СПИСАНИЕ НА МАКЕДОНСКОТО ЛЕКАРСКО ДРУШТВО
Мак. мед. преглед, 2019; 73(2)

JOURNAL OF THE MACEDONIAN MEDICAL ASSOCIATION
Mac. Med. Preview, 2019; 73(2)

UDK: 61+061.231=866=20 CODEN: MKMPA3 ISSN: 0025-1097

Македонски Медицински Преглед

Macedonian Medical Review

Основано 1946
Founded 1946

www.mld.mk

2/19 MMP

Мак Мед Преглед

Списание на Македонското лекарско друштво

Journal of the Macedonian Medical Association

Наслов на Редакцијата и издавачот / Address of the Editorial Office and Administration:
1000 Скопје, Даме Груев 3, Градски ѕид блок 2
тел. 02/3162 577
www.mld.org.mk / mld@unet.com.mk
Жиро сметка / Bank Account
300000000211884 - Комерцијална банка Скопје

Печати: Бранко Гапо графичко производство - Скопје

Македонски медицински преглед се печати три пати годишно. Претплатата за списанието изнесува 10 евра за лекари, 50 евра за установа, странство 80 евра.

Основано 1946 Фоундед 1946

Содржина/Contents

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ММР

Consensus statement

RECOMMENDATIONS FOR THE DIAGNOSTIC ALGORITHM IN LUNG CANCER *CONSENSUS STATEMENT OF THE MACEDONIAN RESPIRATORY SOCIETY AND THE MACEDONIAN ASSOCIATION OF PATHOLOGY*

ПРЕПОРАКИ ЗА ДИЈАГНОСТИЧКА ПОСТАПКА КАЈ БЕЛОДРОБЕН КАРЦИНОМ *КОНСЕНЗУС НА МАКЕДОНСКОТО РЕСПИРАТОРНО ЗДРУЖЕНИЕ И НА ЗДРУЖЕНИЕТО НА ПАТОЛОЗИ НА МАКЕДОНИЈА – НАДГРАДЕНО ИЗДАНИЕ*

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Abstract

Lung cancer is the "number one cancer killer" in the world. Its prevalence is associated withsmoking as the primary cause, although air pollution in general and genetic factors are also important.
The mortality especially from advanced stage lung cancer is still high, although there has been a significant improvement in the past 10 years, mostly due to the introduction of novel compounds such as targeted and immunological treatment. The advances in the treatment of NSCLC have imposed updating of the guidelines for the diagnostic procedure and screening of LC, for the indications for molecular testing as well as for targeted selection of patients who shall benefit the most fromthe novel treatment modalities.
These recommendations shall fulfil their purpose only if implemented in the educational curriculum and if incorporated in the healthcare system strategies.

Keywords: guidelines, non-small cell lung cancer, molecular diagnosis, diagnostic algorithm

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Абстракт

Карциномот на бели дробови е „убиец број еден" во светот! Неговата преваленција е асоцирана со пушењето, како примарна причина, но и загадувањето и генетските фактори играат не помалку важна улога.
Морталитетот, особено кај белодробниот карцином (БК) во напреднат стадиум, сеуште е многу висок. Сепак, последните 10 години се забележува значително подобрување на стапката на смртност, кое најмногу се должи на воведувањето на новите супстанци, како што се целната и имунолошката терапија. Напредокот во третманот на неситноклеточниот белодробен карцином наметна и осовременување на водичите за дијагностичката процедура и скринингот на БК, на индикациите за молекуларно тестирање, како и за прецизна селекција на пациенти кои ќе имаат најголема корист од новите модалитети за третман.
Овие препораки ќе ја исполнат својата цел само доколку бидат имплементирани во курикулумот за едукација на докторите, но и ако се инкорпорираат во националните стратегии на здравствениот систем.

Клучни зборови: Препораки, неситноклеточен белодробен карцином, молекуларна дијагноза, диајгностички алгоритам за белодробен карцином

Introduction

Lung cancer is the most frequent cause of mortality associated with malignancies in the world [1,2]. The prevalence of lung cancer (LC) evolves impressively and is associated withprevalence of smoking inthe specific population. From a rare, sporadic disease up to the beginning of the XXth century, in the 1980-ies, lung cancer became the type of cancer with the highest incidence in the world. The overall incidence of LC in Macedonia is 34.1/100 000 and in males it is as high as 55.7/100 000 (GLOBOCAN 2018)(3,4).A global problem and one of the main reasons for the high mortality of LC is the diagnosis of the disease in the advanced stages, when the effects of the treatment are limited.
Implementation of ***active screening*** for LC contributes to detection of the disease in the early stages, when treatment has a higher probability for success, followed by decreasing of mortality for 20-25% [5,6].The current recommendations determine that screening with

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low-dose helical computerized tomography of the chest should be done in all active heavy smokers (≥30 pack/ year history of cigarette smoking [7], or smoking cessation ≤15 years if a former smoker), at age from 55 to 80 years. Other diagnostic methods, such as chest X-ray, sputum cytology, etc. are not recommended for LC screening.

The exact ***histological classification and molecular analysis*** of LC determines the predicted survival, resectability, type of recommended treatment and prognosis of the disease, provides important information on the epidemiologic prevalence of various types of LC and their association to the risk factors, and is a milestone for determination of prognosis and further treatment of patients. The collaboration between clinicians and pathologists as well as the standardized processing of biopsy samples is of great importance inreaching correct and precise diagnosis.

Numerous molecular investigations of LC samples have detected various mutations, relevant because of the possibility of their targeting with immunomodulatory and targeted therapy, which significantly changes the prognosis and survival of patients. The mutations detected in non-small cell LC (NSCLC) are of exceptional importance, especially in adenocarcinoma, found in about 40% of all LC.

In the squamous type of NSCLC, it is important to detect the presence of the aberrant expression of PD-1 receptor (receptor for programmed death) and its ligands PD-L1 and PD-L2[8].Specific place in the treatment of patients with LC is dedicated to the targeted treatment towards mutations of EGFR, PTEN, PI3K, DDR2, and other genes with thyrosine kinase activity [9]. A large number of driving mutations hasalready been determined in lung adenocarcinoma, for which the contemporary pharmaceutical industry has developed corresponding targeted agents. The most investigated are the mutations of the gene for the Epidermal Growth Factor (EGFR), such as mutations L858R, deletion of the exon 19 (Del-19) and T790M [10-16].

For the aforementioned molecular defects (deteriorations), targeted therapy (compounds that inhibit the activity of receptor or tyrosine kinases) is available and shows very good treatment effects in clinical practise (first generation tyrosine kinase inhibitors-TKI, erlotinib and gefitinib, second generation afatinib, and osimertinib and rociletinib as third generation TKI targeted to the T790M mutation). In 4-5% of the confirmed adenocarcinomas, rearrangement of the Anaplastic Lymphoma Kinase (ALK) gene is detected. The current guidelines and available studies confirm the effects of crizotinib (first generation ALK inhibitor), alectinib and ceritinib (second generation), whereas the novel formulations such as brigatinib and entrectinib are in finalstage of investigation. ALK inhibitors are often efficient in patients with ROS1 mutation, occurring in 1-2% of Caucasian patients [17,18]. The increasing number of detected genetic alterations and driver mutations found in lung cancer patients, and the possibilities for targeted treatment, lead to introducing a new, molecular classification of NSCLC [19].

The development and advances in molecular oncology, oncogenesis and targetedtreatment for NSCLC have introduced novel challenges in the treatment of LC, but also higher expectations, especially with regards to the prognosis and course of the disease. The plead of very well defined genetic mutations (mutations of EGFR, ALK, ROS1, KRAS, PD-1, PD-L1, etc.), the wider availability of the methods for molecular testing and the development of new generations of targeted compounds dictate the need of creation and optimization of diagnostic algorithms and recommendations determining the exact place for their application in clinical practice [20]. Acknowledging the information on the signifycant positive effects of targeted therapy on the survival rates and the quality of life of LC patients, the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP)updated their guidelines concerning the indications and procedures for molecular testing of patients with lung cancer [21,22]. These new data in the field of molecular diagnosis and targeted treatment for NSCLS imposed the need for revising and updating the 2011 Guidelines of the Macedonian Respiratory Society for Diagnosis of LC [23].

Recommendations for the diagnostic steps in lung cancer- clinical aspects

1. Clinical presentation and imaging methods

The clinical manifestations of the disease are determined by the localization and distribution of the tumor. The most frequent symptoms and signs are: *cough, wheezing, stridor, dyspnea, atelectasis, pleural effusion, chest pain, pain in the arm and shoulder, dysphonia,* important especially if persistingfor more than three months. The incidence of *hemoptysis, Horner's Syndrome* (ptosis, myosis, enophtalmus) in smokers over 40 years of age is highly associative for LC.

The patient should be **urgently referred** to a specialist (pulmologist or internal medicine specialist) in case ofpresentation with bloody sputum, dysphonia, superior vena cava syndrome, and stridor or if chest X-ray shows changes indicative for LC.

2. Performance status of the patient

Classification of patients according to the Karnofski scale is recommended for estimation of the prognosis of each individual patient, and estimation of the effect of treatment. Lower Karnofski score is associated with worse performance, prognosis and survival of the patient [24,25].

3. Non-invasive imaging methods

We recommend the following imaging methods for defining the macro-morphologic features of LC:

1. Chest X-ray (two projections)
2. Computerized tomography (CT) of the chest shows a high level of sensitivity for early diagnosis, screening, and is necessary for determining the further evaluation and diagnostic steps.
3. Chest ultrasound (using a combination of high and low frequency, linear, curved and phase-array probes) -the method represents a useful tool for detection and targeting peripheral and mediastinal tumor masses, pleural effusion and pneumothorax.
4. Positron emission tomography with low dose CT (PET-CT) is a complementary method for defining the stage and further treatment in selected patients, only if indicated by*multidisciplinary boardfor lung cancer*.

4. Determination of the clinical stage of NSCLC and TNM classification

Determination of the clinical stage of NSCLC is crucial for estimation of the recommended treatment and prognosis of the disease. The classification is calculated by combining the results obtained with high resolution chest CT enhanced with contrast, magnetic resonance of the central nervous system, PET-CT, endobronchial ultrasound guided transbronchial needle aspiration (EBUS/EUS-TBNA), and/or mediastinoscopy (onlyby indication of the boardfor LC).

These guidelines recommend that the determination of the stage of the disease and the decision about further treatment ofeach patient should be reached by a ***multidisciplinary boardfor lung cancer***, composed of at least ***an interventional pulmologist, radiologist, pathologist, oncologist and thoracic surgeon.***

Clinical staging should be done using the TNM classification (VIIIth edition), issued by the American Joint Committee-AJCC [26], the Union for International Cancer Control (UICC) [27]and the International Association for the Study of Lung Cancer-IASCL [25,28].

Methods for obtaining adequate sample for analysis

Optimally, efficient diagnostic approach means obtaining sufficient amount of vital tissue from the suspected lesion, as well as recommendations for rational tissue manipulation by the pathologist, with a final goal to determine the diagnosis of the cancer quickly, precisely and efficiently. The invasive procedures for obtaining the sample can be performed by a pulmologist or radiologist (trained for invasive diagnostics), or a surgeon (when the changes are not accessible with the available methods for invasive diagnosis, if conclusive result was not obtained with minimum invasive procedures, or when dealing with a small solitary pulmonary nodule -T1N0M0, when it is expected that the surgical intervention shall provide diagnosis and radical treatment in one act [29].

The education of the interventional pulmonary specialists should be unified and standardized on state level, and should be performed in a referent University Centre for education in the field of interventional pulmology. The centres-candidates for education in interventional pulmology must perform sufficient number of specific interventions on regular basis (determined by international recommendations for achieving and maintenance of skills) and must have experts withthe highest level of proficiency in the field. The competency of these professionals should be regularly estimated according to the international and national educational programs. Education ofyounger candidates should represent a continuous process in everyday practise, organized also through dedicated educational courses in the country or abroad.

The diagnostic methods for obtaining an adequate tissue sample, which should routinely be available,are:

- Cytologic analysis of the sputum;
- Fiberbronchoscopy with bronchial biopsy and/or transbronchial biopsy (TBB) and transbronchial needle aspiration (TBNA), also familiar in Macedonia as "perbronchial aspiration biopsy (PBAP)", according to the old nomenclature;
- Rigid bronchoscopy;
- Trans-thoracic "core"-biopsy (TTB), guided by ultrasound, CT or fluoroscopy and/or fine needle trans-thoracic aspiration;
- Diagnostic pleural thoracocenthesis;
- "Blind" pleural biopsy (by Rammel);
- Medical thoracoscopy;
- Surgical procedures (mediastinoscopy, video assisted thoracoscopy-VATS, open lung biopsy).

Optimally, the number of small bioptic samples obtained by endoscopy (bronchial biopsy, TBB) should be 4-6 (from vital tissue without necrosis). Besides obtaining adequate *biopsy samples*, the intervention should proceed with application of other available techniques, such as *fine needle aspiration biopsy, catheter biopsy, brushing, bronchial aspiration, bronchoalveolar lavage*. If necessary (necrotic tumour, repeated bronchoscopy because of inadequate previous sampling), 4-6 biopsies with cryo-probe should be obtainedin the centres which have adequate equipment and trained personnel.

Trans-bronchial needle aspiration, guided by linear or radial endobronchial ultrasound probe (EBUS or EUS) is a complementary method which can be used for diagnosis as well as staging. At least three passes (punctions) of each targeted region are necessary. We recommend targeting every lymph node with sagittal diameter larger than 10mm, detected on CT (not older than 1 month) or larger than 5mm, visualized with EBUS. If PET-CT is provided, all PET- positive lymph nodes should be sampled. EBUS needles, sized 18G and 19

G are preferred. Samples obtained with 21G needles might not be sufficient for molecular analysis.

*The **indication*** for the intervention must be unequivocal and brought on the basis of previous complete diagnostic workup of the patient. Whenever possible, the type and timing of the intended procedures should be planned before beginning of the intervention. The purpose, type and possible complications of the intervention should be clearly explained to the patient, and signed ***informed consent*** must be provided before the intervention. Within the process of obtaining informed consent, the patient (and his family member/s) should also be familiarized with the risk and consequences from not performing the procedure and thealternative possibilities for obtaining diagnosis. The type of anaesthesia/analgosedation should be discussed, recommenddations should be given for preparation before the intervention and the patient should be informed about theexpectationsof the procedure.

Prior to performing the ***procedure***, it is essential to estimate the safety of performing the planned interventions and the probability of complications (bleeding, pneumothorax, air embolism), taking into consideration the general state of the patient, present comorbidities and the accessibility of support from other specialties (conditions for transport, anaesthesialogy/intensive care unit, thoracic surgeon), according to the International Guidelines for Interventional Pulmology and Bronchology of the British Thoracic Society from 2013. It is minimum standard that before every interventional procedure is that the patient must have at leasta chest CT (not older than 1 month), recent ECG, gas analyses and basic laboratory (blood count, electrolytes, glucose, degradation products) with estimated time of bleeding. The patient should be observed for one hour after bronchoscopy with bronchial biopsy, TBNA, bronchoalveolar lavage and brushing, and at least four hours after biopsies with higher risk for complication (TBB, TTB, pleural biopsy).

The ***standard time*** necessary for realization of endoscopic procedures is from 45 to 60 minutes. In case of need for repeating biopsy (negative or non-conclusive histopathological finding) the repeated intervention should be done by another bronchoscopist.

The ***report*** about the intervention should contain the generaldata of the patient, the correct time when the procedure was performed (start and end time), vital parameters before and after the intervention, type of analgo-sedation and local anaesthesia, data (serial number, type, dimension) of the instrument used,intubation route, detailed description of the macro-morphologic finding and the samples taken, as well as the estimated blood loss. When processing of the material, it should be accompanied by a dedicated form with integrated report from the intervention, together with relevant clinical information on the patient [25].

Recommendations for pathologic diagnostics and molecular testing for lung cancer

The histological diagnosis and molecular classification of lung cancer are multistep processes:

- Tissue processing of the obtained specimen to paraffin embedded tissue sections
- Histological/cytological and histochemical analysis
- Immunohistochemical (IHC)/immunocytochemical (ICC) analysis
- Molecular characterisation of the tumor.

The recommendations for histological diagnosis and molecular testing in lung cancer represent a complex diagnostic algorithm, which sets a lot of challenges for proper management of patients with lung cancer, due to which every institution should establish a multidisciplinary team that will coordinate the optimal approach from sampling to adequate processing, in order to enable fast diagnostics and molecular analyses. Obtaining appropriate sample (biopsy or cytological) of tumor tissue is a basic precondition in the diagnosis of LC.

The aim of the pathological evaluation of the lung cancer varies depending on the type of the sample: 1) biopsy or cytological sample for initial diagnosis in cases of suspected lung cancer; 2) operative material, or 3) material for molecular analysis in previously confirmed diagnosis of NSCLC.

In small biopsies or cytological material intended for *initial diagnosis*, the main aim is: a) to establish the correct diagnosis according to the Classification of the WHO for lung cancer from 2015[30], and b) to save enough tissue for molecular analyses, especially in patients in advanced stage [31, 32].

In small biopsies and cytological material intended for *molecular analyses*, in previously diagnosed cases or when the disease has progressed after targeted therapy, the main aim is: a) to confirm the initial histological type of the tumor with the use of minimal number of immunohistochemical stains in cases with small-cell transformation or different histological appearance, and b) to save enough material for molecular analyses.

The recommendations for histological diagnosis and molecular classification of lung cancer include:

1. Recommended terminology and diagnostic protocols for lung cancer in biopsy and cytological material
2. Recommendations for handling of cytological and tissue samples.

1. Recommended terminology and diagnostic protocols for lung cancer in biopsy and cytological material

WHO Classification from 2015 suggests new standardized criteria for diagnosis and terminology of lung cancer in small biopsies and cytological material [32] and at the same time recommends special stainings (IHC/ICC or histochemical / cytochemical) in order to precisely establish the histological type of the tumor.

Due to the connection between the therapy and the type of the tumor, as well as the need for molecular testing for eligibility to specific therapies, it is recommended to reduce the use of the term "non-small cell carcinoma, not otherwise specified" as much as possible, and to classify the tumors according to their specific histological subtype [31,33].

IHC can be used for differentiation between the primary lung adenocarcinoma and squamous carcinoma, metastatic carcinoma and primary pleural mesothelioma (especially in pleural samples). The need for molecular classification of lung cancer requires minimal immunohistochemical workup for determination of the histological subtype in cases with poorly differentiated tumors without clear differentiation by routine microscopy, and preservation of as much tissue for molecular analyses as possible [33].

It is recommended to use one marker for adenocarcinoma (TTF-1 or mucin) and one squamous marker (p40 or p63). The tumors that clearly stain positive for TTF-1 are classified as NSCLC, favor adenocarcinoma, and tumors positive for squamous markers are classified as NSCLC, favor squamous cell carcinoma [33]. If these stains are negative, additional evaluation is needed for confirmation of the diagnosis or excluding metastasis. In cases where one population of tumor cells stains for TTF-1, and another population is positive for squamous markers, adenosquamous carcinoma may be suggested, although this diagnosis is recommended for operative materials. If the tumor tissue is inadequate for molecular testing, it is necessary to perform rebiopsy in order to obtain enough material for molecular analyses that dictate the therapy in patients [31].

Non-small cell carcinomas without clear morphology of adenocarcinoma or squamous carcinoma or immunoexpression on markers are classified as NSCLC, NOS. The percentage of these cases should not exceed 5% [34].

The diagnosis of large cell carcinoma should not be used for histological or cytological analysis of biopsy or cytological material, and is recommended for use only for surgical specimens, where the tumor is thoroughly sampled and analyzed in order to exclude a differentiated component [33].

Metastatic carcinomas of the lungs are almost always negative for TTF-1, except for metastatic thyroid carcinoma, where both thyroglobulin and pax 8 are also positive.

Neuroendocrine markers (CD56, Chromogranin and Synaptophysin) should be used for confirmation of neuroendocrine differentiation of the tumor, in cases with neuroendocrine morphology [35].

Distinction between lung adenocarcinoma and malignant mesothelioma (epithelioid type) is made by correlating the clinical appearance, clinical data, imaging studies and immunostains sensitive and specific for mesothelioma: WT-1, Calretinin, CK5/6 and D2-40, as well as markers sensitive and specific for lung adenocarcinoma: TTF-1 and Napsin A [36,37].

Other markers that may be helpful for tumor histogenesis are: breast cancer (ER, PR, GCDFP15, GATA3, Mammaglobin) [19,37], renal carcinoma (pax 8) [19,37], serous papillary carcinoma (pax 8, pax 2 and ER) [37], adenocarcinoma from the gastrointestinal tract (CDX2, CK20) [19,37], thyroid cancer (TTF-1, thyroglobulin, pax 8) [37]. p40 may help in differential diagnosis between epithelioid mesothelioma with pseudosquamous morphology and squamous carcinoma.

2. Recommendations for handling cytological and tissue samples

Close collaboration between pathologists and clinicians and information on patient's history is necessary to make the diagnosis, to exclude metastatic disease and to avoid unnecessary consumption of the sample due to the use of wide range of markers to confirm the tumor's histogenesis.

Tissue and cytological samples (including pleural liquid) can be used for diagnostic and molecular analyses. Therefore, it is necessary to notify the diagnostic procedure during which the sample is obtained.

Tissue samples for histological and immunohistochemical analyses

Tissue sample (at least 4-6 biopsy samples) [37] should be placed in fixative immediately, or not later than 60 minutes after the moment of sampling. The samples should be transported on ice and the time-gapuntil beginning of samples fixation should not exceed 1 hour. Vacuum packaging of the surgical specimens may prolong their transportation time (period of cold ischemia) for up to 92 hours, preserving the high quality of the tissue (the vacuum should be sealed and kept at 4^{o}C).

Optimal time of fixation is 24 hours, although minimal and maximal time of fixation varies between 6 and 48 hours. Concentration, pH and presence or absence of buffer in formalin solution used for tissue fixation also **influence the quality and results of immunohistochemical and molecular analyses**. Most antigens give consistent immunoreaction and superior preservation when samples are fixed in 10% neutral buffered formalin (prepared from 4% formaldehyde) with pH value 5 to 7, using phosphate buffer.

Optimal tissue-fixative ratio should be 1:10. The prepared formaldehyde solution should not be older than two weeks.

When preoperative diagnosis is not possible or available, frozen section is recommended for primary diagnosis of lung neoplasms. The frozen section diagnosis should be confirmed on paraffin sections after tissue fixation and adequate standard tissue processing.

Tissue samples should be embedded in paraffin attemperature between 56-57^{o}C. If there are more samples of tumor tissue, they should not be embedded in one

cassette (the tissue should be embedded in two cassettes at least, one of which is used for immunohistochemical analyses, and the other for molecular analyses) [33].
It is recommended to make 10 initial unstained slides in order to avoid the need to returnthe paraffin blocks to microtome, thus reducing waste of material and time for diagnosis:

- 2 sections for standard hematoxylin eosin(H&E) staining (the first and the last section);
- 2-4 sections for diagnostic immunohistchemical analyses for determination of the histological subtype (up to two markers for distinction between adenocarcinoma and squamous carcinoma-TTF-1 and p63 or p40, as well as two neuroendocrine markers in cases where distinction from neuroendocrine carcinoma is needed) [31,39];
- 4-6 sections for molecular testing.

Samples from bone metastases that have been decalcified by acid reagents known to degrade DNA should be avoided for molecular testing, if possible.

Cytological samples

Accurate interpretation of cytological materials depends on several factors:

- The method of sampling;
- Appropriate preservation of fluidspecimens prior to processing;
- Preparation of the material for microscopic analysis;
- Staining and mounting of the cell sample.

Individual cells can be collected by exfoliative cytology (cytology of sputum, bronchoscopic cytological sample, fine-needle aspiration biopsy, FNAB and modifications listed in the text above) and obtained from pleural fluid.Smears are prepared and fixed according to the requirements of the stain that will be used.
Collected fluid (at least 60 mL) [40] should be sent to the pathology laboratory, for further processing (smears and/or cytoblock from the centrifugal sediment) within 30 minutes; or direct smears can be prepared, following the standard procedures.
When using hematological stain, such as May-Grunwald-Giemsa, Diff Quick or Giemsa, the smears need to be air-dried. Rapid stains are particularly useful in preliminary assessment of adequacy of the sample before the patient is released from hospital.
Fixation in 96% alcohol is used for Papanicolaou (PAP) or H&E staining, enabling good visualization of the nuclear chromatin and cell cytoplasm. Smears haveto be prepared quickly, fixed and stained immediately, in order to avoid artefacts that can compromise specimen evaluation by the pathologist. When sufficient material is aspirated, then more smears can be prepared, some of them can be air-dried or some can be alcohol fixed. Additional smears can also be used for special staining or other ancillary techniques. In addition, the smears should be fixed one after the other, subsequently, immediately after their preparation in order to avoid drying. Preparation of so called cytospin smears with a centrifugation procedure enables the use of immunocytochemical methods. The latest system of "liquid-based cytology" opens up new perspectives in the cytological diagnosis of malignant diseases, including lung cancer. Final cytological diagnosis is based on the integration of the clinical findings before the cytology sample collection, the observations during the procedure and the microscopic evaluation. Hence, as with histological diagnostics, the close collaboration between the clinician and the pathologist is required.

Molecular analysis

A correct preanalytical phase procedure, including sample collection, fixation and processing of samples for histological and cytological diagnostics, is required for obtaining high-quality DNA and RNA sufficient for molecular tests.
For that purpose, it is important that the pathologist marks the most appropriate tumor area of the slide so that the optimum tumor content can be extracted from the paraffin blocks or the tumor cells can be removed from the marked portion of the slide using a scalpel in cases when there is very scarce material.
If several tissue blocks are available, tumor tissue with least amount of necrosis, blood, mucus or inflammation should be selected. It is recommended that the material tested for mutations contains at least 20-30% tumor cells, which makes the methods of real time PCR and next generation sequencing (that has sensitivity of at least 10% tumor cells)more favourable than the Sanger sequencing method, whichsensitivity is limited to 50% tumor cells in the material tested for genetic alterations [19,41]. The purpose of this approach is to minimize false-negative results.
In the 2018 Guideline Recommendations from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology [21], some of the recommendations published in 2013 [22] are reconfirmed, but some are updated due to advanced knowledge for pathogenesis of the lung cancer, and the rapid development of target therapy as indicated below. In addition, these recommendations refer to patients with advanced lung cancer (stage IIIB and IV).

1. Along with biopsy material, cytological samples with adequate cellularity and preservation can be used for molecular analysis, especially if cell blocks are available.
2. Low sensitivity technologies that cannot test tumors with less than 50% tumor content should be avoided in order to avoid additional potentially more invasive procedures for patients, solely to obtain a tissue sample with high tumor content.

3. Set of genes that must be available in all laboratories that test lung cancer, as an absolute minimum is: EGFR, ALK, and ROS1. IVD tests should be used.
4. Routine testing of somatic mutations for EGFR and gene rearrangement for ALK in all patients diagnosed with adenocarcinoma and any carcinoma that has a component of adenocarcinoma is recommended [21]. EGFR and ALK testing can be performed in cases of other types of non-small cell carcinoma that meet certain clinical criteria (young patients, non-smokers) [21]. In addition, the immunohistochemically confirmed protein product for ALK is an alternative method of FISH testing for ALK using 5A4 antibodies (Novocastra) or D5F3 (Ventana) and appropriately validated immunohistochemical assays.

It is recommended to performROS1 testing in all patients with lung adenocarcinoma, regardless of clinical characteristics. Immunohistochemical analysis for ROS1 can be used as a screening test in patients with lung adenocarcinoma. Positive immunohistochemical staining for ROS1 should be confirmed by molecular or cytogenetic method, RT PCR and FISH respectively [21].
Molecular or cytogenetic tests for BRAF, RET, ERBB2 (HER2), MET and KRAS are currently not indicated as stand-alone routine assays. These tests can be used as part of larger testing panels or when routine tests for EGFR, ALK and ROS1 are negative [21].
Revised recommendations for the diagnosis and treatment of lung cancer patients have introduced immunomodulatory therapy as a novelty in the treatment of lung carcinoma, which significantly improves the outcome of the disease in a subset of patients with advanced lung cancer. Therefore, this therapy is approved as a second-line therapy for advanced lung cancer patients or as first-line therapy for patients with high level of PD-L1 expression that are negative for EGFR and ALK mutations. Thus, in squamous cell carcinoma patients, it is recommended to provide samples for PD-L1 analysis (1 section for immunohistochemical analysis and 1 section for molecular analysis). Ideally, tissue sections for this analysis contain tumor tissue, intervening stroma and inflammatory cells (T-lymphocytes) [21]. Administration of specific therapy requires evaluation protocols with given cut-off values for each specific drug to be implemented.
Currently it is not recommended to use liquid biopsy (circulating free DNA-cfDNA) for routine diagnosis of primary lung adenocarcinoma. In certain clinical condition when tumor tissue is limited or unavailable to existing diagnostic procedures, cfDNA assay may be used to identify certain mutations. The circulating free plasma DNA assay can be used to identify EGFR T790M mutations in lung adenocarcinoma patients with a progression or with secondary clinical resistance to EGFR targeted tyrosine kinase inhibitors.

Pathological evaluation

The same pathologist (if possible) should evaluate all available material from the same patient, including biopsy and cytology, to select the most appropriate sample for molecular testing. Tissue sections older than 4-6 weeks should not be used for immunohistochemical and molecular assays, in order to avoid non-informative or false-negative results.
It is recommended the report for pathohistological diagnosis, immunohistochemical and molecular analysis is in a form of a single report and the interpretation of the results is understandable and useful to clinicians. It is recommended to describe both the method of analysis and the quality of the sample.
Pathology report should include the size of the sample (mm), size of tumor (mm), the presence of necrosis, inflammatory infiltrate, type and subtype of the carcinoma, in accordance with the nomenclature and criteria of the 2015 WHO Classification of Lung Tumors [32].
The pathohistological diagnosis and molecular testing should be completed within 10 working days.
The pathologist is responsible for education and supervision of the technical staff that is responsible for preparation of samples for molecular analysis.
Laboratories for histological diagnostics and molecular testing should be accredited and participate in external quality tests in order to maintain quality control.
It is necessary for state authorities, Ministry of Health and Health Insurance Fund of the Republic of Macedonia to provide a budget for financing diagnostic procedures and treatment of patients with malignant lung disease in order to prolong survival and improve the quality of life.

Discussion

Patients with LC, especially when the disease is diagnosed in the advanced stage and there is no possibility for radical surgical treatment, have average survival of one year. Still, in cases with adenocarcinoma, where specific molecular alterations are detected, targeted treatment with specific thyrosinekinase inhibitors or immunotherapy provide significantly better prognosis and quality of life. According to the contemporary guidelines, it is necessary to ensure precise molecular diagnosis for every patient with treatable LC mutations, for further planning of the optimal treatment.
The new treatment options, but also the level or their accessibility, the high price of the molecular diagnosis and available treatment have opened a series of questions concerning the exact indications, selection of patients, the time-frame for obtaining the precise diagnosis and the options in case of relapse after the first-line treatment. In searching for answers to these key questions, in 2013, the IASLC issued comprehensive guidelines for the selection of LC patients for treatment with

TKI [22]. Fast evolution of information in this field ledto the necessity of their revision in 2018 [21].
Taking into consideration the limited resources of the health system in Macedonia, we consider it necessary, when defining the diagnostic algorithm for LC in these recommendations, to offer answers to the following relevant key questions:

Key question 1: When should molecular testing be performed?

