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HOW TO RECOGNIZE AND AVOID POTENTIAL, POSSIBLE, OR PROBABLE PREDATORY OPEN-ACCESS PUBLISHERS, STANDALONE, AND HIJACKED JOURNALS

Lenche Danevska[1], Mirko Spiroski[2], Doncho Donev[3], Nada Pop-Jordanova[4], Momir Polenakovic[4]

[1] Central Medical Library, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, R. Macedonia
[2] Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, R. Macedonia
[3] Institute of Social Medicine, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, R. Macedonia
[4] Macedonian Academy of Sciences and Arts, Skopje, R. Macedonia

Corresponding author: Lenche Danevska, MSc, Central Medical Library, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia, e-mail: lencedanevska@yahoo.com

Abstract

Introduction and aim: The Internet has enabled an easy method to search through the vast majority of publications and has improved the impact of scholarly journals. However, it can also pose threats to the quality of published articles. New publishers and journals have emerged so-called open-access potential, possible, or probable predatory publishers and journals, and so-called hijacked journals. It was our aim to increase the awareness and warn scholars, especially young researchers, how to recognize these journals and how to avoid submission of their papers to these journals.

Methods: Review and critical analysis of the relevant published literature, Internet sources and personal experience, thoughts, and observations of the authors.

Results: The web blog of Jeffrey Beall, University of Colorado, was greatly consulted. Jeffrey Beall is a Denver academic librarian who regularly maintains two lists: the first one, of potential, possible, or probable predatory publishers and the second one, of potential, possible, or probable predatory standalone journals. Aspects related to this topic presented by other authors have been discussed as well.

Conclusion: Academics should bear in mind how to differentiate between trustworthy and reliable journals and predatory ones, considering: publication ethics, peer-review process, international academic standards, indexing and abstracting, preservation in digital repositories, metrics, sustainability, etc.

Keywords: predatory publishers, standalone journals, hijacked journals, open access

Introduction

Over the past several years there has been a debate in academic circles on the issue of how to recognize potential, possible, or probable open-access predatory scholarly publishers and how to avoid publishing in so-called hijacked journals.

Researchers, scholars, doctors and academic staff need to publish the results of their work and make them accessible to their colleagues and to the public. Also, academics tend to publish as many papers as possible in order to be promoted within their academic institutions. At this point, they have to cope with the issue of how to choose a relevant, reliable, true, peer-review journal, indexed in a reputable scientific database and then submit their manuscripts for publication. Since the emergence of these potential, possible, or probable open-access predatory publishers and journals this task has be-

come even more daunting for scholars. Online hackers and cybercriminals have built fake or counterfeit websites for journals that actually mimic reputable journals.

Background: In 2008 Jeffrey Beall, an academic librarian and researcher at the University of Colorado, Denver, U.S.A. had received a large number of e-messages inviting him to submit articles to journals he was not familiar with. He then started extensive research on open-access publishers and coined the term "predatory scholarly open-access publishers". Such publishers use a business model where authors have to pay in order to publish their articles. In the *Chronicle of Higher Education* from 2012 Beall gives his definition of *predatory open-access publishing: "Predatory open-access publishers are those that unprofessionally exploit the gold open-access model for their own profit. That is to say, they operate as scholarly vanity presses and publish articles in exchange for the author fee. They are characterized by a various level of deception and lack of transparency in their operations. The open-access publishing model seems like a recipe for abuse: The more articles a publisher publishes, the more money it makes."* [1].

The aim of this paper is to give authors guidelines on how to recognize hijacked journals and to avoid cooperation with potential, possible, or probable predatory open-access publishers; that is, to raise awareness of dishonest publishing practices.

Methods

To accomplish the set aim, we have used information from the relevant published literature and registered websites available to the public, as well as our personal experience, thoughts, and observations.

First of all, we have consulted the blog platform and website of Jeffrey Beall (see: https://scholarlyoa.com) [2].

We have also consulted the list compiled and updated by Dr. Mehrdad Jalalian, a physician, journalist, book publisher and publication ethics researcher, who considers himself the world's leading researcher on the topics of hijacked journals (See: http://www.mehrdadjalalian.com/index.php/updates-of-hijacked-journals) [3].

To further illustrate the topic, we present the characteristics of predatory publishers and hijacked journals (Box 1).

Box 1. Characteristics of Predatory Publishers and Hijacked Journals
Large fees for articles revealed only after papers are submitted
Aggressively campaigning for academics to submit articles or serve on editorial boards
Listing academics as members of editorial boards without their permission
Appointing fake academics to editorial boards
Mimicking the name or web site style of more established journals
Improper use of ISSNs
Fake or non-existent impact factors
Accepting articles quickly with little or no peer review or quality control
Journals are not listed in standard periodical directories (such as Urlich's Periodicals Directory)

Results

Potential, possible, or probable predatory publishers

Predatory publishers use spam email to invite authors to publish their manuscripts, usually indicating large fees after papers are submitted. In fact, predatory publishing uses the open-access publishing business model, where it is very easy to set up an open-access publishing website. These websites can be created by almost anyone who has some knowledge of how to design them [4, 5]. They charge publication fees to authors without providing the editorial and publishing services associated with legitimate journals. Sometimes they even negotiate a lower fee, when a potential author would comment on excessive fees. They indulge in very unethical and unscholarly practices just to collect money for publication. These publishers do not respect any of the policies and guidelines given by the Council of Science Editors [6], International Association of Scientific, Technical & Medical Publishers (STM) Code of Conduct [7], or Committee on Publication Editors (COPE) [8]. Instead, to promote, preserve and make the published material available, these publishers exploit the author-pays model for their own profit. They also call

academics to serve on the editorial boards in order to present an impression that it is a respectable journal. They have little or no peer review in most cases. Some claim to assess submission within 72 hours and digitally publish them upon acceptance and receipt of the fee. *"If the peer-review process were only that simple!"* – says Robert Bartholomew in his paper [9, 10]. Many of them have no digital preservation and they can disappear at any time, which will result in the loss of content.

Declan Butler, in his excellent paper on the explosion of open-access publishing [11], offers a checklist to identify reputable publicshers. He warns authors to perform due diligence before they submit their manuscripts to a journal. He advises authors to: check whether the publisher has verifiable contact information, check whether editorial board list includes recognized experts with full affiliations, check whether the journal prominently displays its policy for author fees, be cautious when receiving e-mail invitations to submit to journals or to become an editorial board member, etc.

Beall has created *Beall's List of potential, possible, or predatory scholarly open-access publishers* (See: https://scholarlyoa.com/publishers/) [12] and he updates it regularly. He published his first list of predatory publishers in 2010 and in 2012 he posted his criteria for determining and evaluating publishers (See: https://scholarlyoa.com/2012/11/30/criteria-for-determining-predatory-open-access-publishers-2nd-edition) [13].

Here is one example of a medical publisher with some problems, which has been recently presented by Beall on his site [14]. It is InnoVison Health Media, which is a Minnesota-based publisher of six online medical journals. Beall has investigated this publisher and discovered that there were many editorial problems, including late issues, poor editing practices, etc. as well as questionable editorial boards. Therefore, Beall recommends that researchers consider publishing their papers in higher-quality journals than in InnoVision's offerings.

Potential, possible, or probable predatory open-access publishers have no transparency in publishing operations, provide insufficient information or hide information about author fees, falsely claim to have their content indexed in legitimate abstracting and indexing services, operate based in a Western country chiefly for the purpose of functioning as a vanity press for scholars in a developing country, copy "authors guidelines" from other publishers, do not use ISSN numbers, DOI numbers or use them improperly, and so forth.

Potential, possible, or probable predatory scholarly open-access journals (standalone journals)

These standalone journals do not have an official publisher behind their work. They act essentially alone, that is to say, on behalf of one or several individuals. Those who publish in predatory journals are, for the most part, young and inexperienced researchers from developing countries [15]. We believe that economic and sociocultural conditions in these developing countries have contributed to the differences found in authorship between predatory and non-predatory journals [16]. These new journals are actually competing for authors and their money and offer little in return [17].

Jeffrey Beall has also created a list of questionable, scholarly open-access standalone journals (See: https://scholarlyoa.com/individual-journals/) [18].

For example, one of the journals on his list is American Journal of Advances in Medical Science (ARNACA) [19]. If you visit this website, you will discover that the chief editor, associate editor, and managing editor are from India, and all the members of the Editorial Board are from Asian countries. Furthermore, all the reviewers are from India, yet the journal title is "*American* Journal of Advances in Medical Science".

Hijacked journals

Hijacked journals are those that try to defraud academics and researchers by using the name and reputation of the original journals. They usually send e-mails to attract their victims who are from certain countries (usually low and middle-income countries).

The journal falsely claims to have an impact factor and to be included in reputable databases. It lacks peer-review, or the corresponding author is asked to suggest reviewers,

who are subsequently used later by the publisher. Hijacked journals are usually not listed in standard periodical directories or are not cataloged in library databases.

The number of hijacked journals has rapidly increased over the past several years. People included in this process have managed to cheat professors and Ph.D. scholars who are in urgent need of publishing their articles in journals that are found on the Web of Science Journal Citation Reports. Hijackers create a journal website and attract authors by indicating an impact factor of the journal, which means that it is a Thomson Reuters indexed journal, and by conducting the peer review process in just a couple of weeks.

Also, many require considerable manuscript processing charges for authors. Such journals are considered to be primarily interested in making quick money and paying little or no attention to peer review [16].

A study by Jalalian and Mahboobi has shown that many of the fake journals have started to imitate the features of respectable scientific journals, and not only some relatively young journals but also such with a long tradition [20]. They even mimic the name of the journals. Among these journals are Wulfenia Journal, Jokull Journal, or Sylwan.

Authors can be easily deceived when they receive an invitation to submit their manuscript to journals whose title or logo closely resembles a highly respected publication [17].

Here is one example: The real Wulfenia journal may be found on the following website http://www.landesmuseum.ktn.gv.at/210226w_DE.htm?seite=15 [21] where there is a warning on the other website where the hijacked Wulfenia Journal is found (Box 2).

Box 2. Warning at the genuine Wulfenia journal regarding websites of the hijacked Wulfenia Journal [21]

The real Wulfenia journal website:
http://www.landesmuseum.ktn.gv.at/210226w_DE.htm?seite=15
Warning about the other website where the hijacked Wulfenia Journal is found:
Warning!
The websites
www.wulfeniajournal.at
www.wulfeniajournal.com
www.multidisciplinarywulfenia.org
are not the official websites of the journal "Wulfenia: Mitteilungen des KärntnerBotanikzentrums" published by the Regional Museum of Carinthia. These websites criminally usurp the identity of the official journal. They fraudulently use false information, a false editorial board, and false publication requirements to encourage authors to submit articles and to transfer page fees to a bank account in Yerevan (Armenia).

The list of hijacked journals created by Jeffrey Beall can be seen at: https://scholarlyoa.com/other-pages/hijacked-journals/ [22]. He updates it regularly.

There is also another list created by Dr. Mehrdad Jalalian, journalist, and researcher, who is particularly concerned with the issue of hijacked journals [23]. His hijacked journal list can be found and consulted from the following website: http://www.mehrdadjalalian.com/index.php/list-of-hijacked-journals-and-fake-publishers/30-hijacked-journal-list-2014-first-edition-june-2014 [24].

We have to emphasize the fact that this issue of hijacked journals is a great threat for medical sciences, that is, for clinical practice and health policy making. Many of the articles published in these journals will appear in the search results when retrieving literature and will be a source of new medical hypotheses that can be used to attack the reliability and validity of future clinical research [25–27].

Mehdi Dadkhah and Giorgio Bianciardi [28] in their paper discuss the possible ranking of predatory journals. First, they present criteria for detection of predatory journals, which include: editorial members' credentials, review process, and publishing, announcements, Open Access policies and publication charges. Further in their paper they present their predatory ranking metric entitled "predatory rate", based on the noted criteria.

Discussion

Open-access is a noble concept by which research is freely accessible to scholars and the

public. It has brought substantial changes to higher education. Many open-access journals are legitimate and contribute to scientific knowledge, but recently a significant number of untrustworthy journals has appeared [9, 29].

New terms have been coined: predatory publishers and predatory journals referring to fraudulent publication practices. In the literature much has been lately written on predatory journals, but not on hijacked journals [5, 16, 30–33]. The intention of those who have dealt with the issue has been to raise awareness among scholars how to recognize and avoid submission of manuscripts to potential, possible, or probable predatory journals and hijacked journals.

This study has purposely been presented in biomedical journals published in Macedonia since we belong to this academic community. It is an imperative of the editorial board of the journals to inform scholars about this new threat on the publishing scene. Academics involved in faculty and staff promotion processes should warn and advise young scholars where to submit their papers for publication and the tenure and promotion review committees should be prepared to conduct a serious assessment of articles published in standalone or hijacked journals. Unethical scientists earn tenure and promotion at the expense of the honest [34]. The higher education sector has to employ academic rigor so as to maintain quality and integrity within publishing practice. Academics have to be more skilled in their own digital skills that will help them to identify fraud on the Internet and "low credibility", counterfeit, and predatory journals [35, 36].

Scientists must be able to recognize publishing fraud. Although there is no real clue to the problem, suggestions have been offered how to combat predatory publishers and journals. There is an ongoing debate over the use of black- and white-lists of journals, as well as over the use of metrics, being identified as a problematic factor and needs further elaboration in some other study [29].

"A black-list is easier to compile and maintain than a white list and by its nature contains more updated information than a white-list could. I often hear criticisms of my lists. Some believe that the predatory publicshing problem is really a small problem, and my highlighting the problem is making it appear bigger than it really is. Others claim that we really need to give these predatory publishers a larger opportunity to succeed, that it is not fair to attack people from poor countries...." – said Jeffrey Beall in Learned Publishing [4, 11, 37].

Open-access associations such as Open Access Scholarly Publishers Association (OASPA) and Directory of Open Access Journals (DOAJ) should have a set of criteria to which publishers and journals must comply with in order to be considered trustworthy. In fact, OASPA was founded in 2008 after facing the challenges of OA journals. DOAJ is continually working to strengthen the journal approval process and it has already tightened up its inclusion criteria, with the purpose of serving as a white-list, as opposed to Beall's black-list. Also, regarding medical publishers and journals, the World Association of Medical Editors (WAME) has collaborated with the Committee on Publication Ethics (COPE), DOAJ and OASPA and has developed *Principles of Transparency and Best Practice in Scholarly Publishing*. Editors of peer-reviewed medical journals should adhere to these principles [38, 39].

Beall said that he engaged himself in this topic partly by his sense of duty, as an academic librarian, to evaluate online resources and to help patrons to "recognize scholarly publicshing scams and avoid them", and partly by the "private and very positive feedback" he receives from researchers and librarians [11]. Thus, academics may consult Beall's weblog and check the credibility of the listed journals. They cannot solely rely on his list, but should make their own evaluations as well.

Some criticize Beall's work or wonder whether it is fair to classify these publishers as "predatory" [40–42] stating that Beall is acting as prosecutor, judge and jury on who is predatory and who is not. Some say that it is an open question whether it is fair to classify these publishers and journals as "predatory" [43]. However, many state that Beall's list is widely read and consulted by librarians and researchers, and they applaud his efforts to reveal shady publishing practices. Some publishers, for example, the Academic Research Publishing Agency publish journals that cover very

broad subject areas. It is difficult to image how a single journal of this publisher, *International Journal of Research and Reviews in Applied Sciences* [44] can validate papers from such a wide range of scientific fields (computer science, mathematics, economics, applied physics, nuclear engineering, chemistry, and many more) [43].

We are absolutely confident that scholars should avoid publishing their papers in hijacked journals; academics should refuse membership on Editorial Boards of such journals and they should not accept reviewing any papers submitted for publication in hijacked journals. The question on whether to accept the already published papers in these hijacked journals during the tenure and promotion processes is still under debate, since not all authors are informed about the existence and identification of these journals. Some authors suggest that already-published papers in the hijacked journals deserve a second chance, that is, these papers can be published in other legitimate journals and cannot be considered as plagiarized papers [31].

The situation is slightly different with the potential, possible, or probable open-access publishers and/or journals. These categories contain different sub-groups that are very roughly categorized in the Beall's list [41]. There is a real possibility that publishers and journals from poor, underdeveloped and developing countries be unfairly compared with those from developed countries. It means that high criteria from highly developed countries are also applied for developing countries creating comparison bias [45, 46]. Therefore, recommendations given for hijacked journals cannot be entirely applied to predatory publishers and/or journals. It is crucial to raise awareness among scholars for their existence and to increase efforts for their recognition and identification as well as to advise the editorial board to improve their quality and to follow the principles of international publishing standards.

In our opinion, everyone should check the following items or look for answers to some questions prior to making his/her decision where to submit the manuscript for publication (Box 3).

Box 3. Items to be checked or questions to be answered prior to making decision where to submit the manuscript for publication
the exact title of the journal
names of the editors
its place of publication and journal's business address
contact information
publication fees
sustainability
indexing databases
ISSN number
statement of publishing ethics, COPE membership
impact factor in the Thomson Reuters list
the quality of the already published papers, evidence of peer review
preservation that is depositing the digital content with a trusted, financially secure library (for example many publishers deposit their digital contents in the British Library)
have leading scholars in the field you are interested in, have already published articles in those journals
consult black- and white-lists of journals
think critically and don't do anything to compromise your career
resist the temptation to publish quickly
share information about fraudulent practices on scholarly social networks

Further studies are necessary in order to cover other aspects of potential, possible, or probable predatory publishers and journals, including the used metrics and financial issues.

Recently, at the meeting of the Annual Assembly of the Macedonian Association of Medical Editors (MAME), held on 13 April 2016, special attention was given to "Critical analysis of publishing in journals with Open Access", emphasizing the journals which should not be considered for submitting papers to them. At the MAME website (See: www.mame.mk) [47] separate links are available to approach "Potential, possible, or probable predatory open access

publishers", "Potential, possible, or probable predatory open access journals", "Hijacked journals" and "Wrong metrics for journals". Predatory publishers and journals were recognized as the most serious problem and threat. It was proposed to inform the Faculty of Medicine and the Ss Cyril and Methodius University in Skopje and other universities in R. Macedonia to adjust the procedure and criteria for election in academic educational and scientific titles so as not to recognize the papers published in journals by publishers whose names can be found in Beall's list [12]. The warning was also directed to hijacked journals that are kidnapped by another publisher to earn huge sums illegally.

Since the issue on predatory publishers and hijacked journals is certainly targeting medical scholars, we decided to publish this paper in a number of Macedonian biomedical journals in order to warn not only young scholars, but also all medical professionals and academic institutions on the threat of being falsely attracted to publish their manuscripts in illegitimate journals.

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Р е з и м е

КАКО ДА СЕ ПРЕПОЗНААТ И ДА СЕ ИЗБЕГНАТ ПОТЕНЦИЈАЛНИ, МОЖНИ ИЛИ ВЕРОЈАТНИ ИЗДАВАЧИ, СПИСАНИЈА-ГРАБЛИВЦИ СО ОТВОРЕН ПРИСТАП И КИДНАПИРАНИ СПИСАНИЈА

Ленче Даневска[1], Мирко Спироски[2], Дончо Донев[3], Нада Поп-Јорданова[4], Момир Поленаковиќ[4]

[1] Централна медицинска библиотека, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[2] Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[3] Институт за социјална медицина, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[4] Македонска академија за науките и уметностите, Скопје, Р. Македонија

Вовед и цел: Интернетот овозможи лесен метод за пребарување огромен број публикации и го зголеми влијанието на научните списанија. Меѓутоа, тој, исто така, претставува и закана за квалитетот на објавените трудови. Се појавија нови издавачи и списанија, т.н. потенцијални, можни или веројатни издавачи и списанија-грабливци со отворен пристап и т.н. киднапирани списанија. Целта на трудот беше да се подигне свеста и да се предупредат научниците, а особено младите истражувачи, како да ги препознаат овие списанија и како да избегнуваат да ги поднесуваат своите трудови во вакви списанија.

Методи: Преглед и критичка анализа на релевантна објавена литература, интернет-извори на информации и лично искуство, размислувања и погледи на авторите на трудот.

Резултати: Најмногу го користевме блогот на Џефри Бел од Универзитетот во Колорадо. Тој е библиотекар во Денверската академска библиотека и на својот блог редовно ги дополнува двете листи: првата за потенцијални, можни или веројатни издавачи-грабливци и, втората, за потенцијални, можни или веројатни грабливи и киднапирани списанија со отворен пристап. Во трудот се претставени и ставовите на други автори во врска со оваа проблематика.

Заклучок: Научниците треба да прават разлика меѓу списанија кои се веќе афирмирани и кредибилни и списанија-грабливци, притоа имајќи ги на ум следниве работи: етика во публикувањето, процес на рецензирање на трудовите, интернационални академски стандарди, индексирање и вклучување во бази на податоци, зачувување во дигитални репозиториуми, метрика, одржливост итн.

Клучни зборови: издавачи-грабливци, списанија-грабливци, киднапирани списанија, отворен пристап

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HORMONE THERAPY REDUCES BONE RESORPTION BUT NOT BONE FORMATION IN POSTMENOPAUSAL ATHLETES

Suzy Y Honisett[1], David Pagliaro[1], Kathy Tangalakis[1], Bronwyn Kingwell[2], Peter Ebeling[3], Robyn Craven[4], Juliana Antonopillai[1], Vasso Apostolopoulos[1], Lily Stojanovska[1,*]

[1] Centre for Chronic Disease, College of Health and Biomedicine, Victoria University, Melbourne VIC Australia
[2] Baker Heart Research Institute, Prahran, VIC Australia
[3] Department of Diabetes, Endocrinology and Medicine, The University of Melbourne, The Royal Melbourne Hospital, Parkville, VIC Australia
[4] Women's Health and Breast Clinic, Freemasons Hospital, East Melbourne, VIC Australia

Corresponding Author: Lily Stojanovska, Centre for Chronic Disease, College of Health and Biomedicine, St Albans Campus, Victoria University, VIC 3021, Australia; tel: +613 99192737; fax: tel: +613 99194565;
e-mail: lily.stojanovska@vu.edu.au

Abstract

Introduction: Independently, hormone therapy and exercise have well-established protective effects on bone parameters. The combined effects of hormone therapy and exercise, however, are less clear. We, therefore, examined the effects of hormone therapy on bone turnover markers in postmenopausal women undergoing regular high intensity exercise.

Methods: In a randomised, double blind study, postmenopausal athletes competing at Masters level, received either hormone therapy (50 μg transdermal oestradiol, 5 mg MPA, n = 8) or placebo (n = 7) for 20 weeks. Women were tested before and after treatment for plasma concentrations of oestradiol, FSH, LH, and serum bone formation marker -osteocalcin (OC); and urine bone resorption markers-pyridinoline (PYD) and deoxypyridinoline (DPD).

Results: As a result of treatment with hormone therapy there were significant reductions in levels of FSH (73.3 ± 13.7 to 48.6 ± 10.5 mmol/L, $p = 0.01$) and bone resorption markers (PYD, 81.9 ± 7.7 to 57.8 ± 3.7 nmol/mmol Cr, $p = 0.001$, and DPD, 18.5 ± 3.1 to 11.8 ± 2.1 nmol/mmol Cr, $p = 0.01$). Oestradiol and bone formation markers were not significantly altered as a result of hormone therapy. There were no changes to any variables with placebo treatment.

Conclusion: Hormone therapy reduced bone resorption, but not bone formation, in postmenopausal athletes. These favorable reductions in bone turnover; therefore, provide an effective treatment in combination with high intensity exercise to further reduce the subsequent risk of osteoporosis and associated fractures.

Keywords: Exercise, menopause, bone turnover, hormone replacement therapy, estradiol

Introduction

Menopause is associated with an increase in bone turnover markers [1–3], and an elevated risk of osteoporotic fractures [1, 3, 4]. Hormone therapy and exercise are 2 interventions commonly prescribed to ameliorate this risk.

Estrogen administration has been shown to slow bone loss in postmenopausal women [5–7], providing protection against the development of osteoporosis and the risk of fracture [8]. This reduction in bone loss is apparent in measures of biochemical markers of bone formation and resorption, with estrogen producing a net reduction in these markers [9, 10].

A number of meta-analysis studies have been conducted that assess the effects of phy-

sical activity on bone loss in pre- and post- menopausal women. High impact exercise increases bone mineral density (BMD) in premenopausal women [11, 12], whereas, 8 months of Astanga yoga program provide beneficial effect on bone formation but did not improve BMD in premenopausal women. One study [13] found that the overall treatment effect of endurance training regimes prevented or reversed almost 1% of bone loss per year in lumbar spine and femoral neck, whilst two other meta analyses [14, 15] found a beneficial effect of impact exercise on the spine in post-menopausal women.

Despite these positive independent associations of exercise and hormone therapy on bone parameters, there are only limited number of studies that have examined their combined effects in a postmenopausal population. The potential benefits of physical activity in synergy with hormone therapy are inconsistent, and the results unclear [16, 17]. Prospective studies have reported improved bone mineral density (BMD) after initiating hormone therapy and exercise in previously sedentary postmenopausal women [16, 17]. However, it is difficult to ascertain in these studies what proportions of the beneficial effects are attributable to initiating exercise or hormone therapy. Although this area is not new, it illustrates the need for a well-controlled approach to this topic, which addresses a direct comparison of exercise with hormone therapy, whereby both interventions are carried out optimally.

Herein, we determined whether hormone therapy would provide further improvement to bone turnover when given to postmenopausal women currently undertaking regular high intensity weight bearing exercise.

Methods

Subjects

Postmenopausal women were recruited from 'Masters' athletic running associations. Sixteen women were measured at baseline and fifteen women completed the study. The criteria for inclusion were: 1–5 years postmenopause; aged between 45–60 years; competing at 'Masters' level in athletics, or vigorously running, jogging or cycling at least 4 times a week; a VO_2 max of greater than 30 ml/min/kg^2 which isconsidered very good to high cardio-respiratory fitness for age. The criteria for exclusion were: taking any form of hormone therapy; previous hysterectomy or oophorectomy; family history of malignancies; established cardiovascular disease; smoking; excessive alcohol use or taking any drug therapy that would affect bone mineral and mineral metabolism. All women were cleared for participation following a full physical examination by a clinician. Recruited women were randomly assigned into two groups using a double blind design. Eight women were assigned into the 'ill group', and commenced using transdermal oestradiol patches (Estraderm 50 μg twice/week, Novartis Pharmaceuticals, North Ryde, NSW, Australia) and oral medroxyprogesterone acetate (MPA, 5 mg / day, Pharmacia Pharmaceuticals, Rydalmere, NSW, Australia). Seven women were assigned to the 'placebo' group and commenced using placebo patches and oral placebo tablets. One woman dropped out of the study due to skin irritation caused by the trans-dermal patch. All women gave informed consent to participate in this study, which was approved by the Human Ethics Committee, Victoria University (991017/CO484).

Protocol

All women underwent a number of measures at baseline (Tl) and at twenty weeks (T2). These included: blood samples for measurement of oestradiol, luteinising hormone (LH), follicle stimulating hormone (FSH), and the bone formation marker osteocalcin (OC), urine samples two hours post-first morning void for measurement of the bone resorption markers pyridinoline (PYD) and deoxypyridinoline (DPD); and aerobic capacity. Height, weight and body mass index (BMI) were also determined for all subjects. The above-mentioned biochemical markers of bone formation and resorption are of value in estimating bone turnover and have been used to reliably and consistently identify rapid bone losses [18]. Cross-sectional studies [3, 19] have shown that bone turnover rates, evaluated by these markers, increase at menopause and remain elevated, whilst bone turnover rates in postmenopausal women correlate negatively with BMD [20]. Participating subjects were requested to maintain their current training routine and diet throughout the study.

Assays

Serum oestradiol was measured using a competitive chemiluminescent immunoassay (Gba-Coming, Medfield, MA, USA). FSH and LH were measured using a two-site chemiluminescent (sandwich) immunoassay (Gba-Coming, Medfield, MA, USA). Serum OC was measured by radioimmunoassay using the osteocalcin RIA kit (Incstar Corp., Stillwater, MN, USA). Total PYD and DPD cross -links of type I collagen were measured in hydrolyzed urine samples by high performance liquid chromatography, corrected for urine creatinine and expressed as nmol/ mmol Cr [1].

Aerobic Capacity (VO_2 max)

The laboratory where the trials were conducted was maintained at a constant temperature (20 ± 1°Q and humidity (44 ± 2%). A 12 lead ECG (Montara, X-scribe, Stress testing System, Milwaukee, USA) was used to monitor subjects' heart rhythm and rate throughout the duration of the test. Subjects exercised on a stationary bicycle ergometer (Cybex Metabolic System, Met 100, Huntsville, USA) at a constant cadence of 70 rpm. Increments in cycle resistance occurred, commencing at 35 watts and increasing by 10 watts every minute. These increments continued until the subject felt they could not continue due to exhaustion, or until the subject experienced discomfort, or the practitioner stopped the test for medical reasons. Each subjects' oral gas expiration was measured via Vacumetric Vista Turbofit Software package (Version 3.2, Ametek, Pittsbergh, USA) to determine their respiratory exchange ratio (RER) and VO_2 max. Both variables were subsequently charted throughout the duration of the exercise to ensure that their aerobic threshold was reached. Subjects were expected to have reached their VO_2 max (a measure of maximal aerobic capacity) when at least two of the following criteria were achieved (a) a plateau of VO_2 max readings, (b) exercising heart rate to within 10 beats of subjects' maximal heart rate, (c) an RER of greater than 1.10.

Statistical Analysis

The data were analysed using Sigma Stat. 2.0 Gandell, USA). The data are reported at baseline as mean ± standard error of the mean (SEM). Changes to variables with treatment are reported as percent change from baseline. A Student's paired t-test was used to determine significance of changes with treatment. At-test was used to determine differences between treatment groups at baseline. Significance was reported as $p < 0.05$.

Results

There were no significant differences between the two groups at baseline for: age, BMI, VO_2 max, serum oestradiol and gonadotrophin levels, OC and DPD (Table 1). Urine PYD was significantly higher in the HRT group compared at baseline with the placebo group ($p = 0.03$) (Table 1).

Table 1

Baseline groups characteristics in the two groups of postmenopausal athletes

Variable	**Placebo N = 7**	**Hormone Therapy N = 8**
Age, years	54.7 ±1.3	53.8 ±1.8
BMI, $kg.m^2$	22.2 ±1.2	23.5 ±1.2
Estradiol, mmol/L	76.1 ±12.1	55.1 ±26.1
FSH, mmol/L	70.8 ±13.5	73.3 ±13.7
LH, mmol/L	38.2 ±6.7	40.9 ±9.1
VO_2 max, ml/kg/min	35.6 ±3.0	35.2 ±2.0
Osteocalcin, U/L	32.5 ±9.9	31.2 ±10.2
PYD, nmol/mmol Cr	64.0 ±7.0	81.9 ±7.3*
DPD, nmol/mmol Cr	13.4 ±1.8	18.5 ±3.1

Values are mean ± SEM. BMI indicates body mass index; FSH, follicle stimulating hormone; LH, luteinising hormone; VO_2 max, maximal aerobic capacity; PYD, pyridinoline; DPD, deoxypyridinoline. * indicates a significant difference, $p < 0.05$, between groups

After 20 weeks of treatment, there were no significant changes in any parameter in the placebo group. However, in the HRT group FSH levels significantly decreased (73.3 ± 13.7 to 48.6 ± 10.5 mmol/L, $p = 0.01$, mean ± SEM) indicating that subjects were compliant with HRT. Medication compliance was verified by medication counts.

VO_2 max was unchanged in both groups (HRT group 35.2 ± 2.0 to 37.0 ± 2.1 ml/kg/min, $p = 0.27$; placebo group 35.6 ± 3.0 to 34.6 ± 3.6 ml/kg/min, $p = 0.66$) indicating that subjects maintained their existing exercise training regimen and fitness level throughout the duration of the trial. The bone formation marker, OC, remained unchanged with HRT administration (31.26 ± 10.2 to 32.3 ± 11.8, $p = 0.53$). However, there were significant reductions in both bone resorption markers after 20 weeks of HRT, with PYD decreasing by 27.3% (81.9 ± 7.3 to 57.8 ± 3.7, $p = 0.001$) and DPD decreasing by 33.0% (18.5 ± 3.1 to 11.8 ± 2.1, $p = 0.01$) from baseline (Figure 1).

