insulin Resistance (HOMA IR) in non-diabetic, treatment naive patients with chronic hepatitis C. Secondary endpoints analysis was the association of IR with genotype, viremia, gender, age, laboratory parameters (transaminases, lipid and carbohydrate status, C-reactive protein-CRP, ferritin and serum iron), histological changes in the liver (inflammatory and fibrotic), body mass index and the presence of steatosis.

Material and methods

In this observational and cross-sectional study, with prospective inclusion of data, a total of 224 non-diabetic patients with chronic hepatitis C were included, in the period from January 2010 to December 2015. The study was approved by the local Ethics Committee.
Inclusion criteria: treatment naïve, hepatitis C virus ribonucleic acid (HCV RNA) positivity patients, confirmed by PCR method.
Patients were excluded from the study if they were: co-infected with other virus (hepatitis B virus-HBV or human immunodeficiency virus-HIV), if they had other liver disease (autoimmune hepatitis, Wilson's disease, hemochromatosis, primary biliary cirrhosis, primary sclerosing cholangitis), signs of decompensation, history of liver transplantation, end-stage renal disease, type 2 diabetes mellitus, alcohol abuse (>20 g/day) and hepatocellular carcinoma.
Blood was taken from all patients and the samples were sent to central biochemical laboratory for analysis of the following parameters: transaminase activity (aspartate transaminase-AST, alanine aminotransferase-ALT), lipid status (triglyceride-TG, total cholesterol, high-density lipoprotein cholesterol-HDL-C; low-density lipoprotein cholesterol-LDL-C), FPG and fasting insulin blood level, hemoglobin A1c-HbA1c, CRP, ferritin and serum iron. Insulin resistance was calculated according to the formula of HOMA-IR: fasting insulin (μU/mL) x fasting glucose in plasma (mmol/L) /22.5.). For the value of ≥2 insulin resistance was confirmed.
The genotyping and the viremia were performed at the Research Centre for Genetic Engineering and Biotechnology "Georgi D. Efremov", Macedonian Academy of Sciences and Arts.
Assessment of inflammatory activity and liver fibrosis was made by a liver biopsy. Knodell scale was used for measuring the degree of inflammation (HAI-histological activity index, which is numbered 1 to 18) and presence of fibrosis). Patients were divided into three groups: group 1-no fibrosis, group 2-evident fibrosis and 3-liver cirrhosis. Ultrasound was used to assess the presence of fatty liver. Patients were divided into three groups: group 0-no steatosis, group 1-mild steatosis and group 2-severe steatosis.
Patient weight was expressed through body mass index-BMI, which was calculated by the formula: weight in kg/height^2 in meters.
Patients in this study were divided into two groups: group 1-patients with hepatitis C virus infection with no evidence of IR and group 2-patients with hepatitis C virus infection with evidenced IR.
These two groups were compared in terms of multiple parameters such as gender, age, genotype, viral load, inflammatory and fibrotic changes in the liver, presen-

ce of steatosis, BMI and laboratory parameters (AST, ALT, TG, total cholesterol, HDL-C, LDL-C, fasting glucose and fasting insulin level in blood, HbA1c, CRP, ferritin and serum iron).

Statistical analysis: The statistical program SPSS 17 for Windows was used. For description of quantitative variables descriptive statistics was used (mean, standard deviation, standard error, median and interquartile range). For description of categorical variables, frequencies and percentages were used. For testing the difference of categorical variables between the two groups, Chi-square test was used. For testing the difference of numerical variables, Mann-Whitney test was used. For all analyzes p value <0.05 was considered statistically significant, and p <0.01 highly significant.

Results

Basic features of patients with chronic hepatitis C are shown in Table 1. Their average age was 33.83±8.24, and the values for FPG, fasting insulin level in blood, HOMA IR and HbA1c were: 5.24±0.74, 12.89±15.60, 2.87±3.44 and 5.22±0,94, respectively. Average value of BMI was 24.47±4.32. Insulin resistance was evidenced in many of the patients with chronic hepatitis C, in 102 patients (45.5%) of a total of 224 patients. The remaining 122 patients or 54.5% were patients with no evidence of IR (Table 2). With regard to gender males predominated in both groups of patients. Still, IR was more often evidenced in females (50.88%), compared to males (43.71%), with no statistical significance between the groups (p=0.348). The age of patients with IR was significantly higher (35.9±9.3) compared to patients with no evidence of IR (32.1±6.7), with significant difference of p=0.0022. In terms of genotype and viral load, no significant difference between the groups was detected (p=0.742 and p=0.900, respectively). Highly significant difference between the groups was obtained regarding inflammatory activity obtained from liver biopsy. Evidently, patients with determined IR had a higher Knodell score (HAI), with a mean HAI of 4.072±2.920, compared to those without IR whose mean HAI was 2.761±2.402 (p=0.001). Patients with evidenced fibrosis or cirrhosis more frequently encountered IR (in 54.05% and 83.33%, respectively), with statistical significance

Table 1. Baseline Characteristics of Patients With Chronic Hepatitis C Infection

Variable	Patients N=224
Age, years, mean ± SD	33.83±8.24
FPG (3.6-5.6 mmol/L), mean±SD	5.24±0.74
Fasting insulin (2-17 μIU/ml), mean±SD	12.89±15.60
HOMA IR, mean±SD	2.87±3.44
HbA1c (4.8-5.9%), mean±SD	5.22±0,94
BMI, mean±SD	24.47±4.32

Abbreviations: SD: standard deviation; FPG: Fasting plasma glucose; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, BMI: body mass index

Table 2. Absence or evidence of insulin resistance (IR) in patients with HCV

Baseline Characteristics	Absence of IR (N=122)	Evidence of IR (N=102)	P value
Sex, No (%)			
Male	94(56.29)	73(43.71)	0.3483 NS[1]
female	28(49.12)	29(50.88)	
Age, years, mean ± SD	32.1±6.7	35.9 ±9.3	0.0022 S[2]
Genotype No (%)			
Subtype 1	37(56.92)	28(43.08)	
Subtype 2	1(33.33)	2(66.67)	0,742 NS[1]
Subtype 3	80(55.56)	64(44.44)	
Subtype 4	1(33.33)	2(66.67)	
HCV viral load (IU/ml), mean±SD	2410226±6702822	2065677±5290369	0,900 NS[2]
Liver biopsy			
Knodell Histology			
Activity Index-HAI, mean±SD	2,761±2,402	4,072±2,920	0,001 S[2]
Presence of fibrosis, No (%):			
No fibrosis	100(60.24)	66(39.76)	
Fibrosis present	17(45.95)	20(54.05)	0,006 S[1]
Cirrhosis	2(16.67)	10(83.33)	
Steatosis, No (%):			
No steatosis	53(59.55)	36(40.45)	
Mild	29(40.85)	42(59.15)	0,015 S[1]
Severe	2(22.22)	7(77.78)	
BMI, mean ± SD	23.9 ±3.9	25.2 ±4.7	0.057 NS[2]