- On initial histopathological diagnosis
- In cases of inadequate response or relapse after initial targeted treatment.

The selection of patients suitable for targeted treatment is "condition sine qua non" of the contemporary approach to LC patients. The presence of sensitive mutations to TKI (although present in a small percent of patients with NSCLC; 32% for EGFR mutations [42]; around 4-5% of ALK rearrangement[43], 1-2% for ROS-1[44], have influence on further treatment, especially the choice of the pharmacological agent. Therefore, it is necessary to perform molecular testing simultaneously with the primary histological evaluation. The initial positive response to TKI usually persists for months, but unfortunately relapse occurs frequently. Re-biopsy in these cases often shows incidence of mutations different from thatinitially detected, which makes molecular retesting necessary.

Key question 2: Which patients should be tested for EGRF mutations and ALK rearrangement?

The compounds for targeted treatment in small cell LC are still in the phase of research, and routine testing for specific mutations is not recommended, except forthe purpose of clinical trials [45].
The consensus of the CAP, IASLC and AMP is clear that molecular profiling is indicated in all patients with advanced stage NSCLC[21, 22]. This especially refers to patients in stage IV, where expected survival is 4-5 months and treatment with TKI gives significantly better results [46].Reflex molecular testing in these patients (when the molecular testing is ordered automatically, by the pathologist, upon diagnosis of NSCLC, without waiting subsequent referral from the clinician) can mean saving precious time, but close communication between the pathologist and the clinician is necessary. Some of these patients might not be candidates for targeted treatment for reasons other than type of the cancer (general clinical parameters, availability, etc.). The actual dilemmas concerning determination of specific mutations in potentially resectable NSCLC patients in TNM stages I, II and III are given in detail in the 2013 IASCL guidelines [22]. They support initial determination of EGFR and ALK, always when possible, because these patients are potential candidates for TKI (either upon disease progression or because of re-staging in a more advanced stage after surgical resection). Initial testing in these cases avoids repeated interventions for re-biopsy and prevents problems of long-term storing of pathologic material and miscommunication between the institutions involved in the process. Besides all, some of these patients shall never become candidates for targeted treatment, which imposes that each country should adapt the guidelines according to the local circumstances. Unfortunately, in Macedonia LC screening is on a very low level, and still not imposed as government or health care policy and most of the patients are diagnosed in advanced stages of the disease. On the other hand, the communication between clinicians and pathologists is well established and efficient, and there are only a few referral centers in the country for pathology and pulmonary interventions. Therefore, we think that reflex testing is an adequate option for our circumstances.
The recommendations for molecular profiling also depend on the histopathological type of LC.

1. **In small cell LC** no molecular testsare established for molecular profiling for specific targeted therapy, and it is not recommended for routine evaluation of patients.
2. **Non-small cell LC, histologically defined as adenocarcinoma (level of evidence A)** [21,22].

- **EGFR** molecular testing should routinely be done in all patients diagnosed with **lung adenocarcinoma**. No patient should be excluded from the opportunity to be tested based on clinical characteristics.
- **ALK** molecular testing should be used for selection of patients for ALK-targeted TKI therapy in all patients diagnosed with **lung adenocarcinoma**. No patient should be excluded from the opportunity to be tested based on clinical characteristics.
- **EGFR and ALK** testing arerecommended in adenocarcinoma and mixed LC with adenocarcinoma component, NSCLC and NSCLC-NOS.
- In limited cases**EGFR** and**ALK** testing can be performed in other histological types (squamous and small cell), where adenocarcinoma component cannot be completely ruled out, depending on the clinical features of the patient (in younger patients, and non-smokers).
- In lung adenocarcinoma, testing for **EGFR** and **ALK** should have priority over other molecular biomarkers.
- **ROS1** testing has to be done in all patients with lung adenocarcinoma, regardless of clinical characteristics.
- Immunohistochemical (IHC) analysis for **ROS1** can be used as a screening test in patients with lung adenocarcinoma; still, positive ROS1 on IHC should be confirmed with a molecular method[9].
- In patients with **nonsquamous LC**, if targetable driver mutations-**EGFR, ALK and ROS rearrangement** are not present, we recommend testing for **PD-L1**, inselection of patients for immunotherapy (for application of each specific treatment, the de-

dicated protocol for evaluation should be applied, with the recommended cut-off values for every specific compound).

- Molecular testing for **BRAF, RET, ERBB2 (HER2), KRAS** and **MET** is not indicated as routine analysis, out of the context of clinical studies. It is adequate to include these analyses as part of wider testing panels, and performed either at the beginning or if routine testing for EGFR, ALK and ROS1 are negative.

3. Non-small cell suamous lung carcinoma

- In patients with proven squamous lung carcinoma testing for **PD-L1** is recommended, in selection of patients eligible for immunotherapy (for each specific compound, the relevant protocol for evaluation should be done and determined cut-off values taken into consideration)[9].

Key question 3: Which methods should be used to perform molecular testing?

- IHC is an equivalent alternative to FISH for testing for ALK.
- Multiple genetic sequential panels are preferred over multiple single-gene tests to identify other treatment options apart of EGFR, ALK and ROS1.
- The laboratories should provide results thatare unexpected, inadequate, biased or with low level of confidence to be configured or resolved by using an alternative method, or sample.

Key question 4: Which tests are recommended for patients with mutations, who relapsed after first-line targeted therapy?

The key mechanism for incidence of secondary resistance to first generation EGFR-TKI (erlotinib and gefitinib) is the occurrence of T790M mutation on the same allele for EGFR, which blocks the inhibition of the mutated protein by the targeted therapy. Patients with sensitized EGFR mutations, who relapsed after EGFR-TKI treatment, may benefit from the treatment with third generation EGFR-TKI [47]. Detection of EGFR T790Mmutation is a parameter for selection of patients who are candidates for this treatment.

- In patients with lung adenocarcinoma, sensitized to EGFR mutations and progressed after treatment with EGFR-TKI, we recommend testing for the presence of EGFR T790M mutation necessary for determining patients eligible for third generation EGFR-TKI treatment.

Key question 5: What is the role of testing for circulating DNA (cf DNA) in LC patients (liquid biopsy)?

- At the moment, liquid biopsy (cfDNA) is not recommended for routine diagnosis of primary lung adenocarcinoma.
- In certain clinical settings, where the amount of tissue is limited and not enough for molecular testing, and/or the patient is not fit for biopsy, cfDNA testing can be used for identification of EGFR mutations.
- Methods for determination of cfDNA can be used to identify EGFR T790M mutations in patients with progression of lung adenocarcinoma or when secondary clinical resistance to EGFR-TKI developed; testing of tumor sample is recommended if plasma result is negative[22].

Key question 6: When should the samples be tested for EGFR mutations and ALK rearrangement?

- The basic, mandatory molecular analyses (ROS1, EGFR, ALK in act) should be done immediately (in the same act) with the histopathological analysis of the material.

Key question 7: How rapidly should the result from molecular testing be obtained?

Unlike treatment with classical platinum-based chemotherapy, the clinical effect and the response to targeted therapy areoften rapid, and side effects smaller [48]. Some authors recommend to startanti EGFR treatment even before detecting the presence of specific mutation, but we think that such an approach is medically and economically inadequate, especially because the types of LC with so called "wild-type" EGFR mutations respond better to platinum treatment [49,50]. It is technicaly possible to complete thehistological and subsequent molecular analyses within 5 to10 days. Commencing the treatment as soon as possible is essential, especially in those patients where LC was detected in late stage, where expected survival counts in weeks. Postponing the treatment in these cases can be fatal, which is why this consensus report recommends that:

- Histological and molecular testing should be concluded within 10 working days.
- The laboratories for molecular testing should be accredited and take part in external quality testing in order to maintain accreditation.
- We recommend that the report for the histopathological diagnosis, immunohistochemistry and molecular analyses should be issued as an integral report and the description of the results should be clearly understandable to the clinicians. The method employed for analysis and the quality of the sample should be described.

In order to simplify the processing of the patients, we are presenting the summary recommendations for planning of molecular typisation in NSCLC as an "**Algorithm for molecular testing"** (Figure 1)**,** adapted fromthe Alas for ALK and ROS1 testing from 2016 [51].

Conclusion

The role of molecular testing in treatment of lung cancer is developing rapidly. This implies the necessity of continuous revision of the basic recommendations,

created on the basis of the analyses of the results from numerous studies published by relevant authorities, such as the College of American Pathologists, the International Association for the Study of Lung Cancer and the Association for Molecular Pathology. After detailed revision and control, most of those recommendations were approved by the American Food and Drug Agency. Thus, the targeted therapy became available for lung cancer patients.

The Macedonian Respiratory Society and the Macedonian Association of Pathology intensively follow the scientific achievments in this field and work on continuous education of physicians for application of evidence-

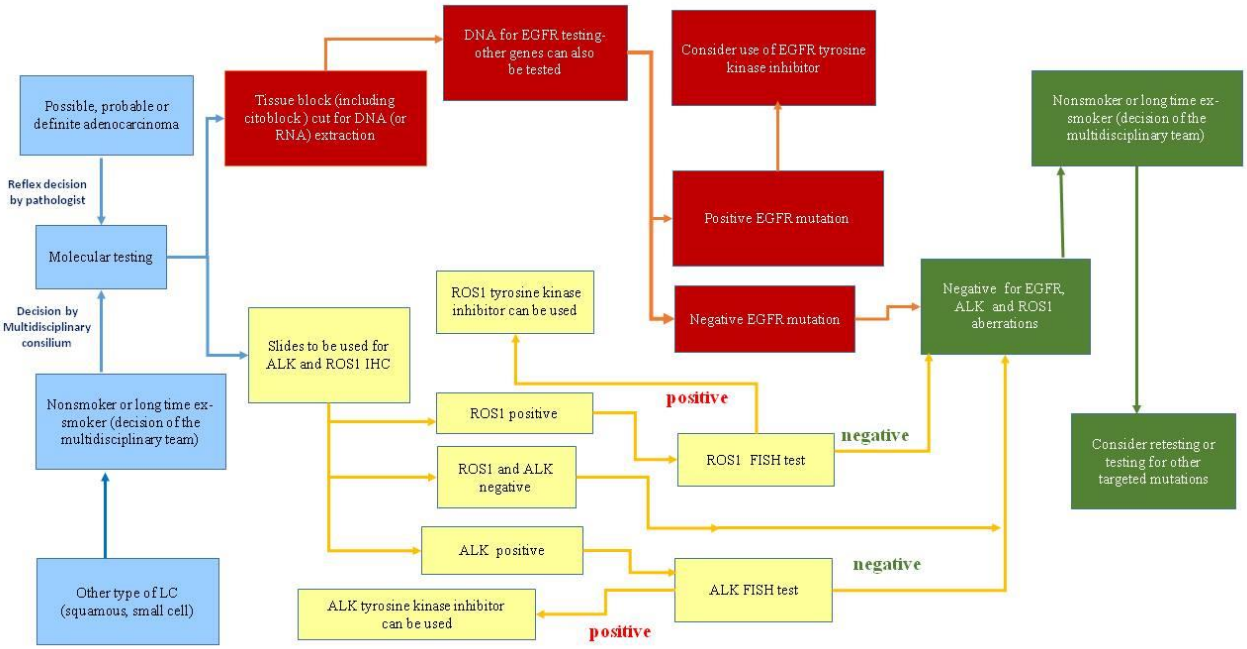

Fig. 1. Algorythm for molecular testing

based medicine, by updating the recommendations for diagnosis and treatment of lung cancer patients. Clearly, it is inevitable that, in order to implementthe contemporary guidelines, it is neccessary to raise awareness of the state authorities about these processes.In order to assure that the Macedonian healthcare follows the European and world standards with the final goal to improve the survival of lung cancer patients, planning of the management of the health care system must assure a dedicated budget for:

1. Early detection of lung cancer by developing and realizing a program for lung cancer screening (according to the recommendations of NLST and NELSON), and
2. Financing of the diagnostic procedures and treatment of patients with malignant lung diseases.

Certainly, there are a lot of unanswered questions, but it is important to begin from a starting point, such as early detection of LC, to obtain adequate and efficient diagnosis, as well as treatment of the patients with advanced LC, which altogether shall enable rational use of the healthcare budget of the country, and provide optimal care for the patients.

Conflict of interest statement. None declared.

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Мак Мед Преѓлед 2019; 73(2): 67-72

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Review

PHARMACOEPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE DATA FOR BACTERIAL INFECTIONS IN HOSPITALIZED CHILDREN

ФАРМАКОЕПИДЕМИОЛОШКИ ПОДАТОЦИ ЗА АНТИМИКРОБНА РЕЗИСТЕНЦИЈА НА БАКТЕРИИ ИЗОЛИРАНИ КАЈ ХОСПИТАЛИЗИРАНИ ДЕЦА

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Abstract

Antimicrobial resistance is a global problem that needs an urgent action. The irrational use of antibiotics is widespread and leads to potential usefulness of medicines and negative therapeutic outcome.In April 2016, WHO stated that the problem of antibiotic resistance is a major clinical problem resulting in treatment failures even in a case ofeasy to treat diseases. Resistance to first line medicines results in huge spending on new generation of antibiotics. In some instances resistance to second- and third-line agents is seriously compromising treatment outcome.Seriousness of the situation requires extensive research and constantly monitoring of the spread of bacteria resistance.Another problem regarding bacteria resistance is the lack of new antibiotics reported by the US Center for Control and Prevention of Disease.A systematic literature search of databasesgave us enough information about the use of antibiotics, most often isolated bacteria and resistance to different classes of antibiotics. According to the official data, bacterial resistance is lowest in the countries where guidelines for prescribing and use of antibiotics are consistently implemented, such as Scandinavian countries, The Netherlands, Germany and Great Britain. It is necessary to create a complete database of bacterial resistance and information on whether patients receive medicines appropriate to their clinical conditionin our country.

Keywords: antibiotics, bacterial resistance, multidrug resistance, bacterial pathogens

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Апстракт

Бактериската резистенција претставува голем здравствен проблем за кој е потребно итно делување. Нерационалната употреба на антибиотици е широко распространета и е една од најзначајните причини за зголемување на резистенцијата инегативни терапевтски исходи. Во април 2016 година, СЗО изјави дека проблемот со отпорноста на антибиотици е голем клинички проблем што резултира со неуспех во лекувањето, дури и во случаи кога станува збор за болести кои вообичаено лесно се лекуваат. Отпорноста на лековите од прва линија резултира со зголемување на потрошувачката на новите генерации на антибиотици. Во некои случаи, отпорноста кон антибиотиците од втора и трета линија сериозно го компромитираат исход од третманот. Сериозноста на состојбата во однос на развојот на бактериската резистенција, бара опширно истражување на овој феномен и постојано следење на резистенцијата на бактериите. Во извештајот објавен од страна на американскиот Центар за контрола и превенција на болести се вели дека дополнителна причина за бактериската резистенција е недостигот од нови антибиотици.Систематското пребарување на литературната база на податоци, ни даде доволно информации за употреба на антибиотиците, најчесто изолирани бактерии и нивната отпорност на различни класи на антибиотици. Според официјалните податоци, бактериската резистенција е најниска во земјите во кои насоките за препишување и користење на антибиотици доследно се спроведуваат, како Скандинавските земји, Холандија, Германија и Англија. Во нашата држава неопходно е да се создаде база на податоци по однос на ова прашање и информациите за рационална фармакотерапија.

Клучни зборови: антибиотици, бактериска резис-

тенција, мултирезистенција, патогени бактерии

Introduction

Bacterial resistance is not a new phenomenon butmajor public health concern. It was recognized in the earlier 1950s as a threat to effective treatment outcome. Since the WHO Resolution, many countries have expressed growing concern about the problem of antimicrobial resistance [1,2]. Reporting about bacterial resistance and monitoring the use of antibiotics today is an obligation for all countries.

The most endangered population for acute infectious disease as a result of bacterial resistance are children. Collective accommodation in kindergartens or schools, unhygienic living conditions, insufficiently effective immune system, as well as irrational, empirical and excessive use of antibiotics, especially unjustified in viral infections are the most common reasons for the frequent occurrence of bacterial infections in children [3-5]. Receiving antibiotics for prophylaxis increases the risk of multidrug resistance among recurrent infections [6]. In hospital acquired infections in children, antibiotic resistance is frequently reported in clinical Gram-negative bacteria. The number of available therapeutic options for treatment of these conditions is limiteddue to the lack of novel active antibiotics [7]. At the end of the 20th century, the attention regarding the threat of antibiotic resistance of the scientific and pharmaceutical companies was focused on multidrug resistant Gram-positive bacteria [8].

In order to understand bacterial resistance, there is a huge need to interpret molecular mechanisms of antibiotic resistance, especially to Gram-negative and Gram-positive clinical pathogens [9-11].

Among the diseases that have been declared as "alarming threat" are severe forms of clostridium-induced diarrhea and gonorrhea infections. But, the most serious infections are those coming from enterobacteria that cause relatively new and rare, but deadly infections resistant to carbapenems. For such infections it is stated that they cannot be cured with so-called spare antibiotics, which confirms the fact that no new antibiotic has been synthesized for a long period of time [12,13]. However, we are now faced with the threat of a post-antibiotic era [14,15].

An interesting fact is that bacteria isolated in children younger than 2 years show a higher percentage of antibiotic resistance compared to bacteria isolated in older children [5]. Also, isolated bacteria in the hospitals are more resistant than those isolated in primary health care [16]. The situation is particularly problematic at the University clinics where patients from other regional hospitals who have already received antibacterial therapy come up. That is the possible reason for the occurrence of resistant and multidrug resistant bacterial strains [17].

Bacterial resistance is a global problem that must be resolved locally, having in mind that there are significant geographical variations in the participation of certain resistant strains, as the triggers of bacterial infections. Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* are the most frequent pathogens who have a significant impact on morbidity and mortality [18].

It is important to highlight that the UN and the WHO organization have developed action plans to reduce antimicrobial resistance in all healthcare settings. They establish the institutional antimicrobial stewardship program as a key intervention to reduce antibiotic consumption in hospitals. The goal was to address high rates of multi-drug-resistant bacteria [19].

Materials and methods

For the purpose of this paper, a systematic literature search of Medline/Pubmed and Embasedatabases was done in order to evaluate the phenomenon of bacterial resistance and multidrug resistance published in the last fifteen years.

A systematic review of Randomized Controlled Trials (RCTs) was made until December 2018 using the following key words: antibiotics, bacterial resistance, multidrug resistance, pharmacoepidemiology. We identified 3287 reports, of which only 32 articleswere subject of evaluation (Figure 1).

Potential analyses, diagnostic criteria, appropriate selection of patients with infections caused by bacteria and therapy were used as criteria for evaluating the study.

In our analysis bacterial strain wasconsidered to be multidrug resistant if it wasresistant to three or more classes of antibioticsat the same time.

Critical review

Three main points were the subject of our research: use of antibiotics, analysis of isolated bacteria and resistance/ multidrug resistance of the most commonly isolated bacteria.

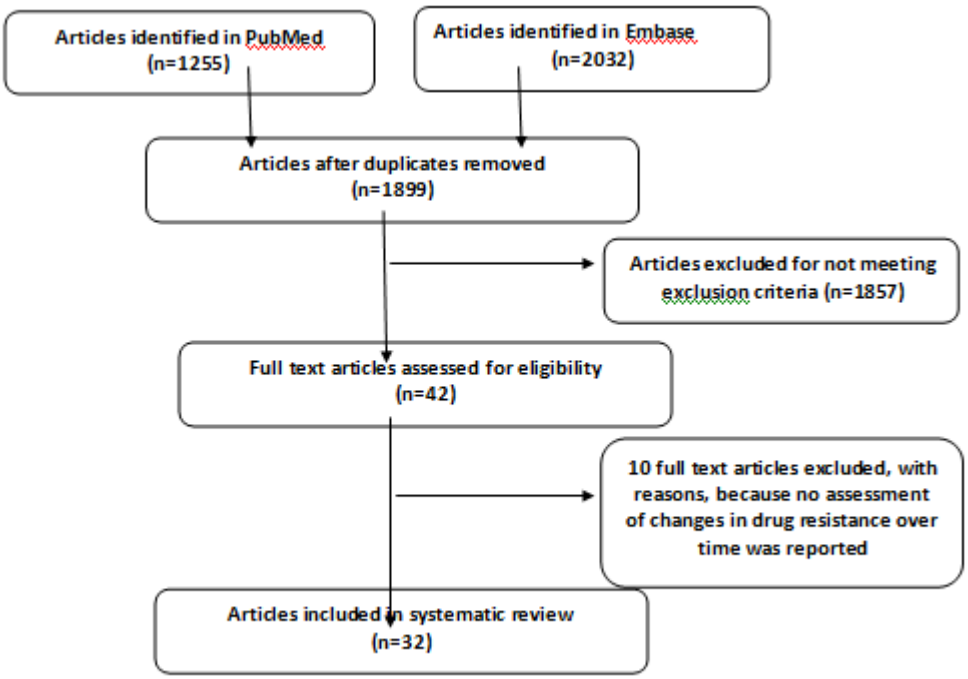

Fig. 1. Flow of Studies Through the Review Process

Use of antibiotics

The analyzed data available from the database for hospitals and general practice within the SACAR project (Specialist Advisory Committee on Antimicrobial Resistance) [20] shows the existence of seasonal variations in prescription of antibiotics. Prescription in the winter period is about 15% higher compared to the summer period. The use of antibiotics was higher in East European countries compared to northern EU countries [20-21].

The use ofpenicillin in hospitals in many European countries is low, although the antimicrobial effect of penicillin is still relatively well preserved [22]. A study conducted at the University of California San Francisco [23] confirms that from 16 classes of antibacterial drugs used, 5 classes are considered safe for pediatric use: beta-lactams+inhibitor, oral and parenteral cephalosporins, macrolides and aminoglycosides. Similar results have also been reportedinone study conducted in China in the period from 2002 to 2006. The most commonly used antibiotics in five children's hospitals in China were amoxicillin+clavulanic acid and parenteral cephalosporins [24]. Penicillins were the most common prescribed in children younger than 5 years in six primary health-care facilities in Burkina Faso, followed by sulfonamides and macrolides [25]. The results of a two-month study [26] conducted at the University Clinic in Bari, Italy, showed that amikacin and a combination of ampicillin + sulbactam were the most used antibiotics in children younger than two years. The most common antibiotics were used for treatment of respiratory infections in children, at the pulmonary departments [5,27]. The results of the multicentric multinational study conducted within the ARPECproject (Antibiotic Resistance and Prescribing in European Children) in September 2011 report a high level of use of antibiotics in the departments for pediatric hematology/oncology. In this one-day data of the use of antibiotics in hospitalized children 73 hospitals worldwide were included [28]. One study conducted in 1992 in three University hospitals in Estonia, Sweden and Spain showed that the use of antibiotics at the surgical departments was 30-50% higher than in other departments [29].

Five primary data collection studies, across the five countries, conducted between 2014-2017 as part of MARVEL (Multi-country economic and epidemiological burden of varicella study), examined empiric data on the appropriate and inappropriate use of antibiotics in the treatment of pediatric varicella patients. In this study 787 patient were included and the proportion of antibiotics prescribed without a probable or confirmed diagnosis of bacterial infection was high (ranged from 45% to 90%) [30].

Analysis of isolated bacteria

Analysis of microbiological isolate along with the antibiogram is one of the preconditions for optimal antibiotic use. For that goal,a sample of patients for microbiological analysis is taken before the start of therapy and the cause of the infection isisolated. The most common samples for bacteriological analysis are blood samples-blood cultures. The most commonly isolated bacteria from the blood cultures are coagulase

negative *Staphylococcus spp.*, which is normally present on the human skin. Therefore, it is very important to properly interpret the bacteriologically positive blood cell finding [31]. A study in England, conducted inneonatal departments in the period from 1992 to 2005, showed an increase of isolated coagulase negative *Staphylococcus spp*. in blood samples, which was partly explained by the increased use of central vascular catheters in these children [18]. In one study conducted in theperiod from 2001 to 2004 inthe Houston Hospital (USA) in respiratory tract samples, a double increase in the number of isolates in which *S. aureus* (771/1562) was reported, were explained by the increased virulence of this bacterium [32]. *E. coli*, the most common cause of urinary tract infections, was the most commonly isolated bacteria from urine samples [33].In urine cultures the most isolated pathogenic gram-negative bacilli are associated with resistance to beta-lactam antibiotics [34].In the samples of the gastrointestinal tract (GIT), *Klebsiella pneumoniae*, *E. coli* (16.5%) and *Pseudomonas aeruginosa* (15.8%) are most often isolated. The results of one retrospective study carried out ina hospital in Caracas (Venezuela) from 1997 to 2003 showed the presence of *Pseudomonas aeruginosa* in 7% of the positive GIT samples [29]. Streptococcus pyogenes was the most commonly isolated bacteria in nasopharyngeal swabs in healthy children (ages of 6 and 7 years) who attended the same primary school. Positive bacterial findings of this pathogenic in healthy carriers are not an indication for antibiotic therapy [35].

Resistance and multidrug resistance of the most commonly isolated bacteria

According to literature data, the level of resistance to bacteria isolated in hospitals is higher comparing tothat in the general practice [36]. Hospitals are often regarded as the focal point for emergence development of resistance and multidrug resistance [37].

E. coli: One-year prospective study conducted in Ankara (Turkey) demonstrated about 30% resistance of*E. coli*, isolated in urinary infections to ceftriaxone. The resistance of ampicillin described in this study was 74.2% [38]. Many authors believe that this high percentage of resistance of *E. coli* is a consequence of irrational, prophylactic and excessive use of antibiotics in general practice [33].

S. aureus: *S. aureus* is usually isolated insamples from the respiratory tract. This bacterium isin a relatively small percentage multidrug resistant (about 10%). Based on the results of five-year retrospective studies conducted in 300 hospitals across the United States, an increase in methicillinresistant *S. aureus* (MRSA) isolated from swabs of the throat was observed [39].

Coagulase negative *Staphylococcus spp.*, according to literature data, is highly resistant and multidrug resistant bacteria, which again is a result ofan inadequate use of antibiotics [40]. Thisbacterium is isolated mainly from blood samples and catheter swabs. The sensitivity of this bacterium remained preserved only on vancomycin and teicoplanin. Regarding fusidic acid it was reported that this bacteriawas almost 30% resistant. Furthermore, isolates from hospital material showed significant resistance to this bacterium tolincosamines and macrolides [41].

*Klebsiella pneumoniae*ismost commonly isolated from samples of the respiratory tractand it is a causative factor for sepsis in neonates. For this bacteriumdata reported from the studies conducted in Asia, Africa and South America shows a high percentage of resistance to many classes of antibiotics: more than 50% tocefotaxime, more than 70% to ampicillin and gentamicin [30]. Isolates of thisbacterium from the blood cultures showed significantly high resistance to aminoglycosides [41]. According to the results of a study conducted in Ankara (Turkey), isolates of *K. pneumoniae* from urinary samples showed about 35% resistance to folate synthesis inhibitors [38].

Penicillin-resistant *Streptococcus pneumoniae* was studied in only one RTC with 35 participants. Before exposure to penicillin, resistance was not significant, but after exposure to amoxicillin the changes in resistance occur and normalized one month after the end of treatment [42].

Pseudomonas aeruginosa is a common cause of hospital infections [43]. It is mostly isolated from samples from the respiratory tract and less from urine and GIT. This bacterium shows 100% resistance to folate synthesis inhibitors and ceftriaxone [44]. It also shows high resistance (about 90%) to antibiotics that are usually prescribed in general practice such as amoxicillin, nitrofurantoin, and cephalexin [45]. 60% of the isolates were sensitive only to quinolones [41].

Acinetobacter spp. has been shown to be highly resistant to most of the includedantibiotics, and appropriate treatment results have been achieved by a combination of a beta-lactam antibiotic and aminoglycoside [46]. Multidrug resistance of Gram-negative bacteria is a major problem in hospitals because it is easily transmitted through contact, and the spread of resistance affects many patients in a small area, especially in immunologically compromised patients [47].

Enterobacter spp. in the developing countries is a common cause of bacteremia and severe complications in newborns. A study analyzing isolates from the respiratory and gastrointestinal tract showed a high percentage of resistance to penicillin and cephalosporins, which is a consequence of the production of beta-lactamase [30]. In Sweden, in adults, this bacterium showed about 20-30% resistance to cefotaxime [48].

Enterococcus spp. takes a more prominent place among the causes of hospital infections [49]. Isolates from urine samples showed the lowest percentage of

multidrug resistance (about 36%), while resistance to GIT, blood and catheter isolates wasmuch higher (around 88%) in all tested classes of antibiotics [50]. According to the results of the international SENTRY project (The SENTRY Antimicrobial Surveillance Program), which included about 70 microbial laboratories around the world, a high percentage of resistance (about 50-65%) has shownthis bacterium to gentamicin [51].

Conclusion

According to the literature data, the highest percentage of antibiotic consumption is at the surgicalclinics. The most commonly used antibiotics in children are parenteral and oral cephalosporins, combinations of beta-lactams+inhibitor (clavulinic acid), macrolides and aminoglycosides. The most commonly isolated bacteria from the samples are *E. coli*, *S. aureus*, coagulase negative *Staphylococcus spp., Klebsiella spp., Pseudomonas spp., Acinetobacter spp., Enterococcus spp.* and *Enterobacter spp*. Multidrug resistance has been confirmed in almost all isolates of *Enterobacter spp*. and *Acinetobacter spp*. In the remaining isolates of *P. aeruginosa*, *Enterococcus spp*., resistance has been confirmed in more than 50% of the isolates. Insignificant multidrug resistance has been identified in isolates of*E. coli* and *S. aureus*.
This data gave us enough information about the use of antibiotics, most often isolated bacteria and resistance to different classes of antibiotics. This will be very helpful in the process of monitoring, completing and comparing data for bacterial resistance in our country.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2019; 73(2): 73-77

ММР

Original article

CORRELATION BETWEEN SERUM LEVEL OF PLACENTAL GROWTH FACTOR IN FIRST TRIMESTER OF PREGNANCY AND SUBSEQUENT PREECLAMPSIA DEVELOPMENT - A PILOT STUDY

АСОЦИЈАЦИЈА НА СЕРУМСКОТО НИВО НА ПЛАЦЕНТАРНИОТ ФАКТОР НА РАСТ ВО ПРВИОТ ТРИМЕСТАР ОД БРЕМЕНОСТА СО ПОДОЦНЕЖНА ПОЈАВА НА ПРЕЕКЛАМПСИЈА - ПИЛОТ СТУДИЈА

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Abstract

Introduction. Preeclampsia (PE) is one of the leading causes for maternal and perinatal morbidity and mortality. The possibility of PE development prediction is essential for adequate management of this pathology, especially in terms of undertaken measures for disease prevention.
Aim. To determine whether there is a correlation between serum levels of Placental Growth Factor (PlGF) in the first trimester of pregnancy with subsequent PE development.
Methods. Study population was consisted of 307 pregnant women in the first trimester of pregnancy who had visited Special Hospital for Obstetrics and Gynecology "Mother Teresa" for aneuploidy screening. During this visit, blood was taken for measuring the PlGF level. The study was prospective-observational-cohort. All pregnant women were monitored for PE development until delivery. The group of pregnant women with PE was matched with that without disease by comparing PlGF values.
Results. From the initial pool, only 283 participants completed the study. Among them, 7 developed PE. The average PlGF concentration in PE+ group was 32.29 pg/ml (1.06 MoM's), and in PE-group 45.42 pg/ml (1.58 MoM's). The difference between the results of the women destined to develop PE in comparison with those who are not, was statistically significant (0.018 and 0.011 for pg/ml and MoM's respectively).
Conclusion. Low serum level of PlGF in the first trimester of pregnancy is correlated with subsequent PE development.