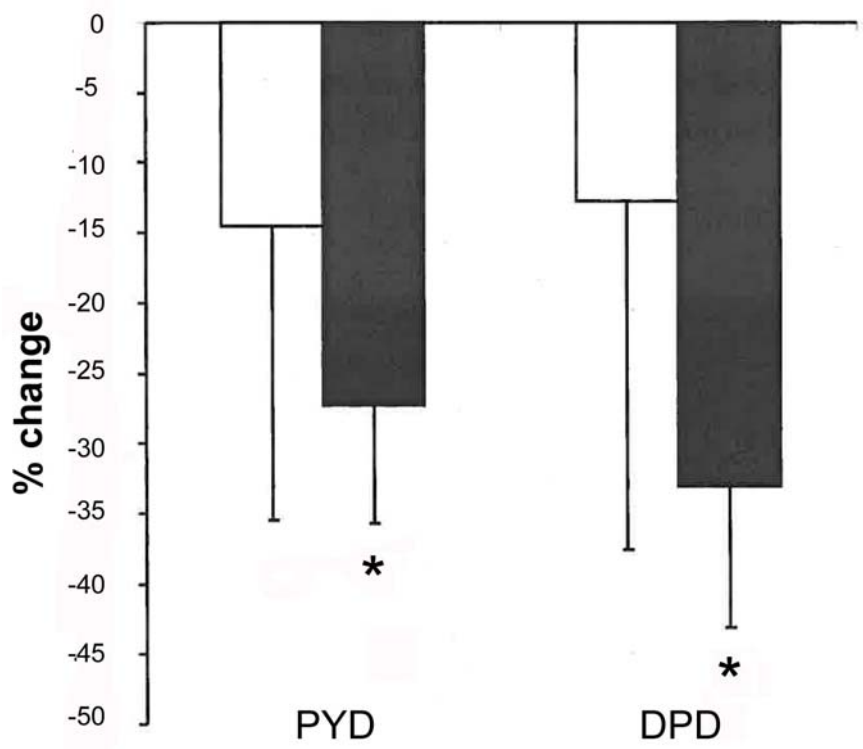

Figure 1 – Percentage change from baseline in resorption markers with placebo (white) and hormone replacement therapy (HRT) (black) treatment in postmenopausal athletes

Discussion

The results of this study demonstrate that athletically trained postmenopausal women are able to significantly reduce bone resorption with the use of hormone therapy; indicating that a combined intervention exceeds the effects of exercise alone on bone.

Many older women are involved in endurance-based exercises such as running, jogging and cycling, and compete at 'Masters' level athletics. For these women skeletal integrity is an important issue that may affect their continued performance in their chosen event after menopause. An improvement in BMD is shown with endurance exercise [13]; however, this form of exercise is not as effective as resistance or weight training [21]. Animal studies have shown that maximal osteogenesis occurs with high-magnitude strains applied with few strain cycles, as performed with resistance training [22–25]. Moreover, in a postmenopausal osteoporosis rat model, exercise significantly increased bone mass by both inhibiting bone resorption and increasing bone formation in trabecular bones [26]. Therefore, to assist in the maintenance and/or improvement of skeletal integrity after menopause, hormone therapy is a viable option.

There have been a number of prospective studies assessing the effect of combined treatment on BMD in postmenopausal women yielded conflicting results [16, 17]. One study identified that weight bearing exercise and hormone therapy independently increased BMD at sites of lumbar spine and proximal femur, while a combined treatment provided an additive effect on BMD at lumbar spine and was synergistic for total body BMD [16]. Another study has shown that combined resistance training and hormone therapy increased BMD at sites of radial mid-shaft, spine and total body, while hormone therapy alone was associated with no change, or bone maintenance, after one year [17]. The effect of exercise alone was not measured in this study making it difficult to assess whether BMD accretion was due to the combination of treatments or due to the effect of exercise alone. In contrast to these studies, another prospective study found moderate exercise alone, including weight lifting, provided no changes in BMD, whilst the combination of exercise and hormone therapy provided no greater benefit in comparison to hormone therapy alone [27]. All of these studies introduced exercise in previously sedentary women, making it difficult to determine whether any changes in bone status were due to the initiation of exercise or the commencement of hormone therapy. None of the studies addressed endurance based exercise regimes. One recent cross-sectional study investigating 'Masters' trained postmenopausal athletes found that the women replacing estrogen had greater BMD of the hip, spine and total body compared with athletes not replacing estrogen [28]. However, years post-menopause were significantly greater in the women not replacing estrogen, possibly affecting BMD values and limiting the conclusions from this study.

The present study investigated women currently involved in endurance-based weight bearing exercises and provided a direct comparison of interventions using a well-controlled approach. This study assessed the impact of hormonal therapy treatment for 20 weeks on bone turnover markers that, to the best of our knowledge, have not been addressed in this population of postmenopausal athletes before.

Interestingly in this population of postmenopausal athletes, hormone therapy for 20 weeks provided added benefit to bone status by significantly decreasing bone resorption but not bone formation, suggesting that these interventions may be highly effective when used in combination to further reduce the risk of bone loss and osteoporotic fracture. Larger controlled prospective studies of longer duration are required to assess effects of hormonal therapy and endurance-based weight bearing exercise on bone density.

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Резиме

ХОРМОНСКАТА ТЕРАПИЈА ЈА НАМАЛУВА КОСКЕНАТА РЕСОРПЦИЈА, НО НЕ И ФОРМИРАЊЕТО НА КОСКИТЕ КАЈ АТЛЕТИ ВО ПОСТМЕНОПАУЗА

Сузи Ј Хонисет[1], Дејвид Палјаро[1], Кети Тангалакис[1], Бронвин Кингвел[2], Петер Ебелинг[3], Робин Крејвен[4], Цулијана Антонопилаи[4], Васо Апостолопулос[1], Лили Стојановска[1]

[1] Центар за хронични болести, Школа за здравство и биомедицина, Универзитет Викторија, Викторија, Австралија
[2] Истражувачки институт „Бејкер Харт“, Прахран, Викторија, Австралија
[3] Оддел за дијабетес, ендокринологија и медицина, Универзитет во Мелбурн, Кралска болница во Мелбурн, Парквил, Викторија, Австралија
[4] Клиника за здравје на жените и дојките, болница „Фримејсон“, Источен Мелбурн, Викторија, Австралија

Вовед: Независно, хормонската терапија и вежбањето имаат добро воспоставени заштитни ефекти врз коскените параметри. Сепак, комбинираните ефекти на хормонската терапија и вежбањето се помалку јасни. Затоа, ги испитувавме ефектите на хормонската терапија на маркери на коскениот метаболизам кај жени во постменопауза подложени на редовни високо интензивни вежби.

Методи: Во рандомизирана, двојно слепа студија, атлетите во постменопауза кои се натпреваруваат на ниво на Мастер, 20 недели при-

мале или хормонска терапија (50 μg трансдермален естрадиол, 5 mg МПА, n = 8) или плацебо (n = 7). Жените беа тестирани пред и по третманот за плазма концентрациите на естрадиол, FSH, LH и серумскиот маркер за формирање на коска – остеокалцин (ОС); и уринскиот маркер за коскена ресорпција – пиридинолин (PYD) и деоксипиридинолин (DPD).

Резултати: Како резултат на третманот со хормонската терапија имаше значително намалување на нивото на FSH (73,3 ± 13,7 до 48,6 ± 10,5 mmol / L, $p = 0,01$) и маркерите за коскена ресорпција (PYD, 81,9 ± 7,7 до 57,8 ± 3,7 nmol / mmol Cr, $p = 0,001$, и DPD, 18.5 ± 3,1 до 11,8 ± 2,1 nmol / mmol Cr, $p = 0,01$). Естрадиолот и маркерите за формирање на коските не беа значително променети како резултат на хормонската терапија. Немаше промени кај кои било варијабли со плацебо третманот.

Заклучок: Хормонската терапија ја намали коскена ресорпција, но не и формирањето на коските, кај атлетите во постменопауза. Овие поволни намалувања на коскениот метаболизам обезбедуваат ефективен третман во комбинација со високиот интензитет на вежбање за натамошно намалување на последователниот ризик од остеопороза и поврзани фрактури.

Клучни зборови: вежба, менопауза, коскен метаболизам, хормонска заменска терапија, естрадиол

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THE EFFECTS OF HORMONAL THERAPY AND EXERCISE ON BONE TURNOVER IN POSTMENOPAUSAL WOMEN: A RANDOMISED DOUBLE-BLIND PILOT STUDY

Suzy Y Honisett[1], Kathy Tangalakis[1], John Wark[2], Vasso Apostolopoulos[1], Lily Stojanovska[1,*]

[1] Centre for Chronic Disease, College of Health and Biomedicine, Victoria University, Melbourne VIC Australia
[2] Royal Melbourne Hospital, Parkville, Melbourne VIC Australia

Corresponding Author: Centre for Chronic Disease, College of Health and Biomedicine, St Albans Campus, Victoria University, VIC 3021, Australia; tel: +613 99192737; fax: tel: +613 99194565; e-mail: lily.stojanovska@vu.edu.au (L. Stojanovska)

Abstract

Introduction: Hormone replacement therapy (HRT) and walking were investigated independently and in combination, to determine which treatment provided most effect on bone turnover in postmenopausal women.

Methods: Using a randomised double-blind pilot study, 10 subjects received HRT (transdermal estradiol, 50 μg/day and oral MPA 5 mg/day) and 12 received placebo for 20 weeks. Following a baseline period of treatment, both groups undertook a graduated walking regimen, which increased in intensity, duration and frequency parameters from weeks 8–20. Measurements of aerobic capacity, female sex hormones, bone formation markers [osteocalcin (OC) and bone alkaline phosphatase (BAP)] and bone resorption markers [deoxypyridinoline (DPD) and pyridinoline (PYR)] were measured at baseline (T1), week 8 (T2) and week 20 (T3).

Results: Age, time of postmenopause, weight or body mass index were no different between each groups. The HRT group had significantly higher estradiol levels compared with the placebo group at T2 and T3. FSH and LH levels were significantly reduced following HRT. DPD and PYR were significantly reduced from baseline levels at T2 and T3 with HRT. No significant changes occurred in OC or BAP levels with either HRT or walking. Walking did not change bone turnover markers in either the HRT or placebo group.

Conclusion: HRT reduces bone resorption, however, walking alone at the intensity and duration prescribed, or the combination of HRT and walking, provided no additional benefit after menopause. Therefore, HRT, but not walking is an effective treatment in reducing bone turnover in postmenopause women.

Keywords: Exercise, Hormone replacement therapy, HRT, Bone turnover, Menopause, Estrogen

Introduction

Menopause is associated with endocrine changes, including significant reductions in estrogen and progesterone levels, and alterations in the pituitary gonadotrophins, follicle stimulating hormone (FSH) and leutinising hormone (LH). Other age associated changes that occur with ageing, that are superimposed on estrogen deficiency include elevated serum parathyroid hormone (PTH) and attenuated calcitriol levels. These changes have been implicated in altered bone metabolism and reduced skeletal integrity after menopause.

Bone metabolism can be assessed by the measurement of biochemical markers that are released into the circulation and urine, providing chemical indices for whole body bone turnover [1–9]. The level of these biochemical markers can predict bone density and rates of bone loss in postmenopausal populations [10].

The relationship of bone turnover markers to changes in bone mineral density (BMD) is inverse in states of altered bone remodeling [11], providing a cost effective, potentially useful tool for the prediction of the bone responses to changes in bone remodeling [11].

Menopause is associated with a 79–97% increase in bone resorption markers, whilst bone formation markers are increased by 37–52%, reflecting an overall increase in bone turnover [12]. Alterations in bone turnover markers precede changes in bone mass and density. BMD is reduced in the initial 3 to 12 months after the last menses by 0.9% at the lumbar spine and 0.7% in the femoral neck [13]. This attenuation of bone is associated with reduced circulating estrogen levels. The bone loss experienced in the initial 6 years after menopause is estimated to be approximately 15% [14], imposing a considerable risk for the development of osteoporosis.

Increasing age is typically associated with reduced levels of physical activity. A reduction in weight bearing exercise, and less frequent involvement in exercise that provides mechanical loading on bones, are potentially associated with lowered bone density and an increased risk of fracture. This is shown in immobility [15] and space flight [16] studies where there is little or no mechanical strain or loading on bone and a subsequent deterioration in bone density occurs.

Hormonal replacement therapy (HRT) containing estrogen, and physical activity are commonly prescribed as independent interventions for reducing the risk of osteopenia after menopause. Estrogen plays a major role in bone metabolism and cytokines production, which maintains the quality of bone and alveolar bone crest [17]. The signal transduction pathways for estrogen and the mechanical strain appear to share common elements [18], and both interventions may be relevant in the prevention of postmenopausal osteoporosis.

The most effective type and intensity of exercise, to provide the greatest benefit to bone whilst fostering compliance, remain to be fully elucidated. Whilst bone mineral density (BMD) and HRT have been extensively researched, there has been little investigation into bone turnover changes as a result of exercise either independently, or combining these 2 interventions in a postmenopausal population. We proposed that moderate exercise in the form of brisk walking would induce a weight-bearing mechanical load on bone, whilst providing an accessible and safe form of exercise for women who do not participate in any regular or structured form of exercise. Herein, we evaluated the effect of low intensity exercise in the form of walking and HRT independently, and when combined, on bone turnover markers in postmenopausal sedentary women.

Methods

Postmenopausal subjects

Twenty eight postmenopausal women were recruited from the general public via advertisements in local papers. Six women withdrew from the study; one woman moved away during the course of the study; two women suffered skin irritation caused from the transdermal estrogen; two women withdrew due to lack of time available to continue involvement; one woman was excluded prior to commencement due to excessive alcohol intake. Twenty two women completed the study. The criteria for inclusion were 1–5 years postmenopause; aged between 45–60 years. The criteria for exclusion were; taking any form of hormonal replacement therapy; previous hysterectomy or oophorectomy; family history of estrogen dependent malignancies; established cardiovascular disease; involved in any regular form of structured exercise; smoking; taking any drug therapy or having any disease that would affect the gain/loss of bone mineral. All women were cleared for participation following a full physical examination by a clinician.

Women were randomly assigned into 2 groups. Ten women were assigned into the 'HRT group', and commenced using transdermal estradiol patches (Estraderm 50 μg, Novartis Pharmaceuticals, North Ryde, NSW, Australia) changed twice weekly, and oral medroxyprogesterone acetate (MPA, 5mg/day) (Pharmacia Pharmaceuticals, Rydalmere, NSW, Australia). Twelve women were assigned to the 'placebo' group and commenced using placebo transdermal patches and oral placebo tablets. All subjects participating in this study were asked to maintain their current dietary habits throughout their involvement in the study. All women gave infor-

med consent to participate in this study, which was approved by the Human Ethics Committee, Victoria University (991017/CO484).

Protocol

Subjects were tested three times over the duration of the twenty week study. Measurements were taken at baseline (Tl), prior to commencement of exercise training, at eight weeks (T2) and on completion of the study, at twenty weeks (T3). Height and weight were determined for all subjects. At each time point, aerobic capacity was determined by measures of $V0_2$ peak. In addition, morning fasting blood samples were taken for measurement of estradiol, leutinising hormone (LH) and follicle stimulating hormone (FSH) and bone formation markers, bone alkaline phosphatase (BAP) and osteocalcin (OC). Urine samples were taken 2 hours post first morning void, at the above intervals for measurement of the bone resorption markers, pyridinoline (PYR) and deoxypyridoline (DPD).

Subjects continued their specified treatment regimen throughout the 20 week period. After 8 weeks, subjects initiated an exercise training program whilst continuing their specified treatment regimen. This program was individually based on each participant's baseline fitness level. From baseline aerobic capacity measures, volume of expired carbon dioxide (ml/kg/min) was plotted against the volume of expired oxygen (ml/kg/min). A parallel graph, plotting ventilation (L/min) against oxygen consumption (ml/kg/min) was determined for the duration of the test. A non-linear fit was assessed for each graph and the maximum of the second derivative was determined as the inflection point, noted as the anaerobic threshold. The heart rate at anaerobic threshold was used to calculate the training heart rate. Individuals underwent a 12 week walking program. Exercise intensities assessed by heart rate monitoring commenced at 70% of the baseline anaerobic threshold and increased by 5% every second week to 95% of anaerobic threshold over the following 12 week period. The participants achieved the increments in heart rate by increasing the speed of walking, or walking on an incline. The duration of exercise at the specified heart rate commenced at 10 minutes and increased by 5 minutes duration every second week to a total of 35 minutes at twenty weeks. A 5 minute warm up and cool down preceded and followed each walking session. Lastly, the frequency of exercise sessions performed per week commenced at three, and increased by one session per week on a monthly basis. Two women suffered skin irritation caused from the transdermal estrogen, whilst there were no adverse effects of involvement in the walking program.

Assays

Estradiol was measured using a competitive chemiluminescent immunoassay (Ciba Coming, Medfield, MA, USA). FSH and LH were measured using a two-site chemiluminescent (sandwich) immunoassay (Ciba-Coming, Medfield, MA, USA). BAP was measured using the standard autoanalyser ELISA technique (Alkaphase B96, Metra Biosystems). Serum OC was measured by radioimmunoassay using the osteocalcin RIA kit (Incstar Corp., Stillwater, MN, USA). Free PYR and DPD cross links were measured on morning void urine samples by an enzyme linked immunosorbent assay (ELISA) using rabbit antipyridinoline (Pyrilinks, Metra Biosystems, Palo Alto, CA, USA) and expressed as pyridinoline/creatinine ratio (PYR nmol/mmol Cr).

Aerobic Capacity

Aerobic fitness was determined via a $V0_2$ peak test. This test measures oxygen consumption per minute during exercise to peak levels. Measurement to maximum levels was deemed less safe within this population. Hence, a $V0_2$ peak test was used instead of a $V0_2$ max protocol. The laboratory where the trials were conducted was maintained at a constant temperature (20 ± 1°C) and humidity (44 ± 2%). A 12 lead electorcardiogram (ECG; Montara, X-scribe, Stress testing System, Milwaukee, U.S.A.) was used to monitor subjects' heart rhythm and rate throughout the duration of the test. Subjects exercised on a stationary bicycle ergometer (Cybex Metabolic Systam, Met 100, Huntsville, USA) at a constant cadence of 70 rpm. Increments in intensity occurred each minute, starting at 35 watts and increasing by 10 watts each minute. These increments continued until the subject was exhausted, or until the subject experienced discomfort, or the practitioner observed significant ECG changes. Each subjects' oral gas expiration was measured via Vacumetric Vista Turbofit Software package (Version 3.2, Ametek, Pittsbergh, USA) to de-

termine their respiratory exchange ratio and aerobic capacity. Subjects were deemed to have reached their $V0_2$ peak when at least two of the following criteria were achieved (a) a plateau of $V0_2$ peak readings over 3 consecutive recordings, (b) exercising heart rate to within 10 beats of subjects' maximal heart rate, calculated as 220 minus age, (c) a respiratory exchange ratio (RER) of greater than 1.10.

Statistical Analysis

Data were analysed using SPSS (10.0). Data reported are mean ± standard error of the mean. Repeated measures analysis of variance was used to determine significance. Post-hoc analysis of group differences was carried out using a Student's unpaired *t-test*. Significance was reported at $p < 0.05$.

Results

There was no significant difference in age (51.1 ± 2.1 vs. 51.6 ± 1.7 years) and time postmenopause (2.3 ± 1.1 vs. 1.6 ± 0.2 years) between groups taking hormonal replacement therapy and those taking placebo. Similarly, there were no significant differences in weight or body mass index (BMI) between or within groups for the duration of the study (Table 1). Estradiol was significantly higher in the HRT group in comparison to the placebo group at T2 and T3. FSH and LH were significantly decreased at T2 and T3 compared with baseline levels in the same group (Table 1). The increased estradiol and simultaneous significant reduction in plasma FSH and LH ascertain that subjects were compliant with HRT, which was supported by medication counts. Bone resorption markers (DPD and PYR) were both significantly reduced from baseline levels with HRT administration (T2) (Table 1, Figures 1 and 2) and were significantly lower than the placebo group at T2. Bone resorption markers were unchanged as a result of exercise. Although slightly reduced, neither BAP nor OC showed significant reductions as a result of HRT or exercise. BAP was significantly lower than the placebo group at T3. There were no significant changes to $V0_2$ peak as a result of exercise training in rate, respiratory exchange ratio and exercise duration were unchanged in both groups as a result of the exercise training regimen over a 12 week period.

Table 1

Personal, Hormonal, Fitness and Bone parameters

	Baseline	**Placebo**	**Placebo & exercise**	**Baseline**	**HRT**	**HRT & exercise**
	T1	T2	T3	T1	T2	T3
Weight, kg	69.4 ±4.7	69.7 ±4.9	69.3 ±4.8	71.8 ±7.8	72.1 ±7.6	72.5 ±7.5
BMI, kg/m^2	26.8 ±2.2	26.9 ±2.3	26.7 ±2.2	27.5 ±3.3	27.6 ±3.2	27.8 ±3.2
Estradiol, pmol/l	77.6 ±12.0	89.2 ±17.1	73.4 ±18.3	100.0 ±2 1.5	166.3 ±36.8$^+$	164.9 ±29.9$^+$
FSH, m/u/ml	57.9 ±9.7	51.1 ±9.4	56.1 ±13.5	77.1 ±14.8	39.8 ±15.8*	37.1 ±14.6*
LH, m/u/ml	45.8 ±11.8	33.7 ±7.3	36.2 ±7.2	44.7 ±8.9	18.4 ±6.8*	19.6 ±8.1*
AC, ml/kg/min	20.6 ±1.9	21.2 ±1.7	21.6 ±1.7	21.4 ±2.6	22.3 ±2.7	22.9 ±1.9
BONE FORMATION						
OC, ug.l	17.3 ±2.6`	17.9 ±2.7	17.8 ±2.4	15.7 ±3.0	15.2 ±2.9	14.3 ±2.8
BAP, U/L	13.6 ±2.8	16.8 ±2.9	16.8 ±3.1	11.1 ±3.8	10.6 ±4.0	9.5 ±4.1$^+$
BONE RESORPTION						
PYR, nmomol/mmol Cr	81.0 ±16.9	79.6 ±15.9	78.1 ±13.6	75.5 ±15.6	58.6 ±12.08*$^+$	64.9 ±14.0
DPD, nmmol/mmol Cr	15.6 ±3.1	16.0 ±3.3	14.1 ±3.0	13.7 ±3.2	9.9 ±2.4*$^+$	9.5 ±2.7

Abbreviations: BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinising hormone; AC, aerobic capacity: OC, osteocalcin; BAP, bone alkaline phosphatase; PYR, pyridinoline; DPD, deoxypyridionline.

* ($p < 0.05$) Significantly different from T1 H group.

$^+$ ($p < 0.05$) Significantly different from placebo group at the same time point.

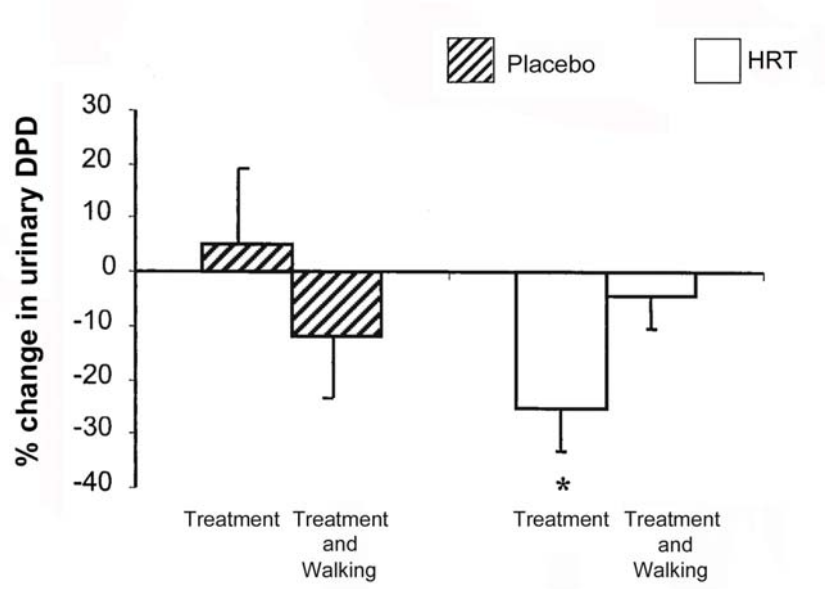

*Figure 1 – Urinary Pyridinoline (PYR) excretion percent change in placebo (n = 12) and HRT (n = 10) groups from baseline levels with hormone or placebo treatment alone and with hormone or placebo treatment and exercise. (*significantly different from baseline levels, $p < 0.05$)*

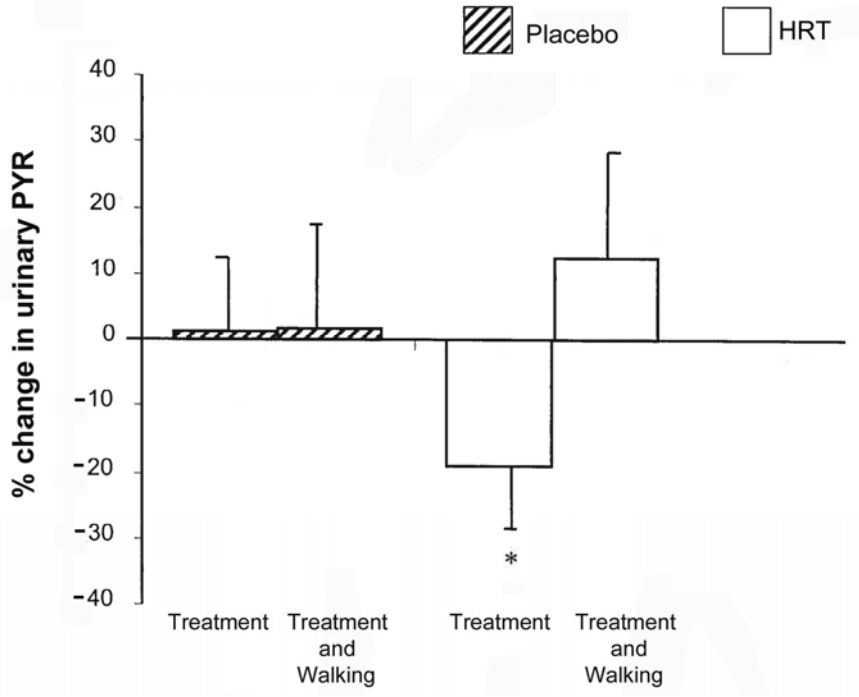

*Figure 2 – Urinary Deoxypyridinoline (DPD) excretion percentage change in placebo (n = 12) and HRT (n = 10) groups from baseline levels with treatment alone and with treatment and exercise (*significantly different from baseline levels, $p < 0.05$)*

Discussion

The main new findings of this study were that bone resorption indices were significantly reduced after 8 weeks of HRT, whilst bone formation markers remained unchanged. Our results are similar to a previous study [19] that found one year of treatment with estrogen significantly suppressed bone resorption, whilst bone formation remained unchanged, preventing overall bone loss in ovariectomised women, measured by dual energy x-ray absorptiometry (DXA). Furthermore recent studies con-

ducted in mice shows decreased trabecular thickness, bone density and bone volume and increased trabecular separation caused by overiectomy were prevented by giving estrogen replacement [17].

Sex hormones play a regulatory role in bone metabolism [20]. Estrogen levels correlated with osteoclastic (bone resorption) activity thus increased estrogen levels directly increase osteoclast apoptosis [21]. Reduced estrogen levels that accompany menopause increase cytokine levels- interleukin-1 and tumor necrosis factor alpha, secreted from peripheral blood monocytes [22]. These factors act on stromal osteoblastic precursor cells, and induce secretion of other cytokines interleukin-6 and interleukin-11, which promote the development of bone resorbing osteoclasts [20, 22, 23]. Thus, estrogen deprivation provides an indirect stimulus as a potent promoter of bone resorption. Combinations of cytokines – interleukin-1, tumor necrosis factor alpha, interferon gamma- increases osteoblastic nitric oxide (NO) production [24], which suppresses bone resorptive osteoclast development and activity, and subsequently reduces resorption. The production of NO by osteoblasts directly regulates osteoclast activity and is perceived to play a regulatory role in conditions of bone such as osteoporosis [l 9l. Estrogen replacement down regulates interleukin-6 production [25, 26] providing at least one molecular mechanism for the inhibitory effect of hormonal therapy on postmenopausal bone loss.

Exercise effect

Weight-bearing exercise provides a stimulus of mechanical loading on bone cells, which induces bone formation [27]. The effect of physical activity on human bone has been well documented in a variety of population groups [28–32]. Younger athletes have a greater bone mass in comparison to sedentary controls [30]. Dominant arms of male and female tennis players exhibit up to 28.4% greater bone thickness and bone mineral content in comparison to non-dominant arms [31, 32]. The bone response to exercise after menopause, however, has provided varying degrees of both positive results [33, 34], results indicating that varying types and intensities of exercise provide no training effect on bone [35–37]. In this study, moderate weight-bearing exercise alone in the form of walking did not alter any bone formation or resorption indices. The intensity, frequency and duration of exercise are important parameters when considering an exercise training regimen. Wolff's law proposes that bone remodeling is directly dependent upon the mechanical load placed on the bone [38]. Alternatively, a strain threshold may exist for the stimulation of osteogenesis. An insufficient application of strain or load may be inadequate to reach the strain threshold, indicating that the magnitude of the strain may be more important than strain frequency. Brisk walking alone may provide an insufficient mechanical strain to overcome estrogen deficiency after menopause. This may explain why, in this study, there was no change in bone turnover markers as a result of walking based exercise training regimen with either HRT or placebo treatment.

Type and intensity of exercise

There are numerous studies that assess bone mineral changes as a result of exercise in postmenopausal populations. The results of these studies, however, provide conflicting outcomes. Aerobic exercise, involving brisk walking and lifetime volleyball involvement, were unable to counteract an age and, or, menopause related decline in BMD [35, 39]. BMD primarily at the femoral neck, however, was increased with exercise that involved vigorous walking, jogging, stair climbing, and high impact aerobics [33, 40, 41] whilst, total body BMD was also augmented [40, 42] in a number of these studies. On the other hand, long term vertical jumping exercises using a weighted vest prevented hip bone loss over five years [43]. High intensity aquatic exercise program performed for 24 weeks prevented from reducing BMD by increase bone formation and reduce bone resorption [44, 45]. Resistance and strength training performed for at least one year increased regional BMD at sites of intratrochanter and hip [46], and total body [42, 46]. Similar programs that were performed for one year or less showed no change in total bone mineral content (BMC) [36], BMD at lumbar spine [35, 37, 47] or total hip BMD [37]. It should be noted that it is difficult to compare studies due to the differences in exer-

cise regimen type, intensity and frequency, and subject characteristics such as age, baseline BMD and years since menopause. With increasing age and years postmenopause, there is an accretion in the amount of bone lost; therefore, if women are commencing exercise at lower bone densities there may be a greater effect of treatment.

The age range of the current study was very tight, with all women 49 to 54 years of age, and early postmenopause (1.6 to 2.3 years) and could partly explain the lack of exercise effect in the current study, as women were commencing exercise prior to having lost substantial BMD due to estrogen deficiency. In contrast, previous exercise studies had large ranges for age and the number of years postmenopause, with many studies involving women up to and over 10 years older than women in the current study. The exercise program designed for these women was mild and graduated to minimize the risk that previously sedentary women would not encounter injury throughout the training program. All women remained injury-free for the duration of their involvement. Walking was chosen as the mode of exercise due to the weight-bearing nature and ease of administration. It was also perceived that previously sedentary women would be more compliant with this mode of exercise, which was verified by subjects' diarising involvement in walking sessions. The moderate nature of the exercise program did not increase the $V0_2$ peak of the subjects, nor alter weight or BMI.