Legend: IR: insulin resistance; HCV: hepatitis C virus; SD: standard deviation; NS: not statistically significant; S: statistically significant; BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate transaminase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.
[1] Pearson Chi-square; [2] Mann-Whitney U Test

Table 3. Absence or evidence of insulin resistance (IR) in patients with HCV

Biochemical Characteristics	Absence of IR (N=122)	Evidence of IR (N=102)	P value
AST (10-34 U/L), mean±SD	56.9±46.2	74.9±71.4	0.002 S[1]
ALT (10-45 U/L), mean±SD	87.9±84.1	113.7±87.9	0.001 S[1]
Triglyceride (0.0-2.0 mmol/L), mean ± SD	1.1±0.6	1.3±0.8	0.142 NS[1]
Cholesterol (0.0-5.5 mmol/L), mean ± SD	4.2±1.1	4.2±1.2	0.732 NS[1]
HDL (0.9-2.0 mmol/L), mean±SD	1.2±0.3	1.1±0.3	0.157 NS[1]
LDL (2.2-3.7 mmol/L), mean±SD	2.5±0.9	2.5±1.01	0.801 NS[1]
FPG (3.6-5.6 mmol/L), mean±SD	5.0±0.6	5.5±0.8	0.0001 S[1]
Fasting insulin (2-17 μIU/ml), mean±SD	5.04±2.4	22.3±19.2	0.0001 S[1]
HOMA score, mean±SD	1.02±0.5	5.1±4.1	0.0001 S[1]
HbA1c (4.8-5.9%)	4.9±0.6	7.3±13.1	0.0001 S[1]
CRP	3.1±6.3	2.4±4.2	0.418 NS[1]
ferritin (up to 300 μg/L)	136.9±134.6	165.1±149.1	0.159 NS[1]
iron (7-28 μmol/L)	18.7±8.4	21.6±9.2	0.110 NS[1]

Abbreviations: IR: insulin resistance; HCV: hepatitis C virus; SD: standard deviation; S: statistically significant; NS: not statistically significant; ALT: alanine aminotransferase; AST: aspartate transaminase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FPG: Fasting plasma glucose; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, CRP: C-reactive protein; HbA1c: Hemoglobin A1c. [1]Mann-Whitney U Test

of p=0.006. Also, less severe as well as severe steatosis was more frequently expressed in patients with IR, with significant difference of p=0.015. It is clear that the IR group had higher body weight or higher BMI (25.2±4.7), unlike the other group (23.9±3.9), but there was no statistical difference (p=0.057) between the two groups. Mean value of AST and ALT in the group with IR was 74.9±71.4 U/L and 113.7±87.9 U/L, respectively, while in the group with no IR 56.9±46.2 U/L and 87.9±84.2 U/L, respectively. Highly significant difference between the groups (p=0.002 and p=0.001, respectively) was detected with regard to absence or evidence of insulin resistance. (Table 3).

There was no significant difference between the two groups in terms of triglycerides, total cholesterol and its fractions HDL-C and LDL-C. Fasting glucose level and fasting insulin level were significantly higher in the group with IR, as well as HbA1c. Thus, p=0.0001 refers to all three parameters. In a large number of patients FPG was between 5.6 and 6.9 mmol/L (in 29.9%) and also HbA1c was higher than 5.7% in 17.1% of patients, which means those patients can be included in the group of pre-diabetes according to the criteria of the American Diabetes Association. Analysis of CRP, serum ferritin and serum iron, as markers of inflammation, showed no significant statistical difference between the groups with or without IR (p=0.418, p=0.159 and p=0.110, respectively).

Discussion

The aim of our study was to show the presence of insulin resistance in non-diabetic, treatment naive patients with chronic hepatitis C in the Republic of Macedonia as well its relation with other factors that can further affect disease progression to fibrosis and cirrhosis.

We can clearly see the high prevalence of IR in up to 45.5% of patients. High representation of IR can also be found in other studies, such as the study of Kiran *et al.* (2013), the study of Moucari *et al*. (2008) where IR was found in 35% of patients with CHC, unlike the group with chronic hepatitis B (CHB) where IR was found in 5% of patients [19-20]

The important factors in relation to IR were as follows: age, extent of inflammatory and fibrotic changes in the liver, steatosis, transaminase activity, fasting glucose, insulin level and HbA1c value.

The age of the patients in our study was significantly higher in the group with IR (35.9±9.3), compared to the other group (32.1±6.7), with a significant difference of p=0.0022. This may be due to persistence of viral infection (longer influence of the virus) that contributed to the development of glucose metabolism disorder on one hand, but also evidence of metabolic disorders in the aged population of patients, on the other hand.

In our group of patients genotype 1 and 3 predominated, while the other two genotypes, 2 and 4, were found in a small number, which was not adequate for statistical analysis. Evidence of IR was almost identical in the two most common genotypes 1 and 3 (in patients with genotype 1 IR was found in approximately 43.08% and in patients with genotype 3 in approximately 44.44% of patients). Opposite to our results, there are studies indicating that genotype 1 and 4 were more often associated with IR [20].

In our analyzed group, there were no significant differences between the two groups in terms of viral load, which would mean that the number of virus particles was not associated with the IR, as also shown in the study of Huang *et al*. (2011) [21]. Unlike our study, in the study of Hsu *et al*. (2008) association between viremia and IR was shown [22].

Hepatitis C viral infection leads to the activation and the presence of inflammatory cells in the liver, which are responsible for the progress of the inflammatory condition which in turn causes liver damage and development of fibrosis. In our study more pronounced inflammatory and fibrotic changes in the histological preparation of the liver biopsy were found in IR group, with statistical significance of p=0.001 and p=0.006, respectively. In the study of Hickman *et al.* (2003), insulin was independently associated with fibrosis, but not with the inflammation [23].

There was a statistical significance in relation to the evidenced steatosis in patients with IR (p=0.015). Steatosis is an important co-factor which could result in accelerating the development of hepatic fibrosis and increased necro-inflammatory activity [24]. Steatosis in patients with genotype 3 is considered to be of viral origin ("viral steatosis") and is closely associated with viremia, while in other genotypes, it is associated with the factors of the host (obesity (particulary visceral), IR and type 2 diabetes mellitus ("metabolic steatosis") [25].