Keywords: preeclampsia, prediction, PlGF, first trimester

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Апстракт

Вовед. Прееклампсијата е една од водечките причини за матернален и перинатален морбидитет и морталитет. Фундаментално значење во обидите за справување со оваа патологија би имала можноста на адекватна предикција на болеста, пред сè во контекст на превземање на мерки за превенција на настаување на истата.
Цел. Да се утврди постои ли асоцијација меѓу серумското ниво на плацентарниот фактор на раст (Placental Growth Factor - ПлГФ) во прв триместар од бременоста со подоцнежна појава на прееклампсија (ПЕ).
Методи. Како материјал служеа 307 трудници кои во првиот триместар од бременоста ја посетија СБГА „Мајка Тереза" за скрининг на анеуплоидии. Ним им беше земена крв за одредување на ПлГФ. Студијата беше проспективно-обсервационо-кохортна. Сите трудници беа мониторирани за појава на ПЕ се до породувањето. Групата на трудници со ПЕ се споредуваа со групата на трудници без ПЕ по однос на нивото на ПлГФ.
Резултати. Од првобитниот пул на испитанички, само 283 ја завршија студијата. Од нив 7 развија ПЕ. Средната концентрација на ПлГФ во ПЕ+групата на трудници беше 32.29pg/ml (1.06 MoM), додека средната концентрација на ПлГФ во ПЕ- групата беше 45.42 pg/ml (1.58 MoM's). Разликата меѓу концентрациите беше статистички значајна (0.018 and 0.011зарg/ml и MoM соодветно).
Заклучок. Ниското серумското ниво на ПлГФ во првиот триместар од бременоста е асоцирано со подоцнежна појава на прееклампсија.

Клучни зборови: прееклампсија, предикција, ПлГФ, прв триместар

Introduction

Preeclampsia (PE) is one of the leading causes of maternal and perinatal morbidity and mortality. Among the total number of fatal outcomes, preeclampsia (with contribution of 12%) is settled on the third place as a cause of maternal mortality behind postpartum hemorrhage (PPH) and sepsis [1]. But, regarding the lowest prevalence in comparison with other two (only 3.2% for PE *versus* 10.5% for PPH and 4.4% for sepsis), PE has the highest case-fatality rate i.e. PE can lead to fatal outcome [1]. As for the fetus, every eighth to every forth newborn (12-25%) with birth weight below 10 percentiles has PE-related fetal growth restriction [2]; 15-20% of all preterm deliveries are actually iatrogenic ones in terms of PE treatment; a half of fetal demise cases has a placental insufficiency (the essence of PE) as the underlying cause [2,3].

The possibility for effective prediction of PE would have substantial impact in outcome-improvement on two levels: 1. by establishing intensive antenatal monitoring for the pregnant women recognized as high risk patients and subsequently by providing early diagnosis of PE and opportunity for timed delivery or patient's allocation to tertiary-care medical facility; and 2. by undertaking measures for disease's prevention. Aspirin administration is such measure, which leads to a significant reduction of PE development [4-6] by 62% to 82% [7], but only if its administration has been started before the 16^{th} week of pregnancy. This knowledge clearly highlights the importance of effective PE screening already in the first trimester of pregnancy.

Reduced placental growth factor (PlGF) precedes clinical expression d of PE and therefore can be used as an early marker [8-11], especially when patients do not fulfill "classical" criteria for the diagnosis. These findings give rise to the idea of evaluating the PlGF concentration far before PE development and eventual use of this marker for early PE screening.

Aim

The aim of this study was to determine if there was a correlation between the serum level of PlGF in the first trimester of pregnancy and subsequent PE development, i.e. can this marker (measured in time when adequate prevention of the disease is still possible) be used as a screening tool for PE.

Material and methods

This study comprised pregnant women **with** the following **inclusion criteria:** singleton pregnancy, gestational age between 11^{+1}-14^{+1} weeks, i.e. crown-rump length (CRL) 45-84 mm, maternal age of at least 18 years, and simultaneously **without** not even one of the following **exclusion criteria:** fetal demise, congenital fetal anomalies, serious mental illness of the pregnant woman, communication difficulties.

Recruiting of all pregnant women was done at their first trimester of pregnancy who had visited the Special Hospital for Obstetrics and Gynecology "Mother Teresa" for aneuploidy screening by using Voluson E8 Expert Ultrasound with 4D probe RM6C from GE Health Care manufacturer, USA. The patients that had passed the recruiting, by signing the informed consent participated in this study.

Regarding the design this was a **prospective observational cohort study.**

At **the start point** of the study (subjects in 11^{+1}-14^{+1} weeks of gestation i.e. CRL 45-84 mm), all subjects were questioned about their medical history. The data were collected following the NICE protocol for PE screening. Then, a venous blood was taken. According to a standard protocol the serum was removed and stored at -20^{o} C until further analysis of PlGF concentration. Measuring of concentration was done at the Institute for Immunobiology and Human Genetics, Medical Faculty, Skopje. The specimens were transported to the Institute frozen, in accordance with the cold chain rule. For quantitative analysis of PlGF, commercially available kit Human PlGFQuantikine HS ELISA kit (R&D Systems manufacturer, USA) was used. Enzyme-linked immunosorbent assay was the method, and the measuring was performed on Perkin Elmer Wallac 1420 Victor2 platform (Perkin Elmer manufacturer, USA).

During the study, at the 32^{nd}, 34^{th}, 36^{th} and 38^{th} weeks of gestation all subjects were evaluated for PE. (Additional evaluations were made individually in case of reporting symptoms by the subjects). The evaluation was made according to the following protocol:

- At each control, blood pressure was measured. The measuring was made by digital automatic devise Omron M7, manufacturer Omron Healthcare Co. LTD, Japan, which passed validation of the European and British Society of Hypertension [13]. Manufacturer recommendations for adequate blood pressure measuring were respected. Subjects with blood pressure bellow 140/90 did not undergo additional investigations.
- From subjects with blood pressure ≥ 140/90 mm Hg, measured twice at least four hours apart, 24-hour urine specimen was collected for evaluation of proteinuria and the venous blood was taken for evaluation of: creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and platelets. The biochemical analysis was made according to the standard procedure on fully automatic biochemical analyzer Beckman Coulter AU 480 from the manufacturer Beckman Coulter, USA.
- Clinical evaluation for other systems dysfunction, such as central nervous system (CNS) and liver was made.

- The **study ended** by establishing a diagnosis of PE or by delivery without establishing a diagnosis.
- For diagnosis of preeclampsia the ACOG (The American College of Obstetricians and Gynecologists) criteria were used, which are adopted by the Ministry of Health of the Republic of North Macedonia [14]:
- Hypertension which occurred for the first time after the 20th week of gestation, with values ≥ 140/90 mm Hg on two occasions at least four hours apart, with at least one of the following findings:
- Proteinuria ≥ 0.3g/24-hour urine specimen, or ≥ 0.3 protein (mg/dL)/creatinine (mg/dL) ratio or a urine dipstick protein of 1+.
- Other organs/systems involvement: renal insufficiency (creatinine>90 μmol/L); liver involvement: (liver enzymes at least double concentration above upper limit with or without epigastric or right upper quadrant pain); hematological complications (platelets <100000 mm^{-3}); neurological complications (heavy headache, visual scotomas, blindness, stroke).

For the diagnosis of PE superimposed on a chronic hypertension, stated criteria were followed, while for the diagnosis of PE superimposed on preexisting renal disease several other systems were evaluated.
Preterm termination of the study (drop out) was foreseen in case of: abortion, impossibility of follow–up or informed consent withdrawal.

Results

From the total of 332 first trimester pregnant women who visited the Special Hospital for Obstetrics and Gynecology "Mother Teresa" for aneuploidy screening, 25 were rejected on the following grounds:

- 5 gemelar pregnancies;
- 12 cases with CRL>84 mm;
- 3 patients younger than 18 years;
- 2 cases with NT>4 mm;
- 1 case of missed abortion;
- 1 case without obtained informed consent;
- 1 case of pregnant woman with poor communication.

From 307 subjects who began the study, 24 dropped out the study on the following grounds:

- 13 cases with insufficient monitoring;
- 6 cases of abortion;
- 13 cases of termination of pregnancy due to fetal anomalies.

The remaining 283 subjects completed the study. Seven of them during the monitoring developed PE, 6 developed solely gestational hypertension, and the rest 270 did not show hypertensive disorder. Subjects' characteristics are shown in Table 1.

Table 1. Maternal characteristics of the subjects

Characteristics	PE-	PE+
Number of cases	276	7(2.47%)
Age	28.6(18-43)	33(27-38)
Body mass index (BMI)	25.7(15.8-46)	29.1(24.9-38.4)
Smoking	42(15.2 %)	3(42.8 %)
Parity		
Nulliparous	137(49.6 %)	6 (85.7 %)
Parous		1
Method of conceiving		
Spontaneous	271(98.2%)	7(100%)
Assisted with ovulatory drug	1(0.36%)	0
Assisted with IVF	4(1.45%)	0
History		
Without positive history	218(79.0%)	4(57.1%)
Chronic hypertension	7(2.54%)	2(28.6%)
Diabetes	1(0.36%)	0
Chronic renal disease	0	0
APL Sy; SLE; thrombophilia	5(1.82%)	1(14.4%)
Previous GH/PE	15(5.43%)	0
Family history of PE	30(10.9%)	
Drugs		
None	237(85.9%)	4(57.1%)
Antihypertensive	4(1.45%)	2(28.6%)
Aspirin	24(8.69%)	1(14.3%)
Clexane	11(3.98%)	0
Risk for PE according to NICE		
Yes	39(14.1%)	4(57.1%)
No	237(85.9%)	3(42.8%)

The results obtained by measuring the PlGF concentration are shown below:

Table 2. PlGF concentration expressed in pg/ml and MoM's

PlGF concentration	PE-	PE+
pg/ml	45.42 (7.98-150.86)	32.29 (22.33- 46.92)
MoM	1.58 (0.169- 4.005)	1.06 (0.48-1.699)

For comparison of the results in order to determine statistical significance of difference, Mann-Whitney test was used. Table 3 shows the level of significance.

Table3. Statistical significance of difference (Mann Whitney test)

	Statistical significance of difference $p < 0.05$
PlGF pg/ml	p= 0.018
PlGF MoM's	p=0.011

Discussion

In this prospective study the prevalence of preeclampsia was 2.7%. This result is corresponding to worldwide declared prevalence of preeclampsia for Caucasians [1].
Nulliparous-parous correlation in PE+ group was 6:1, which emphases the "protective" role of previous

pregnancy without PE [15]. The only parous patient in PE+ group had previous pregnancy complicated with PE. PE+ group had higher mean age, higher BMI, positive history for chronic hypertension, positive history for previous pregnancy complicated with high blood pressure, which was again in agreement with findings in the literature [16], but this study failed to show correlation with positive family history.

Among nulliparous patients, NICE protocol predicted risk in 50% of cases. This result is higher than previously reported NICE protocol sensitivity (40%) [15-19], which can be stemmed from small PE+ group.

The concentration of PlGF in pg/ml in PE+ group showed a statistically significant difference in comparison with PE- group, even unfitted to the variables that influenced PlGF concentration. This finding was in agreement with the results presented by other authors [20].

The Fetal-Maternal Foundation calculator was used for including these variables. The final result expressed in MoM's (multiple of the median) was again significantly lower in PE+ group in comparison with PE- group, which was in agreement with previously published studies [21-23].

Conclusion

The results obtained from this pilot study show that there is a correlation between serum levels of PlGF measured in the first trimester of pregnancy and subsequent PE development.

This findings classified placental growth factor as a biomarker for PE screening far before the onset of the disease, in time when effective prevention is still possible, which is PlGF essential value.

Conflict of interest statement. None declared.

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Мак Мед Прег̄лед 2019; 73(2): 78-82

ММР

Original article

ДИСМЕНОРЕА, РИЗИК ФАКТОРИ КАЈ УНИВЕРЗИТЕТСКА СТУДЕНТСКА ПОПУЛАЦИЈА ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

DYSMENORRHEA, RISK FACTORS AMONG UNIVERSITY STUDENTS IN REPUBLIC OF NORTH MACEDONIA

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Abstract

Aim. To find the prevalence of dysmenorrhea and to evaluate the risk factors and evaluation of its characteristics.
Methods. A cross-sectional observational study conducted in 853 young university students. The age ranged between 19 to 22 years. A questionnaire specially designed for the purpose of this study was used.
Results. Prevalence of dysmenorrhea was 72%. Our study results showed that significant risk factors associated with dysmenorrhea were: smoking cigarettes, low or no physical activity, diet without meat, milk and dairy products.
Conclusions. Dysmenorrhea is a very frequent condition which is seldom treated properly both by patients and professionals.

Keywords: dysmenorrhea, risk factors, university students

Апстракт

Цел. Да се утврди преваленцата на дисменореа и да се опишат ризик факторите за нејзино појавување.
Мметоди. Пресечна студија на 853 студентки на возраст од 18-22 години. Алатка за испитувањето беше прашалник специјално дизајниран за потребите на студијата.
Резултати. Преваленцата на дисменореа е 72%. Како резултат на оваа студија, сигнификантни ризик фактори за појава на дисменореа се појавија: пушење цигари, немање или многу ретка физичка активност, исхрана сиромашна со месо, млеко и млечни производи.
Заклучок. Дисменореата е состојба која се јавува многу често, а сепак ретко е соодветно третирана како од страна на пациентите, така и од страна на професионалците.

Клучни зборови: дисменореа, ризик фактори, универзитетски студентки

Introduction

The menstrual bleeding-menses is a benchmark of the menstrual cycle. The peeling of theendometrium results from decay of corpus luteum in absence of pregnancy. A cascade of reactions happen during this process such as prostaglandin production, vessel constriction and myometrial contractions [1]. This is aphysiological process. During dysmenorrhea there is an overproduction of prostaglandins, especially PgF2α, which results in myometrial hypercontractility, excessive vasoconstriction, further is chaemia that triggers release of more prostaglandins, hypersensitisation of nerve endings, which all result inpain. The pain is crampy, sometimes compared with a renal colic; it may present as a lower back pain, may irradiate to the legs or thighs or may be dull aching sensation in lower abdomen. It is commonly accompanied with general symptomatology like nausea, vomiting, sweating, bloating, diarrhea, constipation, urgency to void frequently etc. The symptomatology may vary in its intensity.
The pain is occurring before and/or simultaneously with the onset of the menstrual bleeding and slowly declines in intensity with days of menstruation. It is often associated with positive familial anamnesis and therefore often considered “normal” following the familial pattern [2].
Depending on the cause, dysmenorrhea may be primary (essential) or secondary (acquired) due to pelvic pathology such as fibroids, endometriosis or pelvic inflammatory disease [3]. Primary dysmenorrhea is a condition without underlying gynecological physical finding. Dysmenorrhea is most common of all gynecological symptoms in young adult females and causes inconvenience and disturbances in everydaylife, but due to the perception that it is part of a “normal” process it is underestimated by patients and undertreated by professionals.
In some developed countries studies have calculated the economic burden of dysmenorrhea. They report that only in USA there is a loss of 2 billion US dollars due to dysmenorrhea.

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There are studies about risk factors for dysmenorrhea in many populations, but there is no such study conducted among the population ofRNM. Thus, this is the first study aimed at investigating the prevalence of dysmenorrhea as well as the risk factors that may lead to it in the Republic of North Macedonia.

Material and methods

The study was conducted after the approval of the Ethics Committee at the Medical Faculty in Skopje.
This is a cross-sectional observational study performed during the period of April and May 2015. A self-designed questionnaire was offered to all female students of first and second year of their study at three of the State Universities: Ss.Cyril and Methodius in Skopje, St. Kliment Ohridski in Bitola and Goce Delcev in Shtip. They were studying medicine, computer engineering and security.
The sample size was calculated by having into account several factors: significance and aim of the study, population size, the risk of choosing bad sample as well as acceptable mistake of the sample. For this study the sample size was accepted based on the following two parameters: the confidence interval (margin of error) and confidence level. According to the data from the State Statistical Office of the Republic of North Macedonia, female population aged between 18 and 24 years is around 120.000, for accepted confidence intervalof 5% as well as accepted confidence level/ security of the results of 95%, the calculated sample size is around 400 participants. We performed testing of the questionnaire on the targeted population before the final publishing.
The questionnaire included demographic data, anthropometric data-weight, height, BMI, nutritional habits, lifestyle, smoking, menstrual history, presence of pain, its intensity and management. The perception of pain was self-measured by VAS (Visual Analogue Scale). The participants were asked to mark the intensity of pain on a 10 cm scale where 0 means “no pain” and 10 means “maximal-unbearable pain”. There were questions about management of the pain as well as the need of professional help.
The questionnaire was disseminated during breaks between classes after the researchers explained to the students the essence of completing the questionnaire. Every questionnaire included an informed consent, and only the students who signed it could proceed to answer the questions. The time needed for completing the forms was about 10-15 minutes, and during that period the researchers were available for consultation. This survey was on voluntary basis and only the students who agreed to participate completed the questionnaire. There was neither reward nor negative consequences for those who refused to participate.
The statistical analyses were performed using Microsoft Excel, Statistica, release 7.

Results

The total number of participants was 847. The age of the participants ranged between 18 and 22 years (mean age=19.8).
The number of students that reported painful menses over the past 12 months was 613, indicating dysmenorrhea in72%. Regarding the intensity of the pain 11% described it as mild, with VAS score below 4, 55% answered with score between 4 and 7 which means moderate dysmenorrhea and the rest 34% reported severe dysmenorrhea.
Next we compared the attributes of those with dysmenorrhea to those without dysmenorrhea.
The majority, 65.8%, had normal BMI (between 19 and 24.5), 18.7% were underweight with BMI below 19 and 14% were overweight between 24 and 30. Only 1.5% were reported to be obese. The percentage of dysmenorrhea was lowest between normal BMI-70.6%, and highest in the obese students -83.3%.
Among smokers 80.5% reported dysmenorrhea and 69% of non-smokers had dysmenorrhea. According to their background, almost 72% of students from urban settings had dysmenorrhea against 58.3% of those who came from rural settings and reported having painful menses.
There was no difference in presentation of dysmenorrhea associated with religious beliefs (72% *vs*. 70%) (Orthodox *vs*. Islam).
Practicing physical activity showed asignificant effect on dysmenorrhea incidence.
Dietary habits as well as consumption of coffee, alcohol, soda beverages were also analyzed.
Age of menarche did not show important for occurrence of dysmenorrhea (mean age for both groups was13 years). However, the menstrual pattern showed no differences.
Eighty-three (83%) of dysmenorrhoic girls had regular cycles on 23-35 days *versus* 82% without dysmenorrhea. Most of the girls with dysmenorrhea (89%) had 3-7day duration of their bleeding days. 58% of dysmenorrhoic girls had normal bleeding pattern (10-20 sanitary pads per cycle). Twenty-four (24%) needed less than 10 sanitary pads per cycle. Only 17% needed more than 20 pads.
Regarding the question about familial anamnesis of dysmenorrhea, 49% answered positively, 27% did not have dysmenorrhea in their families and 23% did not know if someone of the female members had dysmenorrhea.

Table 1. Risk factors for dysmenorrhea in Republic of North Macedonia

	Dysmenorrhea			
	Yes, N=613 (72.4%)	No, N=234 (27.6%)	All, N=847	
	BMI			
	Yes, N=579 (72.3%)	No, N=222 (27.7%)	All, N=801	p-value
Underweight	109 (72.7%)	41 (27.3%)	150 (18.7%)	0.03
Normal	372 (70.6%)	155 (29.4%)	527 (65.8%)	
Overweight	88 (78.6%)	24 (21.4%)	112 (14.0%)	
Obesity	10 (83.3%)	2 (16.7%)	12 (1.5%)	
	Smoking			
	Yes, N=611 (72.3%)	No, N=234 (27.7%)	All, N=845	p-value
Smokers	165 (80.5%)	40 (19.5%)	205 (24.3%)	0.02
Non smokers	446 (69.7%)	194 (30.3%)	640 (75.7%)	
	Place of living			
	Yes, N=589 (69.9%)	No, N=252 (30.1%)	All, N=383	p-value
Urban	519 (71.8%)	204 (28.2%)	723 (86.3%)	0.35
Rural	67 (58.3%)	48 (41.7%)	115 (3.7%)	
	Religious beliefs			
	Yes, N=609 (72.3%)	No, N=233 (27.7%)	All, N=842	p-value
Orthodox	493 (72.0%)	192 (28.0%)	685 (81.4%)	0.44
Islam	79 (70.5%)	33 (29.5%)	122 (13.3%)	
Atheist	34 (81.0%)	8 (19.0)	42 (5.0%)	
Other	3 (100%)	0 (0%)	3 (0.4%)	
	Physical activities			
	Yes, N=610 (72.4%)	No, N=232 (27.6%)	All, N=842	p-value
No or once a week	411 (75.7%)	132 (24.3%)	543 (64.5%)	0.02
Twice a week	130 (67.4%)	63 (32.6%)	193 (22.9%)	
3 times and more	69 (65.1%)	37 (34.9%)	106 (12.6%)	
	Dysmenorrhea in family members			
	Yes, N=609 (74.0%)	No, N=214 (26.0%)	All, N=823	p-value
Yes	300 (84.5%)	55 (15.5%)	355 (43.1%)	0.00
No	168 (57.7%)	123 (42.3%)	291 (35.4%)	
Don't know	141 (79.7%)	36 (20.3%)	177 (21.5%)	

Of all girls with dysmenorrhea only 50% visited a gynecologist.

71.5% of the students answered that dysmenorrhea limited their everyday activities.

The 90% of the students with dysmenorrhoea self-medicated and 72% of them used NSAID.

Regarding the alternative methods, 34% of girls used antalgic position and bed rest, 24% used hot pads on the abdomen, 20% drank hot beverages-tea, 11% used massage to relieve pain.

Discussion

The prevalence of dysmenorrhea in our study was72%. The reported prevalence in the literature varies largely, between 16% to 90%. This is so, because the studies were performed on various populations with vast differences in age, parity, culture etc. The study of Andersch and Milsom from Sweden performed on a cohort of 19-year-olds showed prevalence of dysmenorrhea of 72.4%,which is agreement with the results obtained for our population with age ranging from 19 to 22 years [4]. According to literature probably ageing and parity have beneficial effect on primary dysmenorrhea. Our study results showed that significant risk factors associated with dysmenorrhea were: smoking cigarettes, low or no physical activity, diet without meat, milk and dairy products.

The BMI and its association with dysmenorrhea has been a subject of many studies and most of them oppose each other. For example, the Indian study including 400 girls from urban and rural origin showed a higher incidence of dysmenorrhea in the rural population and in the group of underweight girls. This led to the conclusion that improvement of nutritional status should lead to pain reduction [5,6]. Similar to this is the study of Rafique and Al-Sheikh comprising 370 female students in Saudi Arabia [7]. A longitudinal study of Ju H. *et al.* which followed-up a total of 9688 females aged between 20 and27 years in a 13-year-period, found an U-shaped association between dysmenorrhea and BMI, revealing a higher risk of dysmenorrhea for both underweight and obese women. They concluded that maintaining a healthy weight over time may be important for women to have pain-free periods [8]. Our results have confirmed this theory and have shown that the incidence of dysmenorrhea was higher if the BMI was higher than normal (≥24.5). The BMI showed to be a significant factor for dysmenorrhea (p=0.03).

Tobacco smoking is a frequent risk behavior in modern society. A single cigarette contains over 400 chemical compounds, some of which are toxic and carcinogenic

for the female reproductive system[9].Nicotine has a vasoconstrictive effect, which leads to decreased amount of blood flow in the endometrium and its hypoxia that results in tissue degradation and production of prostaglandins causing pain [10]. A meta-analysis about the relationship between smoking and dysmenorrhea concluded that there was a significant association between smoking and dysmenorrhea, thus providing a new approach for prevention of dysmenorrhea for the policy-makers [11]. Our results have confirmed that between smokers there were more cases of dysmenorrhea and concluded that it was a significant risk factor (p=0.03).
According to the origin, rural *versus* urban setting, our subjects from rural places had less dysmenorrhea compared to those from urban setting (58.3% versus 72%). This might be due to environmental, nutritional or other factors, which has to be subject to some further studies. Nevertheless, there was no statistically significant difference (p=0.35).
Our students seldom practiced physical activity! Sixty-four (64%) of them declared they did not visit gym or recreate, or they did that once a week. This might be a result of their time spent at lectures and taking exams, but not having healthy habits as well [12]. Hence, not practicing physical activity proved to be a significant risk factor for dysmenorrhea (p=0.05). This finding is very important from public health point of view. It can be recommended to plan the classes at the universities by inclusion of physical activities and sport.
The nutritional habits of our population showed that consumption of milk and dairy products and meat had an impact on dysmenorrhea prevalence (p-value less than 0.05).Consumption of calcium, magnesium and protein have beneficial effect on dysmenorrhea [13]. Unlike other studies we found no significance in consumption of fish and omega-3 reach products. This leads to conclusion that nutrition is very important in association with dysmenorrhea. Several studies have shown that even rhythmicity of feeding plays a role in occurrence of dysmenorrheal [14].
Analyzing the habits of drinking coffee and soda beverages did not lead to the expected conclusion as a significant risk factor for dysmenorrhea unlike the studies in Serbia and Iran [15,16]. We also did not find an association between drinking alcohol and fast food consumption with prevalence of dysmenorrhea.
Age of menarche was not connected to higher incidence of dysmenorrhea in our study, unlike the studies in Hong Kong, Nigeria, Japan and Serbia [14,17,18].
Familial history of dysmenorrhea appeared to be a very significant factor in our population (p=0.00). This is in agreement with the results of other studies. The reason may be a "learned" behavior from the female family members [19], but a genetic predisposition through metabolic gene polymorphisms as well [20].

Conclusion

Dysmenorrhea is the most common complaint in adolescent and young adult age. It causes repetitive health issues which interfere with the lives of girls, their responsibilities and obligations at school causing absence from activities. By pointing out the problem and making it more visible the gynecologists and the public health policy makers should fight for health education of this vulnerable population. Building healthy lifestyle habits should make benefit for the health in general.

Conflict of interest statement. None declared.

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Мак Мед Прег̄лед 2019; 73(2): 83-87

MMP

Original article

COMPARATIVE STUDY OFARTICLES ON DIFFERENT SURGICAL APPROACHESBY DIVERSE AUTHORS IN TREATMENT OF CUBITAL TUNNEL SYNDORME

КОМПАРАТИВНА СТУДИЈАНАНАУЧНИ ТРУДОВИ НА РАЗЛИЧНИ ХИРУРШКИ ПРИСТАПИ ОД РАЗЛИЧНИ АВТОРИ ВО ТРЕТМАНОТ НА СИНДРОМОТ НА КУБИТАЛНИОТ КАНАЛ

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Abstract

Introduction. Cubital tunnel syndrome is the second most common compressive neuropathy in the upper limb. The diagnosis of cubital tunnel syndrome is primarily clinical. A thorough history should include the onset of symptoms, presence of grip or pinch weakness, numbness and the chronicity of the condition.
Methods. Depending on symptoms and clinical signs, the surgical methods of choice include *in situ* open decompression, submuscular transposition, intramuscular transposition, subcutaneous transposition and medial epicondylectomy. APubMed search was conducted and published articles were comparedusing predetermined criteria. Data collected showed the follow-up of patients' surgical treatment with different surgical approaches. The percentage results are shown as combined good and excellent outcomes.
Results. Despite the different scoring scales used and difficulty comparing studies directly, the bulk of single technique outcomes studies and multi-technique comparative studies demonstrate that all surgical techniques discussed are effective treatment methods for cubital tunnel syndrome, but fail to demonstrate one technique to be uniformly superior to another.
Conclusion. The literature, articles and casereports, state that all of the techniques are generally effective. Comparative studies show no statistical difference in outcomes with any presentedtechnique. One conclusion is obvious that transposition should be performed only when subluxation of the nerve is present. In conclusion, there is no superior technique and no gold standard in treatment ofcubital tunnel syndrome.

Keywords: cubital tunnel, ulnar nerve, decompression, transposition, ulnar neuropathy

Апстракт

Вовед. Синдромот на кубиталниот канал е втора најчеста компресивна невропатија на горниот екстремитет. Дијагнозата на овој синдром е примарно клиничка. Целосната историја на болеста вклучува појава на симптомите, присуство на слабост при зграпчување или штипењесо шаката, отрпнатост и самата хроничност на состојбата.
Методи. Во зависност од тоа какви се симптомите, хируршките третмани на избор се декомпресија на самото место, субмускуларна транспозиција, интрамускуларна транспозиција, поткожна транспозиција и медијална епикодилектомија. Според критериумите за овој синдром се бараа и споредуваа трудови кои се објавени на PubMed. Информациите од оправокот на пациентите после разлнични хируршки третмани на овој синдром се групираа и анализираа. Се разгледуваше процентот на добар и одличен исход од оперативниот третман.
Резултати. Без разлика на начинот на оценување кај различни студии и неможноста да се споредат директно, се приметува дека исходот кај разлчните начини на оперативен третман сите имаат задоволително ниво на опоравување. Не може да се издвои еден пристап кој би бил подобар од другите.
Заклучок. Литературата, трудовите и приказите на случаеви, ни покажуваат дека сите пристапи се генерално ефективни. Не постои статистичка разлика во резултатот од различните хируршки техники. Еден заклучок може да се издвои, а тоа е дека транспозиција треба да се направи кај нерв кој луксира од лежиштето. Не постои златен стандард при третман на Синдромот на кубиталниот канал.

Клучни зборови: кубитален канал, улнарен нерв, декомпресија, транспозиција, улнарна невропатија

Introduction

Cubital tunnel syndrome is the second most common

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compressive neuropathy in the upper limb. Its history dates back to the 1807 when a 14-year-old girl presented to Dr. Henry Earle with a 3-year history of hypersensitivity and pain in the ulnar nerve distribution that prevented sleep. At one point, her pain was so severe that Mr. Earle (1816) transected her ulnar nerve above the medial epicondyle of the humerus. Intraoperatively, he noted that the epineurium of the ulnar nerve behind the medial condyle was firmer and thicker than normal. After surgery, the patient had permanent ulnar nerve deficit but was cured of her pain [1].