Vibration exercise is one of the safest activities for osteoporosis women that prevent falls. Whole-body vibration strategy is used in preventing sarcopenia and osteoporosis [48, 49]. Although no significant improvement in BMD were seen in a 6 month vibration training [50]; muscle strength was improved [49–51].

Combined hormone replacement therapy and exercise

The addition of a brisk walking program to HRT, in this population of postmenopausal women, provided no additional changes to either bone resorption (Figures la. and lb.) or formation indices, indicating that HRT was the primary stimulus for changes in bone turnover. Previous studies focusing on the effects combined exercise and HRT on BMD have been conflicting. Prospective studies [34] have found aerobic exercise in combination with HRT, but not exercise alone, significantly increased BMD. However, current studies have shown high intensity exercise significantly increased BMD in postmenopausal women [44, 45]. Hence, any benefit conveyed by combined treatment appeared to be attributable to HRT. In contrast, HRT and nine months of vigorous weight-bearing exercise were independently beneficial in increasing BMD, whilst combined treatment provided an additive benefit at sites of lumbar spine and Wards triangle [33]. HRT and weight lifting exercises also significantly increased BMD of the total body in women who had had a hysterectomy, but exercise was not assessed independently of HRT [52]. There are no studies that measure bone turnover markers as a result of these combined treatments.

Conclusion

The current pilot study, although in small group numbers indicated that walking alone, at the intensities and duration prescribed, was an insufficient stimulus to reduce bone turnover in early postmenopausal women, whilst HRT was an effective treatment intervention to reduce bone turnover after menopause. The combination of HRT and moderate weight bearing exercise provided no added benefit in comparison to HRT alone. A combination of resistance training and walking may be more effective in reducing bone turnover, in comparison to walking alone. Alternatively, older populations of postmenopausal women, with lower BMD values, may benefit more from walking alone.

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Р е з и м е

ЕФЕКТИТЕ НА ХОРМОНСКАТА ТЕРАПИЈА И ВЕЖБАЊЕТО ВРЗ КОСКЕНИОТ МЕТАБОЛИЗАМ КАЈ ЖЕНИ ВО ПОСТМЕНОПАУЗА: РАНДОМИЗИРАНА, ДВОЈНО СЛЕПА ПИЛОТ-СТУДИЈА

Сузи Ј Хонисет[1], Кети Тангалакис[1], Џон Варк[2], Васо Апостолопулос[1], Лили Стојановска[1]

[1] Центар за хронични болести, Школа за здравство и биомедицина, Универзитет Викторија, Викторија, Австралија
[2] Кралска болница во Мелбурн, Парквил, Мелбурн, Викторија, Австралија

Вовед: Хормонска заменска терапија (HRT) и пешачење беа испитувани самостојно и во комбинација за да се утврди кој третман најмногу влијаел врз коскениот метаболизам кај жени во постменопауза.

Методи: Користејќи рандомизирана двојно слепа пилот-студија, 10 испитаници добија HRT (трансдермален естрадиол, 50 μg/ден и орално MPA 5 mg/ден), а 12 примале плацебо 20 недели. По почетниот период на третманот, двете групи беа подложени на режим на пешачење, што се зголемуваше во интензитет, времетраење и параметри на зачестеност од 8 до 20 недели. Мерењата на аеробниот капацитет, женските полови хормони, маркерите за формирање на коските [остеокалцин (OC) и коскена алкална фосфатаза (BAP)] и маркерите за коскената ресорпција [деоксипиридинолин (DPD) и пиридинолин (PYR)] беа мерени на почетокот (T1), 8. недела (T2) и 20. недела (T3).

Резултати: Возраста, времето на постменопаузата, тежината или индексот на телесна маса не беа различни помеѓу секоја од групите. Групата на HRT имаше значително повисоки нивоа на естрадиол во споредба со плацебо групата во T2 и T3. Нивоата на FSH и LH беа значително намалени по HRT. DPD и PYR беа значително намалени од почетните нивоа на T2 и T3 со HRT. Немаше значајни промени кај нивоата на OC или BAP, со HRT или пешачење. Пешачењето не ги промени маркерите на коскениот метаболизам кај HRT или плацебо групата.

Заклучок: HRT ја намалува коскената ресорпција, сепак, само пешачењето според пропишаниот интензитет и времетраењето, или комбинацијата на HRT и пешачење, не дадоа дополнителна корист по менопаузата. Затоа, HRT, но не и пешачењето, е ефективен третман за намалување на коскениот метаболизам кај жени во постменопауза.

Клучни зборови: вежба, хормонска заменска терапија, HRT, коскен метаболизам, менопауза, естроген

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THE NEED FOR ACCURATE RISK PREDICTION MODELS FOR ROAD MAPPING, SHARED DECISION MAKING AND CARE PLANNING FOR THE ELDERLY WITH ADVANCED CHRONIC KIDNEY DISEASE

Marijke Stryckers, Evi V Nagler, Wim Van Biesen

Renal Division Ghent University Hospital, Ghent Belgium

Corresponding Author: Evi V.T. Nagler, Renal Division, Department of Internal Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Gent, Belgium; tel: +32 9 332 0417; fax: +32 9 332 3847; e-mail: evi.nagler@ugent.be

Abstract

As people age, chronic kidney disease becomes more common, but it rarely leads to end-stage kidney disease. When it does, the choice between dialysis and conservative care can be daunting, as much depends on life expectancy and personal expectations of medical care. Shared decision making implies adequately informing patients about their options, and facilitating deliberation of the available information, such that decisions are tailored to the individual's values and preferences. Accurate estimations of one's risk of progression to end-stage kidney disease and death with or without dialysis are essential for shared decision making to be effective. Formal risk prediction models can help, provided they are externally validated, well-calibrated and discriminative; include unambiguous and measureable variables; and come with readily applicable equations or scores. Reliable, externally validated risk prediction models for progression of chronic kidney disease to end-stage kidney disease or mortality in frail elderly with or without chronic kidney disease are scant. Within this paper, we discuss a number of promising models, highlighting both the strengths and limitations physicians should understand for using them judiciously, and emphasize the need for external validation over new development for further advancing the field.

Keywords: Prognosis; Proportional Hazard models; Logistic Models; Aged; Renal Insufficiency, Chronic

Introduction

The prevalence of Chronic Kidney Disease (CKD) increases with age, but few of the elderly actually progress to End Stage Kidney Disease (ESKD) [1–5]. During the past decade and partly fuelled by the KDIGO classification of CKD, many have started questioning whether in the elderly, decreased estimated glomerular filtration rate (eGFR) should really be labelled as a "disease" at all [6]. Simultaneously, a tendency to start renal replacement therapy (RRT) at higher eGFR thresholds, has resulted in a spectacular increase in older people starting dialysis [7, 8]. Strikingly, the higher incidence of RRT in the elderly has been mirrored by an increasing number opting to withdraw from dialysis [9]. Despite large variation in attitudes between regions [10], the idea of conservative care has been gaining traction [11–13]. As a consequence, both nephrologists and patients are currently struggling with how to approach advanced CKD.

A thematic analysis of disease trajectory experiences in elderly patients diagnosed with CKD revealed different themes that should be addressed to improve the care for this population: patients were shocked with their being labelled as having a serious disease, and were anxious about their prognosis; nephrologists felt uncertain about what and how to explain the complexity of the condition, and how to predict and steer future events; patients were

eager to discuss eventual advanced care planning and/or need for dialysis, whereas nephrologists felt very uncomfortable and tended to avoid discussing likely negative aspects about their future [14].

Counselling older people with advanced CKD (eGFR<45ml/min/1.73m²) requires reliable estimates of an individuals' absolute probability of death within a given time frame, both with and without starting dialysis. Furthermore, it requires accurate assessment of an individuals' absolute probability of progression to ESKD and eventual need for RRT. Predicting risk of progression to ESKD needs to take into account the competing risk of death to provide relevant information, whereas predicting death is hampered by the fact that developing ESKD in itself is a powerful predictor for mortality. Predicting progression is also challenging because GFR decline may not be linear [15], and rapid decline may occur unpredictably if associated with acute kidney injury [16]. This is especially true for older people who are at greater risk of acute kidney injury because of the high prevalence of frailty and other long-term term conditions [17].

First, correctly identifying patients likely to die early regardless of whether RRT is started, may avoid the unnecessary anxiety induced by preparing for dialysis, and the burden of dialysis itself. Conversely, in patients correctly identified as those who will reach ESKD long before dying, shared decisions on management, require counselling patients and families on different treatment options (haemodialysis, peritoneal dialysis, home-based vs hospital-based dialysis modality, conservative care), balancing quality versus quantity of life. Second, a robust method for identifying those at high risk of progression is necessary to focus renoprotective therapy to those who will benefit from it. Last, correctly estimating risk of death after starting RRT may provide a more accurate perception of the desirability of starting dialysis.

Within this paper we will try to construct an algorithm that helps in planning the nephrological care of elderly people with advanced CKD. We will discuss its potential use in clinical shared decision making, and its potential pitfalls and drawbacks.

Shared decision making

Over the last years, shared decision making has been forwarded as an important instrument to improve quality of care [18]. In contrast to the conventional paternalistic approach to medical decision making in which the physician decides what is best for the patient, shared decision making tries to involve the opinion, values and expectations of the patient in the process. For it to be effective, three steps are essential (Figure 1). The first step encompasses *informing* the patient about the different available options. The expected or most likely outcomes and eventual dangers of these different options should be clearly explained. In the setting of the elderly with advanced CKD, prediction of the outcomes 'mortality' and 'progression to ESKD' is prime for informing the process of shared decision making. During the past decade, there have been several attempts at developing risk prediction models, combining multiple demographic (e.g. age, sex) and clinical characteristics (e.g. medical history, physical examination results) to estimate individual risk of for each of these situations in the elderly (see below).

Several aspects might jeopardize information transfer. First, the correct information on the expected outcome of the different options might simply not be available, or might not be available for the specific population the patient belongs to (generalizability, external validity). This might be especially problematic in elderly patients with advanced CKD, as both elderly and patients with advanced CKD are mostly excluded from studies, and trials in these people are scant. Unfortunately, data from the general population, or even from the elderly without CKD or the non-elderly with CKD, are not readily translated to the elderly with CKD. Applying prognostic models developed in the general population to elderly patients with advanced CKD may result in overoptimistic prognosis, and pointless technical investigations and care.

Second, the information can be transferred in a biased, non-neutral way. Some treatment routines are so embedded in the structure and paths of care that everybody accepts them as the only possible way, leaving no room for alternatives. Accordingly, information on the alternative options is coloured by non-verbal (or even verbal) signs of disapproval

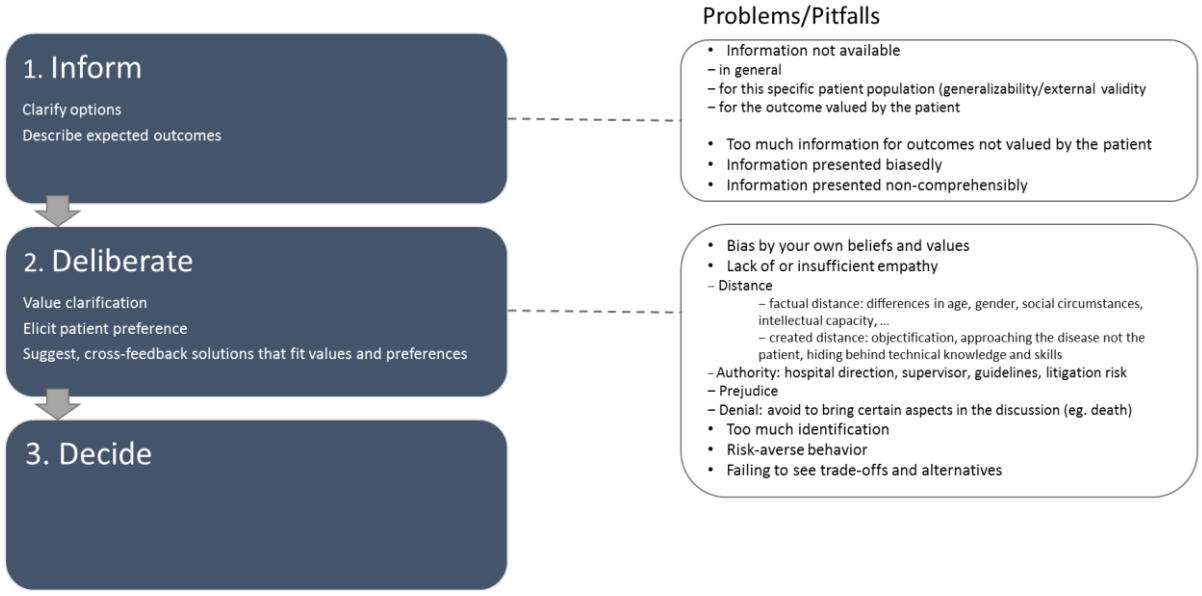

Figure 1 – Shared decision making in steps

Third, the information is often transferred in a way the patient does not understand. It is very difficult to transfer information on probabilities [19] and the uncertainty surrounding them to lay people, and even physicians and healthcare professionals may struggle to understand them [20]. In this era of digitalisation, it is becoming increasingly straightforward to present data in any imaginable graphical format. However, there is limited evidence on which presentations work best, and how these visualisations are processed and understood by the audience [21]. There is some evidence that graphical presentations should be adapted to the target audience, depending upon numeracy and health literacy, and digital tools can help to make these conversions. However, whatever tool or visualisation used, it reduces information from what is already a best guess. The resulting situation is that we are discussing facts that, though objective in themselves, are surrounded by a great degree of uncertainty, leaving ample room for subjective interpretation of the indeterminacy of the future presented. As such, some patients will opt for lowers odds than others. Experiments in the field of decision making on dialysis modality highlight that patients tend to be strongly influenced by stories of other patients [22] and far less by the same information provided by a physician.

Fourth, information on outcomes that matter to the patient might not be available, whereas ample information is provided on outcomes that do not matter to the patient [23]. Recently, the SONG project tried to establish a core outcome set for patients on haemodialysis [24]. SONG clarified that most studies use outcomes that are not patient relevant, whereas outcomes that do have value are rarely studied. In 2011, a comparable initiative was taken by the National Institute of Aging. They proposed that studies in this domain should focus on outcomes including measures of pain, fatigue, physical and mental functioning, social roles, daily activities, disease burden, and caregiver burden [25].

A next step in the shared decision making process is the *deliberation* of the available information. In this step, the physician needs to explore the patients' wishes, expectations, and values and elicit opinion on different potential scenarios. In this stage, empathy or the skill to view the situation through the eyes of the patient, is a necessary property for the medical team. The deliberation can be flawed if sufficient empathy is lacking, and result in the physician's rather than the patient's wish is followed. Empathy can be reduced through *distance*, either factual – substantial difference in age or different social backgrounds – or created, e.g. by a tendency to see the patient as an object or a case rather than as a person with distinct values and experiences. Empathy can also be endangered by *authority*, which not only comprises the supervisor or hospital board expecting the patient to start dialysis, but also

existing guidelines, or fear for litigation. Furthermore, prejudice and denial can compromise open and empathic discussion with the patient. Many physicians find it a major challenge to discuss death with their patients, and will therefore try to avoid these discussions by denying or minimizing risk of death [26], so that patients are presented with unrealistic perspectives. The last step is the actual decision making. Studies indicate that most patients want to be informed about the different options available, but that most will state in one form or another, that the physician should take the decision for them (*doctor, what would you do*). As discussed above, this does not imply that physicians can simply do as they please, but rather that they should take into account patients' desires and values, and use their clinical expertise to propose the solution or treatment most likely to result in an outcome the patient desires. This process requires not only empathy, but also insight in how available evidence is applicable to the particular situation of the individual patient, taking into account known comorbidities. It is flabbergasting to realize that for most provided guidance, there is a complete lack of external validity of the underlying evidence for most subpopulations the guideline refers to [27].

Risk prediction models: definition and assessment pitfalls

In a model of shared decision making, accurate and unbiased information on the fate of the individual is essential.

Risk prediction models aim to objectively predict the risk of a future outcome, e.g. mortality or ESKD, based on a set of variables available at the time the prediction is made. Essentially, each variable is awarded a weight – or coefficient – and combined in a mathematical rule to predict an outcome of interest. Similar to weather-forecasts though, it is not because intricate models exist, that they produce reliable estimates of what will happen in reality.

Evaluating the quality, generalizability and utility of risk prediction models poses certain challenges that are both interesting from a methodological point of view and crucial to understand for the clinician wanting to use such models for informing their clinical practice. Some of the methods for assessing model performance can be quite daunting and lead to misinterpretation or overly confident conclusions around accuracy, reliability and generalizability of the predictions (Table 1).

Table 1

Risk prediction model assessment: pitfalls and solutions

Model assessment pitfalls	Limitations	Solution
Model only internal validated	Produces overly optimistic view of model performance	External validation in cohorts similar to target population
C-statistic as measure of discrimination	No practical meaning No differential weighting of misclassification errors Heavily dependent on risk factor distribution	Positive predictive values Negative predictive values
Vague description and dichotomized risk predictors	Classification errors Information reduction and increase in unmeasured variability	Clarity of description Layered risk predictors
Risk scores with coefficients rounded to nearest integer	Information reduction and unmeasured variability	Risk calculators, apps, integration in electronic health record

1/First of all, a model needs to be tested in a group of people that was not used to develop the model, it needs to be externally validated [28]. Why is that? Well of course, developing a model means mimicking the data as much as reasonably possible and so, often the resulting model will be reasonably good at predicting whatever it is that we want it to predict. As a consequence, conclusions based on performance measures calculated in the same cohort as the one that was used to develop the model, will necessarily produce overly optimistic

conclusions of a model's accuracy [29]. Ideally, validation is done by investigators who were not involved in the model development process.

2/The performance of a risk prediction model is commonly assessed by testing its calibration – the agreement of observed and predicted event rates – and discrimination – the ability to distinguish individuals who will develop the outcome of interest from those who will not. Investigators often use the C-statistic as a global measure of model discrimination, ranging from 0.5 (random concordance) to 1 (perfect concordance). The C-statistic can be seen as the area under the curve of the receiver operating characteristic curve, be it with several important limitations [29].

For one thing, it is a single number that does not really have a practical interpretation. Sure, if it equals 1, the model is perfect, and if it equals 0.5, throw it in the bin; but for any number in between, it has no practical meaning attached to it. It does not convey the implications of the misclassification errors that can occur (predicting an individual who experiences an event to be at low risk; predicting an individual who does not experience an event to be at high risk) [29]. For that we need positive and negative predictive values, which do have a direct clinical meaning, but sadly these are seldom reported.

Secondly, the value of the C statistic depends not only on the model being assessed, but also on the distribution of risk factors in the sample to which it is applied. For example, if eGFR is an important risk factor, the same model can appear to perform much better when applied to a cohort with a wide eGFR range than when it is applied to a cohort with a narrow eGFR range. Finally, the C statistic is only a measure of discrimination, so it provides no information regarding whether the overall magnitude of risk is predicted accurately.

For that we need to look at calibration measures, which assess how accurately the model's predictions match overall observed event rates. In other words, we look at the agreement between what we predict and what we observe. Without going into detail, we can safely state that sadly again, calibration measures are often omitted [30, 31].

3/ For a model to be useful in practice, it needs to include variables that are readily available, well-defined and measurable. Clear definitions of individual risk predictors are necessary to ensure inter-rater reliability. The presence or absence of "diabetes", for example, can be interpreted differently by different raters. Does it apply to everyone meeting international criteria, even if transient or perfectly controlled with limited diet restrictions; or is it limited to patients treated with insulin? The same problem arises for many commonly used predictors, such as cardiovascular disease, peripheral vascular disease, cancer or chronic lung disease: if not explicitly described how these categorical variables should be measured, they can induce substantial variation in scoring, and thus importantly influence model performance [32].

4/ For a model to be applicable in practice, it needs to come with an actual equation that allows straightforward calculation of an individual's absolute risk of the outcome of interest. To this day, researchers often still opt to create risk scores, which basically transform the model parameters to integers that can be summed to derive a global risk prediction for that individual patient. A classic example we are all very familiar with is the CHADS-Vasc score, which allocates points for age, hypertension, diabetes etc., and relates that sum to an absolute annual risk of stroke in atrial fibrillation. With smartphones being in everyone's pockets and emergence of companies specializing in developing apps for risk calculation, there seems to be increasingly less virtue in doing that. Instead of simplifying models to allow risk calculation without computer assistance, attention is probably better refocussed to model visualisation to boost uptake and efficient communication of their results [21].

Available risk prediction scoring systems for elderly with advanced CKD

Progression to ESKD

Several prospective [33–35] and retrospective [4, 5, 36–38] cohort studies aimed to develop risk prediction scores based on identified risk factors for progression to ESKD. The Kidney Failure Risk Equation (KFRE) initiative analysed data from Canadian adults with eGFR 10–59 ml/min/1.73m^2 to develop the KFRE equation to predict the risk of ESKD at 2 and 5

years [38]. Using 8 variables (age, sex, eGFR, albuminuria, serum calcium, serum phosphate, serum bicarbonate, and serum albumin) this score proved to have good discrimination capacity, both in development (C statistic = 0.92) and in the validation cohorts (C statistic = 0.84), and reducing the number of variables further down to 4 easily available parameters (age, gender, eGFR and albuminuria) did not substantially alter the discriminative capacity performed similarly (C statistic = 0.91 and 0.84 in development and validation cohorts, respectively) [39]. An external validation was carried out in a Dutch cohort with stage 3–5 CKD, demonstrating that the scores performed well to predict 5-year risk (C statistic 0.89 and 0.88, respectively) and also had a good calibration (difference between predicted and observed risk 4.0% and 7.1%, respectively). In an external dataset with over 700.000 patients, the 4-variable KFRE achieved excellent discrimination (pooled C statistic 0.90 at 2 years and 0.88 at 5 years), although the KFREs tended to overestimate risk in some non-North American cohorts. Addition of a calibration factor improved calibration in 12/15 and 10/13 non-North American cohorts at 2 and 5 years, respectively [40]. These data seem to indicate that the 4 variable KFRE (with the use of a calibration factor for non-American cohorts) is suitable to estimate the risk for evolution to ESKD in this population.

Mortality risk in CKD

Since 2012, two high quality systematic reviews, one including models predicting death in elderly people [41] and one predicting death in people with CKD [42] have been published on the topic. Starting from the search strategies of these papers, we identified 24 publications including 31 risk prediction models, of which 15 models target elderly people in general [43–55], 4 elderly people with CKD 3–5 [5, 56, 57], and 12 elderly people with end-stage kidney disease (ESKD) [58–63]. Only three models were developed or validated in Western Europe. The most commonly included final predictors of death were age, sex, functional status, heart failure, malignancy and diabetes. Although most models included parameters of frailty, only one model was specifically developed within a frail elderly patient group [43]. As a consequence, it would be safe to consider using an additional scoring system for frailty in patients with a low predicted risk for mortality by any of these scores.

Another caveat is that external validation was mostly not available, and as far as it was, it was mostly done by the same investigators that had developed the model, and in patients very similar to the ones included in the development cohort. In addition, presence of comorbidities was based either on self-report or on coding within administrative databases. Both methods can induce misclassification as criteria might not be clear or well described. This can substantially reduce predictive performance, especially upon generalisation to patient groups external to the ones used for model development. In general, model performance was moderate at best, with only 1 model achieving a c-statistic of > 0.8 [49], and confidence intervals were generally not provided. The Bansal risk prediction model predicts the absolute probability of death within five years for older people with CKD stage 3 through 5 not yet treated with dialysis, provides measures of predictive performance and was externally validated in a large cohort of representative patients, except that the overwhelming majority was able to live independently [56]. The model has a reasonable calibration and model discrimination in both the development (0.72; 95% CI 0.68 to 0.74) and validation cohort (0.69; 95% Cl 0.64 to 0.74). As the validation cohort might not be representative for cohorts containing frail patients, and as it has been well established that frailty is a prevalent condition in patients with advanced CKD (eGFR<45ml/min) [64], it is absolutely mandatory to combine the Bansal score with a score for frailty when Bansal score is low, as in this setting mortality risk will be governed by frailty rather than by traditional risk factors. However, it has been advocated that frailty is an additional risk factor for mortality, on top and independent of other traditional risk factors [65]. As such, a high predicted mortality with the Bansal score will deliver a reliable result even in a frail patient.

Mortality risk in ESKD

One risk prediction model based on the REIN-registry data estimates risk of death at

three months in older people with ESKD starting with dialysis [60]. The cohort is representative for elderly patients starting dialysis, both in terms of age (at least 75 years old, with one in five > 85 years) and in terms of comorbidity (heart failure 33% and peripheral vascular disease 25%). This risk prediction model includes 9 easily available predictors: age, sex, history of congestive heart failure, peripheral vascular disease, arrhythmia, cancer, severe behavioural disorder, mobility and baseline serum albumin concentration. The rate of death in the validation cohort increased with the score, indicating good calibration, but discrimination was moderate with a c-statistic in the internal validation cohort of 0.75 (95% CI: 0.74–0.76). The model was further externally validated in a Flemish cohort [39], although the investigators slightly modified the score. Another risk prediction score based on the REIN-cohort data estimates risk of death at six months in older people with ESKD starting with dialysis [59]. The model was further externally validated in an American cohort [58] although again, investigators modified the score [58].

Floege and co-workers also developed a risk prediction model predicting mortality in patients starting dialysis based on the Framingham study model [66]. This model was than validated in an external cohort of the Dialysis Outcomes and Practices. Patterns (DOPPS), showing a moderate discrimination (c statistic of 0.68 to 0.79 depending upon geographic location). Although the score includes age, it has not been developed or validated in a cohort of elderly dialysis patients (mean age 64 ± 14 years), and the cohort did also not include peritoneal dialysis patients. Furthermore, the development cohort includes only patients who survived the first 3 months, whereas the validation cohort of DOPPS includes mainly prevalent patients. Both attributes make that the score is likely to be not very representative for the dilemma whether or not to start dialysis in the frail elderly, where exactly this risk of short term mortality during the initiation phase of dialysis is what needs to be predicted.

Conclusions

In conclusion, reliable, externally validated risk prediction models for progression of CKD to ESKD or mortality in frail elderly with or without CKD are necessary to inform shared decision making in the trajectory of the elderly with advanced CKD, but available models are scant. Physicians need to understand the limitations of these models so that they can be used appropriately. Next to understanding the models themselves, healthcare workers need to translate the available information to patients, and that in a way the patient can understand, and use the information to choose a trajectory of care most likely to achieve his/her goals and expectations, taking into account his individual needs. Rather than developing new models in a search for more sophisticated statistical models, we emphasize the importance of external validation by different investigators of those models in both frail and non-frail elderly patients to test their performance and applicability. In addition, more effort must go to developing strategies for translating the information such that it becomes digestible and understandable for all involved.

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Резиме

ПОТРЕБА ОД ПРЕЦИЗНИ МОДЕЛИ ЗА ПРЕДВИДУВАЊЕ РИЗИК, ЗАЕДНИЧКО ДОНЕСУВАЊЕ ОДЛУКИ И ПЛАНИРАЊЕ НЕГА НА ПОСТАРИ ЛИЦА СО НАПРЕДНА ХРОНИЧНА БУБРЕЖНА БОЛЕСТ

Маријке Стикерс, Еви В Наглер, Вим Ван Бисен

Оддел за нефрологија, Универзитетска болница во Гент, Гент, Белгија

Како што стареат луѓето, хроничната бубрежна болест станува сè почеста, но ретко доведува до краен стадиум на бубрежна болест. Кога ќе се случи тоа, изборот помеѓу дијализа и конзервативна нега може да биде застрашувачки, бидејќи тоа многу зависи од животниот век и личните очекувања од медицинската нега. Заедничкото донесување одлуки подразбира соодветно информирање на пациентите за нивните можности, и олеснување на разгледувањето на достапните информации, така што одлуките се приспособени на вредностите и преференциите на поединецот. Точните процени за ризикот од прогресија до краен стадиум на бубрежна болест и смрт, со или без дијализа, се суштински за да биде ефикасно заедничкото донесување одлука. Формалните модели за предвидување ризик може да помогнат ако се надворешно потврдени, добро калибрирани и дискриминативни; ако вклучуваат недвосмислени и мерливи променливи; и ако доаѓаат со применливи равенки или резултати. Недоволни се сигурните, надворешно потврдени модели за предвидување ризик од прогресија на хроничната бубрежна болест до краен стадиум на бубрежна болест или смртност кај снемоштени стари лица со или без хронична бубрежна болест. Во рамките на овој труд, разгледуваме голем број надежни модели, истакнувајќи ги предностите и ограничувањата што треба да ги разберат лекарите за да ги користат разумно и да ја истакнуваат потребата од надворешна валидација преку нов развој за натамошно унапредување на полето.

Клучни зборови: прогноза, пропорционални модели на ризик, логистички модели, возрасни лица, бубрежна инсуфициенција, хронична

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RENAL REPLACEMENT THERAPY IN PATIENTS WITH HEART AND KIDNEY FAILURE

Dimitrios S Goumenos, Evangelos Papachristou, Marios Papasotiriou

Department of Nephrology and Renal Transplantation, University Hospital of Patras, Greece

Corresponding Author: Prof. Dimitrios S. Goumenos, Department of Nephrology and Renal Transplantation, University Hospital of Patras, 26500 Patras, Greece; tel.: 0030 2613603361, fax: 00302610994424; e-mail: dgoumenos@upatras.gr

Abstract

The incidence of chronic kidney disease (CKD) in patients with chronic heart failure (CHF) is high as CKD and CHF share underlying risk factors such as arterial hypertension, diabetes mellitus and atherosclerosis. Cardiac failure leads to renal hypoperfusion and dysfunction and then fluid overload and need for aggressive diuretic therapy. However, development of diuretic resistance represents a significant problem in the management of these patients.

The role of Renal Replacement Therapy (RRT) is important for patients who do not response to conservative management of fluid overload facilitating the failing heart to restore function. According to the guidelines, venovenous isolated Ultrafiltration (UF) is indicated for patients with refractory congestion not responding to medical therapy with loop diuretics and infusion of dopamine. A systematic review of randomized controlled trials on the effect of UF vs. IV furosemide for decompensated heart failure showed a benefit of UF on total body weight loss and on readmissions due to heart failure in patients with decompensated heart failure and CKD. Peritoneal dialysis (PD) can provide efficient ultrafiltration and sodium extraction in volume overloaded patients followed by decline of hospitalization days, decrease of body weight and improvement of LVEF in patients with refractory heart failure. The continuous draw of ultrafiltrate is followed by a lesser risk of abrupt hypotension and better preservation of the residual kidney function. This represents a significant advantage of PD over intermittent UF by dialysis.

In conclusion, application of UF by dialysis and PD is followed by significant total body weight loss, reduced need for hospital readmissions and better quality of life. PD has a higher probability of preservation of residual kidney function and can be used by patients at home.

Key words: heart failure, chronic kidney disease, ultrafiltration, peritoneal dialysis

Epidemiology, pathophysiology

The incidence of chronic kidney disease (CKD) in patients with chronic heart failure (CHF) is high as both share underlying risk factors such as arterial hypertension, diabetes mellitus and atherosclerosis. There is abidirectional interplay as cardiac failure leads to renal hypoperfusion and dysfunction that is followed by fluid overload and need for aggressive diuretic therapy. However, the effectiveness of diuretics might be hampered by development of diuretic resistance and the use of renal replacement therapy becomes necessary. The decompensated heart failure in patients with CKD leads to prolonged hospitalizations, significant morbidity and increased mortality [1, 2].

Loop diuretics should be used as first-line agents if GFR is below 30 ml/min. Thiazide diuretics are not effective if used alone in patients with GFR < 30 ml/min. Strategies improving loop diuretic responsiveness in CKD patients with heart failure include reduced salt intake (up to 2g/day but not less), intravenous bolus furosemide (up to 2g/day) instead of oral

administration of equivalent dose especially in cases with fluid overload (edema exists also in the gut) and co-administration of hydrochlorothiazide or metolazone in cases with resistance to loop diuretics. Such patients need close monitoring in order to prevent adverse effects such as decrease in renal perfusion, hypovolemia, hypokalemia, hyponatremia and hypomagnesemia [2, 3]. Severe volume overload with very low cardiac output leads to significant impairment of renal perfusion, decrease of GFR and failure to deliver diuretics to their site of action. This is followed by release of norepinephrine, angiotensin II, aldosterone and vasopressin leading to systemic and renal vasoconstriction, renal salt and water retention with further deterioration of fluid overload and decrease of cardiac output [3, 4].