In our study patients deployed to the group of IR had greater weight, actually higher BMI (25.2±4.7) compared to the other group (23.9±3.9), but there were no statistical differences (p = 0.057) between these two groups. In the study of Souza *et al*. (2011) BMI was noted as a factor that was associated with IR, along with age and waist circumference [26]. Increased body weight, especially visceral adiposity along with other metabolic factors such as IR are the factors that lead to a greater degree of inflammatory activity (adipose tissue actually represents an organ where proinflammatory cytokines are excreted), pronounced steatosis and risk of disease progression to fibrosis [24,26].

The transaminases values (AST, ALT) were significantly higher in patients with IR, as opposed to those without IR, for p=0.002 and p=0.001, respectively. In general, increased transaminases value means a greater degree of hepatocellular damage that can be considered as an inviolable part of steatosis. Since our study showed a greater prevalence of steatosis in IR group (p=0.015), it cannot be strictly determined whether the increased transaminase activity in the group of IR patients was due to steatosis or the insulin resistance itself.

Regarding lipids, there was no significant difference between groups (with or without IR), while glucose status, fasting glucose level and fasting insulin level were significantly higher in the group with IR, as well as HbA1c, for p=0.0001 for all three parameters. In a rather large number of patients (29.9%) the value of FPG was between 5.6 and 6.9 mmol/L and also the value of HbA1c was greater than 5.7% in 17.1% of the patients. According to the criteria of the American Diabetes Association these patients enter the group of pre-diabetes, with real risk of developing type 2 diabetes mellitus.

In the situations of developed insulin resistance, increased production of inflammatory cytokines occurs, which improves the inflammatory damage of the hepatocyte. Serum ferritin and the CRP are markers of inflammation that are increased in terms of the inflammatory condition. In our study the values of ferritin and serum iron were higher in the group of IR, but there was no significant difference between the two groups in terms of CRP, ferritin and serum iron (p=0.418, p=0.159 and p=0.110, respectively) [27].

Conclusion

There was a high percentage of evidenced IR by 45.5% in CHC patients who were treatment naive and non-diabetic in R. Macedonia. IR was associated with the age, the inflammatory and fibrotic changes in the liver, with steatosis and transaminase activity or with other words, IR was more prevalent in older patients, those with a more pronounced inflammatory and fibrotic changes of the liver, patients with steatosis and higher transaminase activity. In the future, it has to be proved whether changes in metabolic factors which include IR will influence on the reduction of inflammatory and fibrotic activity and will prevent disease progression.

Conflict of interest statement. None declared.

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ММП

Case report

КАРПЕНТЕРОВ СИНДРОМ - ПРИКАЗ НА СЛУЧАЈ И ТРЕТМАН

CARPENTER SYNDROME – CASE REPORT AND TREATMENT

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Abstract

Introduction. Carpenter syndrome is a polymorphic disorder transmitted by autosomal recessive inheritance, caused by mutations in the RAB23 gene [1]. These genetic disorders are reflected on the biogenesis of intracranial structures. This syndrome was described for the first time in 1900 by the British doctor George Carpenter. It may include congenital heart diseases, mental retardation, hypogonadism, obesity, umbilical hernia, developmental disorder, bone anomalies and frequent respiratory infections. Carpenter syndrome has two main features: craniosynostosis and more than five fingers or toes [2-4].
Aim. To present our experience in treatment of an infant with Carpenter syndrome including trigonocephaly and polydactyly.
Case report. In May 2003, an eleven-month-old male infant with Carpenter syndrome was hospitalized in the Pediatric Department of the University Clinic of Neurosurgery in Skopje, Republic of Macedonia. The infant was referred to our Department from the University Pediatric Clinic because of trigonocephaly and polydactyly with two thumbs on his right hand. The infant had already been twice hospitalized at the University Pediatric Clinic for two recurrent lung infections suggestive of Carpenter syndrome. The diagnosis of trigonocephaly and polydactyly with two thumbs on the right hand was made by physical examination, X-ray of the right infant's hand and computed tomography of the head. According to Oi and Matsumoto classification from 1986 [5], the infant had a severe form of trigonocephaly.
Surgical procedure. Under general endotracheal anesthesia, the infant was placed supine on the operating table, a bifrontal skin incision was made and the scalp flap was created. The bifrontal craniotomy was realized into one bony piece succeeded by a modified Di Rocco's "shell" procedure including frontal translation and transposition rotating the flap for 180 degrees without /touching the orbital rims.
Results. The postoperative period was uneventful except for the expected forehead swelling. The infant was discharged from the hospital on the 7th postoperative day, neurologically intact. Three months after surgery, the head had excellent esthetic appearance, with regular psychomotor development in line with the age of the patient. Six months after the first surgery the patient underwent a second plastic and reconstructive surgery in order to reduce the number of fingers.
Conclusion. The early recognition and multidisciplinary approach could prevent new disabled individuals in the society. Our technique shortens the entire surgical procedure, diminishes the time under anesthesia and its complications, especially in departments where blood saving devices are not available.

Keywords: trigonocephaly, Carpenter syndrome, surgical treatment

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Апстракт

Вовед. Карпентеровиот синдром е полиморфно пореметување пренесено со автосомно рецесивен тип на наследување, предизвикано поради мутација на генот RAB23. Овие генетски пореметувања, меѓу останатото, се рефлектираат и на биогенезата на кранијалните сутури. Опишана за првпат во 1900 година од британскиот доктор Џорџ Карпентер (George Carpenter), овој синдром може да вклучи конгенитални срцеви аномалии, ментална ретардација, хипогонадизам, дебелина, умбиликална хернија, развојни аномалии, аномалии на коските и чести респираторни инфекции. Овој синдром има две главни манифестации: краниосиностоза и зголемен број на прстите на раката или на ногата.
Приказ на случај. На детскиот оддел на Клиниката за неврохирургија во Скопје, Република Македонија, во мај 2003 година било хоспитализира-

но 11 месечно машко бебе со каптентеровиот синдром. Бебето било упатено од Клиниката за детски болести поради тригоноцефалија и полидактилија со две палчиња на десната рака. Претходно бебето елекувано на Детската клиника поради две епизоди на респираторна инфекција, при што е дијагностициран карпентеров синдром. Дијагнозата на тригоноцефалијата и на полидактилијата беше поставена со физикален преглед, нативна рендгенграфија на десната дланка и компјутеризирана томографија на главата. Според класификацијата на Oi и Matsumoto од 1986 се работеше за тешка тригоноцефалија.

Хируршки третман. Бебето беше воведено во општа ендотрахеална анестезија, ставено во дорзална легната положба на оперативната маса, реализирана бифронтална коронарна инцизија на кожата и челен скалпен резен. Краниотомијата беше реализирана во едно парче и проследена со модифицирана Di Rocco процедура, вклучувајќи фронтална транслација и транспозиција, ротирајќи го коскениот флеп за 180 степени без ремоделирање на орбиталните лакови.