The ulnar nerve (C7, C8, Th1) is formed directly from the medial branch of brachial plexus. The nerve is medial to the axillary and brachial artery and medial to the brachial vein until it reaches the medial part of the humerus. The arcade of Struthers is a deep brachial fascial band that joins the intermuscular septum and invests the ulnar nerve approximately 8 cm proximal to the medial epicondyle. About 4 cm distal to the medial epicondyle, the nerve gives a motor branch for the*flexor carpi ulnaris* muscle, and few centimeters distally it innervates the ulnar part of *flexor digitorum profundus* muscle. The ulnar nerve travels posterior to the medial epicondyle and medial to the olecranon to enter the cubital tunnel. The tunnel roof comprises a tight fascial layer that extends from the *flexor carpi ulnaris* muscle (FCU) to the arcuate ligament of Osborne, while the floor is defined by the ulnar collateral ligament. Upon exiting the cubital tunnel, the ulnar nerve travels into the forearm between the ulnar and humeral FCU heads, then deep to the deep *flexor pronator aponeurosis* [2].

The diagnosis of cubital tunnel syndrome is primarily clinical, as electrodiagnostic tests can often be negative despite significant symptoms and exam findings. A thorough history should include the onset of symptoms, presence of grip or pinch weakness, numbness, aggravating and alleviating activities, comorbidities (i.e., diabetes, peripheral neuropathies), and previous elbow trauma. Perhaps the single most important feature of history, however, is the chronicity of the symptoms. Intermittent symptoms elicited by elbow flexion are likely due to transient ischemia of the nerve and will respond well to treatment. Constant numbness or weakness responds less predictably to surgery. Numbness and paresthesias are the most common presenting features in early cubital tunnel syndrome, with pain developing later in the condition. Patient complaints of loss of dexterity suggest intrinsic muscle weakness. There are many diagnostic tests that can determine this nerve entrapmentsyndrome. Usually electromyography is useful because it demonstrates block or slower motor conduction of the nerve at the region of the elbow. Other diagnostic methods are X-ray of the elbow, computer tomography (CT) scan, MRI or ultrasonography. A scale used by McGowan can be used to classify the pain and the dysfunction caused by the ulnar nerve compression, where grade I dysfunction is characterized by transient paresthesias and subjective weakness. Grade II dysfunction presents with intermittent paresthesias and objective weakness. Grade III is defined by constant paresthesias and measurable weakness. There are few clinical signs of unlar nerve palsy. Duchenne's sign or claw or intrinsicminus deformity, is hyperextension of proximal phalanx with flexion of middle and distal phalanges caused by paralysis of lumbricals and interossei muscles. Masse's sign is flattening of the dorsal transverse metacarpal arch caused by hypothenar paralysis and loss of the fifth metacarpal supination. Wartenberg's sign is ulnar deviation and weak adduction of the small finger caused by unopposed pull of *extensor digiti minimi*. Froment's sign is hyperflexion of thumb distal phalanx and supination of index during attempted key pinch caused by atrophy of *adductor pollicis* and first dorsal interosseous muscles. Jeanne's sign is hyperextension deformity of thumb metacarpophalangeal joint caused by compensatory instability [3]. The treatment of this condition depends on the clinical presentation of the patient. If the symptoms are mild or intermittent, patients can be treated non-surgically, such as activity modification, splinting, and physiotherapy, and the outcome is highly satisfactory. Once the symptoms begin to be permanent, surgical treatment should be considered. There are few surgical methods we use when treating this condition. Depending on the symptoms and the clinical signs, the methods of choice include *in situ* open decompression, submuscular transposition, intramuscular transposition, subcutaneous transposition and medial epicondylectomy.

Material and methods

The methods of choice for surgical treatment of cubital tunnel syndrome are described in brief. Approaches for treatment include *in situ* open decompression, submuscular transposition, intramuscular transposition, subcutaneous transposition and medial epicondylectomy.

In situ open (simple) decompression

The first described approach is open *in situ* (simple) decompression. A 6-10-cm incision is made along the course of the ulnar nerve between the olecranon and medial epicondyle. This procedure is using the wide-awake approach (local infiltration without sedation or tourniquet). Field infiltration of local lidocanie and epinephrine is performed beginning 8–10 cm proximal to the medial epicondyle to ensure anesthesia in the medial antebrachial cutaneous nerve distribution. Care should be taken to avoid branches of this nerve during subsequent dissection. Beginning proximally, the arcade of Struthers is released, followed by Osborne's ligament and the FCU fascia. The ulnar nerve is left undis-

turbed in its bed. The elbow is placed through a range of motion to check for any residual compression sites or subluxation of the nerve [4].

Submuscular transposition

With elbow flexion, the ulnar nerve is placed under tension and compression as the cubital tunnel volume decreases. The goal of transposition is to move the nerve anterior to the axis of elbow flexion, thereby decreasing tension on the nerve. Critics of this technique thinkthat dissection of the nerve from its bed compromises the segmental blood supply of the nerve. Transposition may also lead to more local numbness and discomfort than simple decompression due to the sacrifice of a greater number of local cutaneous and articular sensory branches. As in simple *in situ* decompression, the proximal nerve is identified and traced distally following release of the arcade of Struthers. To prevent the formation of a new compression site proximally, a segment of the intramuscular septum is excised; care must be taken to avoid injury to the venous plexus associated with the septum. The nerve is then unroofed to the level of the deep *flexor pronator aponeurosis*. A vessel loop is placed around the nerve to provide gentle traction while the nerve is dissected free from its bed and transposed anterior to the medial epicondyle. The motor branches to the FCU and the FDP are preserved. The *flexor pronator* muscle mass is divided 1–2 cm distal to the medial epicondyle. The median nerve must be identified and preserved. The *flexor pronator* mass is repaired over the transposed nerve with a stepwise lengthening technique to avoid causing a new compression site .

Intramuscular transposition

Intramuscular transposition is another technique used in combination with anterior transposition. Instead of elevating the entirety of the *flexor pronator* muscle mass to maintain the ulnar nerve anterior to the medial epicondyle, the intramuscular technique involves making a groove in the *flexor pronator* mass. Opponents tothistechnique thinkthat the absence of a natural tissue plane results in a scarred bed around the nerve that can itself lead to nerve compression.

Subcutaneous transposition

After anterior transposition, many surgeons prefer to leave the nerve in a subcutaneous position. Instead of elevating the *flexor pronator* mass, the ulnar nerve is maintained in its transposed position by suturing the loose epineurium to the forearm fascia. Alternatively, a small sling can be created by suturing the subcutaneous tissue from the anterolateral skin flap to the fascia overlying the medial epicondyle, or by suturing a strip of elevated muscle fascia to the overlying dermis. To prevent subluxation of the nerve back into its native bed, the roof of the cubital tunnel may be reapproximated [5].

Medial epicondylectomy

In the medial epicondylectomy technique, the nerve is dissected as in a simple *in situ*decompression. The medial epicondyle is exposed in a subperiosteal plane, maintaining the origin of the *flexor pronator* mass with the periosteum. The anteromedial edge of the epicondyle is scored with an osteotome. The epicondylectomy is performed along a plane midway between the sagittal and coronal planes of the humerus, all the while preserving the attachments of the ulnar collateral ligament. The flexor pronator origin is then reattached over the epicondylectomy site.
Many different scoring scales are used across these studies, however most studies group outcomes into Excellent, Good, Fair, Satisfactory, and Poor. Some of the scales used in determination of the condition are the McGowan improvement scale, Bishop score, Lsu (Louisiana State University) grade, Wilson and Krout, Gabel Amadio, MacDermid, Messina classification and of course the subjective assessment and the patient satisfaction. A PubMed search was conducted and published articles were comparedusing predetermined criteria. Data collected showed the follow-up of the surgical treatment of patients with different surgical approaches. The percentage results are shown as combined good and excellent outcomes.

Results

In situ open (simple) decompression

The poorest outcome wasdescribed by Barterls *et al.* with 65.3% of combined good and excellent percentage [6]. Those with the best combined good and excellent percentages outcomes, both limited by notably small sample sizes, were Cho *et al*. and Keiner *et al.* with 100% and 94.1%, respectively [7, 8]. Most of the other studies presentedfrom 78 to 91% of combined good and excellent percentage outcomes.
Complication rates, while not uniformly reported, are generally low with this technique. Most frequent were incisional tenderness, as well as numbness in the distribution of the median antebrachial cutaneous nerve (MACN), followed by the less common superficial infections and wound dehiscence. Incisional length varied widely, but often as long as 8-10 cmor more, which poses a substantial threat of injury to the MACN, as well as increased postoperative pain and healing time, which are established consequences of open surgery and large incisions [9].

Submuscular transposition

Both Gervasio and Gambardella with a combined good and excellent percentage of 87% [10] and Davis and Bulluss with 82.5% of patients improving at least one Louisiana State University grade, [11] have demonstrated good results with this technique, with only one complication of MACN distribution numbness between the two studies. The main advantage of this technique compared tothe other transposition techniques is the protection offered by the overlying muscle, but there have beenno studies that demonstrate any degree of superiority over any of the techniques discussed. Submuscular placement may be preferable when the patient has little subcutaneous tissue to protect the nerve but transposition is necessary due to subluxation of the nerve.

Intramuscular transposition

Kleinman *et al*. retrospectively analyzed 52 procedures in 48 patients, finding a combined good and excellent percentage of 87%. They noted that many detractors of the technique previously were concerned about scarring within the muscle bed or traction forces on the nerve, but these concerns have yet to be proven and no complications were noted in this study [12]. Only one comparative study, was found, that of Emamhadi *etal*., presenting intramuscular transposition to have better motor outcomes than subcutaneous transposition, but equivalent pain and sensory outcomes between the two groups [13]. It was posited by Kleinman that adequate release of the fibrous aponeurosis and intermuscular septum, between the flexor and pronator muscles, in addition to the creation of a 5 mm trough fashioned into the musculature, allows free movement of the ulnar nerve in a well-vascularized bed providing a better environment for healing and protection than the subcutaneous location.

Subcutaneous transposition

In 2015, Lima *et al.* demonstrated 77.7% of combined good and excellent percentage with complications of scar pain, paresthesia and early superficial infection. A recent meta-analysis by Chen *etal*. concluded that outcomes were equivalent between subcutaneous transposition and *in situ* decompression; however, subcutaneous transposition had a significantly higher complication rate [14].

Overall, there is no evidence to suggest subcutaneous transposition to besuperior to *in situ* decompression, and that outcomes are likely comparable between the two techniques. Except in the case of a nerve subluxation on exam, which over time may cause chronic irritation which is relieved by transposition, it may be preferable to perform *in situ* decompression as the *de facto* procedure in order to preserve the vascular supply which is disrupted by transposition. However, many proponents of the procedure argue that the anastomoses between proximal and distal vascular supply to the nerve negates this point. The nerve is more exposed to potential trauma in its post-transposition location, with only the skin and small amount of subcutaneous tissue protecting it from external forces as compared to being protected by the bony structures of the elbow and several layers of overlying tissue in its native position.

Medial epicondylectomy

Twenty-one case series reported on 886 medial epicondylectomies. The mean percentage of patients obtaining improvement of one or more McGowan grade was 79%. The mean percentage obtaining a good and excellent Wilson Krout grade of outcome was 83%. Of six comparative studies, two showed no significant differences in outcomes between medial epicondylectomy and transposition procedures, and three reported better outcomes with medial epicondylectomy. One reported similar outcomes with medial epicondylectomy and simple decompression [15].

Discussion

Despite the different scoring scales used and difficulty comparing studies directly, the bulk of single technique outcomes studies and multi-technique comparative studies demonstrate that all surgical techniques discussed are effective treatment methods for cubital tunnel syndrome, but fail to demonstrate any technique to be uniformly superior to another, except in the case of ulnar nerve subluxation in which transposition is generally preferred. While anterior transposition is widely accepted as the preferred method for treating cubital tunnel syndrome where ulnar nerve subluxation is present, it seems there are no studies specifically comparing *in situ* decompression against anterior transposition in this specific subset of patients. Studies that compared simple decompression against anterior transposition showed that specific group of patients with subluxation of the nerve experienced distinctly better results when treated with anterior transposition rather than with simple decompression, but that overall there was no significant difference between the two groups. Except for these two studies,it seems there is no evidence supporting the widely held belief that transposition is superior for this subset of patients.

Simple decompression has been shown to be effective in treating cubital tunnel syndrome, with results equivalent to those of anterior transposition. Similarly, a retrospective study comparing medial epicondylectomy alone with medial epicondylectomy and anterior subcutaneous transposition showed no differences. Two meta-analyses compared the outcomes of simple decom-

pression and anterior transposition techniques, butfailed to find a significant difference between surgical techniques, although one of the studies did observe a trend toward improved outcomes with anterior transposition. The major limitation of the meta-analyses in cubital tunnel syndrome remains a lack of reliable, reproducible, and valid outcome measures. The posterior branch of the medial antebrachial cutaneous nerve (MACN) is at potential risk of injury during both simple decompression and anterior transposition. Injury to the nerve can result in a painful neuroma and hyperesthesia. Ulnar nerve subluxation following simple decompression can lead to a persistent pain and is addressed by anterior transposition. Medial epicondylectomy is complicated by a persistent elbow pain in up to 45% of patients. Incomplete decompression is effectively addressed through a thorough reassessment for points of persistent compression followed by an anterior transposition. If there is a significant amount of perineural scarring associated with symptoms, the addition of soft-tissue coverage in the form of a muscle flap, fat flap, or vein wrapping may be considered.

Conclusion

The literature, articles and casesreported state that all of the techniques are generally effective.When considering the various techniques with roughly equal efficacy, many authors suggest choosing techniques that will minimize incision size and degree of tissue dissection, operating time, post-operative complication rates. The predominant role has the surgeon, who has to decide which approach to choose. Some surgical approaches are more invasive than others. Less invasive techniques lead to shorter healing times, less pain and decreased operative times. The rates of infection are decreasing. Comparative studies show no statistical difference in outcomes with any technique. One conclusion is obvious that transposition should be performed only when subluxation of the nerve is present. In conclusion, there is no superior technique and no gold standard in treatment ofcubital tunnel syndrome.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2019; 73(2): 88-93

ММР

Original article

ROLE OF CYTOKINES AND THEIR PRESENCE IN THE AMNIOTIC LIQUID AS A SIGN OF EARLY DETECTION OF PREMATURE BIRTH IN PREGNANT WOMEN

УЛОГА НА ЦИТОКИНИТЕ И НИВНО ПРИСУСТВО ВО АМНИОНСКА ТЕЧНОСТ КАКО ЗНАК ЗА РАНА ДЕТЕКЦИЈА НА ПРЕДВРЕМЕНОТО ПОРОДУВАЊЕ КАЈ ТРУДНИЦИ

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Abstract

Introduction. Cytokines play a significant role in the pregnancy. They are very powerful and important mediators of the cell growth as well as regulators of the immune and inflammatory reactions. Several cytokines (IL-1, IL-6, IL-8, TNF- alfa) are of crucial importance during the pregnancy since they are produced by the placentain the amniotic fluid, in case there is intrauterine inflammation. Inpatients with premature birth, the intrauterine inflammation and infection is often present and leads to inflammatory syndrome of the human fetus. The intrauterine infection of the choriodecidual space and the amniotic fluid are the most common reasons for this obstetric complication, hence the most commonly examined etiologic factor.

Aim. The study was conducted in order to prove the ratiobetween the increased level ofIL-6in the amniotic liquid at the beginning of the second trimester (16-22 g.w.) and the premature birth (< 36 g.w.).

Methods. This is a case control study that has included 36 patientsso far.The pregnant women wererecruited fromthe Clinic of Gynaecology and Obstetrics. They all gave a signed consent on being informed about the aims of the study, and following the protocol, they were analyzed and examinedi. e. all patients under went ultrasound examination, vaginal cervicometry; cervical and vaginal swabs were taken and 5 ml. amniotic fluid during the process of amniocentesis.

The study was performed at the Clinic ofGynaecology and Obstetrics, the Institute of Microbiology and Parasitologyas well as the Institute ofImmunology and Human Genetics.

Results. The results obtained inthisstudy have confirmed the role of the cytokines i.e. they have shown an increase when there is inflammation in the intrauterine cavity which could lead in future to premature birth. There was an association between the risk of premature birth and positive cervical and vaginal swabs, length of cervix, and not a single case showed positive amnio-culture.

Keywords: premature birth, amniotic fluid, cytokines, IL-6, amniocentesis,pregnancy

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Апстракт

Вовед. Цитокините играат многу важна улога во бременоста. Тие се многу силни и важни медијатори на клеточен раст и регулатори на имунолошки и инфламаторни реакции. Неколку цитокини (IL-1, IL-6, IL-8, TNF-alfa) се од исклучително значење во бременоста и истите се продуцираат од страна на постелката во амнионската течност, доколку постои интраутерина инфламација. Кај пациентки кај кои настанува предвремено породување, интраутерина инфламација и инфекција е многу често присутна и води до инфламационен синдром и на плодот. Интраутерина инфекција на хориодецидуалниот простор и амнионската течност е најчеста причина за настанување на оваа обстетричка компликација, а со тоа и најчесто испитуван етиолошки фактор.

Цел. Оваа студија беше спроведена за да се докаже соодносот на покаченото ниво на IL-6 во амнионската течност во почетокот на вториот триместар (16-22 г.н.) ипредвременото породување (<36 г.н.).

Методи. Во рамките на студијата досега се обработени 36 пациентки. Станува збор за case control студија. Трудниците се регрутирани на Клиниката за Гинекологија и Акушерство каде по потпишаната информирана согласност по протокол истите се обработени односно на сите пациентки им е направен ехо преглед, вагинална цервикометрија, земени се цервикални и вагинални брисеви и 5 мл амнионска течност при изведување на самата амниоцентеза. Студијата се изведува на Клиниката за гинекологија и акушерство, Институтот за микробиологијаи

паразитологија и Институтот за имунологија и хумана генетика.
Резултати. Во рамки на студијата добиените резултати ја потврдуваат улогата на цитокините односно нивно зголемување при постоење на инфламација во интраутеринатa празнина која во иднина би довела до предвремено породување. Испитуваната група на овие пациенти покажа поврзаност при зголемување на цитокинот IL-6 и предвремено породување. Исто така покажа поврзаност на ризикот од предвременото породување со позитивните цервикални и вагинални брисеви, должина на цервискот, а во ниеден случај не се доби позитивна амниокултура.

Клучни зборови: предвремено породување, амнионска течност, цитокини, IL-6, амниоцентеза, бременост

Introduction

Cytokines play an important role during the pregnancy [1-3]. They are very powerful mediators for the cell growth as well as regulators of immune and inflammatory reactions. They are either polypeptides or glycopeptides which act through specific receptors in the cell it self and the cell membrane [4]. They can be positive or negative regulator of the immune response. They are messengers which together with hormones and neurotransmitters belong to the group of most important communication materials between cells [5]. Cytokines transfer the information to the target cell which coincides with its receptor. That is how activation and change in the target cells occur [7,8].
Cytokines act as powerful molecules which are released from the cells and then transported to other parts of the organism and act to the function of other cells, which leads to numerous biological reactions. Each live cell with a core in the human organism creates cytokines whose type and quantity of secretion depends on the type and extent to which cells differentiate i.e. degree of their activation stage. The creation of cytokines is encouraged by antigen specific activation of lymphocyte T4 [4].
Cytokines include a group of interleukines, tumor factors of growth and interferons. This division is made depending on the biological and structural differences, but onthe similarity of these mediators, too. Interleukins were named after their function in the mutual communication with the leukocytes. Today we know 29 types of interleukins which are marked with numbers from IL-1 untilIL-29 [8].
Interleukin 6(IL-6) belongs to the inflammatory cytokines and is secreted during inflamed conditions. It is created by many immunogenic cells but also by many non-immunogenic cells and organs which help the control of the inflamed reactions [9].
The gestational tissue including placenta, extravillous trophoblast, amnion, and mother's deciduas are produced by cytokines themselves [7]. These cytokines are considered to affect the outcome of the pregnancy [8-10].
It is thought that the increased level of IL-6, IL-1, IL-2, IL-8, TNF- alphain the amniotic fluidleads to a bad outcome of the pregnancy, but depending on the cause of the increase [2,11-13].
The amniotic fluidis a sterile environment in a normal pregnancy [14,15]. It is a complex body liquid which has an important role in every pregnancy. Its functions are nutritive, protective but also diagnostic for the fetus [16]. Its content changes during the progression of the pregnancy. It contains exclusively important and complex substances which are essential for the normal fetal development [17,18].
The amniotic fluidhas been used for a long time for diagnosing the intra-amniotic inflammation which is closely related to the occurrence of premature birth. The indicators that suggest presence of inflammation are: increased level of matrix metalloproteinases (MMPs) (e.g.MMP-9) [21-23], increased interleukins (e.g. IL-6, IL-1), TNF- α, Granulocyte-colony stimulating factor (G-CSF), increasedLe, low glucose level, etc [24]. The main cytokines for identification of the intra-amniotic inflammation, most closely related to premature birth are the (IL-6) interleukins [9].
Premature birth is present in 5 to 18% of the pregnancies and is the main reason for the neonatal morbidity and mortality [25]. It is in fact every birth which occurs after a possible viability of the fetus i.e. the 24^{th} gestational week, but before the full 37^{th}. The spontaneous occurrence of the contractions or premature bursting of the placenta is considered a reason in around two thirds of these deliveries. Each delivery before the 24^{th} gestational week is considered a miscarriage. Currently, the 23^{rd} gestational week is considered to be a grey zone [3,27].
According to the time of occurrence of premature births, there are three gestational periods. Late premature birth from 32^{nd} -37^{th} g.w., early premature week from 28^{th} to 32^{nd} g.w., and extremely early premature birth under the 28^{th} g.w., i.e. from 24^{th} to 28^{th} g.w. [28].
The etiology of occurrence of premature birth varies depending on the gestational age [29].
Inpatients who have premature birth, the intrauterine inflammation and infection are present and can lead to inflammatory syndrome to the fetus. The subclinical intrauterine infection of the choriodecidual space and amniotic fluidis the most common reason for occurrence of this obstetric complication, and it is the most common examined etiologic factor [1,25]. The uterine cavityis normally sterile but the vagina contains normal bacteria flora. Depending on the concentration of bacteria and vagina resistance, bacteria can ascend from the vagina to the cervix and get to fetal membrane. They might activate the decidua in order to produce inflammation, hence to activate inflammatory mediators

that would later increase the prostaglandins; and they directly affect the myometrium and provoke contractions. The placenta around the fetus might weaken and burst. The neonatal sepsis, mother postpartum endometrial histological chorioamnionitis are diagnoses which are significantly more common in premature birth, especially in those occurring before the 32nd g.w [26].
Apart from the infection, there are other reasons for occurrence of premature birth such as: overstretching of the uterine wall, surgical procedures of the genital organs, abnormal uterine cavity, cervical weakness and idiopathic [27].
If the asymptomatic change in the amniotic fluid, i.e. the increased level of the cytokines is discovered on time, it will contribute toearly therapeutic intervention. Until now, there are no official data in Macedonia from the examinations of the amniotic fluidin pregnancy, especially when patients have not had any symptoms and changes [27].
The aimof the study wasto prove the ratio between the increased IL-6 in the amniotic fluidatthe beginning of the second trimester (16-22 g.w.) and premature birth (< 36 g.w.).

Material and methods

The study included 36 patients of the planned 150, during the period from 01.06.2018 to 01.08.2018.All patients wererecruited fromthe Clinic ofGynecology and Obstetrics. Prior to inclusion in the study, the pregnant women gave their written consent to participate in the study. The study was previously approved by the Ethics Committee at the Faculty of Medicine in Skopje. The examination wasa case control study. Pregnant women wereselected to enter the study between their 16-22 g.w. and werebeing followed until they gavebirth. Each pregnant woman underwent an obstetric ultrasound bywhich the gestational week wasdetermined and confirmed that there wereno exclusion criteria for the patient toenter the examined group.
The pregnant women were followed onVoluson 730pro for ultrasonography. The patients presentedmedical results from vaginal and cervical swabs and in the casewhen such examinationhad not been done, they were sent to the Institute of Microbiology and Parasitology – Skopje. Ultrasound cervicometry was done and the length of cervix was measured with a vaginal transducer and the results were recorded on the personal document for the patient. Each patient was taken a detailed anamnesis and information adapted to the needs for the research.
After examining the patients, they were hospitalized at the Clinic ofGynecology and Obstetrics, and thepreparation for the procedure of amniocentesis followed.
The amniocentesis itself took place in the ultrasound and diagnosis ward, within the Department of pathological pregnancy. Each amniocentesis was done in special sterile conditions with highly determined protocol and was controlled by an ultrasound. It was done in the period between 16-22 gestational weeks.Prior tothe intervention, the whole procedure was described to the patients. The amniocentesis was then performed andadditional 5ml amniotic fluidwastaken for further examination.
Each sterile syringe was marked with the name and surname of the patient, immediately after the intervention. Patients were discharged from the hospital the same day.

Inclusion criteria:

1. Single pregnancy
2. Patients who need amniocentesis in their early second trimester due to clinical indication (advanced mother's age, abnormal test of PRISCA I, suspicious anomalies of the fetus, virus infection or mother's wish)
3. Pregnancy from 16-22 gestational weeks
4. Patients who have no signs of miscarriage (spontaneous abortion) while the amniocentesis is being made.

Exclusion criteria:

1. Positive test of amniocentesis- abnormal karyotype.
2. Multiple pregnancies.
3. Patients who will not contacted and there will be no information on the pregnancy outcome.
4. Confirmed fetal anomalies or patients where pregnancy is prematurely terminated due to other reasons not related to the inflammatory processes such as trauma etc.

Biological samples and their analysis:

Amniotic fluid:

In a separated sample of the amniotic fluid, the number of leukocytes and glucose level were measured. These examinations were done in the biochemistry laboratory of the University Clinic of Gynaecology and Obstetrics-Skopje.
The IL-6 concentration in the amniotic fluid was measured by a device-Immulite 2000 HP, Immulite 1000 HP Diagnostic Products Corp, at the Institute for Immunology and Human Genetics.
The realization of this technique and analysis of the results obtained were done in accordance with the instructions from the manufacturer.
An aliquot of 2ml of the sample was sent to the Institute of Microbiology and Parasitology-Faculty of Medi-

cine-Skopje, where the process of coloring a gram and amnio-culture was done, by using standard bacteriological techniques [28].

Statistical analysis

A database in the statistical program SPSSfor Windows 23.0 was createdfor the purpose of analyzing the results obtained in the research.
The numerical, i.e. the quantitative parametersare shown with an average, standard deviation, median and interquarter rank.
Qualitative i.e. attributive parametersare shown by distributing frequencies.
Mann-Whitney test was used for comparing women who gave premature birth and those who gave term birth.
Statistical significant differenceswere set at $p<0.05$.

Results

This study included36 patients who underwent amniocentesis during which 5ml of amniotic fluid was taken for examination of IL-6, amnio-culture, leukocytes and glucose. Also, vaginal and cervical swabs were taken as well as ultrasound examination and cervicometry. All patients were in the period of 16^{th}-22^{nd} gestational week.
Five (13.9%)of the total 36patients gave premature birth (Table 1).
All 5 patients had increased IL- 6 level (Table 1).
Three of the patients had positive primary vaginal and cervical swabs (*Ureaplasmaurealyticum, Gardnerella vaginalis, Candida albicans*). Three patients had shortened cervix, i.e. it was smaller than 30mm and none of them had a positive amnio-culture. Values of leukocytes and glucose were not increased (Table 1).

Table 1. Values of analyzed parameters in women who gave premature birth

Length of cervix	Glucose concentration in amniotic fluidmmol/l	Conc. Of Le in amniotic fluid	Cervical and vaginal swabs	IL-6 concentration in amniotic fluid Pg/ml	Gestational week during giving birth	Amnio-culture
22	1	4	*Ureaplasma urealyticum*	2234	32	Neg.
29	0	2	/	800	35	Neg.
31	1	0	/	867	36	Neg.
30	3	1	*Ureoplasma urealyticum Gardnerella vaginalis*	1322	34	Neg.
28	2	1	*Candida albicans*	922	36	Neg.

The results of this study showed that cervix length was significantly different in women who gave premature birth compared to those who gave birth on time ($p= 0.049$). Significantly shorter cervix was measured in the group of women who gave premature birth.
The average cervix length in this group was 28.0±3.5, median 29, whereas in the other group the average cervix length was 31.03 ± 2.5, and median 31.

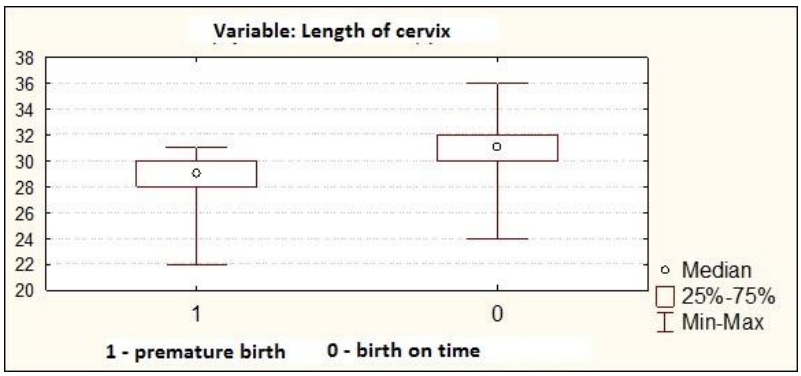

Fig. 1. Correlation of cervical length and time of birth

CytokineIl-6 showed significantly different values in women who gave premature birth and those who gave birth on time ($p=0.00039$). Significantly higher concentration of this inflammatory marker was measured in the group of those with premature birth.
The average value ofIl-6in the group with premature birth was 1229.0 ± 597.5, median 922; average and median value ofIl-6in the group of those who gave birth within their term was 374.52±155.2 and 326 consequently.

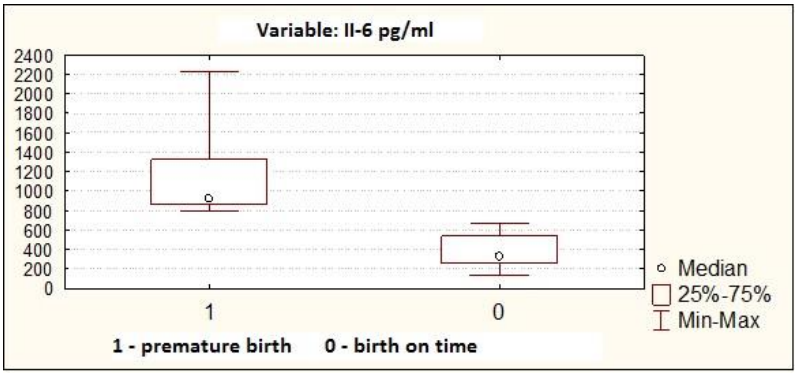

Fig. 2. Correlation of value of IL-6 and time of birth

Discussion

The results obtained in this study, which is still ongoing, support the expected hypothesis that the increased IL-6 in the amniotic fluid, although in asymptomatic patients, still affects the outcome of the pregnancy i.e. its increase leads to premature birth (Figure 1). The examination is more valuable since we know that 5-18% in total of the full number of births in our Clinic belongs to this group. The results have confirmed that risk factors for premature birth include vaginal and cervical infection [29,30], shortened cervix (Figure 1) and presence of increased values of the inflammatory marker IL-6 in the amniotic fluid. In the group of patients who gave premature birth, the average value of gestational week was from32nd to 36th, whereas in the group with normal values, the most common findings showed delivery on time, i.e. in the 37th gestational week (Figure 2). The examinations in which amniotic fluid is used for researches of cytokines, are relatively new and done to a small series of patients [31-33]. In our case, some of these results have been partially analyzed. In the examined group, changes have been observed in other parameters i.e. in vaginal and cervical swabs, in the cervix length, but not in the number of leukocytes, and the values of glucose in the amniotic liquid which suggests that the increased cytokines i.e. IL-6 as a risk factor affect the outcome and time of giving birth. However, only a small number of the examined subjects planned for the whole study has been analyzed, i.e. 36, which means that we should be careful with the interpretation of the results obtained. In the further course of the study, more detailed results will be presented and they will be more representative due to the larger number of included subjects.