Role of renal replacement therapy (RRT)

The role of Renal Replacement Therapy (RRT) is important for patients with no response to conservative management (administration of diuretics) who have fluid overload, pulmonary edema, hyperkalemia, decreased urine output, severe hyponatremia and metabolic acidosis. Fluid removal by extracorporeal therapy has clear-cut benefits in patients with volume overload and pulmonary edema supporting the failing heart to restore function.

Intermittent isolated ultrafiltration (IUF) is a process resembling to conventional hemodialysis. It is performed via a dual lumen catheter inserted in a large central vein in intermittent sessions of 1–4 hours where a goal volume removal is set. Fluid removal is based on a pressure gradient established between blood and UF compartment and in contrast to hemodialysis no dialysate is used [1, 4].

According to the guidelines of the American Heart Association and European Society of Cardiology on the use of ultrafiltration (UF) in heart failure, venovenous isolated UF is indicated for patients with refractory congestion not responding to medical therapy and in particularly if doubling of the dose of loop diuretics and infusion of dopamine do not result in an adequate diuresis and the patient remains in pulmonary edema.

In the RAPID-CHF Trial 40 pts admitted for CHF with evidence of volume overload were randomized to a single, 8 hours UF session in addition to usual care or usual care alone. After 24 hours there was a significant difference in the fluid removal between two groups (4.6L in the UF vs. 2.8L in the usual care groups, $p = 0.001$) but no significant difference in weight loss. This early application of UF for patients with CHF was well-tolerated [5]. In the UNLOAD trial 200 pts hospitalized for HF with more than 2 signs of hypervolemia were randomized to UF or IV diuretics. After 48 hours, weight loss and net fluid loss were greater in the UF group (5.0kg vs. 3.1kg, $p = 0.001$ and 4.6l vs. 3.3l, $p = 0.001$, respectively). At 90 days the UF group had fewer patients rehospitalized for HF (18% vs. 32%, $p = 0.037$) and fewer rehospitalization days (1.4 vs. 3.8, $p = 0.022$) per patient. However, no differences were observed in the mortality rate and serum creatinine between the two groups [6]. In the CUORE trial 56 pts with CHF were randomized to standard medical therapy or UF [7]. The primary endpoint was rehospitalizations for CHF during a 1-year follow-up. Despite similar body weight reduction at hospital discharge in the 2 groups, a lower incidence of rehospitalizations for HF was observed in the UF-treated patients. UF induced benefit was associated with a more stable renal function, unchanged furosemide dose, and lower B-type natriuretic peptide levels. According to this study the use of UF as first-line treatment in patients with HF and severe fluid overload is associated with prolonged clinical stabilization and less need for rehospitalization for congestive HF (7). A systematic review with meta-analysis of randomized controlled trials on the effect of UF vs. IV furosemide for decompensated heart failure in cardiorenal syndrome showed a benefit of UF on total body weight loss in patients with decompensated heart failure and CKD and also on readmissions due to heart failure in patients with decompensated heart failure and chronic kidney disease [8].

The advantages of UF are symptomatic relief due to fluid removal with better gas exchange and decrease work of breathing, improved cardiac function and breaking of the vicious cycle that makes such patients diuretic-refractory, increase of blood pressure, reduction in renal venous congestion and improvement in renal hemodynamics and urine output, higher mass clearance of sodium, decreased

risk of electrolyte abnormalities (e.g., hypokalemia), lack of neurohormonal activation (SNS, RAAS, and AVP), decreased rate of heart failure–related rehospitalizations and decreased hospital length of stay [4, 9].

The disadvantages of UF are hemodynamic stress and instability because of large volume removal in short period of time, lack of protective effect on renal function, lack of effect on markers of mortality (i.e. serum Na and BUN), need for placement of midline or central venous catheter and additional training for staff and physicians, need for anticoagulation, complications related to extracorporeal circuit (e.g., allergic reaction, air embolism, hemolysis, infection, and bio-incompatibility), lack of widely accepted guidelines for its use (e.g., patient population, indications, timing of initiation and termination, and UF rate/volume), lack of knowledge on the long-term outcomes and high cost (device and disposables) [4, 9].

Peritoneal Dialysis (PD) in patients with heart failure

Peritoneal dialysis (PD) can provide efficient ultrafiltration and sodium extraction in volume overloaded patients (especially through the use of icodextrin solution) with simultaneous correction of the metabolic consequences of reduced renal function. The limited experience from trials on PD in patients with heart and kidney failure has shown the safety and efficacy of this therapeutic modality suggesting that it could represent a relevant option for such patients. Several uncontrolled PD studies have favourable results despite the fact that PD was restricted to very ill patients, refractory to alternative options who were not candidates for heart transplant [10].

In a multicenter retrospective study 48 patients with severe HF refractory to maximized drug treatment were treated by PD ultrafiltration (PUF). The patients were proposed for PUF because they had experienced at least 3 hospital admissions in the preceding year for acutely decompensated HF requiring extracorporeal UF. Out of 48 patients, 30 received 1 nocturnal icodextrin exchange, 5 required 2 daily exchanges, and 13 received 2–4 sessions per week of PD. During the first year, renal function remained stable (20.8 vs. 22.0 mL/min/1.73 m^2), pulmonary artery systolic pressure declined ($p = 0.03$) and there was a significant improvement in New York Heart Association functionnal status. Hospitalizations decreased from 43days/patient-year before the start of PUF to 11days/patient-year ($p < 0.001$) whereas patient survival was 85% at 1 year and 56% at 2 years [11]. In another retrospective analysis, 127 patients were treated with PD for management of chronic refractory HF (mean e-GFR: 33.5 ± 15 mL/min/1.73 m^2). Patients with low left ventricular ejection fraction (LEVF) experienced significant improvement after PD. Furthermore PD was associated with a dramatic reduction in the number of days of HF-related hospitalization. During the year before PD initiation, each patient stayed at hospital for an average of 3.3 ± 2.6 days/month whereas after PD the number of hospitalization days declined to 0.3 ± 0.5 days/month ($p < 0.0001$), corresponding to a 91% reduction in hospital stay [12]. Thus, PD therapy could be effective for patients with chronic heart failure in whom conventional therapies have not been associated with significant therapeutic response. These results were confirmed in a recent analysis of 21 published studies on PD for patients with refractory heart failure. After PD, hospitalization days declined, body weight decreased and LVEF improved significantly. This analysis also demonstrated that the yearly average peritonitis rate was 14.5%, and the average yearly mortality was 20.3% [13].

PD continuously draws ultrafiltrate by the osmotic power of the PD solution and therefore has a lesser risk of abrupt hypotension that would exaggerate organ hypoxia and kidney damage. Furthermore better preservation of the residual kidney function by PD represents an advantage of PD over intermittent UF. It should be noted that Na removal is higher than that in urine produced by furosemide since ultrafiltrate Na level is about 100 mmol/L [14]. Use of PD at home for patients with heart failure could potentially lead to significant savings in healthcare expenditure while providing a better quality of life for patients. In patients with significant residual renal function who do not require dialytic support, nocturnal automated PD or a single night time exchange with icodextrin solution could be sufficient to maintain euvolemia. This means that depending on the severity of HF, PD therapy can be used only a few nights a week. The risk of peri-

tonitis that might be a drawback for the use of PD is low nowadays [14, 15]. However, it seems that despite the beneficial effect of PD in patients with HF, it does not alter the natural history of the disease and probably does not influence the survival of these patients. Furthermore the experience on the use of PD in such patients is very limited and this is probably the reason why PD is not yet considered by the cardiology societies as a therapeutic option for HF. Future prospective randomized trials with longer follow-up periods comparing PD with UF by dialysis could address this issue and establish PD as a proper treatment modality for patients with HF [15].

Conclusions

The management of patients with decompensated heart failure and CKD is usually difficult because of the development of diuretic resistance. The application of UF by dialysis and PD is followed by significant total body weight loss, reduced need for readmissions to the hospital and better quality of life. PD has a lesser risk of abrupt hypotension, a higher probability of preservation of residual kidney function and it can be used by the patients at home. Further research with randomized controlled trials is necessary in order to establish PD in the management of patients with HF.

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Резиме

ЗАМЕНСКА БУБРЕЖНА ТЕРАПИЈА КАЈ ПАЦИЕНТИ СО СРЦЕВА И БУБРЕЖНА СЛАБОСТ

Димитриос С. Гуменос, Евангелос Папахристу, Мариос Папасотириу

Клиника за нефрологија и трансплантација на бубрези, Универзитетска болница во Патра, Грција

Инциденцата на хроничната бубрежна болест (ХББ) кај пациенти со хронична срцева слабост (ХБС) е на високо ниво, бидејќи ХББ и ХБС ги споделуваат основните фактори на ризик, како што се артериска хипертензија, дијабетес мелитус и атеросклероза. Срцевата слабост води

кон бубрежна хипоперфузија и дисфункција, а потоа и преоптоварување со течност и потреба од агресивна терапија со диуретици. Сепак, развојот на отпор кон диуретик претставува значаен проблем во лечењето на овие пациенти.

Улогата на заменска бубрежна терапија (ЗБТ) е значајна за пациентите кои не реагираат на конзервативното лечење и за олеснување од преоптоварувањето на срцето со течности, за да се врати на функција. Во согласност со насоките, венски изолираната ултрафилтрација (УФ) е индицирана кај пациенти со рефракторна конгестија што не реагираат на медицинската терапија со диуретици и инфузија на допамин и пациентот останува во белодробен едем. Систематскиот преглед на рандомизирани контролирани испитувања на ефектот на УФ наспроти интравенозен фуросемид за декомпензирана срцева слабост покажа корист од УФ на вкупната загуба на телесната тежина и на повторен прием во болница поради срцева слабост кај пациенти со декомпензирана срцева слабост и ХББ. Перитонеалната дијализа (ПД) може да обезбеди ефикасна ултрафилтрација и вадење на натриумот кај пациенти преоптоварени со волумен, проследено со намалување на деновите на хоспитализација, намалување на телесната тежина и подобрување на LVEF кај пациентите со рефракторна срцева слабост. Постојаното повлекување на ултрафилтратот е проследено со помал ризик од нагла хипотензија и подобра заштита на резидуалната бубрежна функција. Ова претставува значајна предност на ПД во однос на повремената УФ со дијализа.

Како заклучок, примената на УФ со дијализа и ПД е проследено со значителна вкупна загуба на телесна тежина, намалена потреба од повторен прием во болница и подобар квалитет на животот. ПД има поголема веројатност за зачувување на резидуалната бубрежна функција и може да ја користат пациентите дома.

Клучни зборови: срцева слабост, хронична бубрежна болест, ултрафилтрација, перитонеална дијализа

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BODY SIZE AND OUTCOMES IN DIALYSIS AND TRANSPLANT PATIENTS – DOES IT MATTER?

Mehmet Sukru Sever

Istanbul School of Medicine, Turkey

Corresponding Author: Mehmet Sukru Sever, M.D. Istanbul School of Medicine, Turkey

Abstract

The terminologies of "body size", "body mass index (BMI)", "body weight", "large BMI" and "obesity" are controversial for defining the effects of "adiposity" or "fat excess" on the outcomes of dialysis and transplant patients. However, probably these terminologies will be continued to be used in the future as well, because of being older and simpler terminologies.

In the general population obesity is a powerful risk factor for cardiovascular morbidity and mortality, while, it conferred a survival advantage to dialysis patients. However, this may be a oversimplification, since obesity may still be a risk factor in non-sarcopenic hemodialysis patients.

Obesity is associated with early post-transplant adverse effects (i.e. delayed graft function, graft failure, wound infections, also transplant costs) and unfavorable graft and patient survival. However, thanks to safer immunosuppressive protocols, recently graft and patient survival is similar in obese as those of the non-obese patients. On the other hand, morbid obesity may still be a cause of unfavorable patient and graft survival.

Since obese transplant recipients have better life expectancy as compared to wait-listed hemodialysis patients, they should be transplanted as well, while morbidly obese patients should be asked to lose weight before being placed in the waiting lists.

Keywords: obesity, large body mass index, outcome, hemodialysis patients, transplant recipients

Introduction – The Terminology

In the literature, most often, the terminologies of "body size", "body mass index (BMI)" and "body weight" are used interchangeably. Furthermore, vast majority of the studies use "large body size" and "obesity" for describing the effects "adiposity" on the outcomes of dialysis patients and transplant recipients. However, all these terminologies are controversial and, importantly, large BMI and obesity may not be the right wordings for this theme, because BMI is an imperfect metric of fat excess in Chronic kidney disease (CKD) [1]. So the question is: which is the right terminology?

It has been suggested that abdominal obesity, measured by the waist circumference may be better associated with cardiovascular risk in many studies. As an example, in a prospective cohort study on 537 dialysis patients, aged mean 63 years, waist circumference predicted all cause and cardiovascular disease (CVD) mortality, whereas BMI showed an inverse relationship with these outcomes [2]. This controversial finding clearly underlines that abdominal obesity does not always overlap with high BMI. In a WHO report it has been emphasized that measurement of abdominal adiposity (e.g. waist circumference, waist–hip ratio and waist–height ratio) are associated with CVD risk factors and incident CVD events [3]. "On the oher hand, universal cut-off points for BMI and waist circumference are not appropriate for use worldwide given the ethnic or population-specific differences in disease risk for any particular anthropometric measure; while, there may be general a consistency in the cut-off points of waist–hip ratio for predicting CVD risk [3]". Despite all these concerns, probably BMI will continue to be used in the future because it is an older and simpler terminology

[4]. For the sake of simplicity, throughout this review, I will assume that high BMI or obesity simply coincides with high waist circumference, and I will use these terms interchangeably. Also, since devastating weakness is almost always associated with an unfavorable prognosis, most of this review will be dedicated to the effects of obesity/adiposity on the outcomes.

Obesity and Outcomes – General Population

In the general population obesity is a powerful risk factor for morbidity and mortality. It is very well described that obesity is associated with CVD; however, it is rarely considered that obesity is also associated with many other diseases; such as osteoarthritis and even various neoplasia (including, colon, endometrial and breast cancer) [5]. On the other hand, it is not clear whether these concerns are valid for end stage renal disease (ESRD) patients, because no randomized trial, testing the health benefits of intentional weight loss in this population has been performed so far. Therefore, most of the information on this patient population comes from observational studies.

Obesity and Outcomes – Dialysis Patients

In an analysis of the USRDS database, the incidence of obesity were analyzed in more than 660.000 incident adult dialysis patients [6]. The patients were initiated permanent dialysis between 1995 to 2002. Their BMI was calculated with the height and estimated dry weight collected from the Centers for Medicare and Medicaid Services Form. This study showed a significant increase in obesity both in general population and in dialysis patients; however, the slope of increase in dialysis patients far exceeded the increase in general population [6]. This finding underlines that the nephrologists will be faced with more and more obese dialysis patients in the future [7].

Although obesity is assumed to be an unfavorable prognostic indicator in the general population, interestingly, it conferred a survival advantage to human beings till the 19 th century, in eras when food scarcity and infection limited human life to shorter than 40–50 years [8]. Apparently, obese persons were better nourished, and characterized by better immune response against devastating chronic infectious and other diseases, which were more frequent and major causes of death in that particular time period. Then, it can be speculated that, the same protective effect may apply to cancer, heart failure and ESRD patients as well, all of which are devastating illnesses. A USRDS analysis investigated the correlation between body size and outcomes in 418.000 patients over a 2-years average follow-up time [9]. For the purpose of that particular analysis, BMI was divided into 8 categories between 19 to 37. It was found that high BMI was associated with increased survival, even at extremely high BMI, after adjustment for demographic, laboratory, and comorbidity data. High BMI was also associated with a reduced risk of hospitalization in all categories [9]. Similar results have been published in peritoneal dialysis patients as well [10]. However, a recent Japanese registry study, which included 120.000 dialysis patients, pointed out some other interesting findings. This study evaluated the association of basal BMI with mortality and morbidity after a 1-year period [11]. The cases were stratified either by BMI into 4 quartiles or by serum creatinine levels into 3 tertiles. It was shown that obesity paradox occurs in HD patients only when obesity is simply defined by BMI. However, when patients were subdivided by serum creatinine levels in each BMI category, in patients with high serum creatinine, obesity was found to be a risk factor for all cause death similar to general population. Thus, it was concluded that it is not the loss of body fat, but loss of muscle mass (sarcopenia), being responsible from unfavorable outcomes; and obesity may still be a risk factor in non-sarcopenic HD patients. Early identification and intervention for sarcopenia by assessing serum creatinine levels may be more important to improve poor clinical outcomes in prevalent HD patients [11].

Obesity and Outcomes – The Transplant Recipients

Similar to dialysis patients, there is an epidemic of obesity among the kidney transplant recipients over time; there is a decreasing trend in the number of normal weight and underweight patients, while prevalence of obese and morbidly obese patients show a considerable increase [12]. Many studies have underlined unfavorable effects of

obesity both in the short and long term on various parameters after transplantation.

Short term adverse effects include wound infections, delayed graft function, graft failure, CVD and also transplant costs [4, 13–15]. Unfavorable long term risks of obesity have been described as well. For example, an interesting report analyzed the associations of BMI with post-transplant cardiac risk in more than 1.000 patients [15]. Values of BMI were ranked into quartiles, and cumulative post-transplant incidences of congestive heart failure (CHF), atrial fibrillation (AF), myocardial infarction (MI) and a composite of these cardiac diagnoses were assessed. It was found that 5-year cumulative incidence of any cardiac diagnosis rose from 8.6% to 29.3% across the lowest to highest BMI quartiles. Although the rate of MI did not differ by BMI quartile, high BMI was associated with an increased cardiac risk, especially of CHF and AF [15].

Obesity may have adverse effects on graft and patient survival as well. A recent systematic review and meta-analysis on the effects of obesity on kidney transplantation outcomes investigated 21 studies, which included 9296 cases [16]. Overall, graft loss at one year and five years was more favorable in non-obese patients. On the other hand, when studies were subdivided as those published before and after 2003, this beneficial effect was valid only for the studies published before 2003, but not with those afterwards. A very similar pattern was also noted for patient survival; better outcomes in non-obese patients were found in studies only published before 2003. Therefore; 3 major conclusions were drawn: 1. There is (or was) an excess risk for graft loss and death in obese transplant recipients; 2. This increased risk is mostly confined to studies performed before 2000, i.e. before the introduction of safer and more effective immunosuppressives; and 3. In the past, obesity was a risk factor for graft loss and death by CVD and all-cause mortality; however, today, the graft and patient survival is similar as those of the non-obese patients [16].

So the critical question is; can this conclusion be valid for all stages of obesity? The answer is certainly no. A UNOS registry analysis evaluated the outcome of transplantation in super obese (BMI > 50) patients. This study has noted significantly worse patient and graft outcome in super obese patients as compared to any other BMI class in the early and late period after transplantation [17]. Then the second question comes; what is the upper limit of BMI that transplantation can be performed relatively safely? Unfortunately, this is not clear. However, since Bennett et al. [18] has reported a survival advantage of transplantation over dialysis even in the morbidly obese population, it is not possible to define a contraindication for transplantation considering BMI alone.

Management of obese transplant candidates in the waiting lists

Since many studies have shown detrimental effects of obesity early after transplant operation, many transplant physicians are hesitant for transplanting obese cases. Indeed, a recent survey in US has shown that “most centers asked their patients to lose weight so that their BMI was no greater than 35” [4]. However, since there is a definite survival benefit of transplantation as compared to patients who remain in the waiting list, even morbid obesity should not be a reason for excluding obese dialysis patients from the waiting lists. A recent registry analysis from US including more than 200.000 patients between 1995 to 2007 found that risk of death was significantly lower in transplant recipients compared to wait-listed candidates in various BMI strata, confirming that still morbidly obese patients should be transplanted [19]. ERBP guideline [20] recommends that “... patients with a body mass index > 30 kg/m^2 should reduce weight before transplantation”. However, then it adds “There is no consensus on whether obesity should be an exclusion criterion for kidney transplantation and policies differ among transplant centers.” Finally, it has been underlined that “the guideline development group could not make a statement regarding the acceptance or refusal for kidney transplantation based on obesity itself” [20].

To conclude, many studies have shown increased risk of morbidity and mortality of transplantation in obese patients in the early post-transplant period. Although graft and patient survival figures are controversial, obese patients have increased risk of CVD in the long term as well. So, the question is; should we continue to transplant obese dialysis patients? The answer is certainly YES if they have live donors, because transplantation offers better outcomes as compared to dialysis, even in mor-

bidly obese patients. The real controversy stands for management of obese dialysis patients if they do not have live donors. Since there is a scarcity of organs, it has been argued that "... these limited organs should be offered to the persons, who will benefit most" [21]. Thus, in order to guarantee the maximum benefit to the greatest number of patients, "only those patients, who achieve a target BMI should be transplanted", which suggests that non-obese patients should take priority in the waiting lists. On the other hand, considering that obese patients also benefit from transplantation as compared to those in the waiting lists, this argument may give rise to many ethical concerns and puts responsibility on the shoulders of the nephrologists.

It is the personal opinion of this author that obese patients should have the same chance with their non-obese counterparts for deceased donor renal transplantation, while morbidly obese patients should be asked to lose weight before being placed in the waiting lists.

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Резиме

ГОЛЕМИНАТА НА ТЕЛОТО И РЕЗУЛТАТИТЕ КАЈ ПАЦИЕНТИТЕ НА ДИЈАЛИЗА И СО ТРАНСПЛАНТАЦИЈА – ВАЖНО ЛИ Е?

Мехмет Шукру Север

Факултет за медицина во Истанбул, Турција

Терминологиите на „големината на телото“, „индекс на телесна маса (БМИ)“, „телесна тежина“, „голем БМИ“ и „дебелина“ се контроверзни за утврдување на ефектите на „дебелината“ или „вишокот масти“ врз резултатите кај

пациентите на дијализа и со трансплантација. Сепак, веројатно тие ќе се користат во иднина, затоа што се постари и поедноставни терминологии.

Кај општата популација дебелината е моќен фактор на ризик за кардиоваскуларен морбидитет и морталитет, додека таа им дава предност во преживувањето на пациентите на дијализа. Сепак, ова може да е преголемо упростување, бидејќи дебелината сè уште може да биде фактор на ризик кај несаркопенични пациенти со хемодијализа.

Дебелината е поврзана со раните несакани ефекти на посттрансплантот (на пример, одложена функција на графтот, слабост на графтот, инфекции на раните, исто така, и трошоците за трансплантација) и неповолното преживување на графтот и на пациентите. Сепак, благодарение на побезбедните имуносупресивни протоколи, неодамнешните преживувања на графтот и на пациентите се слични и кај дебелите пациенти и кај пациенти што не се дебели. Од друга страна, морбидната дебелина сè уште може да биде причина за неповолно преживување на пациентот и на графтот.

Бидејќи дебелите примачи на трансплант имаат подобар животен век во споредба со пациенти што се на список за чекање за хемодијализа, и ним треба да им се направи трансплантација, додека од морбидно дебелите пациентите треба да се побара да ја намалат тежината пред да бидат ставени на списокот на чекање.

Клучни зборови: дебелина, голем индекс на телесна маса, резултат, пациенти на хемодијализа, примач на трансплант

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SOMATOFORM DISORDERS – A PEDIATRIC EXPERIENCE

Nada Pop-Jordanova[1], Tatjana Zorcec[2]

[1] Macedonian Academy of Sciences and Arts, Skopje, R. Macedonia
[2] University Pediatric Clinic, Skopje, R. Macedonia

Corresponding Author: Prof. Nada Pop-Jordanova, Macedonian Academy of Sciences and Arts, Bul. Krste Misirkov 2, 1000 Skopje, R. Macedonia; e-mail: popjordanova.nadica@gmail.com

Abstract

Somatization in children consists of the persistent experience and complaints of somatic distress that cannot be fully explained by a medical diagnosis.

Working at the Psychophysiological Department at the University Clinic we are dealing with more than 100 children per year manifesting this kind of disorders.

The aim of this article is to summarize some specific characteristics of the somatoform disorder in a group of 243 children, mean age 10.31 (± 2.75) years for both genders, selected randomly. The used psychometric instruments are: CBCL, EPQ for children, and MMPI-201 for mothers.

The obtained results showed high scores for somatization, extroversion and accentuated anxiety for children; as well as a typical Hs-Hy personality profile for mothers.

The treatment with cognitive-behavior therapy and biofeedback showed very positive outcome.

Keywords: somatoform disorders, children, biofeedback, psychology

Introduction

Somatoform disorders are a group of psychological disorders in which a patient experiences physical symptoms that are inconsistent with or cannot be fully explained by any underlying general medical or neurologic condition. This entity is common in pediatric population. It is assumed that more than 50% of patients in a pediatric settings belong to this group. In a general population the somatoform disorders are present in 11% of girls and 4% of boys [1].

This group of disorders can be represented by a wide spectrum of severity, ranging from mild self-limited symptoms, such as stomachache and headache, to chronic disabling symptoms, such as seizures and paralysis. It can be said that the somatoform disorders represent the serious end of a continuum of somatic symptoms [2–5].

It is important to note that these symptoms are not intentionally produced or under voluntary control.

Somatization can be associated temporarily with psychosocial stress and persist even after the acute stressor has been resolved, resulting in the belief by the child and his/her family that the correct medical diagnosis has not yet been found. Thus, patients and families may continue to seek repeated medical treatment after being informed that no acute physical illness has been found and that the symptoms cannot be fully explained by a general medical condition. For the economical point of view, it produces unnecessary expenses in the health care system with heavy utilization of resources through repeated hospitalizations, consultations from different specialists, and ineffective investigations and treatments.

Somatoform disorders are additionally associated with poor school performance and attendance and overall impaired functioning [6–9].

The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)

classifies somatoform disorders in the following diagnoses: somatization disorder, undifferentiated somatoform disorder, somatoform disorder not otherwise specified (NOS), conversion disorder, pain disorder, body dysmorphic disorder, and hypochondriasis. The diagnostic criteria for the somatoform disorders were established for adults and are applied to children for lack of child-specific research base and a developmentally appropriate alternative system [10, 11].

The following criteria are required for a diagnosis of somatoform disorders:

- Four different pain sites (e.g., head, abdomen, back, joints, extremities, chest, rectum) or painful functions (e.g., menstruation, sexual intercourse, urination)
- Two gastrointestinal symptoms other than pain (e.g., nausea, bloating, vomiting, or intolerance of several different foods)
- One sexual or reproductive symptom other than pain (e.g., erectile or ejaculatory dysfunction, irregular menses, excessive menstrual bleeding)
- One pseudoneurological symptom (e.g., impaired balance, paralysis, aphonia, urinary retention)

The so-called "normal" childhood includes an extraordinary range of experiences and adaptive responses. Nevertheless, acute and chronic situations which arise could exceed a child's ability to restore equilibrium. If sufficiently intense or prolonged, these conditions can evoke a variety of biologic and behavior responses. Such responses can lead to the development of a diagnosable disorder. When life is disrupted, often insidiously, worry, sadness, or other unpleasant thoughts and emotions can ensue, along with physical distress presenting as a myriad of bodily symptoms. This is true for both, adults and children [12-15].

It is known that response to different forms of stress is highly individual. One child might have the resilience to move through a difficult life circumstance that overwhelms another, so no single predictable sign or symptom points to the psychosomatic origin of a physical complaint. Table 1 shows the most common pediatric somatic complains related to the age of appearance.

Table 1

Most common somatoform complains in children

Symptom	**Age of appearance**
Recurrent abdominal pain	Preschools children
Headache	Schoolers
Muscle pains	Puberty
Fatigue	Puberty
Neurological symptoms	Puberty

Recurrent complaints often present as diagnostic and treatment dilemmas to the primary care practitioner or a family doctor who is trying to make sense of these symptoms. The doctors may feel poorly prepared and/or may have little time to assess or treat the somatic concerns. While the more disabling somatic complaints are more likely to be referred to a mental health professional, youngsters presenting with these disabling physical symptoms bridge both medical and psychological domains and present a puzzling quandary for professionals from either field if working with them alone. The nature of these symptoms requires an integrated medical and psychiatric treatment approach to successfully decrease the impairment caused by these disorders [16, 17].

The aim of this article is to summarize some psychological specifics of children with somatoform disorders as well to correlate this traits with mother's personality.

Sample and methodology

In this article we evaluate 243 patients with somatoform complains randomly selected from patients treated at the Department for Psychophysiology at the University Pediatric Clinic in Skopje, during a period of 5 years. The mean age of the patients was 10.31 (± 2.75) years for both genders.

The Department for Psychophysiology deals with over 1000 outpatient/year, inpatients comprising 80–100 patients/year, mainly children with somatoform disorders, eating disorders, behavior problems, ADHD, autism, OCD, anxiety disorders etc.

For the evaluated group of patients we applied interviews for mothers and children, Child Behavior Checklist (CBCL) for children

below 12 years, Eysenck Personality Questionnaire (EPQ) for children over 10 years, and Minnesota Multiphase Personality Inventory (MMPI-201) for mothers.

CBCL [18] is designed to obtain the parent's descriptions of their own child behavior in a standardized format. There are 118 behavior problem items plus spaces for parents to write and score additional physical problems with no known medical cause. Two broadband grouping are focused: internalized and externalized. They reflect a distinction between fearful, inhibited, over controlled behavior and aggressive, antisocial, under controlled behavior. The profile can contribute to a formal diagnosis by showing the degree of child's deviance in behaviors that parents are more likely to observe than clinicians, as well as help to structure effective training.

EPQ [19] evaluates the four classical characteristics of the personality: N (level of emotional stability/neurosis); E (dimension of extraversion/introversion); P (psychotic behavior/psychopathy) and L (degree of dissimulation or social adaptability). Our previous experience with this psychometric test confirmed the validity, reliability and discriminability of the obtained results, especially in preadolescents (10–12 years) [20].

MMPI-201 [21] contains ten clinical scales: Scale 1 – Hypochondriasis scale which measures a person's perception and preoccupation with their health and health issues; Scale 2 – the Depression scale measures a person's depressive symptoms level; Scale 3 – the Hysteria scale measures the emotionality of a person; Scale 4 – the Psychopathic Deviate scale measures a person's need for control or their rebellion against control; Scale 5 – Paranoia scale measures a person's inability to trust; Scale 6 – the Psychasthenia scale measures a person's anxiety levels and tendencies for somatization and obsession; Scale 7 – the Schizophrenia scale measures a person's unusual/odd cognitive, perceptual, and emotional experiences, and Scale 10 – the Mania scale measures a person's energy, euphoria or hyperactivity.

Three scales L, F and K are validity scales and measure the readiness of the responders to this kind of examination. L scale refers to rigidity or naiveté of responder's approach to the test material; F scale refers to confused thinking/ lack of understanding the questions or malingering; K scale refers to responses chosen to be socially acceptable.

Raw scores on the scales are transformed into a standardized metric known as T-scores (Mean or Average equals 50, Standard Deviation equals 10), making interpretation easier for clinicians. Before the analysis of the clinical scales, some criteria should be satisfied: L and K scales must be with the score ≤ 70 and F scale ≤ 80. A significant advantage of the MMPI over other self-report and observer rating scales is that it provides valid and reliable estimates of response bias.

The obtained results are statistically evaluated using Statistic 10 package.

Results

The sample comprises 243 children with somatoform disorders, randomly selected. The mean age of the evaluated patients was 10.31 ($\pm$ 2.75) years for both genders. All of them have been outpatients at the Psychophysiology Department of the University Pediatric Clinic in Skopje, the capital of the Republic of Macedonia. The main problems were stomachache 64%; nausea/vomiting 10%; abdominal colic 16% and palpitation/short breathing 5%.

The diagnosis is confirmed using the DMS-IV-R Manuel.

The results obtained for CBCL for boys are presented on Fig. 1. As it can be seen, mothers pointed the internalized symptoms as anxiety and somatoform problems; they are over T-score which is significant.