Резултати. Постоперативниот период помина без компликации, освен лесен оток на челото. Бебето е пуштено дома по седум дена невролошки интактно. Три месеци по операцијата, главата имаше одличен естетски изглед, со нормален психомоторен развој за возраста. Шест месеци по првата операција, пациентот беше подвргнат на втора реконструктивна операција за намалување на бројот на палците.

Заклучок. Раното препознавање и мултидисциплинарниот приод може да спречи појава на нови хендикепирани лица во општеството. Нашата техника дозволува скратување на целата хируршка процедура намалувајќи го времето во анестезија и нејзините компликации, посебно во институции кои не располагаат со апарати за заштеда на крв.

Клучни зборови: Тригоноцефалија, карпентеров синдром, оперативно лекување

Abstract

Introduction. Craniosynostoses represent developmental anomalies of the craniofacial growth in humans, that is, premature adhesion of the sutures of the calvaria, which leads to craniostenosis, obstructing the normal psychomotor development of infants. The consequences of untreated craniosynostosis can be simple esthetic disfigurations of the normal shape of the head, but can also lead to mental disruptions, difficulties in gaining new skills, disturbed behavior, epilepsy, hydrocephalus, headaches, damaging of the cranial nerves (I, II, V, VI, VII), and endocrinopathies [6].

The causes for the craniosynostoses are generally unknown; there are many theories and possibilities: the teratogenic effect of the valproic acid, aminopterin, hydantoin, retinoic acid, oxymethazoline, diseases such as hyperthyroidism, rickettsiosis, thalassemia, sickle cell anemia, thyroid diseases in pregnant woman, shunt-induced after treatment of hydrocephalus, amniotic bands, mucopolysaccharidoses, genetic damages, especially of the genes FGFR1-3, NELL1, MSX2, TWIST and GLI3 [1-6].

The principle of formation of the craniosynostoses has been modified in dependence of the thoughts and observations of the authorities. Virchow (1851) suspected that the craniosynostosis was a primary malformation while the deformity of the cranial base is secondary; Moss (1959) concluded that the malformation of the cranial base is the essence for appearance of premature fusion of the cranial sutures on the calvaria; and Park & Powers (1920) suggested much more acceptable theory that the primary defect was located in the mesenchymal blast tissue that led to anomalies in the cranial vault and the cranial base [4].

The incidence of craniosynostoses estimates approximately 0.1-1 (0.6) from 1000 live babies [1,4].The classification of craniosynostoses distinguishes two group s: non-syndromic (primary, simple) craniosynostoses and syndromic craniosynostoses (conjoined with other developmental anomalies, usually on the extremities) [1-6]. The non-syndromic craniosynostoses are divided depending on the suture that is prematurely closed, respectively, as dolychocephaly (scaphocephaly-head with shape of a boat, the most common-56%), brachicephaly (anterior unilateral-anterior plagiocephaly-24%), turricephaly (head in a shape of tower), trigonocephaly (wedge-shaped head-4%), anterior and posterior plagiocephaly and oxycephaly [1-6]. A total of 150 syndromes have been described accompanied by craniostenosis. The most common syndromic craniosynostoses include the following syndromes: Crouzon, Apert, Pfeiffer, Saethre-Chotzen and Carpenter syndromes [1-7].

Carpenter syndrome is a polymorphic disorder transmitted by autosomal recessive inheritance, caused by mutations in the RAB23 gene [1]. These genetic disorders are reflected on the biogenesis of intracranial structures. This syndrome was described for the first time in 1900 by the British doctor George Carpenter. It may include congenital heart diseases, mental retardation, hypogonadism, obesity, umbilical hernia, developmental disorder, bone anomalies and frequent respiratory infections. Carpenter syndrome has two main features: craniosynostoses and more than five fingers or toes [3-6].

The diagnosis of the craniosynostoses is made with physical examination of the child (inspection-characteristic shape of the cranial vault, palpation-a prominent thicken prematurely fused suture, volumetric measurements, cranial index, cranial perimeter), x-ray, EEG,

computed tomography with 3D reconstructions, magnetic resonance of the brain (for possible associated anomalies of the brain) [4-8]. Treatment of the craniosynostoses is surgical reconstruction, starting with the simple suturectomies going further to the complex cranial vault reconstructions with aim to create enough space for normal development of the brain and the esthetic correction of the shape of the head as well. Others specialists like pediatrics, pediatric psychiatrist, pedagogue, plastic and reconstructive surgeon, orthopedic surgeon, sociologist may be included in the treatment. The best result is achieved if the surgical procedure is realized at the age between 3-7 months of the infant. If the intervention is done before the age of 3 months, there is a high rate of recurrence of the craniosynostosis with a need for additional intervention [5,7].
The **aim** of this paper was to present our experience with this rare form of syndromic craniosynostosis and treatment in order to obtain acceptable results.

Clinical material

In May 2003, an eleven-month-old male infant was hospitalized at the Pediatric Department of the University Clinic of Neurosurgery of the Clinical Center "Mother Theresa" in Skopje, Republic of Macedonia. The infant had been referred to our Department by the University Pediatric Clinic because of trigonocephaly and polydactyly, with two thumbs on his right hand. The infant had already been twice hospitalized at the University Pediatric Clinic disease for two recurrent lung infections suggestive of Carpenter syndrome (Figure 1). The diagnosis of trigonocephaly and polydactyly with two thumbs on the right hand was made by physical examination, X-ray of the right infant's hand and computed tomography scan of the head. According to Oi and Matsumoto classification from 1986 [5], the frontal angle of the axial CT slices showed 89 degrees or severe trigonocephaly.

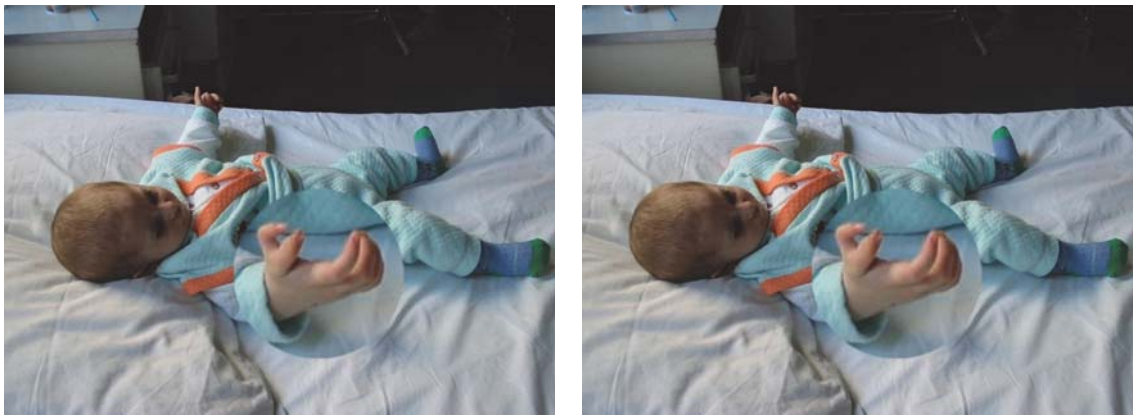

Fig. 1. The infant with trigonocephaly, polydactyly and Carpenter syndrome

Surgical treatment

The procedure was done under general endotracheal anesthesia with the infant placed in supine position (Figure 2). The procedure was started with bifrontal skin incision and creation of the frontal scalp flap.