Conclusion

This study is the first one done in Macedonia aimed at examining any kind of changes in the amniotic fluid, regardless of gestational age. The study has so far confirmed the reason for examining cytokines as a method to discover asymptomatic changes in patients who would give a premature birth. The further course of the study will additionally determine the values and frequency of changes in premature birth. The expected results are those shown in patients who do have certain inflammatory agent (increased IL-6), shortened cervix, presence of microorganisms, and will have more common complications i.e. it would be expected that there is an increased risk of a premature termination of the pregnancy. The benefit of the study lies in detecting asymptomatic cases, so that this complication can be prevented on time. This type of examination would contribute to reduction of premature births, which goes along with a high rate of morbidity and mortality as well as high costs at the Clinic regarding these complications. It would be useful to create an algorithm for multidisciplinary treatment of these patients.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2019; 73(2): 94-100

ММР

Original article

COMPARATIVE ANALYSIS OF RECONSTRUCTIVE TECHNIQUES: FULL - THICKNESS SKIN GRAFTS VERSUS COMPOSITE SKIN GRAFTS (DERMIS AND SUBCUTANEOUS ADIPOSE TISSUE), USED FOR CLOSURE OF DEFECTS AFTER REMOVAL OF NON MELANOMA SKIN CANCERS ON THE NOSE

КОМПАРАТИВНА АНАЛИЗА НА РЕКОНСТРУКТИВНИ ТЕХНИКИ, ТРАНСПЛАНТАТИ СО ЦЕЛОСНА ДЕБЕЛИНА НАСПРОТИ СЛОЖЕНИ ТРАНСПЛАНТАТИ (ДЕРМИС И ПОТКОЖНО МАСНО ТКИВО), ПРИМЕНЕТИ ЗА ЗАТВОРАЊЕ НА ДЕФЕКТИ ПО ОТСТРАНУВАЊЕ НА НЕ МЕЛАНОМСКИ КОЖНИ КАРЦИНОМИ ВО ПРЕДЕЛОТ НА НОСОТ

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Abstract

Introduction. Excision of malignant lesions of the skin located on the face, especially the nose, followed by appropriate reconstruction is a challenge for every surgeon. Non-melanoma skin cancer (NMSC) is the most common malignant lesion in the world and its incidence is increasing. Surgical treatment, when possible, gives the lowest rates of cancer recurrence and is the gold standard for treatment.

Methods. This is a prospective, clinical, longitudinal study. It included 80 subjects, patients operated on at the University Plastic and Reconstructive Surgery Clinic, 40 patients with a malignant skin lesion in the upper half of the nose, and 40 with a malignant skin lesion in the lower half of the nose. Patients were further subdivided into two subgroups of 20 patients, depending on the reconstructive technique used: a full-thickness skin graft (Wolfe) and a composite skin graft consisting of dermis and subcutaneous adipose tissue.

Results. In this study the aesthetic effect of both reconstructive techniques was analyzed through the assessment of the appearance of the scar by the surgeon and the patient. For this purpose POSAS scar assessment scale was used, which contains scores from 1 to 10, where score 1 indicates normal skin, and score 10 the worst scar. The statistical analysis has shown that the aesthetical appearance of full-thickness skin graft has better results in the upper half of the nose compared to the aesthetical appearance of patients where composite skin graft has been used, which on the other hand, appears to be a dominant technique for closing the defects in the lower half of the nose if the skin graft is a technique of choice.

Conclusion. Composite skin graft has been neglected in recent years and has been rarely used. Studies of the use of this graft have been published in the last 20 years, but there is still no single common opinion regarding its use. Composite skin graft as such has been treated for many years as very contradictory. It has been hypothesized that skin adipose tissue stands as a barrier to vascularization of the graft. It is for this reason that the composite skin graft was not accidentally used as a reconstruction technique in this study. The results obtained showed no comparable dominance over the aesthetic effect, especially after six months of surgery, indicating and recommending its use for reconstruction in the lower half of the nose.

In conclusion, the evaluation made by both the surgeon himself and patients on the POSAS scale showed that the aesthetic appearance of the scar following effective technique and technique of choice was better with the full-thickness skin graft for the upper half of the nose compared to the composite skin graft, which was a dominant technique for closing the defects in the lower half of the nose.

Keywords: full-thickness skin graft, complex skin graft, upper nasal half, lower nasal half, POSAS scar assessment scale

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Апстракт

Вовед. Ексцизија на малигни лезии на кожата лоцирани на лицето, а посебно на носот проследени со соодветна реконструкција претставуваат предизвик за секој хирург. Не меланомскиот карцином на кожата (НМКК) е најчеста малигна лезија во светот и нејзината зачестеност се зголемува. Хируршки третман, кога е можно, дава најниски стапки на повторување и претставува златен стандард за третман.

Методи. Истражувањето претставува проспективна, клиничка, лонгитудинална студија. Во него беа вклучени 80 испитаници, пациенти од Клиниката за Пластична и реконструктивна хирургија, 40 со промена во горната половина на носот, 40 со промена во долната половина на носот. Пациентите понатаму беа поделени во две подгрупи од по 20 пациенти, во зависност од користената техника на реконструкција: техника на кожен трансплантат со целосна дебелина (wolfe) и сложен кожен трансплантат составен од дермис и поткожно масно ткиво.
Резултати. Во истражувањето беше анализиран естетскиот ефект од двете реконструктивни техники, преку проценка на изгледот на лузната од страна на истражувачот и од страна на пациентот. За таа цел беше користена ПОСАС скала за проценка на лузни, која содржи скорови од 1 до 10, при што скор 1 означува нормална кожа, скор 10 означува најлоша лузна. Од статистичката анализа се доби резултат во однос на отценката на самиот истражувач и пациентите по ПОСАС скалата за естетскиот изглед на лузната по ефикасна техника и техника на избор претставува трансплантат со целосна дебелина за горната половина на носот во споредба на сложениот трансплантат кој се покажува како доминантна техника за затворање на дефекти во долната половина на носот.
Заклучок. Сложениот кожен трансплантат во последните години е запоставен и се поретко се употребува. Во последните 20 години објавени се студии за употребата на овој трансплантат но сеуште нема единствен заеднички став за негова употреба. Сложениот кожен трансплантат како таков долги години се третирал како многу контрадикторен. Се претпоставувало дека покожното масно ткиво стои како бариера за васкуларизација на трансплантатот. Токму заради ова сложениот кожен трансплантат не случајно е употребен како техника за реконструкција во оваа студија. Резултатот покажува не споредбена доминација во однос на естетскиот ефект посебно после 6-тиот месец од операција што дава насока и препорака за негова употреба за реконструкција во долната половина на носот.
Како краен заклучок може да се каже дека во однос на отценката на самиот истражувач и пациентите по ПОСАС скалата за естетскиот изглед на лузната по ефикасна техника и техника на избор претставува трансплантат со целосна дебелина за горната половина на носот во споредба на сложениот трансплантат кој се покажува како доминантна техника за затворање на дефекти во долната половина на носот.

Клучни зборови: трансплантат со целосна дебелина, сложен кожен трансплантат, горна половина на нос, долна половина на нос, ПОСАС скала за проценка на лузни

Introduction

Excision of malignant lesions on the skin, especially on the nose, followed by proper reconstruction is a challenge for every surgeon. Non-melanoma skin cancer (NMSK) is the most common malignant lesion in the world and its incidence is increasing [1]. The most common cancer is basal cell carcinoma (BCC) presented in 75% of all cases, followed by spinocellular cancer (SCC)-20% of malignant lesions and malignant melanoma 5% [2]. NMSK occurs in people of all ages, but are more common after the fifth decade of life. Risk factors include sun exposure, especially in childhood, then people that have light skin, patients who are receiving immunosuppressive therapy and patients with genetic predisposition to skin cancer [3]. Surgical treatment, when possible, gives the lowest recurrence rates and is the gold standard for treatment. NMSK is considered as high risk when it is located on the patients' ear, or central part of the face [3]. Non-melanoma skin cancers are most commonly seen on the nose. This is due to the fact that this part of the face is more exposed to the sun than the other parts of the face. Chronic exposure to sunlight greatly increases the risk of skin cancer. Therefore, the incidence of skin cancer is increased in both long-hours and open-field professionals (such as farmers). In addition, the longer the duration of exposure to the sun, the greater the incidence of the disease [4].
Primary treatment for NMSK is surgery. The result of defects' repair varies depending on when the defect is repaired, the region and size of the defect, as well as the characteristics of the surrounding skin. The way of reconstruction varies, starting from local geometric flaps, free skin grafts, direct suture for small skin defects ending with free flaps for large defects [5]. Due to the existence of many ways of repairing skin defects that occur after removal of the NMSK in the nose area, researches are dedicated to compare the techniques with a goal-aesthetically dominant solution for the reconstruction of the nose with minimal complications and maximum comfort and patient satisfaction.
This paper compares the aesthetic effect of a full thickness skin graft (Wolfe) compared to a composite skin graft consisting of skin and subcutaneous adipose tissue. The aesthetic effect was evaluated and compared with the use of the patient and observer scar assessment scale (POSAS).

Material and methods

This is a prospective, clinical, longitudinal study. It included 80 subjects, patients from the University Plastic and Reconstructive Surgery Clinic; 40 with a malignant lesion in the upper half of the nose, and 40 with a malignant lesion in the lower half of the nose. The nose is divided depending on the differences in the quality and thickness of the skin that propose different

ways of reconstruction in terms of defect localization [10]. Patients were further subdivided into two subgroups of 20 patients, depending on the reconstruction technique used: a full-thickness skin graft technique (Wolfe) and a composite skin graft technique consisting of dermis and subcutaneous adipose tissue. Inclusion criteria were: patients above age 85, a skin malignant lesion in the nose area for which surgery was indicated, patients who had been examined by an anesthesiologist and could be operated, patients who had voluntarily signed an informed consent, patients who were prepared and willing to collaborate-attend and complete all controls and check-ups on the 13th day, on the 3th and 6th month after surgery.

Exclusion criteria included: patients younger than 18 years, skin defect less than 1 cm, patients with proven metastasis, patients with scar on the nose and a perinasal region because of injury or prior surgical intervention, composite skin defects (including cartilage or bone), patients with anesthesia contraindication for surgery.

Prior to the diagnostic approach and the operative treatment, all patients signed an informed consent for participation in this study. The study was carried out in accordance with the provisions of the Helsinki Declaration and the Declaration of Human Rights of the European Union. Initial evaluation of patients included: history, clinical, and anesthesiological examination. After proper preparation, patients were operated on and local anesthetics with i.v. pain relief and sedation was used. A prophylactic oral dose of antibiotic was given for 5 days.

The following parameters were noted:

1. Localization of the malignant lesion in the area of the nose was marked and evaluated in relation to the two regions according to the skin thickness cover - upper portion (a thin, mobile skin) composed of dorsum and lateral walls, and a lower portion (thick adnexal skin) composed of tip and wings of the nose.
2. The excision of the malignant lesion was made in cancer-free skin margins. According to literature, primary basal cell carcinoma should be removed at least 4 mm from the skin cancer border guaranteeing high percentage of cancer-free skin margins that have been histologically confirmed in 80% of cases [6,7]. In most of the spinocellular carcinomas found on the nose, as one of the high-risk sites, according to literature, the excision line should extend to at least 6 mm in width [8]. In-depth SCC should be removed along with subcutaneous tissue, as in 30% of cases these cancers invade this area [8].

For the best possible security in this study, all malignant lesions were removed over at least 6 mm in width from the visible border of the tumor along with the hypodermis, thus guaranteeing security for free edges of malignant alteration in 95% of cases of both types of cancers [7-9,11].

3. The surgeon paid special attention to the reconstruction techniques. All defects were primarily reconstructed with full-thickness skin graft (Wolfe) and composite skin graft consisting of skin and subcutaneous adipose tissue.

Full-thickness grafts are characterized by ease of use and ability to be placed in any region. The condition is to have a vascularized base on which the transplant will be placed. In this study we present results of using a full-thickness retroauricular skin graft.

The stitches were removed the 13th day after surgery and the scar appearance was evaluated with a POSAS scarring scale. Composite skin grafts were treated in the same way, and were used from post-auricular region. Donor site defect in both cases was closed with direct suture.

Statistical analysis

Statistical data processing was performed in SPSS for Windows 23.0 statistical software, with $p < 0.05$ required for statistical significance.

Shapiro-Wilks W test was used to test the normality of the data.

The categorical parameters are shown with frequency distributions and relative numbers, and quantitative variables with average and standard deviation.

Independent parametric and nonparametric tests (Chi-square test, Fisher exact test, Student t- test) were used to compare subjects undergoing full thickness and complex graft transplant.

Results

The study involved 80 subjects, patients from the University Plastic and Reconstructive Surgery Clinic; 40 with a malignant lesion in the upper half of the nose, 40 with a malignant lesion in the lower half of the nose. The patients were further subdivided into two subgroups of 20 patients, depending on the reconstructtion technique used: a full-thickness skin graft technique (Wolfe) and a composite skin graft technique consisting of dermis and subcutaneous adipose tissue.

Table 1 shows gender distribution of patients with a malignant lesion in the upper and lower half of the nose. After the removal of the malignant lesion the skin defect was reconstructed using a full-thickness and composite skin graft reconstruction technique.

In the group of 40 subjects with upper nasal defect, both reconstructive techniques were more commonly used in male patients-80% (16) *versus* 55% [11], while in the group of 40 subjects with lower nasal defect, full-thickness skin graft technique was more commonly used in female patients-65% [13], and composite skin graft was more common in male patients-60% [12].

The statistical analysis showed an insignificant difference according to gender and type of reconstruction technique used full thickness skin graft or composite

Table 1. Gender distribution of patients with a skin defect in the upper and lower half of the nose reconstructed with full thickness skin graft and composite skin graft

Gender	Upper half of the nose			Lower half of the nose		
	Type of reconstruction technique					
	n	wolfe n (%)	Composite graft n (%)	n	Wolfe n (%)	Composite graft n (%)
male	27	16(80)	11(55)	19	7(35)	12(60)
female	13	4(20)	9(45)	21	13(65)	8(40)
p value	X2=2.85 p=0.09			X2=2.51 p=0.11		

p (Chi-square test)

Table 2. Age distribution of patients with a skin defect in the upper and lower half of the nose reconstructed with full thickness skin graft and composite skin graft

Reconstruction technique	Descriptive Statistics (age)					
	Upper half			Lower half		
	n	mean ± SD	min - max	n	mean ± SD	min – max
Wolfe	20	63.60±11.5	29-87	20	64.15±13.7	32-89
Composite graft	20	64.65±12.6	41-87	20	66.10±12.1	30-84
p value	t=0.27 p=0.78			t=0.48 p=0.64		

p (Student t-test)

skin graft (p=0.09, p=0.09, p=0.09, p=0.09)].

Patients with a defect localized in the upper and lower half of the nose, and reconstructive technique used for defect closer did not differ significantly with respect to age (p=0.78, p=0.64, respectively). This is shown in table 2.

The aesthetic effect of both reconstruction techniques was also analyzed in this study, by assessing the scar appearance both by the surgeon and by the patient. For this purpose, a POSAS scar scoring scale was used, consisting of scores from 1 to 10, with score 1 indicating normal skin, and score 10 indicating the worst scarring.

In the group of patients with a malignant lesion in the upper half of the nose, the surgeon significantly differentiated the appearance of the scar in all analyzed time points depending on the reconstruction technique used (p=0.013, p=0.000001, p<0.001 respectively). After removing the stitches, the 13th day after the intervention, the appearance of the scars in patients operated on by full thickness skin graft (Wolfe) technique was rated by the surgeon as significantly better than that in patients operated on with composite skin grafts-at the lowest score 3, the surgeon assigned 90% (18) of patients to a full-thickness skin graft (Wolfe) reconstruction technique and 55% (11) to a composite skin graft technique. By the third month after surgical reconstruction, patients where composite skin graft was used as a reconstruction technique had significantly better scar appearance-at the lowest score 2, and the surgeon assigned one patient to the Wolfe and 55% (11) to the composite graft technique. At the last control, 6 months after the intervention, a significant difference between the two techniques again supported the better outcome of the skin appearance reconstructed with the full-thickness skin graft method - at the lowest score of 4, the surgeon assigned 90% (18) of patients to a full-thickness skin graft and 55% (11) to a composite skin graft reconstruction technique.

In the group of patients with a malignant lesion in the lower half of the nose, the surgeon evaluated the appearance of the scar in patients operated on by full-thickness skin graft (Wolfe) and composite skin graft technique 13 days after the reconstruction (p=0.18), and three and six months after surgical reconstruction (p<.05). By the third and sixth months of surgical reconstruction, patients where composite skin graft technique was used, were evaluated and it was concluded that the appearance of the scar was significantly better. On the third month the lowest score 2 was assigned to 35% (7) of patients operated on by the composite skin graft technique, and to none operated on by the full-thickness skin graft technique. On the sixth month after surgery, the lowest score 3 for the scar appearance was assigned to 50% (10) of patients operated on by the composite graft method and to none operated on by the full-thickness skin graft technique. The aesthetic effect evaluated by the surgeon for both reconstructive techniques in the upper and lowers half of the nose on the 13th day, 3th month, and 6th month after surgery is shown in table 3, and for better preview graphical presentation of the data is shown in Figure 1.

Next evaluation was made by the patients, and they were giving scores according the POSAS scar assessment scale. In the group of patients with a malignant lesion in the upper half of the nose, patients operated on by the full-thickness skin graft (Wolfe) technique, and composite grafts were significantly different in the appearance of the scar only at the first control, when removing the stitches on the 13th day after surgery (p=0.02). The appearance of the scar in patients reconstructed with the split thickness skin graft technique was better; with the lowest score of 3, assigned to 30% (6) of pa-

Table 3. Aesthetic effect evaluated by the **surgeon** for both reconstructive techniques in the upper and lower half of the nose

Observer		Upper half			Lower half		
		Reconstruction technique					
Time	POSAS scar score	n	wolfe n (%)	Composite graft n (%)	n	Wolfe n (%)	Composite graft n (%)
13th day	3	29	18(90)	11(55)	16	9(45)	7(35)
	4	11	2(10)	9(45)	19	9(45)	10(50)
	5				3	0	3(15)
	6				2	2(10)	0
p value	X2=6.14 p=0.013				Fisher p=0.18		
3th month	2	12	1(5)	11(55)	7	0	7(35)
	3	16	16(80)	0	9	9(45)	0
	4	12	3(15)	9(45)	24	11(55)	13(65)
p value	X2=27.33 p=0.000001				Fisher	p<0.001	
6th month	3				10	0	10(50)
	4	29	18(90)	11(55)	12	2(10)	10(50)
	5	9	0	9(45)	14	1(5)	0
	6	2	2(10)	0	8	8(40)	0
	7				9	9(45)	0
p value	Fisher p<0.001				Fisher	p<0.001	

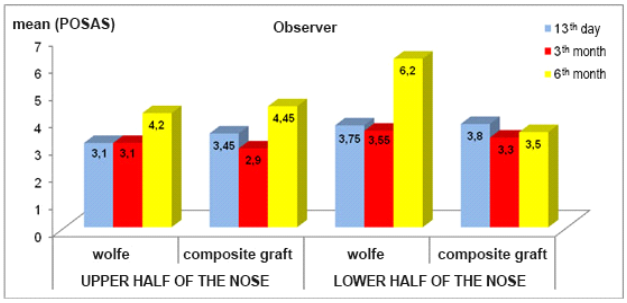

Fig. 1. Aesthetic effect evaluated by the **surgeon** for both reconstructive techniques in the upper and lower half of the nose

tients, and to none of patients reconstructed with composite skin graft technique.

In the group of patients with a malignant lesion in the lower half of the nose, patients operated on by the full-thickness skin graft (Wolfe) technique, and composite grafts were significantly different in the appearance of the scar only at the last control, after 6 months of intervention ($p<0.001$), as a result of significantly better appearance of the scar in patients where composite skin graft technique was used; with the lowest score of 3, assigned to all patients operated on by the composite skin graft technique and to none by the full-thickness skin graft (Wolfe) technique. The aesthetic effect evaluated by the patients for both reconstructive techniques in the upper and lower half of the nose on the 13th day, 3th month, and 6th month after surgery is shown in table 4, and for better preview graphical presentation of the data is shown in Figure 2.

Table 4. Aesthetic effect evaluated by the **patient** for both reconstructive techniques in the upper and lower half of the nose

Patient			Upper half			Lower half	
				Reconstruction technique			
Time	POSAS scar score	n	Wolfe n (%)	Composite graft n (%)	N	wolfe n (%)	Composite graft n (%)
	4	6	6 (30)	0			
13th day	5	34	14 (70)	20 (100)	35	18 (90)	17 (85)
	8				5	2 (10)	3 (15)
p value	Fisher p=0.02				Fisher p=1.0		
	2	39	19 (95)	20 (100)	34	17 (85)	17 (85)
3th month	3	1	1 (5)	0	3	3 (15)	0
	4				3	0	3 (15)
p value	Fisher p=1.0				Fisher p=0.054		
	3	1	1 (5)	0	20	0	20 (100)
6th month	4	38	18 (90)	20 (100)			
	5	1	1 (5)	0	2	2 (10)	0
	6				18	18 (90)	0
p value	Fisher p=0.487				Fisher	p<0.001	

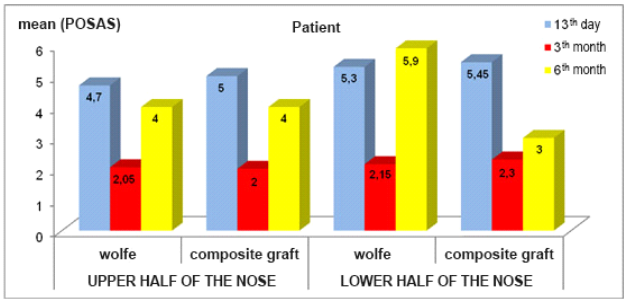

Fig. 2. Aesthetic effect evaluated by the **patient** for both reconstructive techniques in the upper and lower half of the nose

Discussion

Surgical treatment of NMSC is the gold standard for treating these types of malignant lesions. With its position in the facial region, the nose is the most anatomically exposed part and as such has a great signifycance. Any deformation of the nose disturbs the harmony of the face. Disruption of the normal anatomy, on the other hand, can cause nasal obstruction, inability to breathe normally, and can affect the sense of smell, too. Each skin defect in the nasal area should be individually assessed in order to determine and apply the best reconstruction technique from a functional and aesthetic point of view. For these reasons, repairing the nasal skin defects is a challenge for every surgeon. Analyzing the results of the aesthetic appearance of the scar in relation to the POSAS Observer and Patient Scale, it can be concluded that in the group of patients with a malignant lesion in the upper half of the nose, the appearance of the scar was significantly different depending on the reconstruction technique used. After removing the stitches, the 13th day after the surgical reconstruction of the upper half of the nose, the appearance of scars in patients undergoing full-thickness skin graft technique was rated by the surgeon to be significantly better than the scar appearance in patients undergoing composite skin graft reconstruction technique. By the third month after surgery, the surgeon graded as significantly better appearance of the scar reconstructed with composite skin graft technique than with full-thickness skin graft. At the last check-up, 6 months after surgery, again a significant difference between the two techniques was found, and full-thickness skin graft technique was found to be a better option. In the group of patients with a malignant lesion in the lower half of the nose, the surgeon evaluated the appearance of the scar as not significantly different in patients undergoing full-thickness skin graft and composite skin graft technique reconstruction 13 days after surgery. At the third and sixth month after surgery, in patients with malignant lesion in the lower half of the nose, the surgeon graded as significantly better appearance of the scar reconstructed with the composite skin graft technique than with full-thickness skin graft.

In the group of patients with a malignant lesion in the upper half of the nose, patients evaluated the appearance of the scar as significantly different only at the first control, when removing the stitches-first check up (p= 0.02). Patients rated the scar as better when full-thickness skin graft was used as a reconstruction technique.
In the group of patients with a malignant lesion in the lower half of the nose, patients evaluated the appearance of the scar as significantly different only at the last control, six months after surgical reconstruction. Patients rated the scar as better when composite skin graft technique was used.

Conclusion

In conclusion, the evaluation of the surgeon himself on one hand and patients on the other hand, using the POSAS scale, in terms of the aesthetic appearance of the scar following effective reconstructive technique and the technique of choice showed the full-thickness skin graft for the upper half skin defects of the nose to present better result. Composite skin grafts on the other hand are shown to be a dominant technique for closing the defects in the lower half of the nose. The use of composite skin graft technique has been neglected in recent years and is rarely used [12]. Studies of the use of this graft have been published in the last 20 years, but there is still no single common opinion regarding its use. Composite skin grafting as such has been treated for many years as very contradictory. It has been hypothesized that the underlying adipose tissue stands as a barrier to vascularization of the graft itself. It is for this reason that the composite skin graft was used as a reconstruction technique in this study. The results showed no comparable dominance over the full thickness skin graft according to the aesthetic effect, especially after six months of surgery, hence imposing and recommending its use for skin defects reconstruction in the lower half of the nose.

Conflict of interests. Not declared.

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Мак Мед Прегled 2019; 73(2): 101-105

ММР

Original article

A NOVEL METHOD OF TREATING OVARIAN INFERTILITY: IS PLATELET-RICH PLASMA A NEW PROMISING THERAPY IN THE FUTURE?

НОВ МЕТОД ВО ЛЕКУВАЊЕ НА ОВАРИЈАЛЕН ИНФЕРТИЛИТЕТ: ДАЛИ ПЛАЗМА БОГАТА СО ТРОМБОЦИТИ Е ТЕРАПИЈА ШТО ВЕТУВА?

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Abstract

Introduction. In today's modern society, the treatment of patients with poor ovarian reserve presents a medical challenge of increased clinical importance. The use of platelet-rich plasma (PRP) is a new hope that improves pregnancy chances. Increased use of the PRP in a number of *in vitro* centers around the world as well as publication of thefirst experience in *in vitro* fertilization entailed the need for this systematic review.
Methods. PubMed, Cochrane and Ovid Medline were searched between 2000 and 2019 under the following strategy: [<PRP or plasma-rich with platelets> and <ovaries with reduced reserves or function> and <ovarian rejuvenation>]. Fourteen original articles published in medical scientific journals were analyzed in this study. The evidence level and quality assessment were made based on the most up-to-date, reliable, scientific evidence as well as from the number of additional relevant citations.
Results. Taking the current available proof and evidence into consideration, we can conclude that the PRP method improves the ovarian function and increases the chances of clinical pregnancy. In addition, we assume that, over time, the PRP method objectively improves the ovarian reserves. Recent studies support the theory of increasing the number of preantral follicles, followed byappropriate growth and reduction of follicular atresia.
Conclusion. The improvement of the quantity and quality of oocytes with the intra-ovarian application of PRP potentially suggests a new concept of ovarian aging, where the ovarian microenvironment plays a crucial role.

Keywords: ovarian rejuvenation, poor ovarian reserve, platelet-rich plasma

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Абстракт

Вовед. Во денешното современо општество третманот на пациентки со намалени оваријални резерви преставува медицински предизвик со зголемено клиничко значење. Употребата на плазма збогатена со тромбоцити (PRP) е нова надеж со кој се подобруваат шансите за клиничка бременост. Зголемената употреба на PRP во поголем број на ИВФ центри, ширум светот, како и објавувањето на првоте искуства во инвитро фертилизација ја наметнува потребата од овој преглед.
Методи. PubMed, Cochrane и Ovid Medline беа пребарувани помеѓу 2000 и 2019 година според следната стратегија: [<PRP или плазма збогатена со тромбоцити> и <јајниците со намалени резерви или функцијата> и <оваријално подмладување>]. Во студијата вклучени се 14 оргинални статии објавени во медицински научни списанија. Нивото на докази и проценката на квалитетот беа направени врз основа на најсовремени, веродостојни, научни докази, како и од бројот на дополнителни релевантни цитати.
Резултати. Досегашните достапни докази покажуваат дека PRP ја подобрува функцијата на овариумите и ги зголемува шансите за клиничка бременост. Со текот на времето, исто така, се чини дека објективно ги подобрува оваријалните резерви. Неодамнешните студии ја подржуваат теоријата за зголемување на бројот на преантрални фоликули, пратено со соодветен раст и намалување на фоликуларната атрезија.
Заклучок. Подобрувањето на квантитетот и квалитетот на ооцитите преку интраоваријална апликација на PRP, потенцијално сугерира нов концепт на стареење на јајниците, каде оваријалната микросредина има значајна улога.

Клучни зборови: оваријална рејувенација, намалени оваријални резерви, плазма збогатена со тромбоцити

Introduction

Ovarian rejuvenation thorough history

Oogenesis, the production, and development of the oocyte, is the principal role of the ovarian tissue. It was believed for a long time that women are born with certain reproductive potential which is in direct correlation withthe number of primordial follicles produced during the time of embryological and fetal period and which progressively decrease to reach a number circa 300 000 primordial follicles until the time of menarche [1]. These claims were altered by Tilly *et al.* by demonstrating the existence of germ cells in the ovarian tissue [2]. He examined new concepts for how oocytes and their precursor cells might be altered metabolically to sustain or increase ovarian function and fertility in women [2]. This has led to the development of different methods and techniques through which the scientific circles are making endeavors to find the corresponding therapeutic method for patients with decreased ovarian reserves.

The first research presented the use of dehydroepiandrosterone (DHEA) as a food supplement that can have therapeutic benefits in treatment ofpatients with decreased ovarian reserves [3]. The success of DHEA has beenclinically proven in many studies and it remains one of the first noninvasive medical treatments for ovarian rejuvenation. The use of precursors of testosterone can improve the ovarian microenvironment. DHEA reaches a maximum in people aged between 20 to 30 years and decreases approximately 2% per year [4]. A similar concept brings the use of coenzyme Q 10. The role of this molecule is not conclusive in the improvement of the mitochondrial function caused by ageing of the tissue. The androgen supplements can also have positive effect on mitochondrial function [5]. Doctorsin multiple IVF centers in the USA recommend the transvaginal trauma of ovarian tissue, i.e. piercing, as a method for ovarian rejuvenation. This method is accompanied by changes in hormonal status, local immunological response and increased vascularization in the ovarian tissue. The trauma initiates growth factor production, which promotes tissue regeneration [6]. Furthermore, Bukovsky recommended novel methods of treating premature ovarian failure and ovarian infertility. In hisstudy he has concluded that the follicular renewal is also dependent on the support of circulating blood mononuclear cells. The circulating mononuclear cells as a part of the immune system regulate the function of almost all tissues in the body, which leads to temporary rejuvenation of the endocrine and immune system in the ovarian tissue. Namely, the immune system plays a crucial role in the modulation of ovarian function, as it regulates ovarian development, follicular maturation, ovulation and formation of the corpus luteum [7, 8].