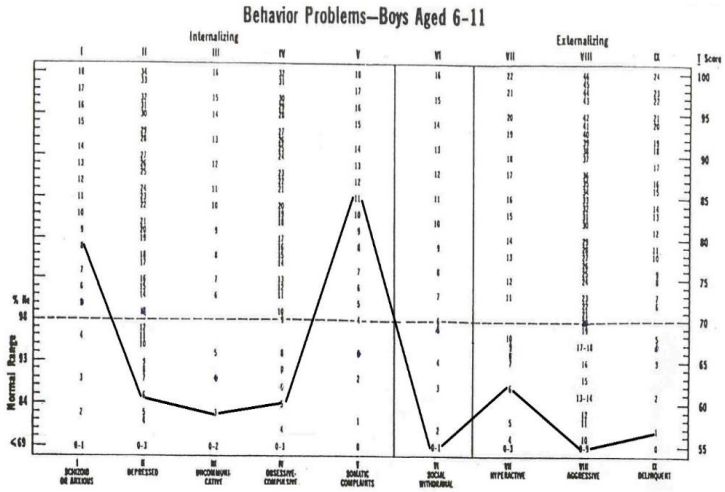

Fig. 1 – Obtained profile for boys on CBCL

For girls, the obtained profile is shown on Fig. 2. Similarly, somatoform complains are dominant and over T-score.

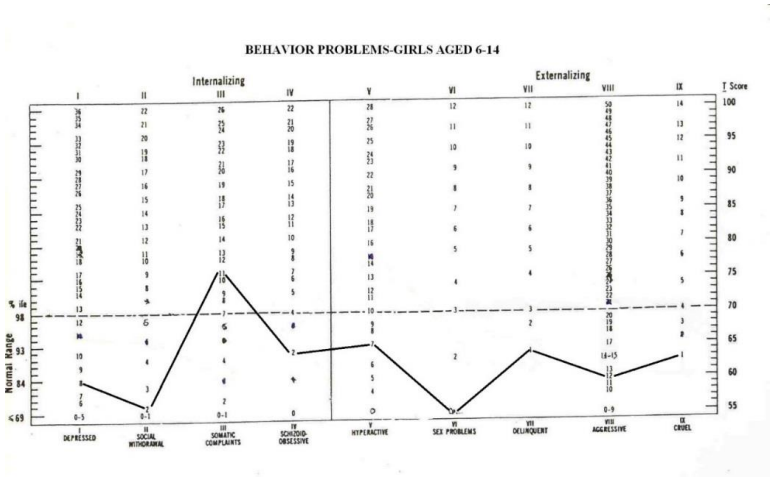

Fig. 2 – Obtained CBCL profile for girls

The EPQ profile for boys (Fig. 3) shows accentuated neurotic tendencies ($p < 0.05$), as well as extroversion ($p < 0.05$).

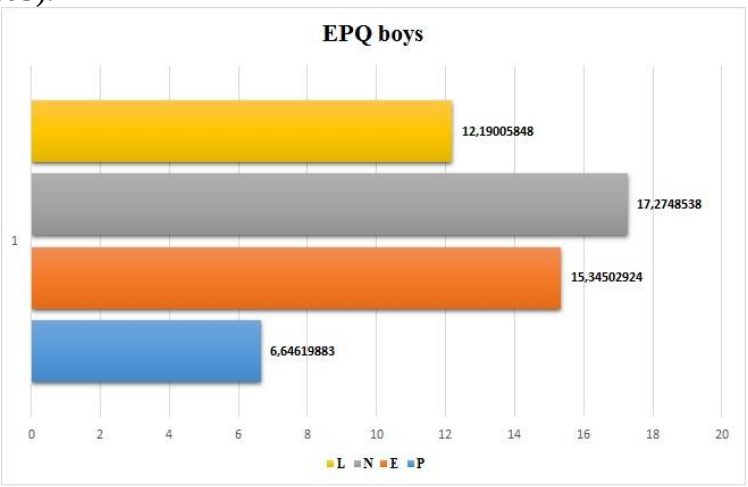

Fig. 3 – EPQ results for boys

For girls (Fig. 4) the EPQ results confirm the much accentuated extroversion ($p < 0.05$), while other personality traits are in normal values.

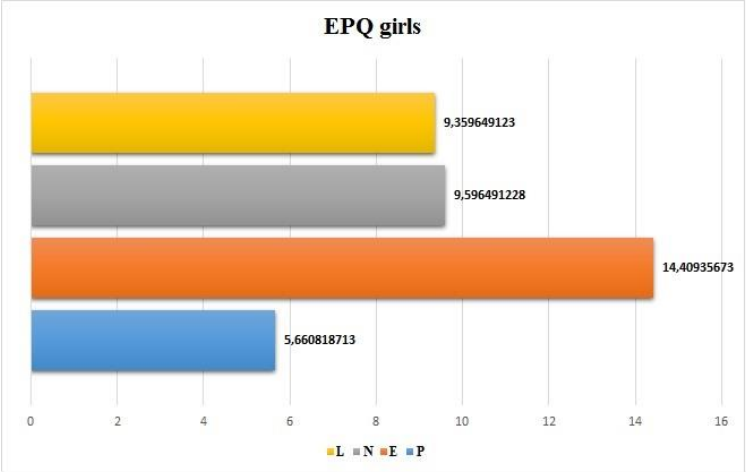

Fig. 4 – EPQ results for girls

The EPQ results are compared with the control group of healthy children at the same age (N = 25).

Having in mind that mothers are the most important personalities for child development, we tested mothers with MMPI-201. The obtained group profile is shown on Fig. 5. The typical **Hs-Hy** profile confirms that mothers of these children are hypersensitive, anxious and react similarly as their children with somatization. The influence of this type of mother as a model for children's behavior is very important not only for the diagnostics but also for the treatment strategies.

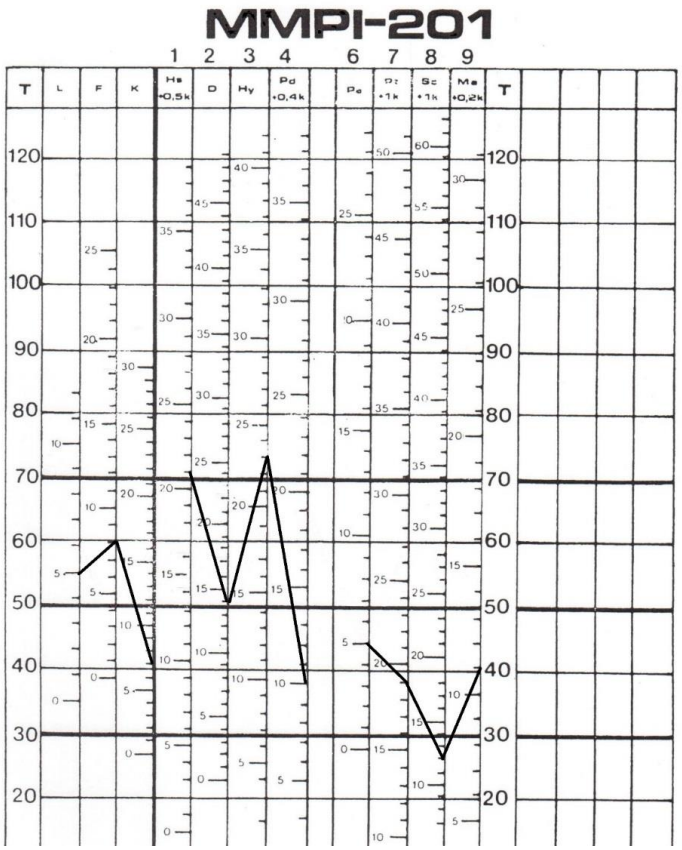

Fig. 5 – Obtained MMPI-201 profile for mothers

The treatment strategies we used are: cognitive-behavioral therapy, family therapy, and peripheral and neurofeedback therapy. As biofeedback modalities we applied electro dermal response (skin conductance) training comprising 10 sessions, organized one a week. Concerning neurofeedback, we used also 10 session of SMR training in Cz positions. SMR means somatosensory rhythm (12–15 Hz.) which is needed for calming the patient and to produce better cognitive abilities. The obtained results for biofeedback are very satisfactory (Fig. 6 and Fig 7).

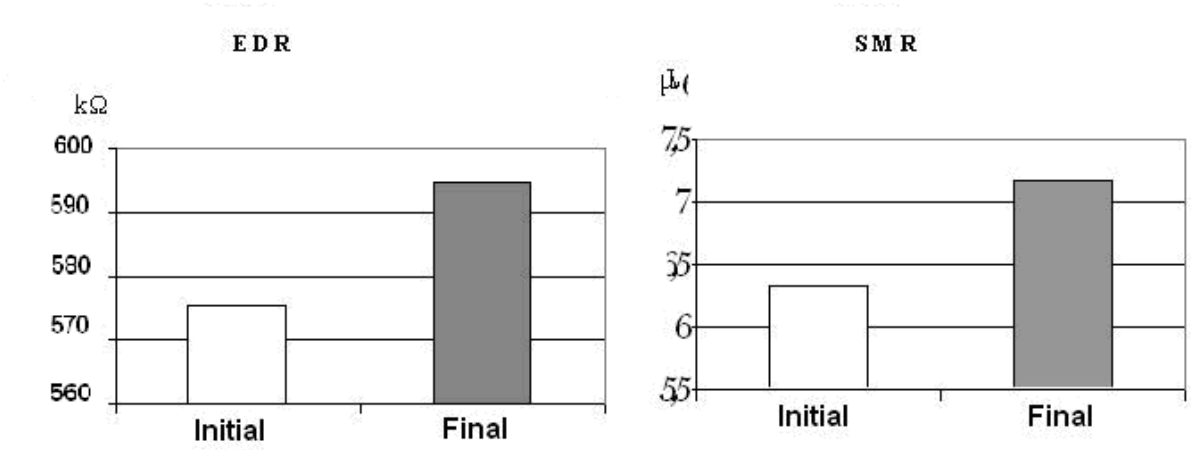

Fig. 6 – Results for EDR and SMR training

The calculated ***t*-test 10.05 ($p < 0.01$)**** for both biofeedback results shows significant improvement.

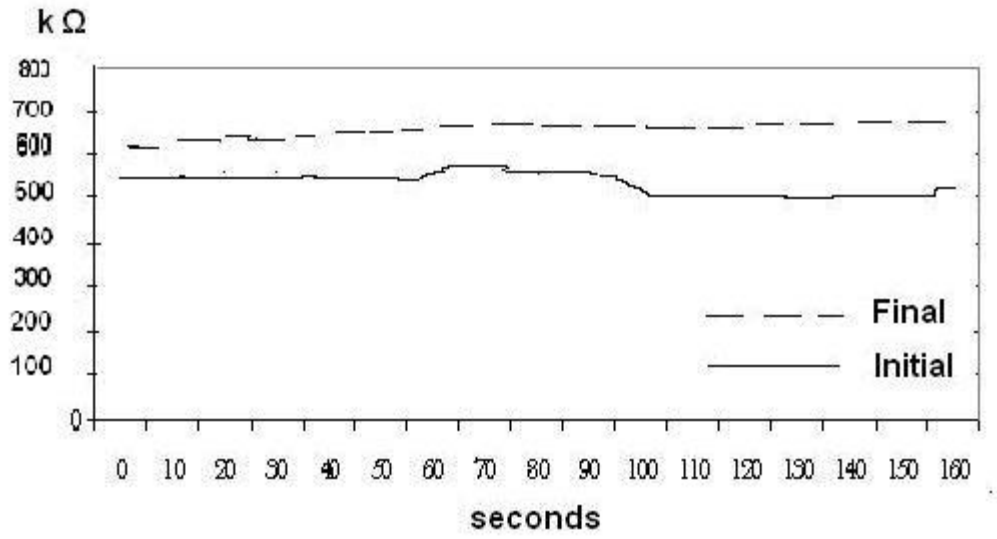

Fig. 7 – Changes for electro dermal activity in initial and final biofeedback session

We have a previously positive experience with the biofeedback therapy [22].

Discussion and conclusion

Being a puzzle between somatic and psychological illness, somatoform disorders can be comorbid with anxiety disorders (separation anxiety, posttraumatic stress disorder) or depression. In this context, psychological/psychiatric interventions are needed [23–29].

It is confirmed that somatoform disorders follow a developmental sequence in which young person experience some affective distress and react in some form of somatic sensations [30–32]. When the child is younger, the most dominant symptoms are recurrent abdominal pain, but later headache, neurological symp-

toms, fatigues, and sleep problems emerge. The difficulty expressing emotional distress verbally is widely thought to underlie the presentation of physical symptoms that cannot be explained in medical terms.

In an early childhood the male to female ration of the somatoform disorders is practically equal, but in adolescence, girls report nearly twice more symptoms than boys.

Krishnakumar and colleagues [30] believe that having more negative affect, being more sensitive to change in the environment, and not persisting in the completion of tasks elevates the risk of developing a conversion disorder in childhood. In addition to using ineffective coping strategies, children with recurrent somatic symptoms tend to focus more intently on the bodily sensations and have heightened emotional responses to stress.

Stuart and Noyes [33] have hypothesized that somatizing behavior is best understood as a unique form of interpersonal behavior driven by an anxious and maladaptive attachment style. Poor coping styles and reinforcement-seeking behavior may also place an individual at risk for developing a somatoform disorder. Youth with more complaints of pain and physical symptoms not only report being angry more often, but also use less-effective strategies to cope with their anger.

Some evidence suggests that medically unexplained symptoms are related to the prior experience of illness in the family and previous unexplained symptoms in the individual. This may reflect a learned process whereby illness experiences lead to symptom monitoring. In this context, the personality profile of mothers could serve as a model for manifestation of symptoms [31–36].

As conclusion we can say that the somatoform disorders in childhood represent a common diagnostic issues. The pediatrician must be aware of the psychological basis of the etiology. A multidisciplinary approach (pediatricians, psychiatrist, and psychologist) is needed.

As therapeutic strategies, the cognitive-behavior therapy, and especially the biofeedback modalities (electro dermal response and neurofeedback) are very useful.

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Резиме

СОМАТОФОРМНИ РАСТРОЈСТВА – ПЕДИЈАТРИСКО ИСКУСТВО

Нада Поп-Јорданова[1], Татјана Зорчец[2]

[1] Македонска академија на науките и уметностите, Скопје, Р. Македонија
[2] Универзитетска клиника за детски болести, Скопје, Р. Македонија

Соматизацијата кај децата се карактеризира со постојано искуство и поплаки за соматски проблеми за кои не може да се најде објаснување преку позната медицинска дијагноза. Работејќи на Одделот за психофизиологија на Клиниката за педијатрија, се справувавме со над 100 деца годишно кои манифестираа вакви проблеми. Целта на овој труд е да се сумираат некои специфични карактеристики на соматоформните растројства во група од 243 деца, средна возраст 10.31 (± 2.75) години кај обата пола, избрани случајно. Користени се психометриските инструменти CBCL, EPQ за децата и MMPI-201 за мајките.

Добиените резултати потврдија високи скорови за соматизација, екстроверзија и анксиозност, додека кај мајките е добиен карактеристичен Hs-Hy профил. Лекувањето со когнитивно-бихевиорална терапија и биофидбек покажа позитивни резултати.

Клучни зборови: соматоформни растројства, биофидбек, деца, психологија

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FACTORS ASSOCIATED WITH LETHAL OUTCOME IN PATIENTS WITH SEVERE FORM OF INFLUENZA

Marija Cvetanovska[1], Zvonko Milenkovik[1], Valerija Kirova Uroshevik[1], Ilir Demiri[1], Vlatko Cvetanovski[2]

[1] Clinic of Infectious Diseases and Febrile Conditions, Clinical Center, Skopje, R. Macedonia
[2] General Hospital Remedika, Skopje, R. Macedonia

Corresponding Author: Marija Cvetanovska, Blvd. AVNOJ 18-1/4, Skopje, R. Macedonia; tel: 022462106; e-mail: mcvetanovska2001@yohoo.com

Abstract

Introduction: Clinical manifestations of influenza range from relatively mild and self-limiting respiratory infections to severe clinical manifestations with significant morbidity and mortality. The awareness of predictive indicators for the lethal outcome of influenza is of particular significance in making timely and exact decision for adequate treatment. The *aim* of this study was to identify the factors in patients with a severe form of influenza, resulting in lethal outcome.

Materials and methods: The investigation was a prospective group comparison conducted at the University Clinic for Infectious Diseases in Skopje, R. Macedonia in the period from January 01, 2012 to January 01, 2015. The study included adult patients with a severe form of influenza who were further categorized into a group of either survived patients or a group of deceased patients. Demographic, clinical and biochemical data were noted in all patients included in the study on admission. The variables of the univariate analysis that showed a significant difference in terms of the outcome were used for creating multivariate logistic and regression analysis of the outcome as dependent factors. The independent predictors for lethal outcome in severe cases of influenza were identified by using logistic regression.

Results: The study included 87 patients with a severe form of clinical and laboratory confirmed influenza. The patients were divided in two groups: survived (n = 75) and deceased (n = 75). The overall mortality was 13.79%. Multivariate analysis conducted on admission to hospital identified cardiovascular comorbid diseases (p = 0.014), urea values higher than 8.3 U/L (p = 0.045) and SAPS score (p = 0.048) as independent predictors of the outcome in patients with severe form of influenza. Influenza patients with cardiovascular diseases had 2.024 times greater risk of death from influenza in comparison to the patients having influenza without history of such a disease (OR = 2.024 95% CI 1.842–17.337). Patients with serum urea values higher than 8.3 U/L had 1.89 times higher chance of death compared to patients with normal values (OR = 1.89 95% CI 1.091–11.432). The increase of the SAPS score in one point increased the chance of death in patients with influenza by 1.2% (OR = 1.12 95% CI 1.01–2.976). The ROC analysis indicated that cardiovascular diseases, increased urea values and SAPS score in combination act as a good prognostic model for the fatal outcome. The global authenticity of this predictive model to foresee lethal outcome amounts to 80%, sensitivity being 82%, and specificity 70%.

Conclusion: Cardiovascular diseases, increased values of urea over 8.3 mmol/l and SAPS score are independent predictive indicators for lethal outcome in severe influenza. Early identification of the outcome predictors in patients with severe influenza will allow implementation of adequate medical treatment and will contribute to decreasing of mortality in patients with severe form of influenza.

Keywords: severe influenza, predictive indicators, lethal outcome

Introduction

Clinical manifestations of influenza range from relatively mild and self-limiting respiretory infections to severe clinical manifestations with significant morbidity and mortality [1]. During seasonal epidemics from 3 to 5 million severe cases and about 250.000–500.000 lethal cases are registered worldwide [2]. Until now there has not been a laboratory test which has served as a potential marker for identification of patients with a high risk of developing severe clinical forms of influenza and lethal outcome [3, 4]. It is known that patients with different comorbid conditions such as diabetes mellitus, chronic cardiovascular and pulmonary diseases, immunosuppressive conditions, adult patients and other conditions are at higher risk of developing severe clinical course of the disease and lethal outcome [5]. Although the influenza virus is primarily a respiratory pathogen, the severe clinical forms of the disease are manifested as systemic infections with multisystem organ affection, and even 10–30% of the diseased need intensive treatment [6, 7]. Pneumonia, delayed antiviral treatment, severe hypoxemia and multisystem organ failure are most commonly referred as leading risk factors for lethal outcome [8]. The largest number of studies has evaluated isolated risk factors leading to lethal outcome and only a few of them have been focused on the complete palette of predictors for development of a severe form of the disease and lethal outcome [9–12]. From the clinical practice point of view, the awareness/recognition of the risk factors and predictors for lethal outcome of influenza is of particular importance in bringing timely and exact decision for hospitalization, treatment or undertaking special measures for intensive monitorring of these patients.

Severe influenza is defined by signs for respiratory weakness (dyspnea, tachypnea, hypoxia, cyanosis) that is arterial PaO2 < 70 mmHg (< 9.0 kPa) and/or need of mechanical ventilation or signs of ARDS (PaO2/FiO2 ≤ 200), intensive care, severe complications, exacerbation of the existing chronic disease.

The aim of this study was to identify the risk factors that lead to lethal outcome in patients with severe form of influenza.

Materials and methods

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

The study was clinical, prospective, group comparison and it was performed at the Clinic for Infectious Diseases and Febrile Conditions in a three-year-period (01.01.2012–01.01.2015).

A total of 87 patients with severe forms of clinically and laboratory confirmed influenza were analyzed. The patients were divided into two groups:

Group 1 contained 75 patients who survived and

Group 2 contained 12 patients who had lethal outcome

Criteria for inclusion in the study:

All patients with clinical and laboratory confirmed severe form of influenza.

- Age ≥ 16 years

Criteria for exclusion of the study:

- Patients were excluded if they died in the first 24 hours of their inclusion in the study. Those that did not receive approval for inclusion

On admission of patients, the following parameters were noted: demographic characteristics, comorbidities, clinical signs of the disease and laboratory-biochemical characteristics.

For determining the presence of the influenza virus nasopharyngeal smear was used. In the Laboratory of virology and molecular diagnosis at the Institute for Public Health from the previously isolated RNA (ribonucleic acid) real time **RT-PCR** (**reverse transcriptase/ion-polymerase chain reaction in real time**) was performed on the apparatus IQ (BioRad) for detection of matrix gene of influenza A and influenza B. The samples positive to influenza A were subtyped by the same method, by RT-PCR, with a specific set of primers for highly conserved regions of X1, X3 and X1 pdm (pandemic).

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

metric tests (t-test for independent samples, Analysis of Variance) were also used non-parametric tests for independent samples (Mann-Whitney U test, Chi-square test, Fisher-exact test).

Regarding the determination of the prognostic factors of death in patients with influenza the method of multivariate analysis was used (Logistic Binary Regression), by which the relation of probability of exposure (OR) was determined as an approximate value of the real risk (RR). The statistical precision of (OR) was obtained by calculation of the confidence intervals (CI) about the estimated values.

The value of $p < 0.05$ was considered to be statistically significant, and the value of $p < 0.01$ highly significant.

Results

The study included 87 patients with a severe form of clinically and laboratory confirmed influenza, who were treated at the Clinic for Infectious Diseases and Febrile Conditions in the period from 01.01.2012 to 01.01.2015. Twelve (13.79%) of them died.

Our results showed that women died insignificantly more often than men (16.13% vs 12.5% ($p = 0.64$).

The age had significant influence on the disease outcome ($p = 0.019$). The mean age of the deceased patients was 65.58 ± 17.5 years, opposite the mean age of the survived patients which was 53.04 ± 16.8 years.

The place of living of the patients had no significant influence on the outcome ($p = 0.44$), that is, patients from the rural environment died insignificantly more often than patients from the urban environment (22.22% vs 12.82%) (Table 1).

Table 1

Demographic characteristics of patients regarding outcome

variable	Severe influenza			p value
	Total n = 87	Survived n = 75	Deceased n = 12	
Sex [n (%)]				
women	31(35.63)	26(83.87)	5(16.13)	[a]0.64
men	56(64.37)	49(87.5)	7(12.5)	
Age (mean ± SD)				
	54.77 ± 17.3	53.04 ± 16.8	65.58 ± 17.5	[b]0.019*
Place of living [n (%)]				
city	78(89.65)	68(87.18)	10(12.82)	[a]0.438
village	9(10.34)	7(77.78)	2(22.22)	

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

The patient who was vaccinated against influenza overcame the disease whereas 13.95% of the patients who were not vaccinated died. ($p = 1.0$).

Prior to hospitalization 77.78% of survived patients 22.22% of patients who died were treated with osaltamivir ($p = 0.6$). The duration of health problems prior to hospitalization differed significantly between the survived and deceased patients ($p = 0.05$). The mean duration of symptoms prior to hospitalization was 5 days in the group of survived patients and 7 days in the group of deceased patients (Table 2).

Table 2

Vaccination, use of osaltamivir, days prior to admission in relation to outcome

variable	Influenza			p value
	Total n = 87	Survived n = 75	Deceased n = 12	
Vaccine [n (%)]				
yes	1(1.5)	1(100)	0	[c]1.0
no	86(98.85)	74(86.05)	12(13.95)	
Use of osaltamivir prior to admission [n (%)]				
no	78(89.65)	68(87.18)	10(12.82)	[c]0.6
yes	9(10.34)	7(77.78)	2(22.22)	
Days prior to admission (median IQR)				
	5 (3–7)	5 (2–7)	7 (4–7)	[d]0.05

[a]p (Chi-square test) [c](Fisher exact test) [d](Mann-Whitney U test)

The highest mortality rate of influenza was registered at the Intensive Care Unit (22.5%) (p < 0.001). Statistically significant difference was also registered in the outcome of patients who were and who were not treated with mechanical ventilation where significantly dominated the deceased patients who underwent mechanical ventilation (41.67% vs 9.33% p = 0.01) The results of our study have demonstrated that patients with comorbid conditions died more often than those without these diseases (15.38% vs 9.09%) (p = 0.72). The cardiovascular diseases had a significant impact on the outcome of influenza (p = 0.011). All other analyzed comorbid conditions such as: chronic pulmonary diseases (survived 92.31% vs deceased 7.69%) (p = 0.68), neurological diseases (80% vs 20%) (p = 0.62), renal diseases (60% vs 40%) (p = 0.14), endocrinological diseases (88.24% vs 11.76%) (p = 1.0), hematological diseases (83.33% vs 16.67%) (p = 1.0) were insignificantly associated with outcome in patients with influenza. Small number of patients who had previous immunological disease (1), hepatic diseases (1) and obesity (2) survived in spite of being presented with severe form of influenza. Two pregnant patients also survived. SAPS 2 score which was calculated in the first 24 hours of admission, was significantly associated with lethal outcome (Table 3).

Table 3

Stay at the Intensive care unit, days on intensive care and mechanical ventilation, comorbid conditions, SAPS 2 score in relation to outcome

variable	Total n = 122	Influenza Survived n = 75	Deceased n = 12	p value
Intensive [n (%)]				
no	47(54.02)	46(97.87)	1(2.13)	[a]0.0006
yes	40(45.98)	29(72.5)	11(27.5)	
Days at intensive (median IQR)				
	7 (3–11)	6 (3–10)	8 (2–12)	[d]0.72
Mechanical ventilation [n (%)]				
no	75(86.21)	68(90.67)	7(9.33)	[c]0.01
yes	12(13.79)	7(58.33)	5(41.67)	
Comorbid conditions [n (%)]				
no	22(25.29)	20(90.91)	2(9.09)	[c]0.72
yes	65(74.71)	55(84.62)	10(15.38)	
Cardiovascular disease [n (%)]				
no	44(50.57)	42(95.45)	2(4.55)	[a]0.011
yes	43(49.42)	33(76.74)	10(23.26)	
SAPS 2 score (mean ± SD) median (IQR)				
	36.4 ± 29.1 med 26(17–42)	33.7 ± 28.9 med 23(16–37)	53.4 ± 24.9 med 46.5(40–53)	[d]0.00038**

[a]p (Chi-square test) [c](Fisher exact test) [d](Mann-Whitney U test)

Out of all the laboratory–biochemical analyses conducted on admission, only urea > 8,3 mmo/l, showed significant association with a lethal outcome (survived 25.33% vs deceased 66.67%) (p = 0.007).

Tables 4 and 4a present the results from Univariate Logistic Regression analysis in determining the analyzed demographic, clinical and biochemical variables that have confirmed to be predictors of the lethal outcome.

The results of the multivariate analysis as independent predictors of lethal outcome, from the analyzed demographic, clinical and biochemical parameters have confirmed the following: cardiovascular comorbidities (p = 0.014), urea values higher than 8.3 U/L (p = 0.045) and SAPS 2 score (simplified acute physiology score) (p = 0.048).

Patients with influenza and cardiovascular diseases had 2.024 times higher risk of death by influenza when compared to patients with influenza without history of cardiovascular comorbidity (OR = 2.024 95% CI 1.842–17.337).

Table 4

Univariate Logistic regression analysis for prediction of lethal outcome in patients with influenza

variable	**Crude OR 95% CI for OR**	**p value**
Demographic variables		
age	1.05 (1.006– 1.095)	0.025*
Men vs women	0,743 (0.214– 2.573)	0.639
Village vs town	1.943 (0.353–10.698)	0.445
Tamiflu prior to admission	0.515 (0.093– 2.834)	0.445
Comorbidity	1.818 (0.366– 9.025)	0.465
Number of comorbidities	3.167 (0.903– 11.102)	0.072
cardiovascular	6.364 (1.304– 31.055)	0.022**
Clinical variables (symptoms)		
temperature >37.8°	0.364 (0.105–1.259)	0.11
dyspnea	1.067 (0.292–3.859)	0.928
cyanosis	1.056 (0.258–4.324)	0.94
Chest pain	1.109 (0.303–4.057)	0.876
pulse >80	1.313 (0.149–11.555)	0.806
SAP <120	0.8 (0.076–8.474)	0.853
SAP >120	1.077 (0.112–10.369)	0.949
respirations >20	1.25 (0.247–6.318)	0.787
SAPS	1.15 (1.07–3.18)	0.039*
RTG finding		
consolidation	1.091 (0.268–4.438)	0.903

Table 4a

Univariate Logostic regression analysis for prediction of lethal outcome in patients with influenza

variable	**Crude OR 95% CI for OR**	**p value**
Biochemical variables		
leukocytes > 9	1.027 (0.304–3.474)	0.966
thrombocytes <140	2.0 (0.492–8.129)	0.333
thrombocytes > 250	0.737 (0.136–3.992)	0.723
glycemia > 6.3	1.891 (0.473–7.569)	0.368
urea > 8.3	5.89 (1.593–21.807)	0.008**
creatinine > 110	2.222 (0.627–7.87)	0.216
potassium < 3.5	0.429 (0.05–3.672)	0.492
potassium > 5.5	1.714 (0.306–9.599)	0.54
sodium < 135	0.653 (0.126–3.372)	0.611
sodium > 145	1.175 (0.216–6.388)	0.852
ALT > 52	1.706 (0.457–6.362)	0.426
AST > 47	1.885 (0.552–6.431)	0.312
LDH > 618	3.152 (0.644–15.422)	0.156
CPK > 170	1.083 (0.32–3.655)	0.898
bilirubin > 17	1.05 (0.204–5.407)	0.953
bicarbonates	1.893 (0.583–6.653)	0.32

Patients with serum urea levels higher than 8.3 U/L had 1.89 times greater chance for dying compared to patients with normal values (OR = 1.89 95% CI 1.091–11.432).

The increase of SAPS score for one score increases the chance of death in patients with influenza by 1.2% (OR = 1.12 95% CI 1.01–2.976).

Table 5

Multivariate Logistic regression analysis for prediction of lethal outcome in patients with influenza

variable	**Adjusted OR 95% CI for OR**	**p value**
Cardiovascular	2.024 (1.842–17.337)	0.014*
urea > 8.3	1.89 (1.091–11.432)	0.045*
SAPS score	1.12 (1.01–2.976)	0.048*

ROC analysis has demonstrated that the combination of cardiovascular diseases, the increased urea values and the SAPS score are a good prognostic model of the lethal outcome. The area under the ROC curve, that is, AUC was 0.755, with 95% confidence interval from 0.587–0.923 suggesting that the probability of combination of these two predictors for death in influenza patients was 75.5%.

The global precision of this predictive model to foresee the lethal outcome was 80%, sensitivity 82%, and specificity 70%.