After elevation of the periosteum, epidural dissection of the free edge of the frontal bone at the great fontanel was performed, followed by bifrontal craniotomy with one-piece free bony flap. The upper edge of the bony flap contained the coronal suture, spreading laterally downward to the both temporal fosses. The lower edge

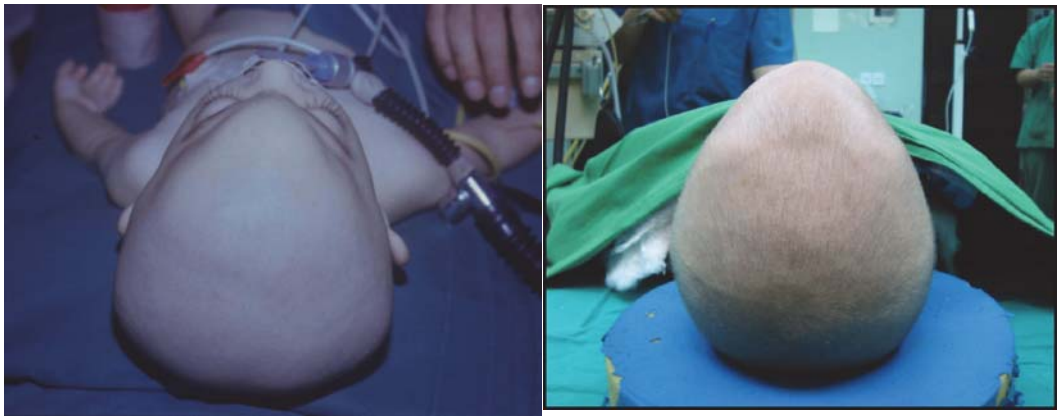

Fig. 2. The infant with trigonocephaly, polydactyly and Carpenter syndrome at surgery

of the bony flap was made just over the supraorbital rims, after creating a "burr-hole" using diamond drill over the frontonasal suture where the biggest thickening of the metopic suture was observed. The bony flap was diminished for 1 cm and rotated for 180 degrees.
The most prominent and thick part of the bone was excised, the midline of the bony flap was fractured in fashion of "green-stick" fracture and radial osteotomies were done for complete remodeling of the forehead. The bony flap was fixed forward and distal to the most frontal part of the cranial base through small bone holes on the free edge of the base with interrupted 2-0 silk sutures. Scalp flap closure was done with interrupted Blair-Donatti 4-0 polypropilene sutures, without using epicranial drainage.

Results

The postoperative period was uneventful except for the expected forehead swelling. The baby was discharged from the hospital one week after surgery. Three months after surgery, the head had excellent esthetic appearance, with regular psychomotor development in line with the age of the patient (Figure 3). Muscular tonus was better after reconstruction and correction of the craniostenosis, no further resistant respiratory infection and no opisthotonus were observed. Six month after the first surgery, the patient underwent a second plastic and reconstructive surgery in order to reduce the number of fingers. The child has been followed for 13 years and the "long term" results showed an excellent esthetic effect and normal psychomotor development, normal IQ, scolarity and socialization.

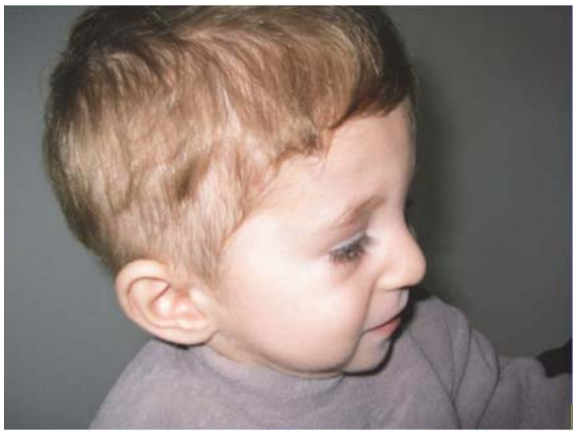

Fig. 3. The infant with syndrome 18 months after surgery

Discussion

The trigonocephaly (premature fusion of the metopic suture) takes 4% of all simple non-syndromic craniosynostosis [8]. The clinical appearance is typical, with wedge-shape, triangular forehead, flattened supraorbital rims, thickened metopic suture and cranial index with normal value. The computed tomography of the brain is also typical, with flattened bilateral frontal lobes, small anterior cranial fossa and small frontal angle [5].
The only treatment for trigonocephaly is surgical correction of the deformity. There are various number of operative interventions in which the common principle is reconstruction of the whole frontal bone, even including complex reconstructions with corrective osteotomies of the roof of the orbits and the lateral ends of the supraorbital rims for advancement and enlargement of the anterior cranial fossa.
Braid and Proctor [7] suggest that the operative correction is done between 6 and 12 months of age of the infant for open reconstruction of the anterior part of the vault because of the associated bigger blood lost, the durability of the intervention and high rate of recurrence if the intervention is done before the age of 6 months of the infant.
Raimondi's [9] opinion is to undertake surgical intervention at the age of 7 months with follow-up period until 13 months of age, presenting his excellent esthetic effect and no signs of recurrence of the deformity, with normal psychomotor development. Our patient was 11-month-old.
All open surgical procedures include bifrontal craniotomy, creation of free bony flap in one or two pieces, excision of the nasal extensions of the frontal bone and frontal extensions of the nasal bones, lateral advancement of the superior orbital ridges by pivoting on their sectioned or green-stick fractured medial edges, replacement of the frontal bony flaps after modified their edges, curvature and orientation. The variations include creation of a free orbital bar and its replacement after opportune remodeling, the insertion of a bone graft in the midline gap resulting from the removal of the upper part of the nasal bones to correct hypotelorisme [3].
Di Rocco's personal surgical technique [3] accomplishes all mentioned goals through a procedure named "shell" surgery because of the characteristic form of the frontal bony flap. In fact, the procedure consists of a frontal craniotomy in order to remove the deformed frontal bone and part of the parietal bones from a line 2 cm above the orbital ridges to the anterior fontanel. The flap is remodeled with the drilling of the thick ridge of the metopic suture and anterior displacement of its lateral aspects. Radial osteotomies converging downwards and towards the midline (so mimicking the lines of the shell) diminish the resistance of the bone and allow modifying its curvature. The nasal processes of the frontal bone and the upper part of the nasal bones are removed. The roof and the lateral walls of the orbits are sectioned and the lateral borders of the superior orbital ridges pushed forward in order to compensate for the hypoplastic orbital cavity. The pushing maneuver is made using the medial borders of the superior orbital ridges, cracked only partially, as pivots. The advance-