In recent years a new approach has emerged in the treatment of ovarian infertility based on the use of plasma rich with platelets (PRP) with or without stem cells as a method for ovarian rejuvenation and follicular reactivation [9,10].

Material and methods

We conducted a systematic search of PubMed, Cochrane and Ovid Medline databases from 2000 to 2019 using the following keywords: platelet-rich plasma, ovarian reserve or diminished ovarian reserve, ovarian function or diminished ovarian function, poor responders, reproductive age, IVF. This search yielded 110 studies, of which 14 original articles and reviews were included. All publications were reviewed by the authors of this manuscript, who agreed onthe analysis and interpretation of data. Some of the studies have addressed the question of whether these approaches of transvaginal intraovarian application of PRP may be beneficial in poor responders with respect of clinical pregnancy and live birth rates in patients. Studies were eligible if they met one of the following criteria: primary evidence (clinical trials) that assessed the effectiveness of a procedure correlated with an outcome measurement (pregnancy, IVF, live birth rates); meta-analyses; and relevant articles from bibliographies of identified articles. The level of evidence was evaluated using the grading system available online and wasassigned for each reference in the bibliography. The quality of evidence was evaluated using the grading system, adapted from Johns Hopkins Nursing Evidence-based Practice Grading System.

Results

The first description of PRP was introduced as a MeSH (Medical Subject Headings) term in 2007 as: “a preparation consisting of concentrated platelets in a limited plasma volume. It is used in various regeneration procedures of surgical tissues, where growth factors from platelets can affect the speeding up of healing and regeneration of the wounds” [11]. The growth factors (GFs) contained in platelet alpha granules are a major part of the PRP. They induce, through appropriate transmembrane receptors in target cells, a whole range of intracellular processes leading to proliferation, differentiation, matrix formation, osteoid production, collagen synthesis, haemostasis, and everything that leads to tissue recovery and regeneration. It is noted that the mitogen effects of PRP are only limited to augmentation of the normal healing process and is theoretically not mutagenic, as the GFs released do not enter the cell or its nucleus, but only bind to the membrane receptors and induce signal transduction mechanisms (Table 1) [12].

Table 1. Platelet growth factors and their specific characteristics

Platelet Growth Factor Type	Growth factor Source	Biological Actions
Platelet derived growth factor (a-b)	Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells	Mitogenic for mesenchymal celss and osteoblasts, stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells, regulates collagenase secretion and collagen synthesis, stimulate macrophage and neutrophil chemotaxis
Transforming growth factor TGF(alpha –beta)	Platelets, extracellular matrix of bone, cartilage matrix, activated TH1 cells and natural killer cells, macrophages/monocytes and neutrophils	Stimulates undifferented mesenchymal cell proliferation ; regulates endothelial, fibroblastic and osteoblastic mitogenesis ; regulates collagen synthesis and collagenase secretion, regulates mitogenic effects of growth factors, stimulate endothelial chemotaxix and angiogenesis, inhibits macrophage and lymphocyte proliferation
Vascular endothelial growth factor, VEGF	Platelets, endothelial cells	Increases angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells
Epidermal growth factor, EGF	Platelets, macrophages, monocytes	Stimulates endothelial chemotaxis / angiogenesis, regulates collagenase secretion, stimulates epithelial /mesenchymal mitogenesis
Fibroblast growth factor, FGF	Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts	Promotes growth and differentiation of chondrocytes and osteoblasts, mitogenic for mesenchymal cells, chondrocytes and osteoblasts
Connective tissue growth factor CTGF	Platelets through endocytosis from extracellular environment in bone marrow	Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion
Insulin like growth factor – 1 IGF -1	Plasma, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells, osteoblasts, bone matrix	Chemotaxis for fibroblasts and stimulates protein synthesis. enchances bone formation by proliferation and differentiation of osteoblasts

(Principles and Methods of Preparation of Platelet-Rich Plasma: a Review and Authors Perspective: 2014. Journal of Cutaneous and Aesthetic Surgery – Oct-Dec 2014, Volume 7, Issue 4)

In recent years the effect of autologous platelet-rich plasma has beenincreasingly used in the treatment of any injuries of the soft and connecting tissues as well as the bone grafts. The use of PRP is a significant part of the speed-up healing process of the injured tissue, angiogenesis and tissue remodeling - with this, the use of PRP has become a routine treatment in orthopedics, dermatology, and specific autoimmune diseases [13, 14].
Many clinicans have noticed that the use of PRP improves the function of the target organ, which resulted in an enthusiastic use of PRP in patients with ovarian insufficiency. Studies have shown that many innovative techniques find their way from theory to practice. Namely, the use of PRP in restoring both the reproductive and endocrine functions of the ovary was presentedin a study from 2017 by Ljubic. He described the first case of ahuman embryo obtained after autologous ovarian *in vitro* activation with orthotopic retransplantation [15]. The novel application of PRP in the ovarian cortex was pioneered by Pantos [9]. The idea of potential therapeutic use of autologous PRP in the renewal of egg cells and follicular reactivation was published in 2017, via the first abstract and detailed oral report on PRP as a method of renewal of egg cells atthe ESHREmedical conference.
During the following year, 2018, an increase in oocytes and embryo quality was also confirmed inScott's study [10]. This pilot study was focused on the intra-ovarian injection of the autologous plasma enriched with thrombocytes and the effect of PRP on the ovarian microenvironment and the creation of oocytes of decent/high quality. Melo's study has confirmed the efficacy of using an intracortical ovarian injection of PRP that significantly improves ovarian reserve and subsequent reproductive outcome in infertile women [16]. The effects of autologous PRP were observed in the treatment of repeated implantation failure in IVF cycles in order to improve pregnancy outcome [17]. It is considered that one of the mechanisms bywhich PRP results in changes in the ovarian reserves and the activation of the primary preantral follicles is through the synergy connection of the factors for growth contained in the PRP with the usage of gonadotropins for ovarian stimulation.
In women considered to be poor ovarian responders, there is insufficient evidence forrecommending or rejecting intraovarian injection of autologous PRP before IVF treatment.

Discussion

It remains essential to understand the physiological basis of ovarian aging to interpret the mechanisms of action. With the use of platelet-derived growth factors (PDGFs), dysfunctional ovarian tissue is believed to be supplied with essential factors necessary for ovarian regeneration. In this context, it is necessary to mention angiogenesis and follicular vascularization and their important role in the aging of the follicles. Receptors for growth factors are present on granulose cells confirming their association with the activation process of the primordial follicles. The most important component in PRP is the transforming growth factor-beta family (TGF beta) that plays a significant role during the developmental phases of the follicle [18]. Confirmation of all the above statements is also obtained from the

Hosseini study [19]. This study evaluated the effects of platelet-rich plasma (PRP) on the growth and survival of isolated early human follicles in a three-dimensional culture system. The conclusion was that media supplementation with PRP could better support the viability and growth of isolated human preantral follicles *in vitro*.
On the other hand, the presence of OSCs on the surface of the ovarian tissue, under certain conditions, can produce *de novo* primordial follicles and thus the appearance of new antral follicles. It is noteworthy to mention that only a fraction of OSCs undergoes meiosis culture to form oocytes. It remains unknown why only a few cells express Stra8 and undergo differentiation [20]. Alternatively, it is possible that OSC aging is a result of impaired DNA double-strand break repair, a recently identified cause of aging in mammalian oocytes. Elucidating the mechanisms that cause OSCs to age could lead to new treatments that could delay ovarian aging and slow infertility. Also, several questions about the PRP's mechanism of action remain unanswered.

PRP activation and intraovarian application

According to the results in the literature different protocols which describe the optimal conditions needed for preparation of autologous PRP are detailed. The relative centrifugal force, time temperature, the use of anticoagulants and the method of activation of the PRP can influence the preparation of the autologous PRP [13, 14]. The process of the activation of α granules of the platelets is one of the key steps that could influence the availability of the released biomolecules and consequently the quality of the PRP [21]. Thrombin and/or calcium chloride is used as the most frequent activator before the administration of PRP in the damaged tissue [22]. The secretion of the active biomolecules begins 10 minutes after the activation of the platelets, and 95% of the growth factors are released in a period of 1 hour. Through the method of the activetion, the amount and the kind of the biomolecules is determined, which directly influences the tissue healing. The PRP activation strategy is determined by the type of procedure (open or laparoscopic), and the desired biological effect that is expected from the biological tissue [23].
PRP for the treatment of ovarian infertility is a lower concentration (2.5x3 times) system. The process is carried out under strict aseptic conditions as well as optimum temperature regulations i.e. 21-24^{o}C. PRP is prepared according to the manufacturer's guidelines. In the last step, the volume immediately above the erythrocyte layer is collected. Calcium gluconate is used as an activator. After activation, in a period less than 2 min, approximately 3-5 ml of the PRP isinjected into the ovaries under transvaginal ultrasound guidance. Intervention is made following a protocol set by the IVF department. The entireintervention lasts 15 to 20 minutes. The method of obtaining PRP is simple, minimally invasive and low cost. High concentration of growth factors and cytokines from PRP in the damaged tissue leads to balance between the anabolic and catabolic processes, optimizing tissue environment and favoring the process of tissue regeneration.

Predicting the effectiveness of intraovarian PRP application

FSH, estradiol and AMH levels are predictable of treatment outcomes after PRP utilization. The treatment can lead to increasing the preantral follicles and in general, may enhance ovarian function by increasing follicles recruitment.

Limitations

Even though PRP has a widespread usage in multiple medical spheres, unfortunately, there is still a lack of controlled clinical studies regarding the process. The limitation of this format should be taken into account i.e. sample size, RCT, design, etc.
The main limitation of this study was the absence of previous data attesting the safety of PRP injection into human ovaries.Future larger trials would be required to corroborate the efficacy of PRP injection for treatment ofovarian infertility, which would confirm the findings of this study.

Conclusion

The authors reviewed the best available evidence for the usage of PRP in actual medical practice. Because the diagnosis of patients with lower ovarian reserves often leaves limited time for treating, the patients need to be given a choice. There is fair evidence that the clinical and live birth rates are substantially different after the intraovarian application of PRP before IVF treatment. Further research is required to investigate whether decreasing the level of FSH and increasing the number of antral follicles following intraovarian PRP injection is sustained.
Future studies need to evaluate whether this approach may be especially beneficial in a specific subset of patients.

Conflict of interests. Not declared.

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Мак Мед Прегled 2019; 73(2): 106-111

Original article

PROGNOSTIC VALUE OF MATRIX METALLOPROTEINASE-2 (MMP-2) AND -9 (MMP-9) IN PATIENTS WITH COLORECTAL CANCER

ПРОГНОСТИЧКА ВРЕДНОСТ НА МАТРИКС МЕТАЛОПРОТЕИНАЗА-2 (ММП-2) И -9 (ММП-9) КАЈ ПАЦИЕНТИ СО КОЛОРЕКТАЛЕН КАРЦИНОМ

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Abstract

Introduction. Finding prognostic markers to better identify patients with higher risk for poor survival would be valuable in order to customise pre- and postoperative treatment as well as to enable closer follow-up ofthe cancer patients. Matrix metalloproteinases are produced by tumour cells, hence, they may be associated with tumourprogression including invasion, migration, angiogenesisand metastasis.

Aim of our study was to examine MMP-2 and MMP-9 serum levels and correlated them with pathological data such as stage of the colorectal cancer (CRC) and patients outcome.

Methods. The investigation has been made on 82 patients with operable CRC without distant metastases, who had underwent blood tests in order to determine the MMP-2 and MMP-9 serum levels in the following points of time: preoperatively, 3, 6, 9 and 12 months postoperatively.

Results. The values of the investigated MMPs decrease postoperatively and start to increase 6 months later in CRC patients of all stages, reaching the highest value 12 months postoperatively with statistically important differences of MMP-2 and MMP-9 serum levels in terms of disease staging and defined points of time. Analysis of the results showed that MMP-2 serum levels preoperatively, at 3th and 12th month postoperatively, as well as MMP-9 preoperatively and at 3th, 9th and 12th month postoperatively are in a significant correlation with the poor outcome of the CRC patients. According Kaplan-Meier survival curve, 2,65% of the followed CRC patients survive more than 60 months; 52% of the CRC patients in Stadium II survive more than 48 or 60 months and 28% of patients in Stadium III survive more than 55 or 60 months.

Conclusion. The MMP-2 and especially MMP-9 serum values are important indicators for diagnosis of the CRC patients and for monitoring of their disease progression.

Key words: colorectal cancer, matrix metalloproteinases, staging, prognosis.

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Апстракт

Вовед. Наоѓањето прогностички маркери за подобра идентификација на пациентите со повисок ризик за полошо преживување може да биде драгоцено во односна одредување односно прилагодување на пред- и постоперативниот третман како и овозможување на подобро следење на пациентите заболени од карцином. Матрикс металопротеиназите се произведени од туморските клетки, па оттука, тие можат да бидат поврзани со туморската прогресија вклучувајќи инвазија, миграција, ангиогенеза и метастазирање. Целта на нашето истражување беше да се испитаат серумските нивоа на ММР-2 и ММР-9 и истите да секорелираат со патолошките податоци како што стадиумот и исходотна пациентите со колоректалниот карцином (КРК).

Методи. Истржувањето беше направено на 82 пациенти со операбилен КРК без далечни метастази, на кои им беа земени крвни примероци со цел да се утврдат серумските нивоа на ММП-2 и ММП-9 во следните временски точки: предоперативно, 3, 6, 9 и 12 месеци постоперативно.

Резултати. Вредностите на испитуваните ММПбеа намалени постоперативно и почнаа да се зголемуваат 6 месеци подоцна кај пациентите од сите стадиуми со КРК, достигнувајќи највисоки вредности 12 месеци постоперативно со статистички значајни разлики во серумските нивоа на ММР-2 и ММР-9 и во однос на стадиумот на болеста и на дефинираните временски точки. Анализата на резултатите покажа дека серумските нивоа на ММП-2 добиени предоперативно, во 3-ти и 12-ти месец постоперативно, како и ММР-9 предоперативно и во 3-ти, 9-ти и 12-ти месец постоперативно се во значајна ко-

релација со лошиот исход на пациентите со КРК. Според Kaplan-Meier кривата на преживување, 2,65% од следените пациенти со КРК преживеале повеќе од 60 месеци, 52% од пациентите во Стадиум II преживеале повеќе од 48 до 60 месеци и 28% од следените пациенти во Стадиум III преживеале повеќе од 55 до 60 месеци.
Заклучок. Серумските вредности на ММП-2, а особено на ММП-9 се важни индикатори за дијагностицирање на КРК и за следење на прогресијата на болеста.

Клучни зборови: колоректален карцином, матрикс металопротеинази, стадиум, прогноза

Introduction

In all tissues, the extracellular matrix (ECM) provides a structural and biochemical framework for cell supporting and scaffolding, with a range of functions important for regulating both inter- and intra-cellular signaling, and for cellular differentiation, adhesion and invasion. Cancer cells interact with the ECM, and structural remodeling of ECM is important for cell migration from a primary tumor site. Proteins comprising the ECM play critical roles in cell proliferation and migration, and different proteases control ECM remodeling and degradation. One specific group of proteolytic enzymes, matrix metalloproteinases (MMPs), was studied extensively as key mediators of ECM degradation and in the processing of other bioactive molecules [1].
In a variety of different cancers, increased MMP expression and activation generally promote hallmarks of tumor progression including angiogenesis, invasion and metastasis, and correlate with shortened survival. Nonetheless, more recently, some MMPs were shown to have tumor protective effects [2].
MMPs comprise a large family of at least 25 zinc-dependent endopeptidases capable of degrading all components of the ECM and are categorized primarily by their structural features as gelatinases, collagenases, membrane-type, stromelysins and matrilysins. MMPs have a common domain structure including a pro-peptide, a catalytic domain, a hemopexin-like C terminal domain, and a hinge region that links the catalytic site with the hemopexin domain [3]. They are synthesized as secreted or membrane-associated inactive zymogens, and must be proteolytically processed to an active state. This processing involves removal of a cysteine residue that interacts with zinc ions from the active site, thereby resulting in MMP activation.
Among the MMPs, matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9), as members of gelatinase sub-family of MMPs, play important roles in the migration of malignant cells. Due to their ability to degrade type IV collagen and gelatin, they also share proteolytic activity against other extracellular matrix molecules [4,5]. The mechanisms of activation of these enzymes are different. MMP-9 modulates permeability of the vascular endothelium, whereas MMP-2 promotes cleavage of extracellular matrix proteins and is intensively expressed by tumour and stromal components of cancer [6].
Increased levels of matrix metalloproteinase in tumour tissues or in blood circulation have been found to correlate with many cancers, including colorectal cancer (CRC). Several previous studies have shown that MMPs may play an important role as an indicator for apperance of CRC and its progression [7,8].
Colorectal cancer (CRC) is a common disease and it is one of the leading causes of cancer related deaths in developed countries [9]. Despite improvements in surgical techniques, adjuvant and neo-adjuvant chemotherapy, the 5-year survival rate in patients with CRC ranges from 5-90% with tumour progression (stage I: 90-95%, II: 75-85%, III: 50-60% and IV: 0-10%). The prognosis in patients without distant metastasis varies from 50-95% depending on the tumour stage [10].
It is widely recognised that prognostic information based on clinical and histopathological investigation is insufficient, although tumour stage and lymph node involvement are the main prognostic tools in evaluating CRC patients specific survival. Finding prognostic markers to better identify patients with higher risk for poor survival [11,12] would be valuable in order to customise pre - and postoperative treatment as well as to enable closer follow-up of these patients.
In our study, we examined MMP-2 and MMP-9 serum levels and correlated them with pathological data such as stage of the disease and the patients'outcome.

Material and methods

The study included a total of 82 previously untreated CRC patients, 30(36.58%) females and 52(63.41) males (aged 43 to 75 years, mean age of 67.85; SD±9.67) with operable CRC, without detectable distant metastases, who respected the medical instructions and were available for follow-up. All patients underwent a surgical resection of the primary neoplasm at the University Clinic for Abdominal Surgery in Skopje in the period of 2 years (2007-2009).
Blood samples from all patients were drawn before surgical treatment, as well as 3, 6, 9, and 12 month postoperatively in order to examine MMP-2 and MMP-9 serum levels. None of the CRC patients had received chemotherapy before blood sample collection. To standardize clotting conditions, all sera were separated within 1 h after blood collection, aliquoted and stored at -80°C until assayed.
Serum levels of MMP-2 and MMP-9 were determined using an quantitative solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems,

USA) according to the manufacturer's instructions. MMP-2 and MMP-9 technique can detect both pro- and active forms of recombinant human MMP-2 and MMP-9. High concentrations of MMP-2, and MMP-9 were diluted with calibrator, to produce samples with values within the dynamic range of the assay.
The surgically removed specimens were histopathologically analyzed at the Institute of Pathology of the Faculty of Medicine, Skopje, where the pathological stage was defined for every patient according to the International Union Against Cancer (UICC-pTNM) and American Joint Committee on Cancer (AJCC) 2010.
Forty-three patients with stage II B and III (A,B,C) received adjuvant chemotherapy at the Institute for Radiotherapy and Oncology in Skopje.
Correlations were made between the MMPs serum levels and the pathological parameters.

Statistical analysis

Descriptive statistics (mean) are given according to normality of the distribution. Normality of the distribution was determined by Kolmogorow-Smirnov's test. Analysis of variance with Kruskall-Wallis test was first used in the analysis of different sample types. In the case of significant results, the analyses were continued by pairing the variables and analyzing them with Mann-Whitney's U-test. Fisher's exact probability test and Pearson's Chi-Square test (r) were used for testing the association (linearity of the correlation of serum concentrations) between MMPs and major prognostic variables in CRC, such as grade and stage. P-values less than 0.05 ($p<0.05$) were considered as statistically significant. Survival curves were generated by the Kaplan-Meier method, and the log rank test was utilised to compare survival curves. A value of $p< 0.05$ was considered statistically significant. 95% confidence interval (95%CI) was calculated for variables including gender, age, and delay in diagnosis, tumour site and stage.

Results

There have been 17(20.73%) patients in stage I of the disease, 40(48,78%) patients in stage II and 25(30,48%) patients in stage III. Lymph node metastases were substantiated in 25(30,48%) patients and were not find in 57(69,51%) patients with different pT category (Table 1).

Table 1. Staging of the disease in CRC patients according to AJCC

Stage	pTNM	Number of patients (n=82)	%
I	pT1 N0 M0	8	20.73
	pT2 N0 M0	9	
II	pT3 N0 M0	22	48.78
	pT4a N0 M0	18	
III	pT3 N1b M0	7	30.48
	pT3 N2a M0	9	
	pT4a N1b M0	4	
	pT4a N2b M0	5	

Table 2. Average serum levels of MMP-2 and MMP-9 (ng/mL) in terms of disease staging and defined points of time

	MMP-2 (ng/mL)			MMP-9 (ng/mL)		
Stadium	**I**	**II**	**III**	**I**	**II**	**III**
Preoperatively	117.62	147.96	169.72	259,03	313.35	384.34
3months postoperat.	104.85	137.5	154.38	234	249.31	307.54
6 months postoperat.	117.33	162.45	231.9	298.63	358.143	576.86
9 months postoperat.	126.99	186	252	329.43	429.83	717.11
12 months postoperat.	140.73	223.34	271.51	341.11	521.65	846.45

The majority of patients were with pT3N0M0 (26.82%), i.e. patients in stage II A of the disease, and the smallest number of patients were with pT4aN1M0 (4,87%), i.e. patients in stage III B of the disease.
The mean MMP-2 and MMP-9 serum levels in terms of disease staging and defined points of time are shown in Table 2 and Figure 1.
The mean value of MMP-2 serum levels shown in Table 2 and Figure 1, in patients with stage I of the disease, decreased after the operation and started slightly to increase after the 3th month postoperatively.
This might be due to the 6 patients with poor outcome

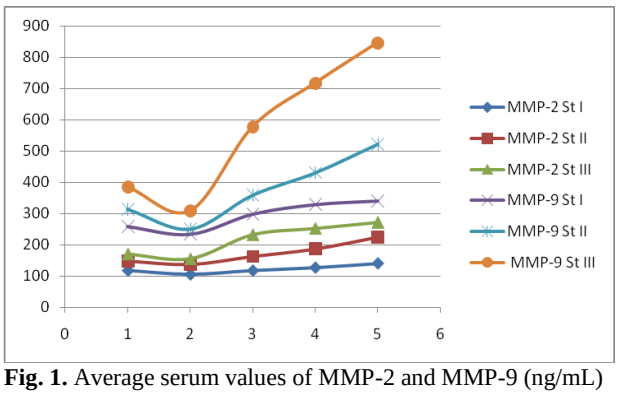

Fig. 1. Average serum values of MMP-2 and MMP-9 (ng/mL) in terms of disease staging and defined points of time

in this group, with mean survival time of 28,5 months.
The curve of the patients in stage II of the disease goes very similarly to the curve of patients in stage I and reaches the peak in the 12th month postoperatively; that may be due to the 19 out of 40 patients with poor outcome, with mean survival of 26,66 months.
The mean levels of MMP-2 in patients in stage III of the disease, decreased after tumour resection, increased abruptly after the 3th month postoperatively and continued to increase slightly after the 6th month postoperatively. This might be due to the poor outcome of more than half of the patients, 18 out of 25 patients with mean survival time of 21,8 months.
There was a significant difference between the mean MMP-2 serum levels before tumour resection and after the operation, i.e. between the preoperative and postoperative levels during defined control points of time in all encompassed CRC stages. There was a significant difference of the MMP-2 serum levels among the stages ($p<0,05$).
There were significant differences between MMP-9 serum levels in all stages ($p<0,01$), as well as between preoperative and postoperative serum levels in all defined points of time.
The number of patients who received chemotherapy and the outcome of all included patients in the study are shown in Table 3.

Table 3. Patients with different stage of the disease who received chemotherapy and the outcome of the disease

Stadium N=82	With chemotherapy	%	Without chemotherapy	%	Poor outcome	%
Stadium I	/	/	17	20.73	6	7.31
Stadium IIA	/	/	22	26.82	8	9.75
Stadium IIB	18	21.95	/	/	11	13.41
Stadium III B	20	24.39	/	/	15	18.29
Stadium III C	5	6.09	/	/	3	3.65
Total	43	52.43	39	47.56	43	52.43

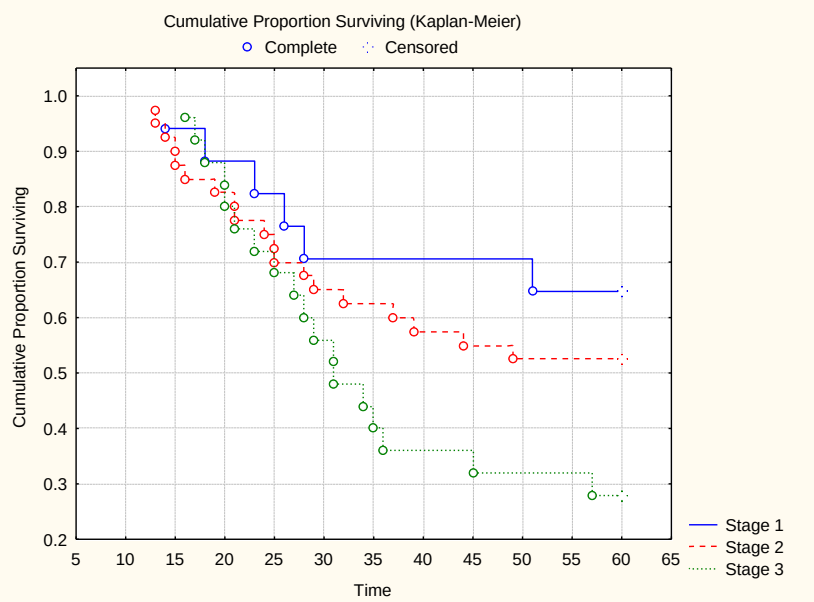

Fig. 2. Kaplan-Meier survival curves for colorectal cancer patients in Stadium I, II and III

According Kaplan-Meier survival curve shown in Figure 2, 65% of the followed CRC patients survive more than 60 months; 52% of the CRC patients in Stadium II survive more than 48 or 60 months and 28% of patients in Stadium III survive more than 55 or 60 months.
We found significant differences in terms of the poor outcome in the CRC patients between stage I and stage II B ($p<0,05$), between stage I and stage III ($p<0,01$) (Figure 3. Log-Rank Test: WW=-5.041; Sum=22.863; Var=5.6428; Test statistic=-2.12226; p=.03382), as well as between stage II A and stage III ($p<0,01$). Associations of the examined parameters and poor outcome are shown in Table 4, where it is shown that MMP-2 serum levels preoperatively, at 3th and 12th month postoperatively, as well as MMP-9 preoperatively and at 3th, 9th and 12th month postoperatively are in a significant correlation with the lethal outcome of the CRC patients.

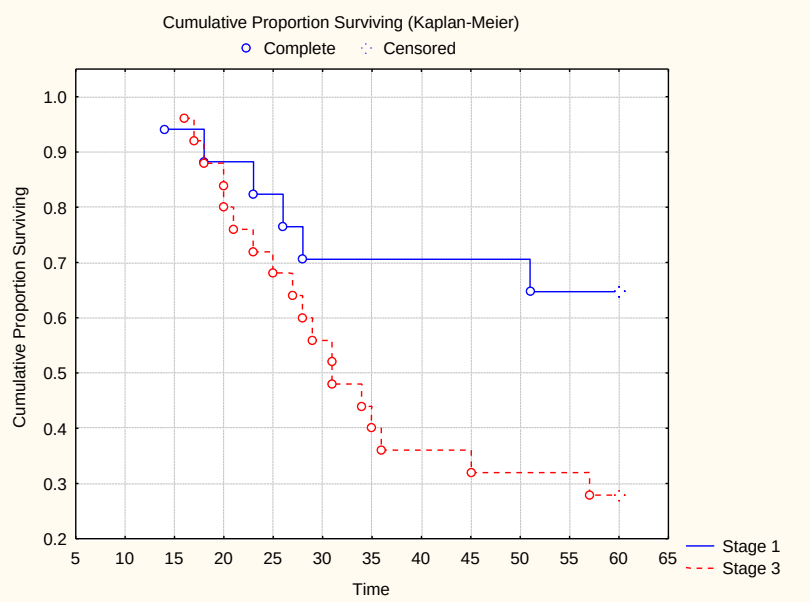

Fig. 3. Kaplan-Meier survival curves for colorectal cancer patients in Stadium I and III

Table 4. Correlations between analyzed parameters and outcome in CRC patients

Parameter	p	R
Stadium	**<0,001**	0,635
pT	<0,005	0,331
pN	<0,005	0,618
MMP-2 preoperatively	**<0,001**	0,156
MMP-2 3 months postoperat.	<0,005	0,793
MMP-2 6 months postoperat.	NS	/
MMP-2 9 months postoperat	NS	/
MMP-2 12 months postoperat	**<0,001**	0,548
MMP-9 preoperatively	**<0,001**	0,619
MMP-9 3 months postoperat	**<0,001**	0,351
MMP-9 6 months postoperat	NS	/
MMP-9 9 months postoperat	**<0,001**	0,219
MMP-9 12 months postoperat	**<0,001**	0,416

Discussion

Several studies confirmed that high preoperative serum or plasma MMP-2, MMP-9 and mainly TIMP-1 antigen levels are strong predictive factors for poor prognosis in patients with CRC [13-17].

The potential tumor marker role of MMPs and TIMPs has also been extensively studied. It has been demonstrated that MMP-9 and TIMP-1 have significant potential as biomarkers in CRC. Diagnostic sensitivity of MMP-9 and TIMP-1 was consistently higher as compared with the conventional biomarkers (CEA or CA 19-9) [18-20].

Some conversation about the optimization of the measurement of blood MMP levels has been engaged already, but Tanus-Santos et al. recommend in their article that plasma samples should be used to measure MMP activities and that serum samples should not be used [21]. There are also some controversial studies about MMP measurements in tumor and plasma samples. Curran *et al*., have shown that tumor tissue MMP profile is an independent prognostic indicator in CRC, but Waas *et al*., did not find any correlation between plasma proMMP-2 and proMMP-9 activities and prognosis of patients with colorectal cancer [22,23].

What is important in marker studies on the clinical aspect is that the same method is used throughout the study. From the clinical point of view, however, no disease-specific level of MMP-9 can be pointed out and if MMP-9 value is used in clinical practice, the measurements should be followed individually only and together with computed tomography scans.

One of the greatest challenges in CRC management is to predict the outcome of each patient so that we can determine who will really benefit from intensified adjuvant therapy. The classical TNM staging system relied heavily on the exact extent of cancer at the time of diagnosis and is greatly predictive in stage I and stage IV tumors. However, it is less informative for patients including stage II and stage III CRC. After curative surgery, stage III CRC patients experience 50% chance of developing recurrence. It is well documented that the overall survival rate of stage III CRC could clearly benefit from adjuvant chemotherapy. In contrast, the role of adjuvant chemotherapy for stage II CRC is still controversial, despite the 20% recurrence in this group. Proteolytic enzymes may help to identify patients who are more likely to have disease relapse and high

risk of death, thus those who are potential candidates to receive aggressive adjuvant chemotherapy [24,25].