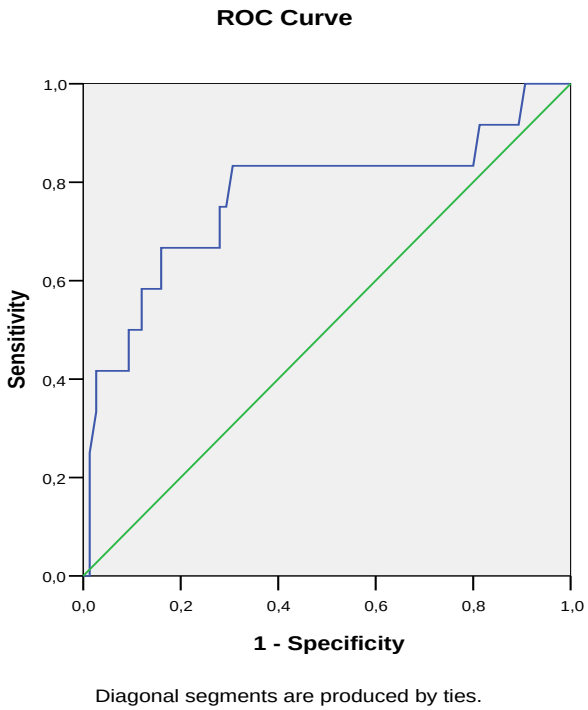

Figure 1 – ROC curve for the influence of cardiovascular diseases, urea and SAPS score in prediction of lethal outcome from influenza

Discussion

The mortality rate of the hospitalized patients with severe influenza infection amounted to 13.79% in our study. The percentage of lethality varies among published studies and it ranges from 10% to extreme 59%, which certainly depends on the various conditions and criteria according to which the patients are analyzed as well as on the criteria for admission to intensive care units [13–15]. Thus, the study performed in China showed that from 60 patients with severe form of influenza 44% were treated at Intensive care unit and the lethality was 14.7% [16].

There was no a significant difference regarding the mortality between male and female patients in our study, although in most of the studies the male sex was identified as a risk factor associated with lethal outcome. [17, 18]. Our results have demonstrated that from the total number of 12 lethal outcomes 5 or (16.1%) were women and 7 (12.5%) were men. Our study is similar to that conducted in Canada where from the total number of 29 lethal out-

comes, 27.6% were men, whereas 72.4% were women [17]. The age had significant influence on the disease outcome in our study. The mean age of patients that died was 65.58 (p = 0.019). The mortality was the highest in patients at the age over 65 (27.2%). These results coincide with almost all studies in the world that identify the old age as an important risk factor for mortality in patients with influenza [19]. The place of living of the patients was not significant in relation to the outcome. Patients from the rural environment exited as patients from the urban environment, 22.2% vs 12.8%.

According to many studies the use of neuraminidase inhibitor within 48 hours from the beginning of the symptoms decreases the risk of progression into a severe form and death of patients with influenza. In addition, the guidelines of the WHO recommend early treatment with oseltamivir for suspected influenza cases and warn that delayed medical attention increases the mortality rate [20]. In our study, 89.6% of patients did not use osaltamivir prior to admission, whereas only 10.35% of them used this medication. In the first group the mortality rate was 12.8% vs 22.2% in the second group (p = 0.06). The answer probably lies in two important issues. The first one is the small group of patients and the second one arises from the existence of resistant forms of the virus [21, 22].

The results of our study have shown that in the group of patients with severe influenza without comorbidity the mortality rate was 9.09% whereas in the group of patients with associated comorbid diseases the mortality rate was higher than in the first one (15.38%) (p = 0.72). The analysis of identified associated chronic conditions have shown that cardiovascular diseases had significant influence on the outcome from influenza (p = 0.011). While patients with a negative history of cardiovascular comorbidity yielded a mortality rate of 4.55%, the patients who did have cardiovascular disease present, yielded a significantly higher mortality rate of 23.26% [5].

All 87 patients with severe influenza in our study had higher body temperature than 37.8º. The mean body temperature between the group of survived and deceased patients showed no statistically significant correlation (38.7 ± 0.7 vs 38.4+ 0.8) p = ns. The other clinical symptoms that were analyzed did not differ among themselves in the group of survived patients and those that died, although the latter group complained on cough, dizziness, dyspnea and chest pain [23–25].

In all of the patients where the diagnostic protocol included chest radiologic procedures in our study, the statistical analysis indicated that 60% of patients that had diffuse bilateral consolidation died contrary to 10.9% of patients who did not have this type of radiologic finding (p = 0.017) [26].

Laboratory and biochemical analyses were performed in patients on their admission to the Clinic, 24 and 48 hours after admission. The statistical analysis showed significantly decreesed values of erythrocyte the second day of the hospitalization (p = 0.009), higher mean value of sedimentation rate (p = 0.02) as well as higher percentage of neutrophils in the group of deceased patients opposite to the survived patients (p = 0.0005). In relation to the remaining biochemical parameters the glycemia was with higher values in the group of deceased patients but statistical significance was confirmed only 48 hours after admission (p = 0.001). In all three measurements the level of urea higher than 8.3 mmol/l was significantly more often in the group of deceased patients (p = 0.007, p = 0.0027 and p = 0.017).

The creatinine level higher than 110 micromol/l demonstrated statistically significant difference between the two groups only in the period of 24 hours after hospitalization. These findings correlate with most of the studies where the increased level of serum createnine was confirmed as a significant statistical factor that had influence on the outcome.

With reference to the mean values of bilirubin, CRP, ALAT, ASAT, CPK, LDH albumins and total proteins analyzed on admission, 24 and 48 hours after admission, the following parameters were statistically significant in the group of patients who died: bilirubin 48 hours after admission (p = 0.038), CRP after 24 and 48 hours (p = 0.01), p = 0.0004), ASAT after 24 hours (p = 0.006) albumins after 48 hours (p = 0.019) and total proteins on admission, 24 and 48 hours (p = 0.007, p = 0.002 and p = 0,002). All these results are in agreement with

a large number of studies that have analyzed the lab-biochemistry parameters [14, 18, 19].

As independent predictors for lethal outcome in patients with influenza, we have identified the following variables: cardiovascular diseases ($p = 0.014$), urea levels higher than 8.3 ($p = 0.045$) and SAPS score ($p = 0.048$). Patients with influenza and cardiovascular diseases had 2.024 times higher risk of death of influenza compared to influenza patients without history of cardiovascular comorbidity. The confidence interval was 98%. Those patients that had urea levels higher than 8.3 had 1.89 times bigger chance for lethal outcome compared to patients with normal values of urea. The increase of SAPS score for one score raises the risk of death in patients with influenza by 1.2%.

Conclusion

In our study the mortality rate was 13.9%. Cardiovascular diseases, the increased urea level over 8.3 mmol/l and SAPS score have been identified as independent variables, which have predicted the outcome in patients with severe influenza on the very admission to the Clinic demonstrating precision of this predictive model of 80%.

The early identification of the outcome predictors in patients with severe influenza will ensure implementation of adequate medical procedures, and also, it will contribute to decreasing the mortality of this disease.

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Р е з и м е

ФАКТОРИ АСОЦИРАНИ СО СМРТЕН ИСХОД КАЈ ПАЦИЕНТИ СО ТЕШКА ФОРМА НА ИНФЛУЕНЦА

Марија Цветановска[1], Звонко Миленковиќ[1], Валерија Кирова-Урошевиќ[1], Илир Демири[1], Влатко Цветановски[2]

[1] Клиника за инфективни болести и фебрилни состојби, Клинички центар, Скопје, Р. Македонија
[2] Општа болница „Ремедика“, Скопје, Р. Македонија

Вовед: Клиничките манифестации на инфлуенца се движат во дијапазон од релативно лесни и самолимитирачки респираторни инфекции до тешки клинички манифестации со сигнификантен морбидитет и морталитет. Познавањето на предикторите за смртен исход од инфлуенца е од посебно значење за донесување навремена и правилна одлука за соодветен третман.

Цел на оваа студија е да се идентификуваат факторите кои укажуваат на смртен исход кај пациентите со тешка форма на инфлуенца.

Материјал и методи: Истражувањето е проспективно групно споредбено и е изведено на Универзитетската клиника за инфективни болести во Скопје, Р. Македонија, во период од 1 јануари 2012 до 1 јануари 2015 година. Во студијата се вклучени возрасни пациенти со тешка форма на инфлуенца кои понатаму се поделени на група преживеани и група починати пациенти. При вклучување во студијата се бележени демографски, клинички и биохемиски податоци. Варијаблите од униваријантната анализа кои покажаа значајна разлика во однос на исходот се употребени за изработка на мултиваријантна логистичка регресивна анализа за исходот како зависни фактори. Со логистичката регресија се добиени независни предиктори за смртен исход од тешка форма на сезонска инфлуенца.

Резултати: Во студијата беа вклучени 87 пациенти со тешка форма на клинички и лабораториски потврдена сезонска инфлуенца. Болните беа поделени во две групи: преживеани (н = 75) и починати (н = 12). Вкупната смртност изнесуваше 13,79%. Мултиваријантната анализа при приемот ги издвои кардиолошките коморбидитетни болести (п = 0,014), вредностите на уреа повисоки од 8,3 У/Л (п = 0,045) и САПС скорот (п = 0,048) како независни показатели кои го предвидуваат исходот кај болните со тешка инфлуенца. Пациентите со инфлуенца и кардиолошки заболувања имаат за 2,024 пати поголема шанса за смрт од инфлуенца, компарирано со пациентите со инфлуенца без историја за кардиолошки коморбидитет (OR = 2,024 95% CI 1,842 – 17.337). Пациентите со вредности на уреа во серум повисоки од 8,3 U/L имаа за 1.89 пати поголема шанса за егзитирање, компарирано со пациентите со нормални вредности (OR = 1,89 95% CI 1.091 – 11,432). Зголемувањето на САПС-скорот за еден скор ја зголемува шансата за смрт кај пациентите со инфлуенца за 1,2% (OR = 1,12 95% CI 1.01 – 2,976).

ROC-анализата покажа дека кардиолошките заболувања, покачени вредности на уреа и САПС скорот како комбинација претставуваат сигурен прогностички модел за летален исход. Глобалната точност на овој предиктивен модел да предвиди летален исход изнесува 80%, сензитивноста е 82%, специфичноста е 70%.

Заклучок: Кардиолошките заболувања, покачените вредности на уреа над 8,3 ммол/л и

САПС-скорот се независни предиктори за смртен исход кај тешка инфлуенца. Раната идентификација на показателите на исходот кај болните со тешка инфлуенца ќе овозможи имплементација на адекватни медицински постапки и ќе придонесе за намалување на морталитетот на болните со тешка форма на сезонска инфлуенца.

Клучни зборови: тешка инфлуенца, предиктори, смртен исход

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ASSOCIATION OF SINGLE-NUCLEOTIDE POLYMORHISM C3435T IN THE ABCB1 GENE WITH OPIOID SENSITIVITY IN TREATMENT OF POSTOPERATIVE PAIN

Vanja Dzambazovska-Trajkovska[1], Jordan Nojkov[1], Andrijan Kartalov[1], Biljana Kuzmanovska[1], Tatjana Spiroska[1], Redzep Seljmani[2], Gjorgji Trajkovski[2], Nadica Matevska-Geshkovska[3], Aleksandar Dimovski[3]

[1] University Clinic for Anesthesiology, Reanimation and Intensive care, Faculty of Medicine, University "Ss Cyril and Methodius", Skopje, R. Macedonia
[2] University Clinic for Digestive Surgery, Faculty of Medicine, University "Ss Cyril and Methodius", Skopje, R. Macedonia
[3] Faculty of Pharmacy, University "Ss Cyril and Methodius", Skopje, R. Macedonia

Corresponding Author: Vanja Dzambazovska Trajkovska, MD MSc, Anesthesiology/ Intensive care specialist University Clinic for Anesthesiology, Reanimation and Intensive care, Faculty of Medicine, University "Ss Cyril and Methodius" Skopje; tel: 00 +389(0)2 070 26 65 36; e-mail: vanjadztrajkovska@gmail.com

Abstract

Background: The minimal effective analgesic concentration of opioids required for satisfactory analgesia may differ significantly among the patients. Genetic factors may contribute to the variable response to opioids by affecting their pharmacokinetics or pharmacodynamics.

Methods: Ninety nine patients undergoing abdominal surgery with colorectal anastomosis because of colorectal carcinoma were enrolled in the present study. C34535T was genotyped in all subjects and the patients were divided into three groups according to their genotype: CC-wild type homozygous, CT-mutant heterozygous and TT-mutant homozygous. Intravenous fentanyl, patient controlled analgesia was provided postoperatively for pain control in the first 24 hour after surgery. Opioid consumption, pain scores and the adverse side effects were evaluated.

Results: Our main result is that the patients in the CC genotype group consumed significantly more fentanyl (375.0 μg ± 43.1) than the patients in the TT group (295.0 μg ± 49.1) and the CT (356.4 μg ± 41.8) group in the treatment of postoperative pain. The patients in the TT group had lower VAS scores at 6h, 12h, 18 h and 24h postoperatively. There were no significant differences in the side effects among the three groups regarding the vomiting and the sedation score. The patients in the TT group had more frequently nausea score 1, than the patients in the other two groups.

Conclusion: Our study indicates that the C3435T SNPs of the ABCB1 gene is associated with differences in the opioid sensitivity. The ABCB1 polymorphism may serve as an important genetic predictor to guide the acute pain therapy in postoperative patients.

Keywords: Fentanyl, ABCB1, Postoperative analgesia

Introduction

Opioids are generally considered as the first line therapy for patients with moderate to severe postoperative pain. The dose of opioids required to achieve sufficient postoperative pain relief is highly variable among patients. The inter-individual variations in response to opioids can partly be attributed to age, gender, weight (BMI), renal or liver functions. However, as each patient often responds differently to specific opioids, providing adequate analgesia for individual patients without concomitant development of adverse effects is still a major challenge.

Each patient may respond differently to specific different opioids. There still exists a need to sort out the multiple explanations for

some variability encountered with the human responses to opioids. The minimal effective analgesic concentration of opioids required for satisfactory analgesia may considerably vary among the patients [1]. Genetic factors may contribute to the variable response to opioids by affecting their pharmacokinetics (drug metabolizing enzymes and transporters) or pharmacodynamics (receptor and signal transduction).

Drug transporters are important structural proteins that can influence the absorption, distribution and elimination of opioids [2]. In the gastrointestinal tract and hepatocytes they have the ability to influence the bioavailability of the orally administrated opioids by restricting or facilitating the intestinal absorption and facilitating presystemic biliary elimination [3, 4]. In particular, the transporter expression at the blood–brain barrier has the potential to significantly influence the clinical efficacy and safety of opioids, whose major site of action lies within the central nervous system [2]. Both efflux and uptake carrier systems have been implicated in the transport of opioids (drugs and peptides), with multiple transporters often functionning in concert to facilitate the efficient transfer of substrates across biological membranes. The 2 major families of drug transporters of relevance to opioid pharmacokinetics are the ATP binding cassette (ABC) superfamily of efflux transporters, and the solute carrier (SLC) superfamily of influx transporters. The ABC superfamily of efflux transporter consist of nearly 50 known human members divided in 7 sub-families. The most characteristic of the ABC transporters is the ABCB1 MDR1, P – glycolprotein (P-gp) efflux transporter which functions at the capillary endothelial cells of the blood brain barrier (with the ABCC family being less well studied). Opioid induced analgesia is increased and prolonged in mice lacking P g-p. Morphine, methadone, loperamide, and fentanyl have all been confirmed as P-gp substrates [1].

The most investigated of the common ABCB1 genetic polymorphisms is the non-synonymous exon 26 SNP, C3434T, which is observed with a frequency of 50–60% in Caucasians, 40–50% in Asians, and 10–30% in Africans. [5–8]. There was a significant relationship between 3435 genotype and the extent of loperamide miotic effects following p-gp inhibition by quinidine [1].

The ABCB1 gene is composed of 28 exons ranging in size from 49 to 299 base pairs, encoding an mRNA of 4.5 kb. The most common polymorphisms found in ABCB1 are 1236C > T, 2677 G > T/A/C, and 3435C > T. It has been suggested that the genetic variations in ABCB1 could be a cause of inter-individual differences in drug response [9]. In this study we investigated the C3435T Single Nucleotide Polymorphisms – SNPs of ABCB1 where C > T. Thus, the major allele is CC, the heterozygous minor allele is CT and homozygous minor allele is TT.

The aim of the study is to evaluate the association between C3435T and the opioid consumption in the acute postoperative period in patients who have undergone abdominal surgery with colorectal anastomosis. Additionally, we explored the association between C3435T and the opioid side effects in the acute postoperative period in the same population.

Methods

Study subjects and analgesia

This was a prospective study approved by the institutional Ethics Committee (No 03-6608/2). A signed informed consent was obtained from all patients. Between July 2013 and February 2016, 100 patients with the American Society of Anesthesiologist physical status of I-III aged 35–75 years, and undergoing abdominal surgery with colorectal anastomosis because of colorectal carcinoma, were enrolled in the present study. The main exclusion criteria included liver and renal disease, history of chronic pain, severe cardiovascular disease, diabetes mellitus, psychiatric disorders, pregnancy, lactation, allergy to opioids, unwillingness to cooperate in the pain assessment, and administration of non-steroidal anti-inflammatory analgetics and/or opioids one week before surgery. All patients underwent surgery under combined general/epidural anesthesia.

After recovering from anesthesia, all patients received fentanyl by intravenous patient-controlled analgesia (PCA) using a PCA pump containing 100 ml saline 0.9%, 1 mg fentanyl. The PCA pump was programmed to give a 20 μg bolus (2 ml) of solution with 5-min lockout time, 5 $\mu g.h^{-1}$ fentanyl background infusion and maximum 145 $\mu g.h^{-1}$ [10]. The delivered fentanyl dose was automatically recorded by the pump. Nausea and vomiting following abdominal surgery are common, therefore all patients were intravenously administered 30 mg metoclopramide divided into three doses. The pati-

ents were monitored closely to prevent fentanyl overdose (pulse oxygen saturation, heart rate and noninvasive blood pressure).

Pain at rest was assessed using 10 cm VAS with a range 0–10, with no pain as zero and the worst possible pain as ten. Successful analgesia was defined as a postoperative VAS pain score ≤ 3. The side effects were recorded every 6 h after completion of the operation, i.e. at 6, 12, 18 and 24 h. Patients rated their nausea on a four-point scale (0-no nausea; 1-mild nausea, 2-moderate nausea; 3-severe nausea). Vomiting was assessed as events occurring in the first 24 h. Sedation was assessed using Ramsay sedation score (0 awake, 6 unresponsive to a strong, painful stimuli). [11–13].

Statistical analysis

The statistical data processing was done in the statistical program SPSS 17 for Windows. The testing of normality in the distribution of the data was used Kolmogorov-Smirnov and Shapiro-Wilk's W test. Categorical traits displayed by absolute and relative representation with quantitative traits mean, SD. For comparison of the three genotypes in relation to the variables analyzed were used the Chi-square test, Fisher exact test, Student's t test, One-way analysis of variance (post hoc Bonferroni test). The correlation between the consumption of fentanyl with age and duration of operative intervention was analyzed with the Pearson's coefficient of linear regression. Concerning the level of significance or importance, the value of $p < 0.05$ was taken, a significant higher value than $p < 0.01$.

Genotyping

Venous blood samples (2 ml) were collected from all patients in the study. Genomic DNA was extracted from whole blood using SaMag Blood DNA Extraction Kit (Sacace Biotechnologies, Como, Italy) on an automatic DNA extractor (SaMag – 12 System, Sacace Biotechnologies, Como, Italy) according to the manufacturer's provided protocol. The quantity and quality of the extracted genomic DNA was determined using NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) with measurements performed at 260 and 280 nm. The ABCB1 C3435T (rs1045642) polymorphism was genotyped using a TaqMan® Drug metabolism genotyping assay (ID C 7586657 20, Applied biosystems, Life Technologies, USA). Amplification reactions were performed in a total volume of 25 µL containing 20 ng genomic DNA, 12.5 µL 2 × Taqman Universal PCR Master Mix and 1.25 µL 20 × Drug Metabolism Genotyping Assay Mix. Thermal cycling was performed according to the manufacturer's recommended protocols using a Stratagene MX3005P real-time PCR system (Agilent Technologies). Both positive and negative controls were included in every genotyping assay.

Results

Out of 100 patients, one did not complete the procedure and was excluded due to problems with DNA isolation. Patients were divided into CC, CT and TT groups after genotyping. Among the remaining 99 subjects, there were 28 wild type homozygotes (CC), 45 heterozygotes (CT), and 26 mutant homozygotes (TT). (Table 1). There were no significant differences in the demographic characteristics among the three genotype groups with regard to sex, age, weight, height, duration of surgery and ASA score (Table 2). In the remaining 99 patients, there were no PCA device failures and no intolerable opioid side effects. There was significant differences in VAS scores between CC and TT groups, and CT and TT. VAS scores 6 h after surgery were CC 4.39 ± 13; CT 3.64 ± 1.3 and 2.1 ± 92.0 for TT genotype group. VAS scores 12h after surgery were 3.18 ± 0.18 in group CC, 3.0 ± 0.7 CT, and 1.73 ± 1.2 for group TT. After 18 h VAS scores for three groups were 2.61 ± 0.6 CC; 2.2 ± 0.7 in group CT and 1.54 ± 0.9 in group TT. VAS scores 24 h postoperatively for three groups were 1.82 ± 0.9 for CC group; 1.56 ± 0.7 among the patients in CT group, and 1.08 ± 0.8 for TT group (Table 3). There were no significant differences in side effects among the three groups regarding vomiting and sedation score. The patients in the TT group had more frequently nausea score 1 than the patients in the other two groups (Table 4). The patients in the CC group consumed significantly more fentanyl (375.0 µg ± 43.1) than the patients in the TT group (295.0 µg ± 49.1) and CT (356.4 µg ± 41.8) group (Table 5).

Table 1

*Genotype distribution of ABCB1 (C3435) in 99 patient*s

ABCB1	**N (%)**
CC	28 (28.28)
CT	45 (45.45)
TT	26 (26.26)
All	99 (100)

CC, wild type homozygous; CT, mutant heterozygous; TT mutant homozygous

Table 2

Demographic and clinical characteristics of ABCB1 genotype groups

Characteristics	ABCB1 CC (n = 28)	ABCB1 CT (n = 45)	ABCB1 TT (n = 26)	p value
Sex n(%)				
Male n = 59	17 (60.71)	26 (57.78)	16 (61.54)	[a]p = 09
Female n = 40	11 (39.29)	19 (42.22)	10 (38.46)	
Age, years				
(mean ± SD)	60.18 ± 9.9	60.2 ± 9.7	56.12 ± 8.7	[b]p = 018
Height (cm)				
(mean ± SD)	170.36 ± 8.4	171.09 ± 8.6	171.19 ± 6.0	[b]p = 091
Weight (kg)				
(mean ± SD)	71.36 ± 12.7	74.84 ± 12.6	77.58 ± 10.6	[b]p = 017
ASA n(%)				
1	6 (21.43)	6 (13.33)	4 (15.38)	
2	20 (71.43)	39 (86.67)	19 (73.08)	[c]0.14
3	2 (7.14)	0	3 (11.54)	
Duration of surgery (min)				
(mean ± SD)	186.25 ± 34.2	198.56 ± 38.6	191.15 ± 35.5	[b]p = 036

- Continuous variables expressed as mean ± standard deviation.
- [a](Chi-square test) [b](Analysis of Variance) [c](Fisher exact test)
- ASA – American Society of Anesthesiologists

Table 3

ABCB1 C3435T polymorphism and postoperative pain in 6h, 12 h, 18h and 24 h postoperatively

Characteristics	ABCB1 CC (n = 28)	ABCB1 CT (n = 45)	ABCB1 TT (n = 26)	p value
VAS (6h)				
(mean ± SD)	4.39 ± 1.3	3.64 ± 1.3	2.19 ± 2.0	[b]p = 0000003**
VAS (12h)				
(mean ± SD)	3.18 ± 0.8	3.0 ± 0.7	1.73 ± 1.2	[b]p < 0001
VAS (18h)				
(mean ± SD)	2.61 ± 0.6	2.2 ± 0.7	1.54 ± 0.9	[b]p = 0000001**
VAS (24h)				
(mean ± SD)	1.82 ± 0.9	1.56 ± 0.7	1.08 ± 0.8	[b]p = 00023**

- Post hoc analysis Bonferroni. VAS (6h) 1вcs3 p = 0.00011** 2vs3 p = 0.0006** VAS (12h) 1вc3 p = 0.0001**
- 2vs3 p = 0.0001** VAS (18h) 1вc3 p = 0.0001** 2vs3 p = 0.0007** VAS (24h) 1вc3 p = 0.0018**
- 2vs3 p = 0.035 **p < 0.05 **p < 0.01
- VAS – visual analogue scale

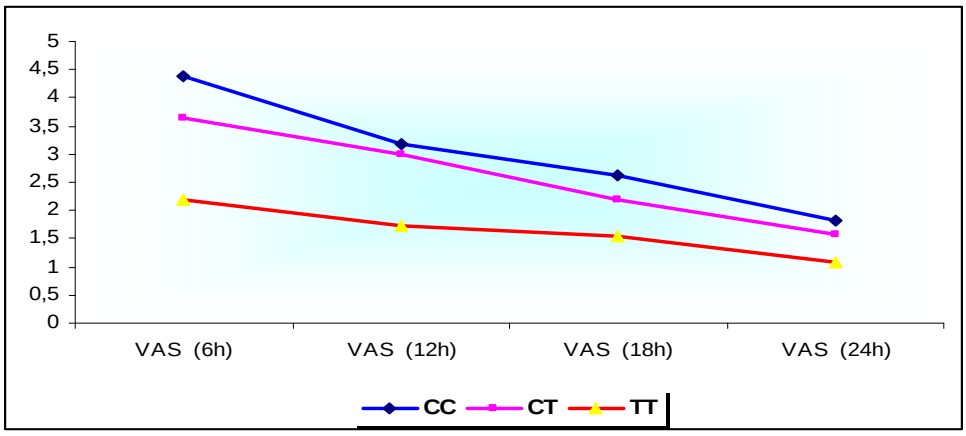

Fig. 1 – Pain assessment by visual analogue scale (VAS). VAS (mean± standard deviation) was recorded at 6h, 12 h, 18h and 24 h after the completion of the operation in three genotype groups CC, CT and TT. CC, wild type homozygous; CT, mutant heterozygous; TT mutant homozygous

Table 4

Side-effects of fentanyl delivered via patient-controlled analgesia (PCA) for patients receiving PCA, in three genotype groups

Characteristics	ABCB1 CC (n = 28)	ABCB1 CT (n = 45)	ABCB1 TT (n = 26)	p value
Nausea – n (%)				
0	24 (85.71)	43 (95.56)	18 (69.23)	[c] 0.014
1	3 (10.71)	2 (4.44)	4 (15.38)	
2	1 (3.57)	0	4 (15.38)	
Vomiting – n (%)				
0	27 (96.43)	44 (97.78)	25 (96.15)	[c] 1 0
1	1 (3.57)	1 (2.22)	1 (3.85)	
Sedation – n (%)				
0	28 (100)	43 (95.56)	23 (88.46)	[c] 0.12
1	0	2 (4.44)	3 (11.54)	

[c] (Fisher exact test)
CC, wild type homozygous; CT, mutant heterozygous; TT mutant homozygous.
Patients rated their nausea using a four –point scale (0, no nausea; 1, mild nausea; 2, moderate nausea; 3 severe nausea). Vomiting was assessed as events occurring in 24 h. Sedation was assessed using the Ramsey sedation score (0, awake; 6 unresponsive to strong painful stimuli).

Table 5

Postoperative consumption of fentanyl (μg) in three genotype groups

Characteristics	ABCB1 CC (n = 28)	ABCB1 CT (n = 45)	ABCB1 TT (n = 26)	p value
Postoperative consumption of fentanyl (μg)				
(mean ± SD)	375,0 ± 43.1	356,4 ± 41.8	295,0 ± 49.1	[b]p < 0.0001

Post hoc analysis Bonferroni **p < 0.01

Discussion

Our main result is that, in Macedonian patients who underwent abdominal surgery with colorectal anastomosis due to colorectal carcinoma, subjects with ABCB1 3435T allele (TT mutant type homozygous) received less fentanyl in the early postoperative period and had lower VAS scores. According to our results subjects in this group are "good respondent" to fentanyl, while patients in the CC group (wild type homozygous) are "bad respondent". The subjects in the CT group (mutant heterozygous) are "moderate respondents". This provides support for potential use of genetic data in predicting the fentanyl doses for adequate postoperative pain control. Some evidence suggests that other variables (e.g., age, sex and type of surgery) may also influence the postoperative pain [14–15]. There are conflicting results in literature regarding the influence of SNPs in ABCB1 gene on both, effects and side effects of opioids. It has been suggested that the TT carriers of C3435T were good respondents to morphine while those with CC or CT were moderate respondents. It has been speculated that the absorption of morphine is reduced in the CC carriers due to the effective efflux by P-gp in gut and/or through the blood brain barrier and consequently reducing the bioavailability of morphine for the receptors in brain. Conversely the TT carriers with abnormal function of P-gp should have a higher concentration of morphine [16]. The C3435T ABCB1 SNP has recently been associated with a different need for morphine in humans. Meineke et al. showed that patients carrying the TT genotype of the C3435T ABCB1 SNP, associated in other tissues with lower P-gp expression, had higher morphine cerebrospinal fluid concentrations than patients carrying the wild type C allele, associated with higher P-gp expression. These pharmacogenetics data obtained in humans are consistent with the involvement of P-gp in morphine brain disposition [17]. Age and prior use of psychotro-

pic agents are associated with postoperative morphine dose requirements. Whether ABCB1 polymorphisms might predict morphine side effects remains to be determined [18]. Previous investigations have observed that opioids are the substrates for P-gp involved in drugs cellular membrane permeability, disposition, and therefore analgesia effect in CNS [19]. In the study of Gong et al., they failed to reveal any significant difference in 24 h opioids doses among the subjects carrying various ABCB1 C3435T phenotypes in patients with cancer pain. However, when they measured using weight-surface area-adjusted-24h-opioids doses instead, TT homozygotes tended to require significantly lower opioids intake dosage than CC/CT carriers. [20]. In the study of Candiotti et al. where C3435T was genotyped in 152 patients undergoing a nephrectomy, authors found an association between the ABCB1 polymorphism (C3435T) and inter individual variations in opioid consumption in the acute postoperative period after nephrectomy. Analyzing the pain scores from 24-hour postoperative period, they observed that the CC genotype demonstrated the highest numerical pain score, the CT group an intermediate score, and the TT genotype the lowest. The same trend was observed in the 6 and 12 hour postoperative pain scores [21]. It has been reported that variants in ABCB1 are associated with the central side effects of opioids such as sedation, confusion, and hallucination in chronic cancer patients [2]. In our study we didn't find association between genetic polymorphisms of ABCB1 C3435T SNP and opioids side effects: vomiting and sedation. Patients in the TT genotype group had more frequently nausea score 2 than CC and CT group. Coulbault et al. suggested that ABCB1 polymorphisms could predict the side effects of morphine remains to be determined [18]. In the study of Candiotti et al. the authors also investigated a possible correlation between morphine related side effects (symptomatic nausea/vomiting) and the ABCB1 gene SNP. Their data showed that the CC genotype demonstrated the numerically lowest usage of emesis medication, the CT genotype demonstrated intermediate usage levels, and the TT genotypes showed the highest usage, but there were no statistically significant differences among the three genotype groups for emesis medications usage at 24 hours after surgery [21]. Wallden et al. also considered that the genetic polymerphism does not explain the nausea and vomiting caused by fentanyl. However, they found that the incidence of nausea and vomiting was higher in patients with inhibit gastric motility. This finding provides another explanation for postoperative nausea and vomiting, namely this may be associated with the opioid-induced changes in gastric motility [22].

Our study has some limitations. This was strictly a gene association study trial and as such opioid levels were not measured in the CNS or blood. Additionally, the study only enrolled patients undergoing abdominal surgery which is very painful. Secondly, the mixed gender study population may have increased variability in postoperative fentanyl requirements, although no statistically significant differences in gender were found between the different genotypes. In addition, only one gene polymorphism was analyzed, leaving a number of gens with functional significance to be assessed in future studies.

Conclusion

Our study indicates that the C3435T single nucleotide polymorphism of the ABCB1 gene is associated with differences in postoperative opioid consumption in patients who underwent abdominal surgery with colorectal anastomosis. The ABCB1 polymorphism may serve as a genetic predictor to guide acute pain therapy in postoperative patients.