ment is maintained by replacing the remodeled frontal bone between the advanced superior orbital rims and the anterior border of the parietal bones [3].
In our case, the lung problems required a shorter surgical procedure and therefore we modified it by translation and transposition of the bone flap without advancement of the orbital rims. The created free frontal bone flap in one piece is osteotomized anterior and distal just above the superior orbital ridges with drilling of the most prominent part of the metopic suture and after that it is rotated for 180 degrees with excision of the most prominent wedge part of the bone flaps. Radial converging linear osteotomies are made on the bone flap with separate green-stick fractures for further enlargement of the intracranial space. The frontal bone flap is repositioned and fixed over the superior orbital ridges with one interrupted 2-0 silk suture on both sides of the forehead. The created reconstruction makes an excellent esthetic and functional effect at 3 months after surgery, especially with the enlargement of the anterior and lateral aspects of the frontal lobes of the cerebrum. Six months after the first surgical cranial procedure, the infant was rehospitalized at the University Clinic for Plastic, Esthetic and Reconstructive Surgery in Skopje in order to reduce the number of thumbs of his right hand.
The possible side effects of the intervention are: bleeding, infection of the wound, with overall incidence under 1%, possible recurrence with further need of additional surgical correction depending on the age of the patient and the type of craniosynostosis. Our long-term results show no complications, no recurrences, and normal development of the child.

Conclusion

The early recognition of these anomalies allows the most adequate treatment according to the conditions of the health system in order to treat deformities of the newborn and infant's head and to prevent abnormal psychomotor development during children's growth.
The multidisciplinary approach could prevent new disabled individuals in the society. Our technique shortens the entire surgical procedure, especially in departments where blood saving devices are not available.

Conflict of interest statement. None declared.

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ММП

Case report

MINOXIDIL OVERDOSAGE: A CASE REPORT

ПРЕДОЗИРАНОСТ СО МИНОКСИДИЛ: ПРИКАЗ НА СЛУЧАЈ

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Abstract

A 64-year-old man ingested about 60 ml 2% of topical minoxidil solution in order tomake his hair grow faster. Twelve hours after ingestion he was brought to the University Clinic of Toxicology with severe hypotension, tachycardia, chest pain and subendocardial ischemia. ECG showed diffuse T-wave inversion and depressed ST segments. He was also oligoanuric at admission. In spite of the intensive hydration with crystalloidsolutions and intravenous dopamine administrationthat resulted in partial hemodynamic improvement and resolution of the ECG changes, kidneyfailure occurred. After two hymodialysis sessions, urea and creatinine levels returned to normal and rebound hypertension appeared. The patient was discharged after 12 days of hospitalization in a good condition. Topical minoxidilsolution is formulation used for treatment of androgenic alopecia. If orally ingested it leads to severe hypotension, acute coronary syndrome, compensatory tachycardia and acute kidneyfailure. Emergency therapeutic approach is a precondition for successful outcome.

Keywords: minoxidil, dopamine, subendocardial ischemia

Апстракт

Маж на 64 години ингестирал околу 60 мл двопроцентен миноксидил, раствор за локална употреба, со цел постигнување побрз раст на косата. Дванаесет часа по ингестијата е донесен на клиника со тешка хипотензија, тахикардија, градна болка и субендокардна исхемија. На ЕКГ детектирана дифузна инверзија на Т бранот и СТ сегмент депресија. Исто така, на приемот беше олугоануричен. И покрај интензивното хидрирање со кристалоидни раствори и со интравенска терапија со допамин, дојде до парцијално хемодинамско подобрување, повлекување на ЕКГ промените, но влошување на бубрежната функција. По изведени две хемодијализи дојде до нормализирање на вредностите на уреа и на креатинин и појава на ребоунд хипертензија. Пациентот е испишан во добра состојба по 12 дена хоспитализација. Миноксидилот за локална употреба е препарат, кој се користи за терапија на андрогена алопеција. Кога ќе се внесе перорално доведува до тешка хипотензија, акутен коронерен синдром, компензаторна тахикардија и акутна бубрежна инсуфициенција. Итниот тераписки пристап е предуслов за добар исход.

Клучни зборови: миноксидил, допамин, субендокардијална исхемија

Introduction

Minoxidil is a known antihypertensive drug, which has recently been approved for treatment of androgenic alopecia. It can be found on the market as 5% and 2% solution. Sixty ml of 2% solution contains about 1,200 mg of minoxidil, which is approximately 12 times greater than the maximum recommended daily dose for controlling hypertension. Correlation between minoxidil ingestion and development ofsubendocardial ischemia, hemodynamic impairment and acute kidney failure (AKF) will be discussed in this paper.

Case report

A 64-year-old man with no prior history of kidney failure and cardiologic diseases was brought as an emergency case into the University Clinic of Toxicology 12 hourspost-ingestion of 60 ml 2% of minoxidil solution (Pilfud, Bosnalijek). He ingested the solution in order to achieve rapid therapeutic effects. He denied use of alcohol or other medications, but said he suffered from a moderate hypertension. On admission he was conscious, orientedto time and place, afebrile, eupneic. His pulse was 110/min, and blood pressure was 60/20 mmHg. The patient experienced chest pain, epigastric discomfort,

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weakness and malaise. ECG showed tachycardia and signs of subendocardial ischemia. Therapy was initiated with resuscitation with 2.5 literof physiological solution given in the first 7 hours, but the patient was anuric. Following administration of 8 mcg/kg/min dopamine the blood pressure increased to 90/60 mmHgat 12 hours after admission, and at 24 hours it reached and remained at 100/70 mmHg in the first three days. The dose of dopamine was gradually reduced to 2 mcg/kg and discontinued after 48 hours. Diuresis was 100/300/700 ml/24 hours on the first/second/third day, respectively, but the values of degradation products increased. The

Table 1.The most characteristic laboratory and clinical parameters

day	hours	CK	CK-MB	CK-MB%	Na	K	urea	creatinine	diuresis/24h	TA
1	09	346	19	5.49	136	4.2	11.1	310	100	60/20
	21	992	14	1.41	141	4.0	15.1	356		90/60
2	09	1098	52	4.74	138	4.8	28.6	601	250	100/70
	21	720	45	6.25	136	4.5		659		90/50
							31.9			
3	09	324	22	6.79	134	4.4	34.6	625	700	90/50
	21				128	4.3	36.5	757*		100/60
4	09	200	14	7.00	134	4.5	21.6	270*	2000	150/85
7	09	170	10	5.88	139	4.8	14.9	124	6400	180/100
10	09	84	8	9.52	140	4.4	4.5	74	9100	200/130
	21									170/110
12	09	75	6	8.00	142	4.5	5.0	82	3000	140/90
	21									130/80

Values of CK, CK-MB, urea, creatinine are expressed in mmol/l, dieresis in ml/24h, blood pressure in mm Hg. *hemodialysis

patient underwent two hemodialysis sessions on the third and fourth day, which resulted in a polyuric phase and decrease of urea and creatinine. Blood pressure started to increase and since the fourth day until the end of the hospital stay ranged from 150/85 to 200/130 mmHg (rebound hypertension).