Conclusion

In our study, we have determined that the MMP-2 and MMP-9 serum levels decrease considerably after the resection of the primary neoplasm; than MMP-2 serum levels preoperatively, at 3th and 12th month postoperatively, as well as MMP-9 preoperatively and at 3th, 9th and 12th month postoperatively are in a significant correlation with the poor outcome of the CRC patients. Subsequently, detection of serum MMP-2 and MMP-9 is feasible and done through a noninvasive technique. They are potential serum markers which can be useful in the CRC detection and in monitoring of the disease progression.

Conflict of interests. Not declared.

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Мак Мед Прегled 2019; 73(2): 112-117

ММР

Original article

FUNCTIONAL RESULTS AFTER SURGICAL TREATMENT OF C2 AND C3 TYPE FRACTURES OF PATELLA ACCORDING TO AO REFERENCES, A PROSPECTIVE COMPARATIVE STUDY OF THREE OPERATIVE METHODS IN THE PERIOD 2015-2018 AT THE UNIVERSITY CLINIC OF TRAUMATOLOGY-TOARILUC, SKOPJE

ФУНКЦИОНАЛНИ РЕЗУЛТАТИ ПО ХИРУШКИ ТРЕТМАН НА ПАТЕЛА С2 И С3 ТИП СПОРЕД АО КЛАСИФИКАЦИЈА, ПРОСПЕКТИВНА КОМПАРАТИВНА СТУДИЈА НА 3 ОПЕРАТИВНИ МЕТОДИ ВО ПЕРИОД ОД 2015 ДО 2018 ГОДИНА НА УК ЗА ТРАУМАТОЛОГИЈА, СКОПЈЕ

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Abstract

Introduction. The kneecap is a particularly large sesamoid bone. Its function in the largest joint in the human body is very important. The patella is one of the key factors fora stable knee joint. The right choice of surgical treatment of C2 and C3 articular complex patella fractures according to AO classification is still controversial among surgeons. We conducted a comparative study of three surgical methods at the University Clinic of Traumatology in the period 2015-2018. Only C2 and C3 fractures were included.

Methods. Eighty-four patients were observed at the University Clinic of Traumatology between 2015 and 2018. The first surgical technique was internal fixation (with screws or tension band-Zuggurtung technique). The osteosynthesis was made in 50 patients with a mean age of 56 years. The second surgical method was partial patellectomy with reinsertion of the patella's ligament. It was made in 30 patients with a mean age of 69 years. The third surgical method was total patellectomy. It was performed in 4 patients with a mean age of 83 years. The inclusion factors were: complex articular patella fracture (C2 and C3 according to AO classification), general good health (morbidities that preclude giving anesthesia) and signed permission for follow-ups. The surgery took time in the first 24 hours after the trauma event in all patients. The patients with advanced gonarthrotic changes, with previous operations on the knee, or previous mobility problems with the knee joint were excluded. All patients wore plaster cast 2 to 4 weeks after surgery. The follow-ups were made on the third day after surgery, on the 12th day, at one month, three months and six months after surgery. All patients were evaluated according to functional test and questionnaires about mobility and pain. All patients underwent physical therapy.

Results. In all three groups the follow-ups were made for functional testing. In all three groups functional testing and questionnaires about mobility and pain were made one month, three and six months after surgery. The functional testing was made according to Tegner-Lysholm knee scoring scale. The questionnaire included issues about the grade of pain, ability for doing everyday activities, surgical complications like contracture of the knee joint, extreme pain, and infections. Revisions were madein 3 patients.

Discussion. Our results have shown that osteosynthesis should be made whenever suitable. However, if the fragments of the fracture are too small and ideal reposition is not achieved, the best solution is partial patellectomy. In elderly female patients where osteoporosis is advanced, partial patellectomy is more appropriated than internal fixation. Total patellectomy is a salvage method with unsatisfactory outcome.

Keywords: patella fracture, knee joint, surgery, follow-up

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Апстракт

Вовед. Чашката на коленото е најголема сезмоидна коска и функцијата во колениот зглоб и е многу важна. Пателата е еден од факторите за стабилност на коленото. Вистинскиот избор за оперативен третман на С2 и С3 комплексни интраартикуларни фрактури според АО класификација е сеуште тема на дискусија помеѓу хирурзите. На Клиниката за Трауматологија се спроведена проспективна компаративна

студија на три хирушки методи во периодот од 2015 до 2018 година.
Методи. На Клиниката за Трауматологија се спроведе проспективна компаративна студија на три хирушки методи во периодот од 2015 до 2018 година. Осумдесет и четири пациенти беа проследени. Првата хирушка техника беше Zuggurtung методата, која беше застапена кај 50 пациенти на средна возраст од 56 години. Втората хирушка техника беше парцијална пателектомија, спроведена кај 30 пациенти на средна возраст од 69 години. Третата хируршка техника беше тотална пателектомија и беше спроведена кај 4 пациенти на средна возраст од 83 години. Инклузивни фактори беа C2 и C3 комплексни интраартикуларни фрактури според АО класификација, позитивна анестезиолошка евалуација, потпишана изјава за согласност за учество во студијата, како и можност за иведување на операцијата во првите 24 часа. Пациентите со гонартроза, претходни операции на колен зглоб, како и претходни потешкотии со мобилност на колен зглоб беа исклучени. Сите пациенти носеа имобилизација 2 до 4 недели по оперативен третман. Пациентите се следеа на 3-тиот ден по операција, 12-тиот ден, првиот, третиот и шестиот месец. Сите пациенти беа евалуирани според функционални скорови и прашалници. Кај сите пациенти беше спроведена физикална терапија.
Резултати. Кај сите три групи функционалните тестирања се направија на првиот, третиот и шестиот месец по операција според Tegner-Lysholm knee scoring scale. Прашалниците беа за степенот на болка, способност за извршување на секојдневни активности. Појава на контрактури и инфекции беа нотирани. Кај 3 пациенти беа направени ревизии.
Дискусија. Остеосинтеза треба да се направи секогаш кога е можно. Доколку фрактурните сегменти се премали за постигнување на анатомска репозиција, подобро е да се направи парцијална пателектомија. Кај постари, посебно женски пациенти, парцијална пателектомија е подобар избор. Тотална пателектомија дава незадоволителни функционални резултати.

Клучни зборови: скршеници на патела, оперативен третман, следење

Introduction

The knee cap is the largest sesamoid bone of the human body and is embedded in the quadriceps tendon. It is one of the few bones without a periosteal surrounding. The patella is one of the key factors to a stable knee joint. Fractures of the patella account for about 1% of all skeletal injuries and can lead to profound impairment due to its crucial function in the extensor mechanism of the knee. According to epidemiologic studies, these fractures occur twice more often in male than in female and are most prevalent within the age group of 20-50 years. The majority of cases are caused by direct injury mechanism. The resulting fracture type depends on the trauma mechanism (i.e. direct or indirect), the energy transmitted to the bone and the bone quality. The most common fracture pattern is a simple 2-part diversion caused by a direct blow (i.e. dashboard injury). As a result of the bony lesion the extensor mechanism of the knee joint can become insufficient. The degree of the insufficiency depends among other factors on accompanying damage to the reserve extensor mechanisms. Additional injuries to the adjacent bones are rare, but can affect the articular surface of the distal femur. The most frequent indirect mechanism is a fall on the feet with eccentrically contraction of the quadriceps muscle. Depending on the velocity of the fall and the resistance of the extensor mechanism, either the patella or the adjacent tendons fail. Most frequent causes are traffic accidents in 78.3%, followed by work-related accidents in 13.7% and domestic accidents in 11.4%. Sports-related fractures of the patella are relatively seldom.
The diagnosis of a fracture of the patella is made on the basis of the injury mechanism, physical examination and the radiological findings. It is suspected in all patients who have sustained a direct impact to the anterior knee and are unable to actively extend their knee after flexion injury or fall.
The right choice of surgical treatment of C2 and C3 articular complex patella fractures according to AO classification is still controversial among surgeons. We conducted comparative study of three surgical methods at the University Clinic of Traumatology in the period 2015-2018. Only C2 and C3 fractures were included.

Materials and methods

Eighty-four patients were observed at the University Clinic of Traumatology. All selected patients were informed about the study and formal consent was obtained. Every fracture of patella was classified by AO surgical references classification. The surgery took time in the first 24 hours after the trauma event in all patients. The first surgical technique was internal fixation (with screws or tension band-Zuggurtung technique). The osteosynthesis was made in 50 patients (36 females and 14 males) with a mean age of 56 years.
The second surgical method was partial patellectomy with reinsertion of the patella's ligament. It was made in 30 patients (24 females and 6 males) with a mean age of 69 years. The third surgical method was total patellectomy. It was performed in 4 patients (2 females and 2 males) with mean age of 83 years.
Inclusion factors were: complex articular patella fracture (C2 and C3); general good health (morbidities that

preclude giving anesthesia), isolated trauma and signed permission for follow-ups. Exclusion factors were: advanced gonarthrotic changes, previous operations on the knee, previous mobility problems with the knee joint and polytraumatised patients.
For each patient a preoperative protocol was followed: chest x-ray, x-ray of the injured knee AP and lateral view, blood count, ASSA evaluation, questionnaire about previous illnesses and mobility.
The first surgical technique was internal fixation (with screws or tension band-Zuggurtung technique). The osteosynthesis was made in 50 patients (36 females and 14 males) with a mean age of 56 years. This procedure was performed with the patient in a supine position with the knee flexed in 30 degrees. A mid-axial longitudinal approach was used. The knee joint and fracture lines must be irrigated and cleared of blood clot and small debris to allow exact reconstruction. The larger fragments were reduced using a pointed reduction forceps or tenaculum. Reduction was held by one or two reduction forceps and was verified by palpation of the retropatellar surface.

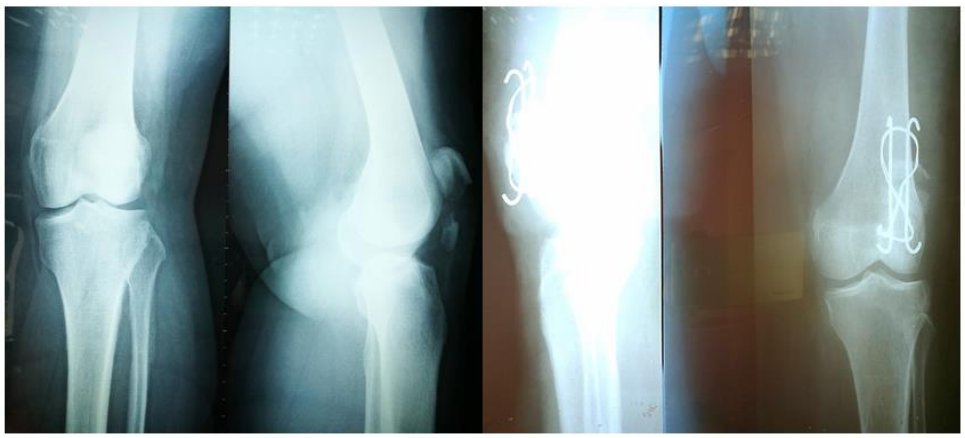

Fig. 1. Male, 49 yr, injured in bike accident. Zuggurtung method. Tegner and Lysholm score of 83 points

Using the outside-in technique, the first K-wire was drilled in an axial direction. The second K-wire was drilled parallel to the first, through the reduced fragments ensuring the K-wires do not enter the joint. It may be difficult to find the right direction and position for the wires. Two parallel K-wires were inserted to give more stable fixation. The position of the wires was checked with fluoroscope before proceeding to insert the tension band. We chose wire of enough strength to withstand the tensile forces generated in the figure-of-eight loop (1.0-1.25 mm diameter). A figure-of-eight is superior in neutralizing tension forces and is therefore preferred by many surgeons. While tightening the figure-of-eight wire with the knee in extension, we always checked the

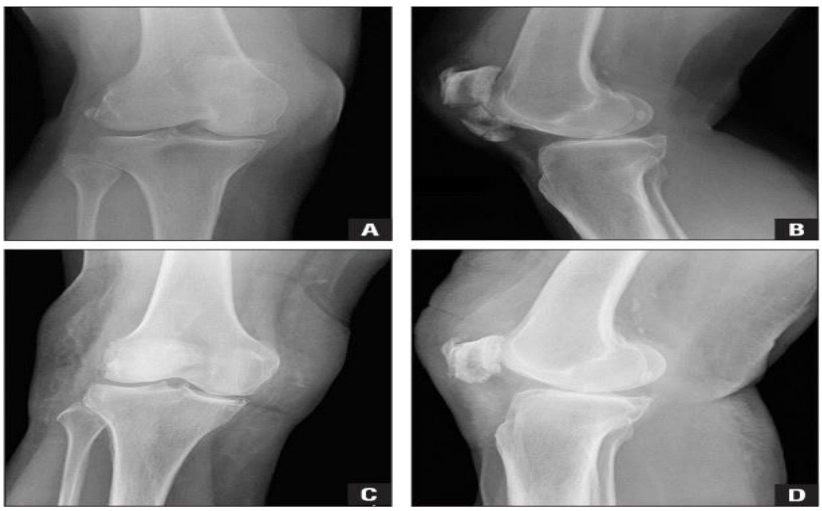

Fig. 2. Female, 46 yr, fall on the knee. Type c3 patella fracture. Partial patellectomy. Tegner and Lysholm score of 90 points

reduction by palpating the retro-patellar surface (Figure 1).
The second surgical method was partial patellectomy with reinsertion of the patella's ligament. It was made in 30 patients (24 females and 6 males) with a mean age of 69 years. This procedure was performed with the patient in a supine position with the knee flexed in 30

degrees. A mid-axial longitudinal approach was made. Debridement of heametoma and little bone fragments was made. Suture was made with non-absorbable suture of the most proximal part of the remaining patellar tendon to the remaining patella using suture anchors. Repairing of lateral and medial parapatellar retinacula was made. (Figure 2).

The third surgical method was total patellectomy. It was performed in 4 patients (2 females and 2 males) with mean age of 83 years. This procedure was performed with the patient in a supine position with the knee flexed in 30 degrees. A mid-axial longitudinal approach was made. It is a salvage method where we excised the remaining patella and suture the proximal extensor mechanism with a non-absorbable suture to the distal extensor mechanism.

The surgery took time in the first 24 hours after the trauma event in all patients.

A tutor cast was applied to each patient after surgery. All patients were on antibiotics, pain killers and therapy with LMWH. All patients wore plaster cast 2 to 4 weeks after surgery. The follow-ups were made on the third day after surgery, on the 12th day, at one month, three months and six months after surgery.

Examination in outpatient settings was made at one month, three months and six months after surgery. Tegner-Lysholm score was noticed at the third month and at the sixth month.

Results

In all three groups the follow-ups were made for functional testing after the third month and sixth month after surgery. In all three groups functional testing and questionnaires about mobility and pain were made at three and six months after surgery (after two or three cycles of physical therapies). The functional testing was made according to Tegner-Lysholm knee scoring scale (Table. 1). From 91 to 100 points the results were considered to be excellent: from 90-84 good results; 65 to 83 fair and from 64 points and less were poor results.

Table 1. Tegner-Lysholm knee scoring scale

Modified Lysholm limp (5 points)		None = 5 Slightly or periodically = 3 Severe or constant = 0	
Support (5 points)		None= 5 Limp = 2 Weight-bearing impossible = 0	
Locking (15 points)		No locking or crepitation sensation = 15 "Catching" sensation but no locking sensation = 10 Occasional locking = 6 Frequently = 2 Locked joint on examination = 0	
Instability (25 points)		Has never presented buckling = 25 Rarely during sports activities or other exertion = 20 Frequently during sports activities or other exertion = 15 Occasionally in daily living activities = 10 Frequently in daily living activities = 5 With every step = 0	
Pain (25 points)		None = 25 Inconstant and slight during severe exertion = 20 Marked during severe exertion = 15 Marked on or after walking 2 km or more = 10 Marked on or after walking less than 2 km = 5 Constant = 0	
Joint effusion/swelling (10 points)		None = 10 On severe exertion = 6 On slight exertion = 2 Constant = 0	
Stair climbing (10 points)		No problem = 10 Slightly impaired = 6 Step by step (one stair at a time) = 2 Impossible = 0	
Squatting (5 points)		No problem = 5 Slightly impaired = 4 Up to 90 degrees = 2 Impossible = 0	
TOTAL COUNT			
Result (first letter)			
Excellent: 91-100	**Good: 84-90**	**Fair: 65-83**	**Poor: < or = 64**

Three months after surgery there was no significant difference between the results of the three groups (Table 2). On the other hand, after six months, group 1 and 2 showed increased Tegner-Lysholm knee score, but the third group did not (Table 3). This confirmed the hypothesis that in total patellectomy the functional results were surprisingly good in the first 2 months, yet after that period the function decreased rapidly.

Table 2. Results fromTegner-Lysholm knee scoring scale three months after surgery

Tegner-Lysholm scoring scale results	Group 1 50 patients treated with osteosynthesis	Group 2 30 patients treated with partial patellectomy	Group 3 4 patients with total patellectomy
Average results	70	67	68

Table 3. Results fromTegner-Lysholm knee scoring scale six months after surgery

Tegner-Lysholm scoring scale results	Group 1 50 patients treated with osteosynthesis	Group 2 30 patients treated with partial patellectomy	Group 3 4 patients with total patellectomy
Average results	89	83.4	55.8

Table 4. Comparative results fromTegner-Lysholm knee scoring scale between female patients above 50 years, six months after surgery

Tegner-Lysholm scoring scale results	Group 1, patients treated with osteosynthesis >50 yr, 20 of 36 female patients	Group 2, patients treated with partial patellectomy >50 yr, 14 patients out of 24 female patients
Average results	77.4	84.2

The Tegner-Lysholm knee score were higher in female patients older than 50 years of the second group than in the first group (Table 4).
Postoperative complications like contracture of the knee joint, extreme pain, infections were evaluated, too.

Revisions were made in 3 patients. All three of them were in the first group treated with Zuggurtung method. Two of them were re-operated because of loosening of the K-wires and one because of severe contracture of the knee joint (Figure 3).

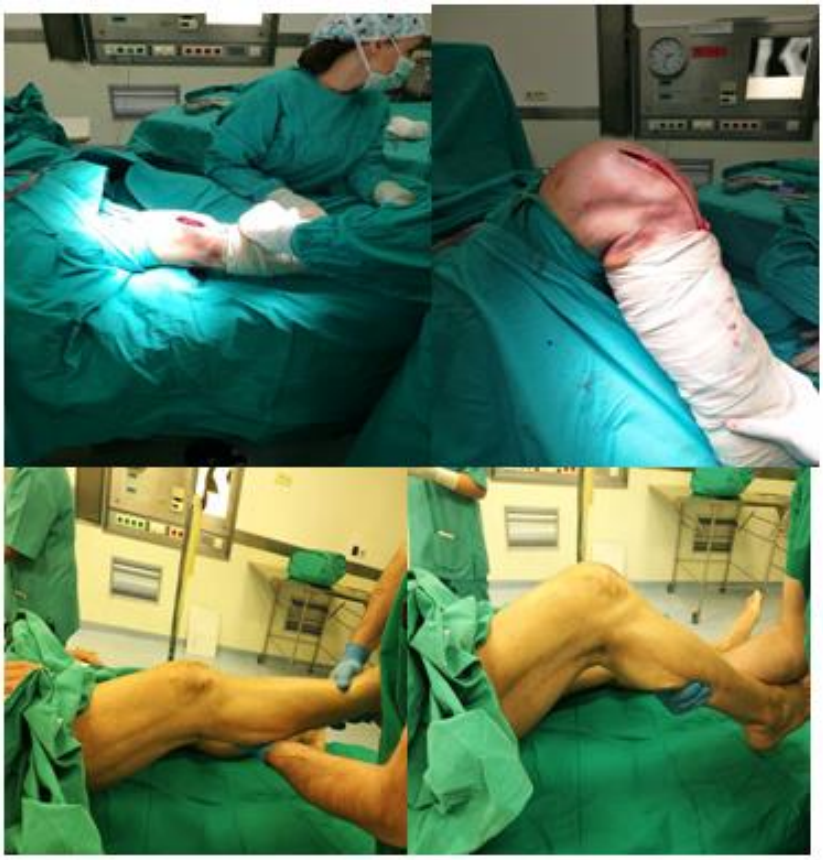

Fig. 3. Complications-revision after tension band fixation in male patient due to contracture

Discussion

Surgical treatment of comminuted patellar fracture involving the articular surface is often complicated and difficult, mainly due to weak patellar bone and more small fragments. The principles of AO trauma references are clear that we should always tend to do anatomical reduction to all joint fractures. Additionally to that principle is that patella consists of a large number of cancellous bones, and the fracture heals quickly. Ho-

wever, resection of all (total patellectomy) or part of the patella (partial patellectomy) may be indicated in certain situations. Biomechanical studies have demonstrated that removal of the patella decreases the quadriceps strength by 50%. Partial patellectomy is indicated for severely comminuted fractures of the superior or inferior pole that comprise less than 40% of the patellar height and are not amenable to fixation.

Lue *et al.* [12] used non-absorbable suture cerclage combined with nickel-titanium patellar concentrator to treat comminuted fracture patterns; 75.8% of the patients have excellent results and 24.2% have good results. These authors proposed that for comminuted patellar fractures, laterally displaced fragments cannot be stably fixed by the nickel-titanium patellar concentrator due to traction of the patellofemoral constructs during knee flexion.

Yangyang Sun *et al*. [13] used the clinical grading scales of Bostman and the average score of the final follow-up was 28.7 (range 20-30). The average score of 32 (84.2%) excellent patients was 29.5±0.7 (range 28-30) and that of six (15.8%) good patients was 24.5±2.2 (range 20-27). The range of knee flexion activity of patients was 130° (range 110-140), and the prognosis was satisfactory.

The studies above suggest good functional results after reduction and fixation of patellar fractures. This confirms our results that after a good anatomical reduction of the joint surface and rigid fixation, with proper rehabilitation a good outcome is very likely to be achieved.

However if a reconstruction is not possible, partial patellectomy is preferred over total patellectomy.

Conclusion

Our results have shown that osteosynthesis should be made whenever suitable. However, if the fragments of fracture are too small and ideal reposition is not achieved, the best solution is partial patellectomy. In elderly female patients where osteoporosis is advanced, partial patellectomy is more appropriate than internal fixation. Total patellectomy is a salvage method with unsatisfactory outcome.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2019; 73(2): 118-121

ММР

Case report

PARACENTESIS-INDUCED ABDOMINAL WALL HEMATOMA: CASE REPORT AND REVIEW OF LITERATURE

ХЕМАТОМ НА АБДОМИНАЛЕН ЗИД АСОЦИРАН СО ПАРАЦЕНТЕЗА: ПРИКАЗ НА СЛУЧАЈ И ПРЕГЛЕД НА ЛИТЕРАТУРАТА

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Abstract

Despite the well-known coagulopathy-associated complications, paracentesis is considered a relatively safe procedure when performed inpatients with liver cirrhosis. We present a case of a large abdominal wall hematoma after paracentesisin a 72-years-old male with decompensated cirrhosis, portal hypertension, refractory ascites and moderately prolonged prothrombin time. Several hours after therapeutic paracentesis wasperformed at the usual point, in the left lower abdominal quadrant, the patient was admitted with severe abdominal pain, circulatory instability and significant blood loss. Ultrasound of the abdominal wall revealed a 10 cm intramural hematoma at the puncture site.In addition to the usual resuscitative measures, the patient required fresh frozen plasma and five units of cryoprecipitate for definitive stabilization. Paracentesis-associated abdominal wall hematoma is a potentially serious, life-threatening complication requiring invasive therapeutic intervention in most cases. In some caseshowever the conservative treatment with cryoprecipitate and fresh frozen plasma can also be quite effective.

Keywords: hematoma, paracentesis, bleeding complications, liver cirrhosis

Апстракт

И покрај добро познатите компликации асицорани со коагулопатијата, парацентезата се смета за релативно безбедна процедура кога се изведува кај пациенти со црнодробна цироза. Презентираме случај на голем хематом на стомачниот зид после парацентеза кај пациент на возраст од 72 години со декомпензирана цироза, портална хипертензија, реф рактерен асцит и умерено пролонгирано протромбинско време. Неколку часа по парацентезата реализирана на вообичаената точка, во долно левиот абдоминален квадрант, пациентот беше хоспитализиран со силна абдоминална болка, циркулаторна нестабилност и значителен губиток на крв. На местото на парацентезата ултрасонографски се утврди присуство на хематом со големина од 10 цм. Освен со примена на вообичаените ресусцитациски мерки, пациентот беше дефинитвно стабилизиран по администрација на свежо смрзната плазма и пет единици криопреципитат. Хематомот на абдоминален зид асоциран со парацентеза е потенцијалносериозна животозагрозувачка компликација за која во повеќето случаи потребна е инвазивна терапевтска интервенција. Сепак, кај некои пациенти конзервативниот третман со криопреципитат и свежо смрзната плазма може да биде прилично ефикасен.

Клучни зборови: хематом, парацентеза, крваречки компликации, црнодробна цироза

Introduction

Liver cirrhosis is accompanied by many abnormalities in primary hemostasis, coagulation and fibrinolysis [1]. For this reason, clinicians have historically been concerned about an increased bleeding risk during invasive procedures in patients with cirrhosis.However, recent data suggest that liver cirrhosis actually creates prothrombotic state [2]. In these patients there is an unstable balance between prothrombotic and antithrombotic processes that routine coagulation tests do not properly show [3-6]. Large volume paracentesis (LVP) is rarely associated with clinicallysignificant bleeding and other procedure-related complications and is considered a relatively safe procedure [7-10]. The incidence of fatal bleeding is approximately 0.2% per procedure, and the incidence of mortality due to bleeding complication is lower than 0.01% [11]. Paracentesis-related bleeding complications (PRBC) are more prevalent after

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therapeutic vs. diagnostic LVP [9], in patients with high CTP and MELD scores [9,13], in renal dysfunction [7,12], in acute-on-chronic liver failure (ACLF) [13] and in patients with low fibrinogen level [13]. More importantly, PRBC are not related to elevated international normalized ratio (INR)[9,12], low platelet count [12] or operator's experience [12]. There are three types of PRBC described in the literature: abdominal wall hematoma, pseudoaneurysm, and hemoperitoneum. Although they are all rare, according to one systematic review abdominal wall hematomas occur mostfrequently [7]. While the bleeding may be due to direct puncture of a superficial abdominal wall vein or a mesenteric varix, in most cases the bleeding originates from a lesion of the inferior epigastric artery or one of its tributaries [7,9]. Most PRBC are minor and can be successfully controlledwithsupportive medical measures such as fluid resuscitation, blood transfusion and correction of the coagulation disorders [12]. Occasionally, severe, life-threatening bleeding can occur and an interventional procedure is oftenrequired in order to control the bleeding.

Case report

We present a case of 73-year-old male withlong-term alcohol consumption and recently diagnosed decompensated liver cirrhosis, admitted to our department after variceal bleeding. Transabdominal ultrasound revealed findings typical for advanced liver disease, aslightly enlarged spleen and alarge amount of ascites. Gastroscopy showed grade III esophageal varices. We performed band ligation of the esophageal varices and after stabilization the patient was discharged on anon-selective beta blocker, lactulose and dual diuretic therapy. In the follow-up period, we noticed his ascites to be diuretic resistant and proceeded to perform uncomplicated large volume paracentesis (LVP).

Several weeks later the patient returned for a second LVP. Before the intervention we performed bothclinical and ultrasound examinations, complete blood count and complete biochemical panel. The results revealed no significant changes in the lab results. The CTP score was 12, MELD score 19, INR 1.7 and the platelet count was $176x10^3$/µl (Table 1). We performed the intervention at the usual site, the lower left abdominal quadrant and we evacuated 5 liters of yellow fluid. The patient felt well and was discharged several hours later. Later that day, the patient returned to our department complaining of severe lower abdominal pain and weakness. Examination revealed hypotension and tachycardia and CBC showed a significant decline in Hgb from its baseline. Ultrasonographic examination of the abdominal wall revealed 10 cm hematoma within the abdominal wall at the puncture site (Figure 1A). During the hospitalization the patient was closelymonitored and treated with supportive measures includingcrystalloid and colloid fluids and fresh frozen plasma. Despite this,he remained hemodynamically unstable and his blood count continued to decline despite multiple blood transfusions. The patient's hemodynamic instability precluded obtaining a dynamic CT scan. Taking into account the worseningclinical course and the limited therapeutic options, we decided to administer five units of cryoprecipitate. After the infusion,striking clinical improvement and definitive stabilization occurred. Shortly after, the patient was safely discharged from the hospital.The hematoma size was markedly reduced at follow-up examination several weeks later (Figure 1B).

Table 1. Complete blood count and biochemical blood analysisduring the hospitalization

DAY	0 Before LVP	0 After LVP	1	2	3	4	5	6	7
HGB (g/dL)	89.00	69	72	58	66	78	99		99
HGB (g/dL)	89.00	69	72	58	66	78	99		99
RBC ($x10^6$ /µl)	2.20	1.83	1.73	1.7	1.98	2.36	2.95		2.89
HCT (%)	25.3	20.9	18.5	17.5	20.2	24	29.5		29.5
PLT ($x10^3$/µl)	176	187	145	92	94	93	112		122
BUN (mmol/l)	12.1	13.7					14.2		11.7
Cr (µmol/l)	88.3	90					94.6		82.6
PT (sec)	18.90								14.1
INR	1.7								1.29
bilirubin (µmol/l)	146								137.9
albumin (g/l)	25								27
Na (mmol/l)	133								134
K (mmol/l)	4.9								3.8
AST (U/L)	117								90
ALT (U/L)	63								49
AP (U/L)	188								168
GGT (U/L)	129								128

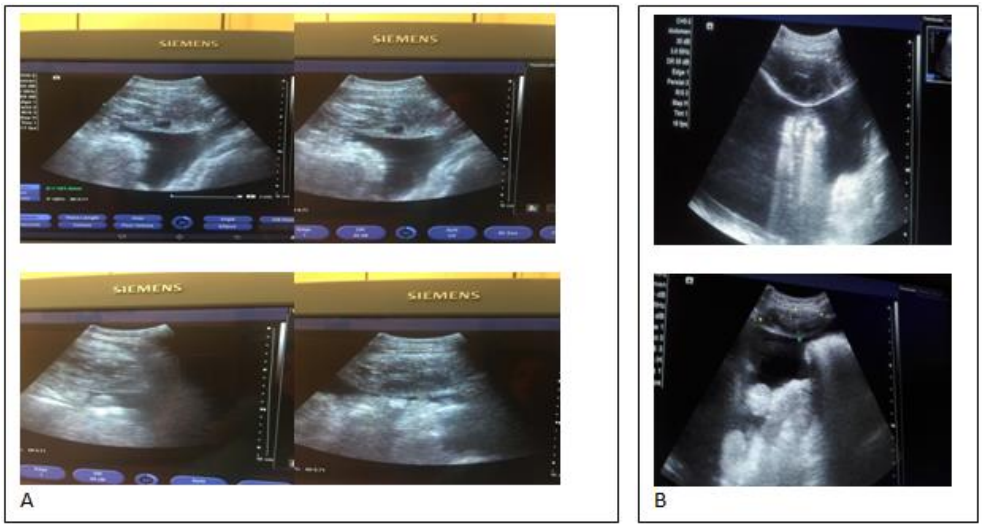

Fig. 1. Ultrasound images of the post-puncture intramural hematoma; A: The ultrasound examination at presentation revealed aclearly visible 10 cm large nonhomogeneous oval formation within the abdominal wall highly suggestive of intramural hematoma. B: six weeks later we registered a significant reduction of the hematoma size

Discussion

Many cirrhosis-associated complicationsare actually a consequence of the complex acquired coagulation disorder that developsin these patients. Previously this condition was mainly thought to involve anticoagulation reflected as an elevated INR. More recent data show disturbances in the concentration ofboth, procoagulants and anticoagulant factors [6]. Compared to healthy subjects, cirrhotic patients have reduced levels of antithrombin, protein C, factor V and factor II, and significant increase of factor VIII [6,14]. The markedincrease of the powerful procoagulant factor VIII and the significantdecrease of the naturally occurring anticoagulant factor protein C, seem to be the most typical coagulation abnormalities in cirrhotic patients [6]. Based on the coagulation tests abnormalities [prolonged prothrombin time (PT), activated partial thromboplastin time (aPTT) and bleeding time (BT), low platelet count] the bleeding complications in cirrhotic patients have been attributed to the presence of coagulopathy and/or thrombocytopenia. Therefore, liver cirrhosis has beenthought to confer an increased bleeding risk. However, there are studies showing that plasma from cirrhotic patients actually generates normal amounts of thrombin [5]. Data obtained in the last two decades have provided substantial evidence strongly suggesting that liver cirrhosis is predominately associated with hypercoagulable state and an increased tendency for thrombotic events compared to healthy controls [2,6]. Also, the increase of the powerful procoagulant Von Willebrand factor is at least partially able to compensate for the disturbance in primary hemostasis due to thrombocytopenia and thrombocytopathy [4,5,14].