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Резиме

ПОВРЗАНОСТ НА ЕДИНЕЧНИОТ НУКЛЕОТИДЕН ПОЛИМОРФИЗАМ НА C3435T ОД ABCB1 ГЕНОТ СО ОПИОИДНАТА ОСЕТЛИВОСТ ВО ТРЕТМАНОТ НА ПОСТОПЕРАТИВНАТА БОЛКА

Вања Џамбазовска-Трајковска[1], Јордан Нојков[1], Андријан Карталов[1], Билјана Кузмановска[1], Татјана Спироска[1], Реџеп Селмани[2], Ѓорѓи Трајковски[2], Надица Матевска-Гешковска[3], Александар Димовски[3]

[1] Клиника за анестезија, реанимација и интензивно лекување, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[2] Универзитетска клиника за дигестивна хирургија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[3] Фармацевтски факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија

Вовед: Минималната ефективна концентрација на опиоиден аналгетик може значително да се разликува помеѓу пациентите. Генетските фактори кои влијаат врз фармакокинетиката и фармакодинамиката на опиоидните аналгетици може да придонесат за различната осетливост на опиоидните аналгетици.

Пациенти и методи: Во студијата беа вклучени 99 пациенти оперирани од колоректален карцином. Кај сите пациенти беше земена

венска крв за ДНК-изолација и генотипизација на C3435T од ABCB1 генот. Според генотипот, пациентите беа поделени во три групи: CC-хомозиготи со див тип алели, CT-хетерозиготи со мутантни алели и TT-хомозиготи со мутантни алели. Кај сите пациенти постоперативно беше даден фентанил на PCA-пумпа (patient controlled analgesia). Степенот на болка, потрошувачката на фентанил и несаканите ефекти беа корелирани со резултатите од генетска анализа.

Резултати: Пациентите со генотип CC консумирале значително повеќе фентанил (375,0 μg ± 43,1), од пациентите во TT генотипската група (295,0 μg ± 49,1) и CT-групата (356,4 μg ± 41,8) во третманот на постоперативна болка. Пациентите во TT-групата имаа понизок степен на болка според VAS-скалата по 6 часа, 12 часа, 18 часа и 24 часа по операција. Нема значајни разлики во несакани ефекти кај трите генотипски групи во однос на инциденца на постоперативно повраќање и седација. Пациентите во групата TT имале почесто гадење степен 1, за разлика од пациентите во другите две групи.

Заклучок: Нашата студија покажува дека полиморфизмите на C3435T од генот ABCB1 се поврзани со разликите во степенот на болка и потрошувачката на фентанил кај пациенти кои биле оперирани од колоректален карцином. Генетскиот полиморфизам на C3435T може да послужи како важен генетски предиктор при третман на постоперативна болка.

Клучни зборови: фентанил, ABCB1, постоперативна аналгезија

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THE ROLE OF SERUM COAGULATION FACTORS IN THE DIFFERENTIAL DIAGNOSIS OF PATIENTS WITH PNEUMONIA AND PARAPNEUMONIC EFFUSION

Sanja Petrusevska Marinkovic[1], Irena Kondova Topuzovska[1], Zvonko Milenkovic[1], Biserka Kaeva[2]

[1] University Infectious Diseases Clinic, Medical Faculty, Skopje, R. Macedonia
[2] University Pulmonology and Allergy Clinic, Medical Faculty, Skopje, R. Macedonia

Corresponding Author: Petrusevska Marinkovic S., University Infectious Diseases Clinic, Medical Faculty, Skopje, R. Macedonia; tel: +389 (0)2 3228 224; e-mail: sanjapm@yahoo.com

Abstract

The aim of this study was to identify the participations of the serum coagulations and fibrinolysis factors that contribute to the differential diagnosis of the patients with community-acquired pneumonia (CAP) without effusion, uncomplicated parapneumonic effusion (UCPPE) and complicated parapneumonic effusion (CPPE).

The coagulations system is fundamental for the maintenance of homeostasis, and contributes to the inflammatory process responsible for CAP and the parapneumonic effusion. The factors of coagulations and fibrinolysis participate in the cellular proliferation and migration as in the synthesis of the inflammatory mediators.

We evaluated the laboratory profile of coagulations and fibrinolysis in the serum of 148 patients with CAP without effusion, 50 with UCPPE and 44 with CPPE. We determined the test of the coagulation cascade which measures the time elapsed from the activation of the coagulation cascade at different points to the fibrin generation. As a consequence, there is an activation of the fibrinolytic system with the increased D- dimer levels measured in the plasma in the three groups.

The patients were with mean age ± SD (53,82 ± 17,5) min – max 18–93 years. A significantly higher number of thrombocytes was in the group with CPPE with median 412 × 10^9/L (rank 323–513 × 10^9/L). The extended activation of the prothrombin time (aPTT) was significantly higher in the same group of patients with median of 32 sec. (rank 30–35 sec). The mean D-dimer plasma level was 3266,5 ± 1292,3 ng/ml in patients with CPPE, in CAP without effusion 1646,6 ± 1204 ng/ml and in UCPPE 1422,9 ± 970 ng/ml.

The coagulations system and the fibrinolysis play important role in the development and pathophysiology of CAP and the parapneumonic effusions.

Keywords: coagulation factors, fibrinolysis, community-acquired pneumonia (CAP), parapneumonic effusion, uncomplicated parapnemonic effusion, complicated parapneumonic effusion, D-dimer

Introduction

Community-acquired pneumonia (CAP) is one of the leading cause of sepsis and death from infectious disease [1, 2]. In the recent years, attention has tuned to other events in the host response to bacterial challenge, notably the coagulation activation [2]. The acute lung injury is associated with both vascular and extravascular coagulation [3, 4]. The relevance of the interaction between the coagulation and inflammation as a response to the severe infection, in its most extreme form manifests as disseminated intravascular coagulation (DIC) and multiple organ failure, and is becoming increasingly clear [5]. It is assumed that there is an explanation about the epidemiologic link between

infection and the higher risk of death, particularly due to acute cardiovascular events [6]. The activation of the host response to infection may persist at hospital discharge when patients appear to have recovered clinically from the infection, and it increases the risk of acute deteriorations of the cardiovascular disease and subsequent death [1, 6]. During an acute infection, the activation of the hemostatic system leads to a prothrombotic state [6]. Abnormalities are common even during less severe infection [1, 6]. The factors of coagulations and fibrinolysis participate in the cellular proliferation and migration as in the synthesis of the inflammatory mediators.

Parapneumonic effusion occurs in 20 to 40% of patients who are hospitalized with pneumonia like most common complication [7]. The mortality rate in patients with parapneumonic effusion is higher than that in patients with pneumonia without a parapneumonic effusion [7, 8]. The evolution of the parapneumonic effusion can be divided into three stages that represent a continuous spectrum [7, 9]. There is: the uncomplicated parapneumonic effusion (UPPE) depending on how sterile the exudative pleural effusion is, followed by a treatment with antibiotic alone [7, 8]. Less become secondarily infected (complicated parapneumonic effusion) (CPPE), and sometimes it requires a drainage in order to be resolved [8]. Bacterial metabolism and neutrophil phagocytosis in the pleural space lead to lactic acid production and increased glucose utilization. [8, 9]. The ongoing infection eventually leads to the accumulation of pus in the pleural space (empyema). After a variable time interval, the pleural infection enters an "organizing" stage characterized by fibroblast proliferation and the development of solid fibrous peel. This inhibit lung re-expansion usually necessitates surgical thoracotomy and décorticatión [8, 10]. The development of infection is associated with activation of the coagulation cascade and inhibition of fibrinolysis within the pleural space [10]. The coagulation system, when chronically activated and in the presence of an inflammatory state, can generate adverse effects such as chronic relies of procoagulant factors (e.g., tissue factor), cellular activation (adhesion molecules), protein modulation (transformation of fibrinogen into fibrin), and even histological changes promoted by cytokines [11].

D-dimer is a metabolic substance produced during the catabolization of fibrin by plasmin and they are fibrin degradation products [1, 2, 11, 12]. D-dimer levels have shown disorders that trigger fibrin production and catabolization; these disorders include pulmonary emboli (PE), deep vein thrombosis (DVT), solid tumors, leukemia, severe infection, trauma or post-operative state, pregnancy, congestive heart failure etc. [1, 12].

In order to recognize the contribution of the coagulation system to the differential diagnosis of patients with CAP without parapneumonic effusion and with parapneumonic effusion, we evaluated the laboratory profiles of the coagulations and the D- dimmer.

Materials and methods

We analyzed the laboratory profile of coagulations and fibrinolysis in the serum of 148 patients with CAP without effusion, 50 with UCPPE and 44 with CPPE. The patients were diagnosed and treated at the University Infectious Diseases Clinic, Faculty of Medicine, Skopje, at the Department of Respiratory Diseases in the period from September 2011 to June 2015. Individuals were excluded from the study because of the cancer and malignant effusion, transudate effusion, vasculitis or sickle-cell anemia, pregnancy, pulmonary emboli (PE), younger than 18 years of age and thromboembolic diseases.

The demographic characteristics, the physical examination findings and the laboratory findings (sedimentation, thrombocytes (platelets), leucocytes, hemoglobin, hematocrit, glucose, sodium, potassium, urea, C-reactive protein (CRP), lactate- dehydrogenases (LHD), albumin and total protein) and the microbiological findings of all study participants were monitored regularly. Initial lung X-rays were taken for all patients at the Institute of Radiology, Medical Faculty in Skopje. After the admission all the patients underwent an ultrasound of the pleura and lung with a three-dimensional echo at the University Infectious Diseases Clinic for diagnosis of the pleural effusions and implementation of the diagnostic thoracentesis if the size of effusion was more than 10 mm. After the verification of pneumonia and pleural effusion, the distinction between the transudate and exudate was done

according to Light's criteria. Exudative pleural effusion is one that meets at least one of the criteria of Light. It is a transudate if the effusion meets all three criteria at the same time (1. To have intercourse protein p/s below 0.5, 2. Intercourse LDH p/s below 0.6 and 3. LDH in pleural fluid under 282 U/L which is the lowest limit in our laboratory. The exudative pleural effusion according to the evolution and on the basis of the pH, glucose and LDH value in the pleural fluid is divided into: – Uncomplicated parapneumonic effusions: pH > 7.2, glucose > 60 mg/dl, LDH < 1000UI/ml; – Complicated parapneumonic effusions: pH < 7.2, glucose < 60 mg/dl, LDH >/= 1000 UI/ml.

The blood sample for coagulations and fibrinolysis factors were taken from the antecubital vein with an injector and placed into citrated tube (with sodium citrate anticoagulant, 3.2%) and transported immediately to the Institute of Transfusion Medicine, Faculty of Medicine in Skopje, where it was evaluated with the quantitative latex coagulation method. We determined the number of Tr (normal value 150–250 × 10^9), activation prothrombin time (aPTT), prothrombin time (PT) and thrombin time (TT). Also, the value of the D-dimmer in the three patients groups was measured. The plasma D-dimer level over 500 ng/ml were considered to be high. Maximum value of D- dimer is 4,500 ng/ml.

Statistical analysis

The statistical analysis was conducted using SPSS 17 for Windows. The testing of the normality in the distribution of the data was done using the Kolmogorov-Smirnov and Shapiro-Wilk's W test. The categorical traits were displayed by the absolute and relative representation with quantitative traits mean, SD, median, minimum, maximum, 25–75 percentiles. For the comparison of the three groups of subjects in relation to the analyzed variables were used the Kruskal-Wallis ANOVA and Mann-Whitney U test (Z). As level of significance or importance was taken the value of $p < 0.05$, a significantly higher value than $p < 0.01$.

Results

In the three group of patients the majority were the male patients (58.11%, 58%, 61.36%) consequently. The difference in the distribution of patients with CAP without effusion, UCPPE and CPPE in terms of their sex was insignificant ($p = 0.9$). The mean age of patients only with CAP was 54.58 ± 17.5 years, in UCPPE 55.5 ± 16.6 and in the group with CPPE was 51.91 ± 18.4 and there was no statistical difference ($p = 0.58$).

In addition, the distribution of participants according to the smoking status was insignificant ($p = 0.25$).

Table 1

Demographic characteristics of three group of patients

variable	CAP N=148	UCPPE N=50	CPPE N=44	p value
sex n (%)				
male	86(58.11)	29(58)	27(61.36)	[a]p = 0.9
female	62(41.89)	21(42)	17(38.64)	
Age (years) mean ± SD, min-max				
	54.58 ± 17.5 18–89	55.5 ± 16.6 21–83	51.91 ± 18.4 18–93	[b]p = 0.58
Smoking status n (%)				
smoker	92(62.16)	37(74)	31(70.45)	[a]p = 0.25
non smoker	56(37.84)	13(26)	13(29.55)	

[a]p(Chi-square test) [b]p (Analisys of variance)

Patients with complicated effusion had significantly higher values of thrombocytes compared to the patients with CAP without effusion ($p < 0.0001$), and compared to the patients with UCPPE ($p < 0.0001$). The median value of the thrombocytes in patients without effusion group with complicated and uncomplicated discharge was 216 × 10^9 (rang 158.5–298 × 10^9), 226 (rang 167–276 × 10^9), 412 × 10^9 (rang 323–513 × 10^9) consequently.

Platelets higher than 400 × 10^9 had 11.8% of the patients with CAP without effusion, 8 % of the patients with UCPPE, and 52.27% of the patients with CPPE.

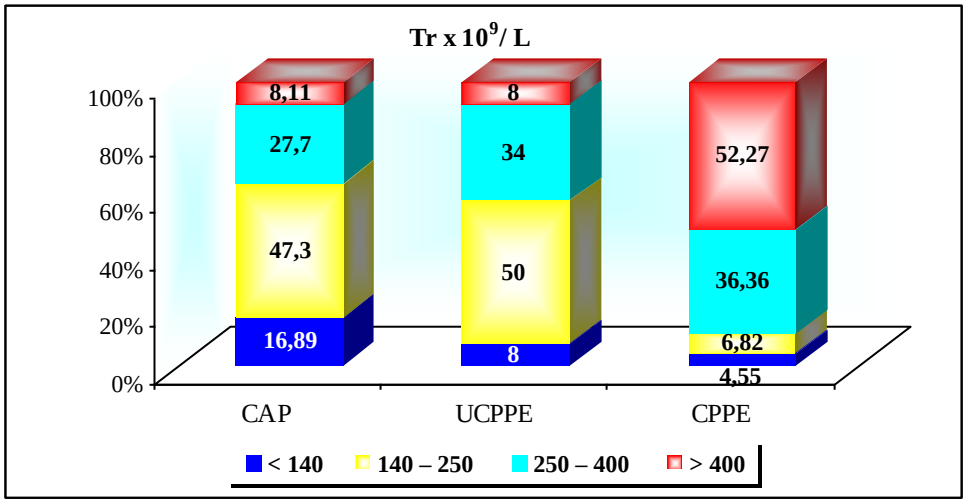

Figure 1 - Distribution of thrombocytes in the three clinical different patient groups

Statistically significant differences were confirmed between the three groups analyzed and compared to the values of the activated partial time (aPTT) (p = 0.0032). This significance is due to significantly higher values of this parameter in the group of patients with CPPE as compared with the group with CAP without effusion – median of 32 sec. (range 30–35 sec.) vs. median 29 (rang 27–33), and as compared with the group with uncomplicated effusion – median 32 (range 30–35) vs 29 (range 26–32).

The measured values of prothrombin (PT) and thrombin time (TT) are not statistically proved different among the respondents from the three analyzed groups (p = 0.092, p = 0.33 accordingly T). These results are shown in Table 2.

Table 2

Test of coagulation cascade in three clinical different group of patients

variable	CAP N = 148	UCPPE N = 50	CPPE N = 44	p value
Trx10^9/ L				
< 140	25(16.89)	4(8)	2(4.55)	
140–250	70(47.3)	25(50)	3(6.82)	
250–400	41(27.7)	17(34)	16(36.36)	
> 400	12(8.11)	4(8)	23(52.27)	
Trx10^9 mean ± SD median (25–75thquartiles)				
	237.67 ± 124	241.86 ± 93.2	452.75 ± 202.2	^{c}p < 0.0001
	216(158.5–298)	226(167–276)	412(323–513)	1vs3 p < 0.0001
				2vs3 p < 0.0001
PT sek mean ± SD median (25–75thquartiles)				
	17.89 ± 45.8	12.52 ± 2.3	13.36 ± 4.0	^{c}p = 0.092
	13(12–14.5)	12(12–13)	12(12–14)	
aPTT sek mean ± SD median (25–75thquartiles)				
	31.09 ± 7.7	30.48 ± 6.5	33.02 ± 5.5	^{c}p = 0.0032*
	29(27–33)	29(26–32)	32(30-35)	1вс2 p = 0.0018**
				1вс3 p = 0.003**
TT sek mean ± SD median (25–75thquartiles)				
	17.12 ± 1.8	17.12 ± 1.7	18.2 ± 4.4	^{c}p = 0.33
	17(16–18)	17(16–18)	17.5(16–19)	

^{c}p (Kruskal-Wallis test) ^{d}p (Mann-Whitney test) *p < 0.05 **p < 0.01

The D-dimer level was with statistically important differences between both group with parapneumonic effusion ($p < 0.0001$), and between the group with CPPE and CAP without effusion ($p < 0.0001$). The median of D-dimer level was higher in CPPE, 3792 ng/ml, (rang 14–4500ng/ml), lower in patients with CAP, 1136 ng/ml (rang 794–2366 ng/ml) and lowest in the group with UPPE 1112 ng/ml (rang 689–1712 ng/ml). The average D-dimer plasma level was 3266.5 ± 1292.3 ng/ml in patients with CPPE, in CAP without effusion 1646,6 ± 1204 ng/ml and in UCPPE 1422,9 ± 970 ng/ml. This statistically significant difference in favor of CPPE is shown in Table 3 and figure 2.

Table 3

Value of D-dimer in three clinical different group of patients

variable	CAP N = 148	UCPPE N = 50	CPPE N = 44	p value
D dimeri ng/ml	**mean ± SD median (25–75thquartiles)**			
	1646.6 ± 1204	1422.9 ± 970.7	3266.5 ± 1292.3	$^{c}p < 0.00001$
	1136(794–2366)	1112(689–1712)	3792(1814–4500)	1вс3 $^{d}p < 0.0001$
				2вс3 $^{d}p < 0.0001$

^{a}p(Chi-square test ^{c}p (Kruskal-Wallis test) ^{d}p (Mann-Whitney test) *p < 0.05 **p < 0.01

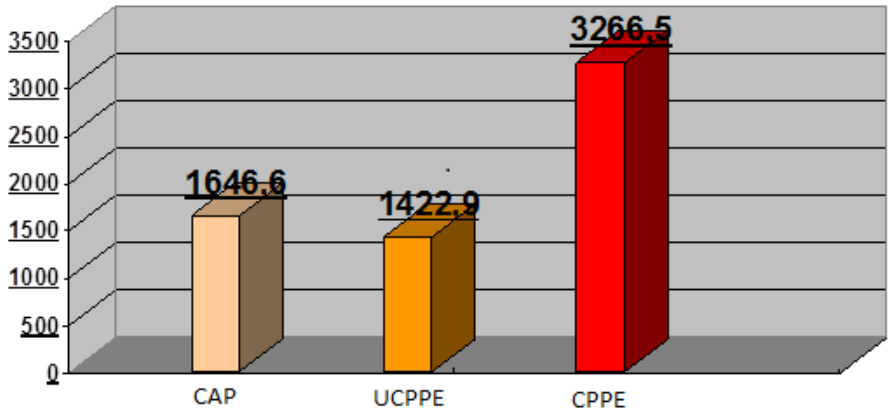

Figure 2 – Mean values of D-dimers in serum in ng/ml

Discussion

Acute lung injury is associated with both vascular and extravascular coagulation [3, 4]. Our study shows that coagulation factors and fibrinolysis in the serum distinguishing patients with CPPE on one side, and patients with CAP without effusion and UCPPE on the other.

CAP without effusion and UCPPE usually treated with antibiotic therapy have a good resolution without the need for additional interventions. UCPPE are sterile exudate and in the coagulation and fibrinolysis tests in the serum there are no significant differences. Differences are found between these two group and CPPE concerning the fibropurulent advanced stage in the development of parapneumonic effusions. The patients with CPPE have more severe clinical picture and they are with higher biomarker of inflammation and higher mortality rate, unlike the patients with CAP without effusion and the patients with UCPPE [10, 11, 13, 14].

The classic coagulation tests had significant difference in the activated partial time (aPTT) that was prolonged in patients with CPPE, unlike the two mentioned groups previously ($p = 0.0032$). The coagulation pathway that occurs in the aPTT test represents the intrinsic coagulation pathway. The extended aPTT shows incising abnormality and deficiency and severe infection associated with the prolonged aPTT without clinical signs of bleeding [14, 15]. Such findings also published Satoshi in a multicenter, prospective study in critically ill patients [15]. Because the prolonged aPTT is

good screening test for factor IX deficiency, in the Kale study the factor IX, thrombin- antithrombin complex, antithrombin and plasminogen activator inhibitor- 1 are with significantly higher value in patients with severe CAP and of older age (after the age of 65). A prolonged aPTT cannot be completely normalized with the addition of normal plasma it can be explained only with the presence of a circulating inhibitor of coagulation. The presence of these inhibitors is almost always acquired, and their exact nature is not always apparent. [12, 13, 15]. From a clinical point of view, the most common inhibitors should be considered the antithrombins. These compounds inhibit the activity of thrombin on the conversion of fibrinogen to fibrin. One of the two most common inhibitors of prolonged aPTT is heparin [15–17]. The measured value of prothrombin (PT) and thrombin time (TT) is not statistically proven to be different among the respondents from the three analyzed groups ($p = 0.092$, $p = 0.33$ accordingly). They are not specific test for coagulation disorders caused by infection [15–17].

Patients with complicated effusion had significantly higher values of thrombocytes compared to the patients with CAP without effusion ($p < 0.0001$), and compared to the patients with UCPPE ($p < 0.0001$). The median of Tr was 412 $x10^9$/L (rank 323–513 $\times 10^9$/L). Thrombocytes contribute to hemostasis and consist of vascular platelet phase of hemostasis. With their adhesion, activation and aggregation they form platelet plug (primary hemostasis) which is associated with the activation of the coagulation cascade with resultant fibrin deposition and linking (secondary hemostasis) [16–18]. In 2008 Chalmers et al. in prospective observational study of patients with CAP, analyzed 92 patients about the development of CPPE. With multivariate logistic regression, the value of the thrombocytes of more than 400×10^9 was identified as the independent predictor of the subsequent development of CPPE and empyema and it is part of the scoring system with "good" performance for predicting the development of CPPE and empyema in patients with CAP(18).

As a consequence, there is activation of the fibrinolytic system with increased levels of fibrin degradation products, including the D-dimer, which was measured in the plasma in the three patients groups. The value of D-dimer in not measured in the pleural fluid because some authors suggest that there is no significant difference between the value of D-dimer in the exudative effusions of different etiologies [11, 17, 19, 20]. Apart from this fact they found significant differences between the value of the D-dimer between the exudative and the transudate pleural effusion with higher value in the exudative effusions [11, 19, 20]. The D-dimer like part of the fibrinolysis has higher value in the infection and the value correlated with the severity of the infection [1, 5, 6, 11, 12, 21, 22]. Our conducted study confirmed expectations of statistically higher values of D-dimer in patients with CPPE than those with CAP without effusion and UCPPE. But, we have statistically important differences also between both group with parapneumonic effusion ($p < 0.0001$). The average D-dimer plasma level was 3266.5 ± 1292.3 ng/ml in patients with CPPE, in CAP without effusion 1646.6 ± 1204 ng/ml and in UCPPE 1422.9 ± 970 ng/ml.

The level of the D-dimer was lower in the group with UCPPE, probably because of the less severe clinical condition of the patients with UCPPE, than of the patients with CAP without effusion. The D-dimmers in a growing number of studies has been proven a robust biomarker that indicates the severity of the clinical condition and disorders in fibrinolysis as part of a cascading process of coagulation which is important for maintaining the homeostatic mechanism. They are biomarker which indicated the severity of CAP [1, 6, 21, 22]. Lately there are studies that indicate it as a significant marker for the differentiation of malignant, parapneumonic and tuberculous effusion [11, 17, 19]. In this study patient are not divided according to the severity of the clinical picture, which is the lack of this study.

Yet the differences that exist among the aforementioned coagulation factors and D-dimer indicate significant disorders which may have contributed to the demarcation of patients with pneumonia with or without pleural effusion, especially when it comes to CPPE. Coagulation disorders can lead to death in a patient as a result of developing pulmonary embolism [12, 23]. Different disorders can lead to dysfunction of various organs as a result of the

interplay of inflammation, coagulation and organic dysfunction [13, 23].

The study of Yende and the associates from 2011 in a large cohort of patients hospitalized with CAP, show that hemostasis makers are elevated during recovery, as evident with the higher thrombin – antithrombin complex (TAT) and D-dimer levels at hospital discharge. The higher concentration of these hemostasis markers was associated with the higher risk of death over 1 year, particularly due to the acute deterioration of the cardiovascular disease [6, 25]. This suggests that a persistent prothrombotic state even after infection may explain the epidemiologic link between the infection and the higher risk of cardiovascular disease [6, 26]. Thus, interventions, such aspirin and statins, with beneficial effects on resolution of the prothrombotic state and inflammation, should arguably be investigated to improve the long-term outcomes after pneumonia [25].

The study can help into understanding the physiological mechanisms in patients with CAP with and without parapneumonic effusion and may help to define new diagnostic and therapeutic approaches.

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Резиме

УЛОГАТА НА СЕРУМСКИТЕ КОАГУЛАЦИОНИ ФАКТОРИ ВО ДИФЕРЕНЦИЈАЛНАТА ДИЈАГНОЗА КАЈ ПАЦИЕНТИ СО ПНЕВМОНИЈА И ПАРАПНЕВМОНИЧЕН ИЗЛИВ

Сања Петрушевска-Маринковиќ[1], Ирена Кондова-Топузовска[1], Звонко Миленковиќ[1], Бисерка Каева[2]

[1] Универзитетска клиника за инфективни болести, Медицински факултет, Скопје, Р. Македонија
[2] Универзитетска клиника за пулмологија и алергологија, Медицински факултет, Скопје, Р. Македонија

Цел на оваа студија е да се идентификуваат факторите на коагулација и фибринолиза кои имаат придонос во диференцијална дијагноза на пациенти со вонболнички здобиена пневмонија без излив, пациенти со некомплициран и со комплициран парапневмоничен излив.

Коагулациониот систем кој е фундаментален за одржување на хомеостазата учествува и во воспалителниот процес кој се јавува кај вонболнички здобиена пневмонија и парапневмонични изливи. Факторите на коагулација и фибринолиза учествуваат во клеточната пролиферација и миграција, како и во синтеза на инфламаторни медијатори.

Евалуиравме лабораториски профил на коагулација и фибринолиза во серум кај 148 пациенти со вонболничка пневмонија без излив, 50 пациенти со некомплициран парапневмоничен илзив и 44 со комплициран парапневмоничен излив. Ги одредувавме коагулационите тестови кои го мерат времето поминато од активација на коагулационата каскада со осврт на различните начини на генерација на фибрин. Како последица на оваа активација на фибринолитичкиот систем се зголемува нивото на Д-димери во плазма кое е одредувано во сите три групи пациенти.

Пациентите беа со средна виозраст ±SD (53,82 ± 17,5) мин.-макс. 18–93 години. Сигнификантно повисоки вредности на тромбоцити беа во групата со комплициран парапневмоничен излив со медијана 412 × 0^9/L (ранг 323–513 × 10^9/L). Продолжено активирано протромбинско време (аПТТ) беше сигнификантно со повисоки вредности во истата група пациенти со медијана 32 сек. (ранг 30–35 сек.). Средни вредности на Д-димери во плазмата беа 3266,5 ± 1292,3 ng/ml кај пациенти со комплициран парапневмоничен излив, кај оние со вонболничка пневмонија без излив 1646,6 ± 1204 ng/ml и кај пациенти со некомплициран парапневмоничен излив 1422,9 ± 970 ng/ml.

Коагулациониот систем и фибринолиза имаат значајна улога во развојот и патофизиологијата на вонболничка пневмонија и парапневмоничните изливи.

Клучни зборови: коагулациони фактори, фибринолиза, вонболничка пневмонија, парапневмоничен излив, некомплициран парапневмоничен излив, комплициран парапневмоничен излив, Д-димери

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MOLECULAR BIOLOGY AND GENETIC MECHANISMS IN THE PROGRESSION OF THE MALIGNANT SKIN MELANOMA

Sofija Pejkova[1], Gjorgje Dzokic[1], Smilja Tudzarova-Gjorgova[1] Sasho Panov[2]

[1] University Plastic and Reconstructive Surgery Clinic, Medical Faculty, University Ss. Cyril and Methodius, Skopje, R. Macedonia
[2] Molecular Biology and Genetics Laboratory, Faculty of Natural Sciences and Mathematics, University Ss. Cyril and Methodius, Skopje, R. Macedonia

Corresponding Author: Ass. Dr. Sofija Pejkova, Address: University Plastic and Reconstructive Surgery Clinic, Medical Faculty, Ss. Cyril and Methodius University, Vodnjaska 17, MK-1000, Skopje; e-mail: pejkova@gmail.com

Abstract

Malignant skin melanoma is a tumor deriving from transformed skin melanocytes as a result of complex interactions between genetic and environmental factors. This melanoma has a potential to metastasize early and very often it is resistant to the existing modalities of the systemic therapy. As in any other neoplasms, certain types of melanoma may skip certain stages of progression.

The progression from one stage to another is accompanied by specific biological changes. Several key changes in the melanoma tumorogenesis influence the regulation of the cell proliferation and vitality, including the RAS-RAF-ERK, PI3K-AKT, and $p16^{INK4}$/CDK4/RB pathways. A key role in the dissreguarity of the RAS-RAF-ERK (MAPK) pathway in the malignant melanoma development have been demonstrated by many studies. To date, the molecular genetic alterations during melanoma development have been partially known. In the pathogenesis of the malignant melanoma, there are mutations of various genes such as NRAS, BRAF, and PTEN and mutations and deletions of CDKN2A.

In the past years, great advance has been made in the insights of the molecular aspects of the melanoma pathogenesis. However, this field yet poses a challenge to discover new details about the melanoma molecular characteristics. The research results are focused towards the improvement of the melanoma patients prognosis by introducing personalized targeted therapy.

Keywords: melanoma, NRAS, BRAF, and PTEN, prognosis factors

Introduction

Malignant skin melanoma is a tumor deriving from the transformed genetically altered skin melanocytes as a result of complex interactions between genetic and environmental factors. The melanocytes located in the basal epidermal layer synthesize and transfer the pigment melanin to the surrounding keratinocytes and thus protect these cells from the harmful effect of the UV rays [1]. A rapid increase of malignity has been observed in melanoma. The latest epidemiological analyses have shown that melanoma incidence is higher than the rest of the skin malignity [2, 3]. The malignant skin melanoma has a potential to metastasize and very often it is resistant to the existing modalities of the systemic therapy. This is the reason due to which the increased incidence is related to the high mortality of patients with this disease.

Progression of Melanoma

According to the results from the clinical and histological studies, the melanoma development and progression are defined in several stages: stage 0, normal melanocytes; stage 1, congenital and acquired nevus in presence of

structurally normal melanocytes; stage 2, dysplastic nevus with atypical architecture; stage 3, melanoma in situ (MIS) and radial stage of growth (RSG), i.e. primary melanoma without metastatic competency; stage 4, vertical stage of growth (VSG), i.e. primary melanoma with metastatic competency, and stage 5, metastatic melanoma. As in any other neoplastic system, certain types of melanoma may skip certain stages of progression [5].

The progression from one stage to another is accompanied by specific biological changes. The transition from a mature melanocyte while forming a nevus is characterized by the disorder of the cell-cell cross-talk between the melanocytes and keratinocytes, which results in exiting of the melanocytes from the keratinocyte regulatory mechanisms. The nevus cells are characterized by the limited proliferation and they do not have significant chromosome aberrations. Nevus can develop not as a result from a certain stimuli only, but also because of the loss of the keratinocyte control mechanisms over the melanocytes. The progression from melanocytic to dysplastic nevus or RGP melanoma is most probably accompanied by the occurrence of the genetic aberrations. The cells are cytologically atypical and can separate from the basal membrane without the apoptosis process, and the whole lesion is architecturally atypical. The lesion cells with RSG in vitro have biological properties with medium benign to malignant features. The primary melanoma with VSG have nodular changes that penetrate deeply into the dermis. The primary melanoma with VSG are especially aneuploidy. Biologically, the cells of these melanoma types are characterized by relative plasticity, while some of them have metastatic competency. There is a high level of genetic instability and phenotypical plasticity in the metastatic cells depending on the environment and all selective factors that affect the cells [5, 6]. The metastatic cells are quite mobile and independent of the growth factors and have acquired capacity for invading other tissues and organs.