Laboratory analysis showed leukocytosis, increase of CK (creatine kinase) and CK-MB, which did not surpass 10% of the CK value, and troponine was negative. Urea and creatininepeaked at36.5 mmol/l and 757 mmol/l, respectively, on the third day and returned to normal on the 10th day. The remaining laboratory findings were unspecific (Table 1).

The first day ECG revealed diffusely inverted T-waves with depressed ST segments in V2-V6. These changes withdrew and ECG stabilized on the third day (Figure 1, Figure 2).

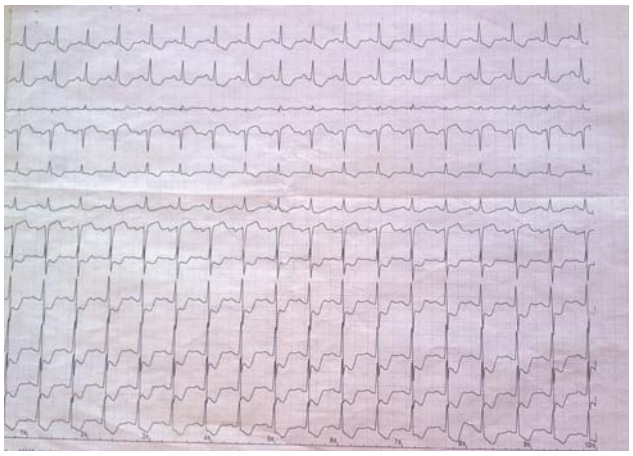

Fig.1. First day ECG

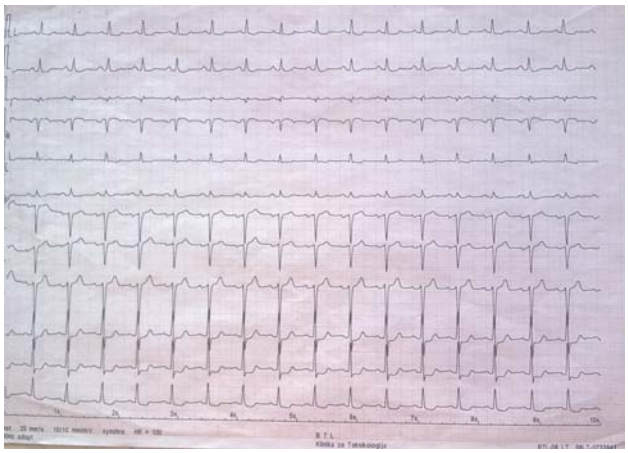

Fig. 2. Third day ECG

Discussion

Minoxidil, which was originally used for treatment of hypertension, recently has been approved for treatment of male pattern baldness [1]. Adverse effects of minoxidil local application are rare and minor. Most commonly it can cause itching and irritation on the affected area and other dermatologic complications and minor systemic effects due to its small resorption. The systemic application of minoxidil is associated with more serious complications.

Minoxidil is activated in the liver and its action isto relax vascular smooth muscle by opening cell surface potassium channels causing an efflux of potassium, hyperpolarization and relaxation of smooth muscle cells.

Minoxidilproduces systemic hypotension by a direct arteriolar vasodilatation and is associated with a reflex

increase in cardiac output and myocardial contractility mediated by the sympathetic nervous system. Maximal concentration in the blood is achieved 1 hour after oral administration, but due to delay of active metabolic formation, the maximal therapeutic effect appears much later. The serum half-life is 3 to 4 hours, but the effect can last 24 hours or longer [2]. Minoxidil is eliminated mainly by hepatic metabolism.

There are reports thatminoxidil does not cause hypotension in normotensive individuals [3]. However, many authors report prolonged hypotension post-ingestion, which lasts two to four days after admission to the hospital [4,5].

Various cardiovascular manifestations resulting from different doses of minoxidil have been reported. Lower doses produce hypotension and successive increase in doses leads to tachycardia and myocardial ischemia. This tachycardia and resultant myocardial ischemia are probably a compensatory mechanism for severe hypotension. These cases are treated with combination of crystalloids, dopamine [6] and phenylephrine infusion [7] guided by the cardiovascular parameters.

The greater the contractility, the more oxygen the myocardium consumes. Increased heart rate (HR) leads to increased myocardial O2 consumption.

In our patient, in addition to prolonged hypotension and tachycardia coronary syndrome developed along with reverse ECG changes and negative CK-MB and troponine. Subendocardial ischemia is believed to be caused by an increased myocardial oxygen demanddue to secondary catecholamine overload that increases myocardial contractilityand decreasedcoronary perfusion that is due to tachycardia and hypotension acute minoxidil intoxication. Similar transitory ECG changes when larger doses of minoxidil (about 3 grams) had been given were described by other authors [8,9]. Some authors have presented development of nontransmural infarction as a result of ingestion of similar amount of minoxidil solution, associated with pleural effusion and good response to conservative treatment [7].

Besides coronary syndrome, the patient developed AKF that did not respond to the conservative treatment, but hemodialysis was required for returning the degradation products to normal values. In our patient the cause for kidney failure was dishemodynamicand was probably a result of delayed hospitalization (12 hours post-ingestion) and prolonged kidney hypoperfusion. In other case reports a smaller degree of oliguria was registered as well as a smaller increase in degradation products with more rapid response to drug treatment as a result of the urgent hospitalization (two hours post-ingestion at the most) [5,7,9]. There is a lack of literature on minoxidil direct nephrotoxicity that results in decreased kidney function in a state of acute overdosage. Therefore, we assume there was a preranal failure in our patient, which was caused by hemodynamic insufficiency and suffered renal hypoxia.

The so-called rebound hypertension was registered in our patient, which appeared on the 4th day of the hospital stay, that is, following hemodialysis and establishment of diuresis.