The presented case highlights several important issues. Thepatient's prior therapeutic paracentesis went well, without any bleeding complications. Moreover, he had no history of complications related to coagulopathy. Lab analysis on the day of the paracentesis including complete blood count and routine coagulation tests were not indicative of an increased bleeding risk. Nonetheless, the patient had advanced liver disease and significant, potentially life-threatening bleeding that was only successfully controlled with fresh frozen plasma and five units of cryoprecipitate. This indicates that in patients with liver cirrhosis there are probably numerous subtle abnormalities in the haemostatic systemthat routine tests of coagulation do not capture. Also, these tests are not able to point out to an increased bleeding risk in the way they do in the general population. Moreover, the complex interaction between pro- and anticoagulant factor and also the mutual compensation between different abnormalitis in cirrhotic patients further complicates the assessment of bleeding risk

Despite the well-established haemostaticabnormalities in patients with liver cirrhosis, the relevant literature suggests that the bleeding complications that occasionally occur in such patients are not always due to derangement in the coagulation process [12]. Most studies have indicatedthat the prolonged PT and elevated INR were not related to increased risk for bleeding complication [9,12,13]. For example, Lin *et al.* showed that severe hemorrhagic complications more frequently occur in patients with ACLF [13]. Also, most of the patients included in one systematic review that analyzed PRBC (90% had liver cirrhosis) confirmed some form of renal function impairment in 70% of patients [7]. This means that the hemorrhagic complications that occur in advanced liver disease could be more closely related to some liver disease-associated complications and acute events than to a distinct form of acquired coagulation disorder resulting from the liver dysfunction.

Hemodynamic alterations related to portal hypertension, bacterial infections, endothelial dysfunction or renal failure may all potentiallyplay a certain role in determining the bleeding risk in these patients [3,5,7].

Conclusion

Despite increasing awareness that chronic liver disease is not an anticoagulated state [1,14], invasive procedures in cirrhotic patients may in a selected population be associated withsignificant, often life-threatening bleeding complications. Paracentesis-associated bleeding is a rare, but serious complication requiring an invasive therapeutic intervention in most cases. However, in some cases the conservative treatment with cryoprecipitate and fresh frozen plasma can also be quite effective.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2019; 73(2): 122-128

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Case report

METABOLIC CONTROL OF GP-LED HYPERTENSIVE TYPE 2 DIABETES PATIENTS ON ORAL ANTIDIABETIC DRUGS

МЕТАБОЛНА КОНТРОЛА НА ПАЦИЕНТИ СО ДИЈАБЕТЕС ХИПЕРТЕНЗИВЕН ТИП 2, СО ОРАЛНО АНТИДИЈАБЕТИЧНИ ЛЕКОВИ,ВОДЕНИ ОД ОПШТИТЕ ЛЕКАРИ

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Abstract

Aims. To assess the metabolic status of hypertensive type 2diabetes patients on oral antidiabetic drugs (OADs) while managed bygeneral practitioners (GPs).

Methods. Eighty-two GPs agreed to provide patient data for retrospectiveanalysis. The patients' inclusion criteria were: 1/ Age ≥40 years; 2/Type 2 diabetes for >1 year; 3/ Treatment with OADs >3 months; 4/Arterial hypertension for >1 year; 5/Stable doses of antihypertensivedrugs for >3 months. Exclusion criteria were type 1 diabetes and injectableantidiabetic treatments. Data were introduced in a specific electronic registry by the GPs themselves. An IBM SPSS 19.0 package was used for statistical analysis.

Results. A total of 5 926 patients' records met the entry criteria. 7.6% were aged 40 to 50 years; 22.6% - 51 to 60, 39.4%-61 to 70; 53.75% were women. Normal BMI, overweight and obesity grade I - III were found in 16.2%, 42.1%, 27.6%, 9.7% and 4.2%. The waist circumference was <94cm in 26.4% of men; and <80 cm in 10.7% of women. Fasting plasma glucosewas ≤6.0 mmol/l in 19.2%, and ≤7.0 mmol/l in50.4%. Glycated hemoglobin was ≤7.0% in 62.9%, and ≤8.0% in 87.0%. The LDL-C was<1.8 mmol/l in 9.1%, and 1.8 to 2.6 mmol/l in 19.6%. The bloodpressure was <140/90 mm Hg in 77.4% of the patients.

Conclusions. The data about the glycated hemoglobin and the control of bloodpressure are reassuring. An improvement is needed in controlling obesity anddyslipidemia.

Keywords: type 2 diabetes, arterial hypertension, metabolic control, epidemiology, GP

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Апстракт

Цели. Да се процени метаболичкиот статус на хипертензивните пациенти со дијабетес тип 2 на орални антидијабетични лекови(ОАЛ),додека се водени од општ лекар(ОЛ).

Методи. Опфатени се осумдесет и два општи лекари кои се согласија да дадат податоци за пациентите за ретроспективна анализа.Критериумите за вклучување на пациентите беа: 1. Старост >40 г. 2. Тип 2 дијабетес >1 година, 3. Третман со ОАД > месеци, 4. Артериска хипертензија >1 година, 5. Стабилни дози на антихипертензивни лекови >3 месеци. Критериуми за ексклузија беа тип 1 дијабетес и антидијабетични третмани со инекции (инсулин). Податоците беа внесувани во специфичен електронски регистар од страна на самите општи лекари. За статистичка анализа беше искористен пакет IBM SPSS 19,0.

Резултати. Вкупно 5926 пациенти ги исполниле критериумите за вклучување во анализата. 7,6% биле на возраст од 40 до 50 години, 22,6% - 51 до 60, 39,4% - 61-70,53. 75% биле жени. Нормален БМИ, прекумерна тежина и дебелина,градуирани од I - III,епронајден во 16,2%, 42,1%, 27,6%, 9,7%, и 4,2%. Обемот на половината беше <94 см кај 26,4% од мажите и <80 см кај 10,7% од жените. Плазма глукозата на гладно беше <6.0 ммол/л во 19,2% и <7.0 ммол/л во 50,4%. Гликолизираниот хемоглобин беше <7.0% во 62,9% и <8.0 % во во 87,0%. LDL - С беше <1,8 ммол/л во 9,1 % и 1,8 - 2,6 ммол/л во 19,6%. Крвниот притисок беше <140/90 во 77,4% од пациентите.

Заклучоци. Податоците за гликолизираниот хемоглобин и контролата на притисокот се задоволителни. Потребно е подобрување на контролата на дебелина и дислипидемија.

Клучни зборови: дијабетес тип 2, артериска хипертензија, метаболична контрола, епидемиологија, општ лекар

Introduction

The projections of type 2 diabetes prevalence show an approximate increase of 40% in the following 25-30 years (IDF Atlas, 2015). Two nation-wide representtative Bulgarian studies in 2006 and 2012 showed a rise in the prevalence of diagnosed diabetes from 5.0 to 7.1% and of undiagnosed one-from 3.3 to 2.5% (Borissova *et al.,* 2007; Borissova *et al.,* 2012). The CODE-2 study in Europe showed a doubling of hospitalization and tripling of annual costs per type 2 diabetes patients in the presence of micro- or macrovascular complications (Williams, Van Gaal&Lucioni, 2002). Recent intervention trials such as STENO-2 and ADVANCE-ON have shownthat intensive multi-factorial treatment of diabetes could heavily reduce the incidence and progression of both micro- and macrovascular complications (Gaede *et al.,* 2016; Mohammedi *et al*., 2016; Wong *et al.,* 2016).The constant improvement in the diabetes treatment paradigm has led to substantial decreases in the rates of myocardial infarction, stroke and amputation as shown in an analysis of diabetes-related complications in the United States, 1990-2010 (Gregg *et al.,* 2014). The NHANES data analysis 1999-2012, however, registered an increasing trend of diabetescombined with hypertension orhypercholesterolemia, despite efforts to achieve better metabolic control (Song *et al.,* 2016).
General practitioners (GPs) play a substantial role in the management of type 2 diabetes patients. The BULPRAKT-HEART Study conducted in 2004 gathered data on levels of glycated hemoglobin (HbA1c), while a registry-based analysis of diabetes patients treated by GPs for 2003 yielded information about the fasting plasma glucose (Palaveev, Dimitrova, Christov, Petrova& Ilieva, 2005; Christov, Kamenov, Georgiev, 2006). A recent publication describing the diabetes care in Slovenia, Croatia, Serbia, Bulgaria and Romania found low rates of HbA1c measurements in the Balkans with over 60% of patients on oral antidiabetic drugs (OADs) not achieving good control (Cokolic *et al.,* 2017).

The aim of the present study was to assess the metabolic status of type 2 diabetes (glycemic control, lipids, blood pressure, BMI and waist circumference) in a population-based representative sample of patients with type 2 diabetes and coexisting hypertension managed by GPs.

Materials and methods

Design
This retrospective registry-based cross-sectional observational nation-wide study was approved by the responsible authorities. One hundred and twenty general practitioners were invited to participate. They were selected by 2 criteria: 1/ The location of their practice in order to cover both urban and rural patient practices and to represent the national patient population as close as possible; 2/ Their usualpatient population should includepredominantly adult patients (> 40 years) with type 2 diabetes and/or arterial hypertension. Eighty-two general practitioners agreed to participate.

Data collection

The included patients' data had to be selected in a consecutive order from the electronic databases of the individual GP practices. The patient's inclusion criteria were: 1/ Age ≥ 40 years; 2/ Type 2 diabetes mellitus (T2DM) diagnosed according to the local guideline for the management of diabetes by GPs, with at least 1 year duration (Boyanov *et al.,* 2012); 3/ The diabetes treatment should include diet, physical activity and oral antidiabetic drugs (OADs) without changes for at least 3 months; 4/ Presence of arterial hypertension for at least 1 year according to the local guideline for the management of arterial hypertension by GPs (Kirov &Gotcheva, 2014); 5/ Treatment with stable doses of antihypertensive drugs for at least 3 months prior to data chart review. Exclusion criteria were: age below 40 years, presence of diabetes type 1 and use of any injectable antidiabetic treatments (GLP-1 analogs, insulin). To avoid possible bias the participating GPs were asked to provide all the patient data present in their database that met the inclusion/exclusion criteria.

Measures

The patient data were entered into an electronic database: patient's age, gender, type of residence (urban/rural), duration of type 2 diabetes and hypertension, concomitant diseases (asthma, chronic obstructive pulmonary disorders, dementia, depression, and others), presence of macrovascularor microvascular disease. Information on cardiovascular risk factors includedpositive family history for CVD (<65 years in female relatives; <55 years in male relatives), waist circumference, body mass index (calculated as body weight in kg divided by the height squared in meters), recently measured systolic/diastolic blood pressure (SBP/DBP), fasting plasma glucose (in mmol/l), glycated hemoglobin A1c (in % and in mmol/mol), total cholesterol (TC), HDLand calculated LDL-cholesterol (Friedewald formula), triglycerides. All laboratory data were based on records from the local laboratories. OADs included metformin, sulphonylureas, pioglitazone, alpha-glucosidase inhibitors (acarbose), glinids (repaglinide), DPP-IV inhibitors, SGLT-2 inhibitors or combined. The antihypertensive treatment included ACE inhibitors /ARB blockers, calcium channel antagonists, beta-blockers, diuretics, com-

bined drugs; the lipid-lowering drugs-statins, fibrates, ezetimibe.

Sample size considerations and analysis

In 2012 approximately 577 124 people (9.6% of the population aged 20 years and older) - 337 980 men and 239 144 women in our country were expected to have diabetes mellitus [15].More than half of them were known to have concomitant arterial hypertension (approximately 300 000). Half of those hypertensive type 2 diabetes patients were expected to be on oral antidiabetic medication (OADs) and not using injectable therapies or insulin (approximately 150 000). Four percentageof all hypertensive type 2 diabetes patients on OADs selected on a random basis was regarded a sufficiently-powered sample to study their metabolic control.
Data were first reviewed for completeness and validity and patients with missing data were excluded from the analyses. An IBM SPSS19.0 for Windows platform was used for data processing (IBM SPSS Inc., Chicago, IL). After checking the numerical data distribution, descriptive statistics was performed and frequency tables were built. The thresholds for some numerical parameters were defined according to the national guidelines for the management of diabetes by GPs and endocrinologists (Boyanov *et al*., 2012; Borissova *et al.*, 2016). BP ≤140/90 mm Hg and triglycerides ≤1.7 mmol/lwere regarded as optimal. Optimal LDL-cholesterol levels (in mmol/l) were ≤1.8 mmol/l in the presence of both macrovascular disease and diabetes; and ≤2.6 mmol/l in the presence of uncomplicated diabetes. HbA1c(in %) strata were: ≤6.5, 6.6-7.0, 7.1-8.0, 8.1-9.0, and ≥9.1%. A two-tailed Student's t-test and ANOVA were applied. Statistical significance was set as p≤0.05, the power of the study-at 80%.

Results

The data files of 161 132 patients were reviewed. 142 287 were aged ≥18 years; 15 933 of them (11.19 %) had type 2 diabetes and 45 393 (31.9%) arterial hypertension. 62.99% of all patients with type 2 diabetes had also hypertension, while only 22.11% of the hypertensive patients had concomitant type 2 diabetes. From those, only 5 926 patients with type 2 diabetes and hypertension had valid and complete data according to the inclusion/exclusion criteria. Their age distribution was as follows: aged 40 to 50 years-7.6%; 51 to 60-22.6%, 61 to 70-39.4% and ≥ 71 years-30.4%. 53.75% of the study sample werewomen.
The studied population with diabetes and arterial hypertension had the following distribution according to BMI: 16.2% had normal BMI (12.6% of men and 19.4% of women), 42.1% had overweight (46.5% men/ 38.3 % women respectively), 27.6 % had obesity grade I (28.3 % men / 27.2% women), 9.7% - obesity grade II (9.0% men / 10.0% women) and 4.2 had obesity grade III (3.6% men / 4.8% women).
The waist circumference (WC) was below 94 cm in 26.4% of men; and below 80 cm in 10.7% of all women. The WC was ≤94 cm in 18.9% of men aged 40-50 years, in 22.4% of those aged 51-60, in 26.4% between 61 and 70 years; and in 33.4% of those ≥ 71 years. The WC was ≤ 80 cm in 13.5%, 10.7%, 8.3%, and 13.0% of women in the same age groups.
The proportions of patients with morning FPG below different thresholds are shown according to age and gender in Table 1. Fasting plasma glucose values were below 6.0 mmol/l in less than one fifth of the participants, and below 7.0 mmol/l in around half of them.

Table 1. roportions (in percentages) of patients with fasting plasma glucose values below 6.0, 7.0 and 8.0 mmol/l according to age and gender

Age group (years)	FPG ≤ 6.0 mmol/l		FPG ≤ 7.0 mmol/l		FPG ≤ 8.0 mmol/l	
	Men	Women	Men	Women	Men	Women
40 – 50	13.5%	23.7%	44.4%	50.3%	68.4%	75.2%
51 – 60	17.1%	17.6%	49.4%	50.6 %	72.1%	73.4%
61 – 70	16.7%	22.1%	46.9%	53.0%	70.7%	75.5%
≥ 71	19.3%	21.7%	51.2%	53.0%	75.4%	75.7%
Total	17.1%	21.1%	48.3%	52.2%	72.0%	75.2%

Table 2. Distribution of HbA1c in different age groups (men + women)

Age group (years)	HbA1c ≤ 6.5%	HbA1c 6.6- 7.0%	HbA1c 7.1 - 8.0%	HbA1c 8.1 - 9.0%	HbA1c ≥ 9.1%
40 - 50	41.5%	19.8%	23.0%	6.2%	9.6%
51 - 60	38.3%	23.3%	25.7%	6.5 %	6.2%
61 - 70	39.8%	22.7%	24.4%	7.5%	5.6%
≥71	42.0%	23.0%	23.0%	6.4%	5.7%
Total	40.2%	22.7%	24.1%	6.8%	6.0%

Valid data for HbA1c measurements were available in 5 154 patients (87.0%) and are shown in Table 2. 62.9% of all patients had a glycated hemoglobin ≤7.0%; and 87.0% - ≤8.0%.

The calculated LDL-C fraction was <1.8 mmol/l in 9.1% of the studied population; and between 1.8 and 2.6 mmol/l in 19.6%. Therefore, 81.3% of the participants had LDL-C levels >2.6 mmol/l.The distribution of triglycerides and LDL-C values in different age groups is displayed in Table 3.

Table 3. Proportions (in percentages) of the studied population with LDL-C and triglycerides within the target levels

Age group (years)	Triglycerides < 1.7 mmol/l (150 mg/dL)	LDL-C < 1.8mmol/l	LDL-C < 2.6 mmol/l (100 mg/dL)
40 – 50	31.9%	5.8%	21.5%
51 – 60	36.5%	8.9%	26.9%
61 – 70	43.4%	9.3%	29.0%
≥ 71	51.5%	9.9%	31.5%
Total	43.4%	9.1%	28.8%

Table 4. Blood pressure in the target zone (< 140/90 mm Hg) according to age and gender

Age group (years)	Systolic BP< 140 mm Hg		Diastolic BP< 90 mm Hg	
	Men %	Women %	Men %	Women %
40 - 50	62.1%	71.8%	68.9%	73.5%
51 - 60	58.6%	63.9%	67.7%	71.7%
61 - 70	57.4%	59.4%	70.2%	71.6%
≥ 71	61.2%	59.4%	76.8%	76.2%
Total	59.1%	61.0%	71.1%	73.3%

The reported blood pressure was <140/90 mmHg in 77.4% of the studied population, while it was <140/85 mmHg in only 64.0%. The distribution of good BP control according to age is displayed in Table 4.

All these data show that blood pressure and glycemia were well controlled in the majority of patients while LDL-C, triglycerides and body weight were above the target in most of them.Table 5 shows how many patients met the combined criteria for glycemic + lipid control, or glycemic+ BP control, or all three together. Asmall minority of the patients metall target values, leaving room for further therapy improvement.

Table 5 -Percentages of patients meeting combined criteria for glycemic + lipid control, or glycemic and BP control, or all three together

Age group (years)	HbA1c < 7.0% andSBP/DBP< 140/90 mm Hg		HbA1c < 7.0%, LDL < 2.6 mmol/l, and TG< 1.7 mmol/l		HbA1c < 7.0%, LDL < 2.6,andTG< 1.7 mmol/l, and BP < 140/90mm Hg	
	Men %	Women %	Men %	Women %	Men %	Women %
40 – 50	8.9%	8.3%	1.9%	1.5%	1.5%	0.0%
51 – 60	9.5%	11.9%	2.6%	1.6%	2.1%	1.0%
61 – 70	9.0%	10.2%	2.8%	1.9%	1.5%	1.1%
≥ 71	12.2%	9.9%	4.1%	2.5%	2.8%	1.5%
Total	9.9%	10.3%	3.0%	2.0%	3.0%	1.2%

Discussion

We performed a study based on GP-led diabetes outpatient-clinics in order to assess glycemic, lipid and blood pressure control in patients with type 2 diabetes on OADs and treated hypertension in a nation-wide representative sample. Our results are reassuring when glycated hemoglobin and blood pressure are examined separately-almost ⅔ of the patients had achieved good control. However, the situation with the lipid profiles looks quite differently with less than a half with normal triglycerides and only ¼ with normal LDL-cholesterol, despite treatment. If all three parameters are examined in a combined fashion, less than 15 % will have optimal metabolic control (glycemia + blood pressure + lipids). If body weight (BMI) and waist circumference were added, less than 10% would meet the combined criterion. These results show a treatment gap leaving room for improvement in the field of obesity and lipid abnormalities.

The metabolic control of diabetes has a profound impact on the prevention and delay of micro- and macrovascular complications. New analyses proved a clear and fast benefit of lipid- and blood pressure lowering strategies, while the role of glycemic control remains postponed in time and mainly on the microvascular outcomes (Brugtsetal., 2009; Atkins & Rodgers 2016; Giorgino, Home &Tuomilehto, 2016). The combined effect of all three interventions was proven in interventional trials such as the STENO-2 and the ADVANCE-ON studies (Gaede *et al.*, 2016; Hamet 2012).

Our data should be reviewed in the light of previous publications coming from international and national studies although bearing in mind that patients were not

similar in terms of age, duration of diabetes, medications used etc.In across-sectional analysis on 5382 type 2 diabetic patients in the primary care setting in Spain between 2011and 2012, 17.1 and 67% applied to ADA/ EASD recommendation of HbA1c target of <7 and <8% (Miñambres, Mediavilla, Sarocca& Perez, 2016). A Chinese study examined the data of 9065 adult T2DM outpatients (5035 men) between 2010 and 2012 and found glycemic control rate in only 32.6%, with the triple control rate for glycemia, blood pressure, andlipidemia in only 11.2% (Chen *et al*., 2015). Specialist-led diabetes practices are also not always able to achieveoptimal glycemic control in the majority of patients. Thiswas shown in a Canadian registry-based study including 10 590 patients with T2DM (Aronson *et al*., 2016). In this study mean HbA1c was 7.6%, with 38% of patients meeting theCanadian Diabetes Association target of ≤7.0%. An Indian study found the following percentages of T2DM patients at target: for HbA1c-45%, for BP <130/80 mm Hg-27%, and for LDL <100 mg/dl -37%(Menon&Ahluwalia, 2015). A large sample of 4926 T2DM patients was reviewed in the NationalHealth and Nutrition Examination Surveys (NHANES) from 1988-1994, 1999-2002, 2003-2006, and 2007-2010 (Stark Casagrande *et al*., 2013). In 2007-2010, 52.5% of people with diabetes achieved A1C <7.0%, 51.1% achieved BP <130/80 mmHg, 56.2% achieved LDL <100 mg/dL, and 18.8% achieved all three ABCs. These levels of control were perceived by the authors as significant improvements. Looking at these data we might be reassured about our results, which are quite similar.

We also compared our data to previous publications based on large population samples in our country. The mean fasting plasma glucose of 130 829 T2DM patients in the year 2000 was 8.14±2.79 mmol/l, and it was below 6.5 mmol/l in only 25.9% of the participants (Palaveev, Dimitrova, Cgristov, Petrova, Ilieva 2005). The situation today looks slightly better (see also Table 1). The National Examination of glycated hemoglobin evaluated32 356T2DM patients during the year 2003 (Christov, Kamenov, Georgiev 2006).Approximately 40% of the included patients had a HbA1c <6.5%, approximately 50% between 6.5 and 9.5 %, and 9 % between 9.51 and 12.5%. Again, the situation nowadays looks much better. The data on BP and lipid control in T2DM in our country are very scarce. A nation-wide epidemiological study reported data on lipids, obesity and BP coming from the whole population in 2012 (Borissova *et al*., 2015, 82-92; Borissova *et al*., 2015, 76-81; Borissova *et al*., 2015, 163-173; Borissova *et al*., 2015, 153-162; Borissova *et al*., 2015, 144-152). Arterial hypertension was found in 38.9% of the studied subjects (766 of a total of 1967), and was more prevalent in men (45.1%) than in women (33.5%) (Borissova *et al*., 2015, 76-81). These figures are quite similar to the percentages of T2DM patients achieving optimal BP control in our study. In the nation-wide epidemiological study 46.9% ofmen had hypertriglyceridemia *versus* 22.2% of women, while low HDL was found in 35.7% of women and 29.7% of men (Borissova *et al*., 2015, 163-173). These data unfortunately did not allow separate analysis for diabetes patients. In these series of studies the prevalence of normal BMI was 28.1%, 37.0% of overweight, and 33.2% of obesity (Borissova *et al*., 2015, 153-162). Waist circumference was >94 cm in 61.6% of men and >80 cm in 63.2% of women (Borissova *et al.*, 2015, 144-152). In conclusion, looking at the metabolic situation of the general population, we might feel reassured about the GP-led diabetes patients. Their metabolic status looked not much worse than that of the whole population.

Major limitations of our study are the cross-sectional design and the inclusion of a subgroup of type 2 DM patients-those on OADs with prevalent hypertension being managed by GPs. The cross-sectional design cannot detect temporal changes in cardiovascular risk factors. As an example, a population-based CVD prevention program in Sweden found that between 1991-1995 and 2006-2010, mean age-adjusted cholesterol and systolic blood pressure declined (the former by around 0.5 mmol/l and the latter by 3 mm Hg) with corresponding decreases in the age-standardized prevalence of hypertension and hyperlipidemia (Long *et al.*, 2013). Mean age-adjusted 2-hour plasma glucoseand BMI increased (by 0.2mmol/l and by 0.6-1.1 kg/m^2) with increases in the age-standardized prevalence of diabetes and obesity (Long *et al*., 2013). A similar trend was noted in the Tromso study assessing CV risk factors in relation to the diabetes status (Joseph, Svartberg, Njølstad&Schirmer, 2012). During the 14 years of follow-up the subjects with DM2 had decreasing levels of total and HDL-cholesterol andblood pressure (BP), and increasing levels of triglycerides, BMI, and antihypertensive treatment.

The second limitation of our study is that we assessed a targeted subgroup of hypertensive type 2 DM patients. Our main hypothesis was inspired by the increasing trend of diabetescombined with hypertension orhypercholesterolemia as reported in the NHANES data analysis 1999-2012 (Song *et al.*, 2016). In this specific analysis the treatment goal was achieved in 20.1%in the subgroup withconcurrent diabetes and hypertension-a finding very close to our results (Song *et al.*, 2016).

The major strength of our study is that it allows an up-to-date assessment of the metabolic control and CV risk factors in a subgroup of T2DM patients at particularly high risk due to the concurrent hypertension. It showed some improvements in metabolic control during the last 12-13 years with a reassuring trend inglycated hemoglobin and blood pressure. Unfortunately, it showed also a treatment gap leaving room for improvement in the field of obesity and dyslipidemia.

Summary and conclusions

The management of hypertensive type 2 diabetes patients by GPs in our country is quite adequate to contemporary guidelines for CV risk factor prevention. However, targeted efforts are needed to invert the negative trends in the prevalence of obesity and atherogenic diabetic dyslipidemia.

Acknowledgments. We acknowledge the support of the eighty-two GPs providing valid data for this study as well as Mr. Borislav Nanovski for his statistical expertise.
This work was supported by Pfizer Bulgaria as part of a joined project under the umbrella of the National Association of General Practitioners in Bulgaria.The funding sources had no involvement in the study design; inthe collection, analysis and interpretation of data; in the writing of the report; and in the decision tosubmit the article for publication.

Conflict of interest statement. None declared.

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In Memoriam

Прим. д-р Андон Г. Кочев

(30.09.1930-04.12.2019)

Прим. д-р Андон Г. Кочев, еден од првите специјалисти по општа медицина. Својот работен век го помина во родниот град Радовиш. Д-р Андон Кочев беше еден од основоположниците и столб на модерната медицина во источна Македонија. Изборот на доктор по медицина како кариера е комбинација од склоп на околности и особини на д-р Андон. Неговата докторска големина ја почувствувале речиси сите негови сограѓани. Не само што се грижеше за нивното здравје туку беше и дел од многу срекни и тешки моменти во нивните животи. Во 70-десетите години го изградил првиот современ здравствен дом во Македонија кој подоцна ќе биде за пример и во други градови во Македонија. Здравниот дом е пример за успешна здравствена организација за цело време на неговото директорување.

Во современите услови за работа создава тим на доктори од цела Македонија со кои несебично и пожртвовано работи. Уште тогаш обезбедува специјалистичко-консултативна амбуланта од сите специјалности во соработка со докажани специјалисти. Негова цел мегу другото е и зголемување на бројот на новородени деца што покажува забележани резултати. Скоро 90% од породувањата се обавуваат во пододилиштето во Радовиш. Тое е и еден од неговите специјалистички трудови. Со звањето Примариус се здобива мегу првите доктори специјалисти во Македонија.

Докторската кариера на д-р Андон Кочев не завршува тука. Во 1992 година ја формира првата приватана специјалистичка ординација по општа медицина во Македонија која успешно работи до денес. Својата љубов и посветеност кон медицината ја пренесува и на своите деца и внуци продолжувајки ја традицијата во медицината веќе трета генерација. Неговата прагматичност, секојдневното присуство, неизмерната љубов го направија неговото постоење безвременско во животите на неговата фамилија, пријателите и неговите колеги. Засекогаш ке го паметиме по убави работи.

Прим. д-р Љубин Шукриев

УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

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

Списанието ги има следниве рубрики и категории на трудови:

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

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

1. ТЕКСТ НА РАКОПИСОТ

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

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

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

Изворните трудови и **соопштувањата** го имаат следниов формален редослед: насловна страна, извадок на македонски јазик (вовед, методи, резултати, заклучок) со клучни зборови, извадок на македонски јазик со клучни зборови, вовед, материјал и методи, резултати, дискусија и заклучоци, литература и прилози (табели, графици и слики) и легенди за прилозите во еден фајл.

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

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

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

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

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

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

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

Заклучоците треба да не бидат подолги од 150 зборови.

2. ПРИЛОЗИ

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

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

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

Микрофотографиите може да содржат посебни ознаки во вид на стрелки или симболи. Покрај описот на сликата, мора да се наведе и зголемувањето и видот на боењето на препаратот (ако тоа веќе не е направено во секцијата *материјал и методи*).
Сите ознаки на фотографиите мора да бидат доволно големи, за да може јасно да се распознаат и по смалувањето во печатницата, при нивното вклучување во печатената страница на списанието.

3. ЛИТЕРАТУРА

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

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

б) заеднички автор

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

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

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

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

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

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Известување за членовите на МЛД

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

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

Известување за рецензентите за ММП

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