By definition rebound hypertension is an increase in blood pressure in response to stopping or reducing high blood pressure medication. Severe cases can result in a very large increase in blood pressure which requires prompt treatment to avoid complications such as organ damage. Hypertension in our patient was a result of blood pressure establishing without therapy, when minoxidil, which is dialyzable, was completely eliminated and kidney function was improved. Althoughprazosin was included in the therapy on the seventh day, peak value was reached on the 10th day of hospitalization (200/130 mmHg), and it returned to normal until the discharge day by dose adjustmentof prazosin in the therapy. There are no case reports in the literature presenting with rebound hypertension after minoxidil intoxication. However, this phenomenon has been described in children treated with several hypertensive drugs after discontinuation of minoxidil therapy due to the development ofhypertrichosis. Rebound hypertension was manifested with hypertensive encephalopathy in those children in whom minoxidil was withdrawn rapidly. The occurrence ofrebound hypertension correlated well with the cumulative dose of minoxidil and the rapidity with which minoxidil was withdrawn [10]. Thus, we think this phenomenon can appear also in patients intoxicated with minoxidil, who had a history of hypertension and can be an additional risk factor for onset of other co-morbidities if it is not expected and not treated.

In summary, we have presented a case of severe poisoning after ingestion of 2% of topical minoxidil solution. This is the first case in our clinical practice, which was manifested with severe hypotension, tachycardia, subendocardial ischemia, AKF and rebound hypertension. As the use and availability of these solutions for local application is increasing, a greater awarenessof systemic toxic effect of minoxidil is also necessary as well as immediate and adequate treatment.

Conflict of interest statement. None declared.

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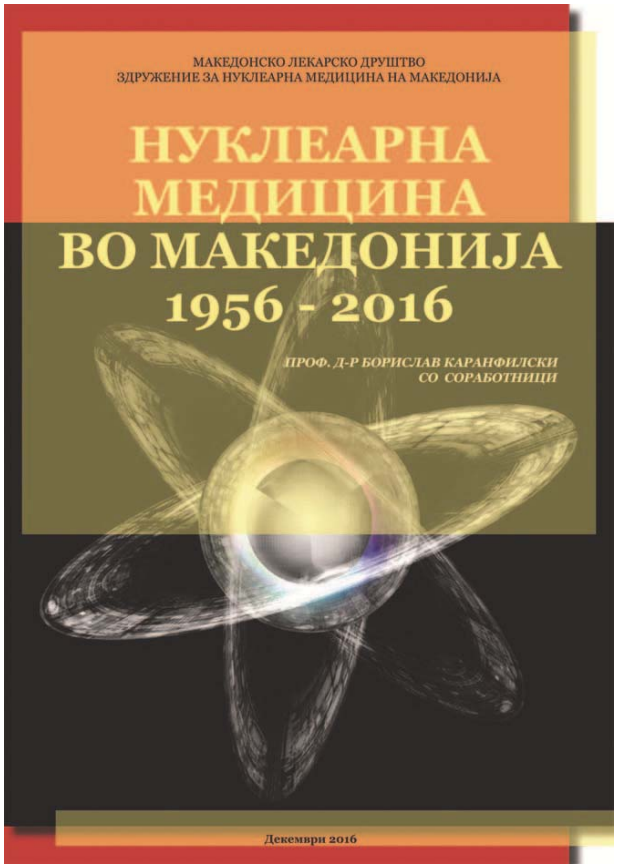
П Р Е Д Г О В О Р



На просторот на Република Македонија, нуклеарната медицина ги прави своите први чекори пред речиси шест децении, само неколку години по етаблирање на првите радиозитопни лаборатории во САД, кога и почнува употребата на радиоизотопите во хуманата медицина. Институтот за патофизиологија и нуклеарна медицина имаше значајно влијание врз развојот на оваа медицинска гранка не само во Република Македонија, туку и во другите центри во поранешна Југославија, во балканските земји и пошироко.

Сето тоа се должеше на ентузијазмот и на посветеноста на вработените на Институтот, но најмногу на визионерската мисла на лекарот, академик доктор Исак Салис Таџер. Целиот свој долг и плоден работен век го посвети на Медицинскиот факултет на Универзитетот „Св.Кирил и Методиј“ во Скопје, но и на македонската медицинска едукација, наука и практика. Професорот Таџер, повеќе од 50 години придонесуваше во работата на Македонското лекарско друштво, како долгогодишен уредник и член на редакцијата на Македонски медицински преглед.

Сиот ангажман во создавањето, востановувањето и развојот на нуклеарната медицина немаше да биде проследен, одбележан и славен доколку не се инвестираше во создавање високостручен, целосно посветен на работата и творечки настроен кадар, кој и денеска го продолжува делото на пионерите на нуклеарната медицина во Република Македонија академик проф.д-р Исак Таџер, проф. д-р Борислав Каранфилски, проф.д-р Вера Долгова-Корубин, проф. д-р Никола Серафимов, проф. д-р Нина Симова, проф. д-р Бранислава Георгиевска, проф. д-р Георги Шестаков и виш стручен соработник Вукосава Бубалова.

Институтот за патофизиологија и нуклеарна медицина, денеска е современа, признаена и ценета институција од областа на конвенционалната нуклеарна медицина, не само во балканските земји, туку во Европа и остатокот од светот. Повеќе од половина век претставува значаен сегмент во додипломската и постдипломската едукација на Медицинскиот факултет, високо стручна апликативна дејност од областа на тироидологијата, како и на радионуклидната дијагностика и терапија, но исто така и на научно истражувачка медиицинска дејност во Република Македонија.

Јубилеите претставуваат убави свечени моменти, кои треба да останат забележани за идните генерации, но истовремено претставуваат и обврска да се продолжи традицијата и да се биде уште подобар и поквалитетен. Современата нуклеарна медицина во Република Македонија со опременоста, посветеноста и ентузијазмот на стручниот кадар, тоа го може и го прави како за медицинската едукација, така и за високостручната апликативната дејност, како и за развојот на македонската медицинска научна мисла.

Доц. д-р Горан Димитров
Претседател на Македонското лекарско друштво

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.

Уплата за испечатен труд во списанието ММП изнесува 3.000, 00 денари и се уплаќаат на жиро сметката на: Македонско лекарско друштво
300000000211884 – Комерцијална банка
со цел на дознака : уплата за стучен труд

Адресата на Редакцијата
Даме Груев бр. 3
Градски ѕид блок II,
1000 Скопје,
Тел.: ++ 389 02 3162 577

Електронска адреса (Е-маил): mld@unet.com.mk

Известување за членовите на МЛД

Сите што сакаат и натаму да го добиваат списанието треба да ја имаат уплатено членаринатa за 2016/2017 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.