Risk Factors in Melanoma

Similar to the most types of cancer, melanoma has two types of risk factors, important for its development. These factors are typical for the host and the environment. Epidemiological studies have identified the host risk factors, important for melanoma development. These include family history, changes in the gene susceptibility extent to melanoma, nevus number and type, skin type, and pigmentation [7]. Melanoma occurs more frequently in persons with light complexion, blue or green eyes, red or blond hair, lots of sunspots, and in persons that react to light by being sunburnt rather than getting suntanned.

UV radiation is the most important environmental factor for melanoma development. Occasional repetitive exposure to sunlight from childhood has been epidemiologically proven as the main reason for melanoma development. It has been experimentally proven that UVB rays, which are a small component of the sunlight that reaches the earth, are the cause of skin cancer in animals. UVB rays can cause DNA damage, especially of the cyclobutane pyrimidine dimers (CPD) and photoproducts that can cause mutations of epidermal cells, that leads to the development of cancer [4–6].

It is known that UVB rays regulate the gene expression through intracellular pathways for signal transduction, which can contribute to the development of skin cancer during the stage of the tumor progression. Further, it has been experimentally demonstrated in animals that the local or systemic action of the UVB rays causes suppression of immune reactions and induction of antigen tolerance. It is believed that these three effects of the UVB rays on the skin are the causes of skin cancer development in humans [8].

UVA rays present the major part (approx. 95%) of the UV light that penetrates the earth's surface and many epidemiological studies have confirmed that these rays can be the cause of benign and malignant skin tumor development. UVA rays through the epidermis penetrate deep into the dermis. The cell damage by the UVA rays occurs primarily through the formation of the reactive oxygen species (ROS). After the exposure to UVA rays, molecular oxygen, H_2O_2 (hydrogen peroxide), superoxide, and hydroxyl free radicals are formed. The interactions among them may cause damage to the cell proteins, lipids, and saccharides. UVA rays may directly cause structural damage to the DNA where 8-

oxo-guanin is the most common lesion that inhibits DNA reparation, while at the same time, it has an effect on various pathways for signal transduction and leads to disorder of the immune system processes [9].

Pathways Relevant to the Cancer Biology

Several key changes of the melanoma tumorogenesis influence the regulation of cell proliferation and vitality, including the RAS-RAF-ERK, PI3K-AKT and $p16^{INK4}$/CDK4/RB pathways [10, 11]. There are many studies that have proved that the RAS-RAF-ERK (MAPK) pathway plays a key role in the malignant melanoma development [11–14].

RAS signaling pathways

The family of *RAS* genes is one of the most commonly activated oncogenes in the human cancer development. RAS proteins are small monomer GTP-ases that have an important role in the process of the growth signals transduction from the cell surface to the nucleus. By activating certain mutations in *RAS*, the cell transformation is promoted through the effect of the growth factors of the independent stimulation of the cell proliferation and the survival. There are three genes identified in humans: *HRAS*, *NRAS*, and *KRAS*. As in other GTP-ases, the functions of the RAS proteins are regulated by GDP-GTP binary switches. The extracellular signals are received by receptors, bound to the cell membrane, such as the receptors bound to the G protein (GPCR) and the tyrosine kinase receptors (TKR). These receptors activate the guanine factors for exchange that cause transitory activation of RAS. The activated RAS-GTP alleviates the binding and activation of the effectors. The RAS signaling is interrupted by RAS GAP-mediated stimulation of hydrolysis of GTP into GDP and frees itself from the bound effector [15]. The most common mutations of *RAS* in tumors occur at the critical RAS regulation points. Single-nucleotide mutations at codons 12, 13, 59, and 61 completely interrupt the GAP-induced GTP hydrolysis of RAS. Unlike the normal RAS, the oncogenic RAS protein remains constitutionally in the active GTP-bound form. Therefore, the properties for transformation of the oncogenic *RAS* are based on continuing the activation of its effectors [16].

There are three main RAS effectors, RAF kinase, RAL-GEF, and PI3K, which bind to the same region of RAS-GTP, i.e. the domain 32-40. All of these effectors increase their *in vivo* activity after binding with RAS [17].

RAS-RAF-ERK signalization

The best known RAS stimulated pathway, which is directly related to the actions that promote growth, begins with the activation of the serine/threonine kinase of the RAF family. There are three members of the RAF class: ARAF, BRAF, and CRAF. The latest studies have shown that RAS interacts with the amino-terminal part of RAF, located in the cytoplasm as part of the 14-3-3 protein, which is an essential cofactor of the RAF kinase action [18]. This interaction causes conformational changes in RAF by which one or more of the phosphorrylation and stabilization residues of the new catalytic active RAF are un/masked. After these conformational changes, RAS binds to the plasma membrane. The RAF-RAS binding is transitory and the moment it binds to the membrane the RAF action becomes independent from RAS and is no longer dependent on the dominantly negative RAS mutations. The phosphorylated RAF activates a series of kinases in cascade, which through the increase of the low cell signals modulates the activity of several cytoplasmic and nucleus factors [19]. During this activity, the signals transferred to the nucleus define the activation of the transcription factors, such as the members of the Ets family [20]. These transcription factors influence the expression of the specific genes responsible for the proteins included in the control of the cell proliferation and/or differentiation [17].

RAS-PI3K-AKT signalization

Another well-known RAS effector is PI3K, with a role in the cell proliferation and survival processes. PI3-kinases are lipid kinases that participate in the process of phosphorrylation of 3'-OH position of inositol phosphorlipids. RAS-GTP may bind and activate the catalytic subunits of this enzyme which produces PI P3 (phosphatidylinositol tri-phosphate) through phosphorylation of PI P2 in 3-position. PI P3 acts directly as second messenger, binding

itself to cytoskeleton protein kinases and thus makes modulation of their activity through conformational changes and/or translocation of the membrane. PI3K class I is made of 110 kDa catalytic subunits and one 85 kDa regulatory subunit. These are activated by RAS or RTK. The catalytic subunit p110 contains RBD (a domain that binds to RAS), to which RAS-GTP is binded. The catalytic subunit p85 has a binding and kinase domain, too. The main downstream goal of PI3K is the serine/threonine kinase AKT (or PKB). In mammals, there are three different isoforms of three different AKT (AKT1, 2, 3). This protein regulates the extracellular growth signals using the phosphatidylinositol-phosphate (PIP3) as one intracellular second messenger. Under the action of the growth factor signals, the intracellular level of PIP3 increases, which leads to phosphorylation of AKT, which promotes the progression of the cell cycle and inhibits apoptosis. PTEN is a negative regulator of PI3K-AKT pathway [21]. PTEN regulates the PIP3 levels, inactivates the results from PIP3 accumulation, makes hyperphosphorylation of AKT and improves cell survival and cell proliferation [22]. PI3K-AKT pathway is hyperactive in melanoma. It must be noted that the increased levels of phospho-AKT has a negative correlation to the survival of melanoma patients [21].

RAS-RAL signalization

Another class of RAS effectors is the GEF family (RalGDS) which is an activator of the small monomers, RAL GTP-ases. In RAL, there is an interaction with Cdc42 and RAC-GAP. Rho, RAC, and Cdc42, which are members of another monomer family, G proteins with an important role in remodeling of cytoskeleton and activation of the kinase action that regulates the activity of various transcription factors. The signaling activity of the RAS GTP-ases begins not only through the action of the direct effectors, but through the activation of other GTP-ases, especially other members of the RAS sub-family (for e.g. Rap) and members of the Rho subfamily (for e.g. RhoA, Rac1, and cdc42). This hierarchical network among various isoforms of RAS is partially controlled by the interactions of GEF, GAP, and the downstream effectors. For instance, Ra1GEF are especially important for the process of RAS-mediated transformation. Ra1GEF, as well as Ra1GDS, link the RAS signalization for activation of the small GTP-ases, Ra1A, and Ra1B. In humans, the RAS mutant effector branch activates Ra1GDS, after which they enter the process of cell transformation [23].

Other Effectors in RAS signaling pathway

Other potential effectors are AF-6, protein kinase C-zeta (PKC-zeta), and Nore1. RAS uses the AF-6 effector for modulation of the intracellular binding and communication. PKC-zeta shows RAF homology. The recent studies have shown that PKC-zeta may activate the RAS pathway regardless of RAS [24]. It has to be also mentioned that recent studies have discovered members of RASSF (RAS association domain family protein) gene family that have effect on tumor suppressive genes. The loss of expression of Nore1 (novel RAS effector 1) and RASSF1, members of the RASSF gene family, are found in various types of cancers [25]. The interaction between RAS and Nore1 has an effect on the apoptosis regulation [26].

RB and p53 pathways

In mammals, the *INK4A-ARF* locus decodes two different proteins, $p14^{ARF}$ and $p16^{INK4A}$, whose actions have an effect on the control of the cell cycle and tumor suppression, and are included in two different protein-protein interactions: $p16^{INK4A}$-RB и $p14^{ARF}$-p53. These two gene products with the transcription process are initiated by various promotors and decode in two different frameworks: $p16^{INK4A}$, which refers to INK4A, and $p14^{ARF}$, which refers to ARF. *INK4A* positively regulates the tumor suppressor RB through *CDK4* suppression, while the ARF protein forms a complex with HDM2 and p53 and blocks their outing from the nucleus, leading to p53 stabilization and its activation in the nucleus [27].

Mutations in the Oncogenes and Suppressor Genes in Melanoma

To date, the molecular genetic alterations during melanoma development have been only partially known. In the pathogenesis of malignant melanoma, there are mutations of various

genes, such as *NRAS*, *BRAF*, and *PTEN*, and mutations and deletions of *CDKN2A*. Selected genetic alterations in malignant melanoma are presented in the Table 1 [25].

Table 1

Genetic alterations in melanoma

Gene Type	Gene	Alteration Frequency in Melanoma	Alteration Type
Oncogenes	*BRAF*	50–70%	Mutation
	NRAS	15–30%	Mutation
	AKT3	43–60%	Expression
	CCND1	6–44%	Amplification
	MITF	10–16%	Amplification
Tumour-suppressors	*CDKN2A*	30–70%	Deletion or Mutation
	PTEN	20–40%	Deletion or Mutation
	APAF-1	40%	Loss
	TP53	10%	Loss or Mutation

Modified by Dohmem et al., 2007 [61]

***RAS*:** *RAS* genes are among the commonest mutated genes in human cancers with various range of *NRAS*, *HRAS*, and *KRAS* mutations. In human melanoma, the commonest are *NRAS* mutations (5–36%), where 90% of these mutations are located in code 61 [28–30]. *HRAS* and *KRAS* rarely mutate. *NRAS* mutations are found in 10% of nevus [31, 32] and in 81% of congenital nevus [26–32], while these lesions do not exist in *BRAF* mutations [33]. Albino and Fountain have shown that there are *NRAS* alterations in 24% metastatic and 12% primary metastatic melanoma tumors, while other authors reported higher frequency of these mutations in primary tumors and suggest that these mutations influence the disease progression and metastases development [34]. Results from other studies present that there are *NRAS*-gene mutations in 33% of primary and 26% of metastatic melanoma tumors [35, 36]. The activated *NRAS* mutations, in correlation to the sun exposure, are found in nodular melanoma tumors [37–40]. The presence of *NRAS* mutations in melanocyte lesions and lesions in radial growth phase (RGP) or melanoma in situ, suggest that the *NRAS* activation in the early phase of melanoma development [35, 36].

***RAF*:** The commonest mutated component of the RAS-RAF-ERK pathway in melanoma is BRAF. *BRAF* mutates in 50–70% of melanoma cases, the most frequent mutation being the substitution of valine with glutamine acid in position 600 (V600E) [36, 41]. This mutation is found in 80% of benign nevus, while it is not present in uveal melanoma tumors [42]. The *BRAF* gene is found in chromosome 7q34 region, very often amplified in melanoma tumors [43].

***PI3K*:** *PIK3CA* mutations are rarely found in melanoma tumors, i.e. in 1% of primary melanoma tumors and 3% in metastatic melanoma tumors, without data for enforcement of any PI3K subunit in primary melanoma [44, 45].

***AKT*:** The activation of *AKT* is a potent oncogene for the set off of melanocyte transformation [46]. AKT3 is the main isoform found in melanoma. *AKT3* locus is found in the DNA copy of melanoma, while selective activation of AKT3 is found in 40–60% of tumors [47]. The latest research have shown that the activetion of various AKT isotypes have an effect on the cell proliferation and survival. For instance, out of the three AKT isotypes, AKT3 is in strong correlation to the tumor progression, while targeted depletion of AKT3 causes apoptotic signalization [47].

***PTEN*:** *PTEN* is another important element in the transduction signal disorder process in human melanoma. *PTEN* is a tumor suppressor candidate from the chromosome region 10q23–24, which is most often deleted in glioma and melanoma [48, 49]. Cytogenetic research show that the loss of 10q is often found in melanoma. *PTEN* decodes a protein which has an extensive homology and specificity to protein phosphatases, such as RAS, and enters pathways for apoptosis control through AKT.

PTEN is a negative regulator of PI3K-AKT pathway. By AKT phosphorylation, it gains more properties, but the main property is antagonism to the apoptosis process. Some research have shown that the loss of *PTEN* leads to the apoptosis process disorder. In melanoma, the loss or altered expression of *PTEN* is found in 20–40% of tumors [21, 50], while there are rarely somatic mutations and homozygous deletions. Functionally, the ectopic expression of *PTEN* in *PTEN*-deficient melanoma cells may interrupt the phosphorylation of AKT, induce apoptosis, and suppress the growth process, tumorigenicity and metastases [51, 52]. Interestingly, *NRAS* and *PTEN* act mutually in melanoma, i.e. *NRAS* mutations and *PTEN* alterations in melanoma have mutually covering/overlapping actions [53]. On the other hand, *BRAF* mutations and PTEN alterations coexist in same melanoma cell lines, which suggests that BRAF activation and *PTEN* loss may mutually activate *ERK* and *AKT* in melanoma [10].

***c-KIT*:** Immunohistochemical studies show that the transition of benign nevus into primary or metastatic melanoma tumors comes from the progressive loss of *c-KIT* expression [54]. Some studies also prove the recurrent mutation of L576P in *c-KIT*. In 153 examined cases, Willmore et al. identified strong expression of *c-KIT* in 4 metastatic melanoma tumors, where L576P mutation with selective loss of normal allele was found in 3 of them. L576P is a GIST-associated mutation that binds to the juxta membrane domain, where the *KIT* cluster mutations are activated [55, 56]. A study with 102 primary melanoma tumors reports that the mutation process is increased in cases with *c-KIT* presence, in 39% of the mucous, 36% acral, and 28% in melanoma tumors with chronically exposed skin to sun, but not in skin melanoma tumors that were not chronically exposed to sun. In 79% of tumors with mutations and 53% with multiple copies of *KIT* there are an increased levels of KIT protein [57].

***p16*^{INK4A}:** Sporadic melanoma tumors and melanoma tumors with family history are associated with mutations, loss of heterozygosity and deletions of *CDKN2A* locus, factors that are especially important for normal progression of the cell cycle. The somatic inactivity of *CDKN2A* (*p16*^{INK4A} and *p14*^{ARF}) is very frequently found in the melanoma cell lines [58]. Study results from metastatic melanoma tumors show that biallelic CDKN2A deletions are found in 45% and are associated with bad prognosis, while this fact emphasizes the importance of this locus in the disease progression [59].

The expression of p16^{INK4A} is in negative correlation to the aggressive melanoma tumors. It must be pointed out that mutations of this locus are found in normal melanocytes, too, as well as in cells of benign nevus without signs of clinical or histological atypia [27].

***P53* and *p1*^{ARF}** *TP53* (*p53* gene) mutations are involved in the pathogenesis of many neoplasms, but their role in the melanoma development has not been proven yet. *TP53* mutations are rare in human primary melanoma and this is the reason why a correlation has not been confirmed between the restructuring of *TP53* or altered expression of p53 protein and the melanocytic lesion progression. Nevertheless, some authors suggest that p53 may have a very complex role in the melanoma pathogennesis through its action of effector genes, such as *HDM2*, *GADD45*, and *CIP1*/*WAF1* [27]. *ARF* is considered to be predominantly positive regulator of p53 tumor suppressor through inhibition of HDM2. Therefore, *ARF* loss may be the cause of TP53 mutation shortage in melanoma tumors. However, it has been experimentally proven that ARF acts as a tumor suppressor through p53 sequence induction. According to the research with *ARF*- and *TP53*-deficient mice, there were no identical tumor phenotypes, ARF entered in interaction with various proteins, including E2F1, Myc, NF-B, and it may act independently of p53 biosynthesis in ribosomes, in DNA destruction, apoptosis, and autophagia [60].

Conclusion

Recently, considerable advance has been made in the insights of the molecular genetic alterations during melanoma development. However, this field yet demands a challenge to discover much more new details about melanoma molecular characteristics. The research is focused on mutations and other epigenetic and genetic alterations at particular genes which may be an important target for specific anticancer prevention and therapy in melanoma patients.

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Резиме

МОЛЕКУЛАРНА БИОЛОГИЈА И ГЕНЕТСКИ МЕХАНИЗМИ ВО ПРОГРЕСИЈАТА НА МАЛИГНИОТ МЕЛАНОМ НА КОЖАТА

Софија Пејкова[1], Ѓорѓе Џокиќ[1], Смиља Туџарова-Ѓоргова[1] Сашо Панов[2]

[1] Универзитетска клиника за пластична и реконструктивна хирургија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје
[2] Лабораторија за молекуларна биологија и генетика, Природно-математички факултет, Универзитет „Св. Кирил и Методиј“, Скопје

Малигниот меланом на кожата претставува тумор кој потекнува од трансформираните генетски алтерирани меланоцити на кожата како резултат на комплексни интеракции помеѓу генетските фактори и факторите на околината. Малигниот меланом на кожата има потенцијал

да метастазира и многу често е резистентен на постоечките модалитети на системска терапија. Како и кај секој друг систем на неоплазија, кај некои меланоми може да дојде до прескокнување на фазите во нивната прогресија.

Прогресијата од една кон друга фаза е придружена со специфични биолошки промени. Неколку клучни промени во туморогенезата на меланомот влијаат на регулацијата на клеточната пролиферација и виталност, вклучувајќи ги RAS-RAF-ERK, PI3K-AKT и p16INK4/CDK4/RB патеки. Постојат многу студии кои докажуваат дека RAS-RAF-ERK (MAPK) патеката има клучна улога во развојот на малигниот меланом. До денес делумно се познати молекуларните генетски алтерации за време на развојот на меланомот. Во патогенезата на малигниот меланом се среќаваат мутации на различни гени, како што се NRAS, BRAF и PTEN и мутации и делеции на CDKN2A.

Во последниве години постои голем напредок во сознанијата за молекуларните аспекти на патогенезата на меланомот, но ова поле сè уште претставува предизвик за откривање нови детали од молекуларните карактеристики на меланомот. Резултатите од овие истражувања се насочени кон подобрување на прогнозата кај пациенти со меланом преку воведување персонализирана таргетирана терапија.

Клучни зборови: меланом, NRAS, BRAF и PTEN, прогностички фактор

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MATERNAL LIPIDS MAY PREDICT FETAL GROWTH IN TYPE 2 DIABETES MELLITUS AND GESTATIONAL DIABETES MELLITUS PREGNANCIES

Brankica Krstevska[1], Sasha Misevska Jovanovska[1], Slagjana Simeonova Krstevska[2], Valentina Velkoska Nakova[3], Vladimir Serafimoski[4]

[1] Clinic of Endocrinology, Diabetes and Metabolic Disorders, Medical faculty, Ss Cyril and Methodius University, Skopje, R. Macedonia
[2] Clinic of Obstetrics and Gynecology, Medical faculty, Ss Cyril and Methodius University, 1000 Skopje, R. Macedonia
[3] University of Medical Sciences, Goce Delchev, Shtip, R. Macedonia
[4] Macedonian Academy of Sciences and Arts, Skopje, R. Macedonia

Corresponding Author: Brankica Krstevska, Clinic of Endocrinology, Diabetes and Metabolic Disorders, Medical Faculty, Ss Cyril and Methodius University, 1 000 Skopje, R. Macedonia; tel. + 389 070 35 90 72;
e-mail: branakrstevska@yahoo.com

Abstract

Aim: During diabetic pregnancy, complex metabolic changes occur in the lipid profile. The aim of the study was to determine the predictive values of maternal serum lipid levels on large-for-gestational age newborns during the third trimester in pregnancies of women with type 2 diabetes mellitus (DM2) and gestational diabetes mellitus (GDM).

Material and methods: Data of forty three pregnancies of women with DM2 and two hundred women with GDM were analyzed. The analysis encompassed the following parameters: age, body mass index (BMI), lipid parameters, HbA1c in first, second and third trimester of pregnancy, preeclampsia and baby birth weight.

Results: DM2 and GDM groups showed statistically significant differences in the following variables: total lipids, triglycerides, total cholesterol, BMI, age, baby birth weight, incidence of SGA and preterm delivery (9.4 ± 2.3 vs. 11.0 ± 2.3 mmol/L, 2.4 ± 1.4 vs. 3.4 ± 1.6 mmol/L, 5.5 ± 1.2 vs. 6.4 ± 1.4 mmol/L, 30.6 ± 5.4 vs. 26.9 ± 5.2 kg/m^2, 34 ± 7.8 vs. 31.5 ± 5.6 years, 3183 ± 972 vs. 3533 ± 699 g., 20% vs. 7.5%, 27.9 vs. 14%, respectively, $p < 0.05$). Linear multiple regression analysis demonstrated that triglycerides, LDL-C and total cholesterol were independent predictors of LGA ($p < 0.05$).

Conclusion: Triglycerides and LDL-C in the third trimester of pregnancy are independent predictors for fetal macrosomia in DM2 and GDM pregnancies. Thus, the maternal serum triglycerides and LDL-C levels determined in the maternal blood taken in the third trimester of pregnancy may indentify women who will give birth to LGA newborns.

Key words: lipid parameter, triglycerides, type 2 diabetes mellitus, gestational diabetes mellitus, fetal macrosomia

1. Introduction

Macrosomia still complicates a signifycant proportion of diabetic pregnancies. Studies show that variations in birth weight which occur more often in diabetic pregnancies are not determined only by the maternal glycemic state, but other metabolic factors as well, such as lipids, may have important influence [1–4].

Maternal lipid metabolism is altered during normal and diabetic pregnancy with increased insulin resistance combined with increased peripheral adipose tissue lipolysis. It re-

sults in increased maternal lipoprotein concentrations [5, 6]. The abnormal lipid metabolism has been associated with preterm delivery, preeclampsia and macrosomia. Furthermore, Freinkel et al. [7] proposed that "mixture" of maternal nutrients (glucose, amino acids and lipids) changes the metabolic environment of the fetuses and these changes not only that affect the fetal growth and development but also influence future obesity, diabetes and neurocognitive development in the offspring ("fuel mediated-teratogenesis"). Lipids treatment is the key therapeutic target in non-pregnant diabetic settings. There are no recommendations for clinic management of lipids in pregnancy complicated by diabetes. Studies show lipid changes in the first, second and third trimester both in normal [8] as well as in diabetic pregnancies [9]. There is an association between insulin resistance and serum lipid levels in pregnancy, as adaptive mechanism allowing fetal grown. Serum lipids are higher in obesity, gestational diabetes mellitus (GDM), type 1 diabetes mellitus (DM1), and type 2 diabetes mellitus (DM2). More, but not all studies have reported the same lipid profiles in well-controlled DM1 as in normal pregnancies, high triglyceride and low HDL-C are found in DM1 women with metabolic syndrome in pregnancy, and those diabetic women who develop preeclampsia have high LDL-C and cholesterol levels. In pregnant GDM women frequent founding are the elevated triglycerides while in DM2 pregnancies the lipid profiles are between those observed in DM1 and GDM [9, 10]. Hence, we decided to explore lipids in DM2 and GDM pregnancies because the latter is a form of type 2 diabetes and they share the same underlying pathophysiology (insulin resistance and beta cell dysfunction).

The aim of the present study was to determine the contribution of maternal lipids in predicting (large-for-gestational age) macrosomic newborns in pregnant women with DM2 and GDM, with emphasis on the intergroup differences and development of macrosomia.

2. Material and methods

2.1. Study subjects

The study was conducted at the University Clinic of Endocrinology, Diabetes and Metabolic Disorders. Data of 43 pregnant women with DM2 and 200 women with GDM were analyzed. All were with singleton pregnancies, and neonates were delivered at the University Clinic of Gynecology and Obstetrics. The diagnosis of DM2 was made when patients have been treated with oral lowering medication before conception, were switched to insulin before or at early pregnancy, and without evidence of ketoacidosis. GDM was generally diagnosed by OGTT in the second part of pregnancy. The OGTT was performed in the morning after an overnight fasting from 8 to 12 h. The criteria for diagnosis of GDM were at least one abnormally high out of three plasma glucose value measurements during the 75 g OGTT (normal values: a fasting level < 5.1, 1-hour level < 10.0, 2-hour level < 8.5 mmol/l). The venous blood glucose levels were measured using the glucose oxydase method (Glucose Analyzer; Beckman, Brea, CA). The glucose tolerance was classified by the latest criteria of the International Association of Diabetes in Pregnancy Study Group (IADPSG) [11].

All patients gave informed consent to participate in the study after a careful explanation of the testing protocol. The study was made in accordance with the Declaration of Helsinki.

2.2. Measurements

The analyzed outcome measures were: age, BMI, lipid parameters in the third trimester, HbA1c in the first, second and third trimester of the pregnancy, preeclampsia, and baby birth weight. The maternal glycemic parameters included glycosylated hemoglobin (HbA1c) in all three trimesters. HbA1c was measured with an ionexchange HPLC instrument (DS5; Drew, USA) with a reference range of 4.2–6%. In the third trimester the fasting maternal total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) were measured in both groups. The lipids assessment was taken after the overnight fasting. The blood samples for lipoproteins were analyzed using Cobas Integra 700, according to the standard methods. Total cholesterol and triglycerides were determined with the full enzymatic methods (TCH-CHOD-POD-PAP and triglyceri-

des-GPO; Cobas Integra 700, Hoffmann-La Roche, Basel, Switzerland). The high density lipoprotein cholesterol (HDL-C) was measured using the polyanion precipitation method, while LDL-C was calculated using the Friedewald formula. The low density lipoprotein cholesterol (LDL-C) was fractioned using ultracentrifugation in the cases of triglycerides exceeding 4mmol/l. The body mass index (BMI) of women with type 2 diabetes and GDM was calculated by dividing the weight with the height squared (kilograms/metar^2). The BMI of 26 kg/m^2 was defined as overweight and 30 kg/m^2 as obesity.

The baby birth weight was measured immediately after delivery. The macrosomia was defined as birth weight greater than 4000 g; the large for gestational age (LGA) was defined as weight greater than the 90th percentile for gestational age and sex. The small for gestational age (SGA) was defined as birth weight lower than 2700 g. or the 10th percentile for gestational age and sex. The gestational age was estimated from the date of the last menstrual period. The pre-term delivery was the birth before 37 weeks of pregnancy.

The patients with DM2 were treated only with insulin. The patients with GDM were treated with diet, metformin or insulin according to glycemic profiles.

2.3. Statistical analyses

The statistical analysis was performed using Statistics 7.0 version for Windows. The normal distribution of all variables was verified using Kolmogorov-Smirnov test. The data were presented as mean values, median and percentage. The comparisons between the two groups were determined with the t-test for independent samples. The differences in proportions were compared with the Chi-square test. The correlations were determined by the Pearson correlation test. To estimate the independent effect of analyzed variables on LGA, linear multiple regression analysis was done. $P < 0.05$ was considered to be statistically significant.

3. Results

The study group consisted of 43 women with DM2 and 200 with GDM. The maternal characteristics of the diabetic pregnancies are listed in Table 1. The investigated groups were statistically different in the following variables: total lipids, triglycerides, total cholesterol, BMI, age, baby birth weight, and incidence of SGA (Table 1).

Table 1

The comparison of analyzed variables between the groups (DM2 and GDM)

	DM2	GDM	p value
Total lipids (mmol/L)	9.4 ± 2.3	11.0 ± 2.3	< 0.01
Triglycerides (mmol/L)	2.4 ± 1.4	3.4 ± 1.6	< 0.01
Total cholesterol (mmol/L)	5.5 ± 1.2	6.4 ± 1.4	< 0.01
HDL-C (mmol/L)	1.4 ± 0.3	1.5 ± 0.4	NS
LDL-C (mmol/L)	3.1 ± 1.0	3.5 ± 1.2	NS
HbA1c I trimester (%)	6.6 ± 1.5	7.1 ± 2.2	NS
HbA1c II trimester (%)	5.9 ± 1.1	5.8 ± 0.9	NS
HbA1c III trimester (%)	5.8 ± 1.0	5.8 ± 1.0	NS
BMI kg/m^2	30.6 ± 5.4	26.9 ± 5.2	< 0.05
Age (years)	34 ± 7.8	31.5 ± 5.6	< 0.05
Baby weight (g.)	3183 ± 972	3533 ± 699	< 0.05
Preeclampsia	5/42 (11.9%)	8/155 (5.2%)	NS
LGA	9/35 (25.7%)	44/187 (23.5%)	NS
SGA	7/35(20%)	14/187 (7.5%)	< 0.05
g.w. of delivery (median)	37.5	39	NS
preterm delivery	12/43 (27.9%)	28/200 (14%)	< 0.05

All data are presented as mean±SD, median and percentages; DM2: type 2 diabetes mellitus; GDM: gestational diabetes mellitus; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; HbA1c: glycosylated hemoglobin; BMI: body mass index; LGA: large for gestational age; SGA: small for gestational age; g.w.: gestational week; NS: not significant.

The mean age of patients with DM2 and GDM were 34 ± 7.8 and 31 ± 5 years, respectively, showing statistically significant difference ($p<0.05$). The patients with DM2 were heavier (mean BMI 30.6 ± 5.6 kg/m^2) in comparison to the GDM patients (mean BMI 26.9 ± 5.2 kg/m^2) and the difference was statistically significant ($p < 0.05$).

3.1. Glycemic control

The maternal HbA1c values were similar in the DM2 patients and the GDM patients; there was no difference between the groups during the first, second and third trimester (Table 1), yet all values indicated generally acceptable glycemic control.

3.2. Lipids

The plasma total lipids were lower in the DM2 (mean 9.4 ± 2.3 mmol/L) in comparison to the GDM patients (mean 11.0 ± 2.3 mmol/L), this difference was significant ($p < 0.01$). Statistically significant differences were found between the GDM patients and the DM2 patients in the mean plasma triglycerides concentrations (3.4 ± 1.6 and 2.4 ± 1.4 mmol/L, respectively), and the mean total cholesterol concentrations (6.4 ± 1.4 and 5.5 ± 1.2 mmol/L), ($p < 0.01$), whereas HDL-C and LDL-C concentrations were similar in both groups, using the National Education Program criteria in the non-pregnant state.

3.3. Neonate size

The pregnancy outcomes are shown in Table1. The babies who were born by mothers with GDM had a mean birth weight of 3533 ± 699g, they were heavier than those of mothers with DM2 (3183 ± 972g) and the statistical analyses showed significant differences between the two groups ($p < 0.05$) (Table 1.) The rate of macrosomia was comparable between two groups: 25.7% and 23.5% in DM2 and GDM groups, respectively.

3.4. Interrelationships between metabolic control, lipids and neonatal size (macrososmia /large-for-gestation age)

Triglycerides concentrations were directly related to the HbA1c levels ($r = 0.18$, $p < 0.05$) and the HDL-C concentrations were associated with the values of HbA1c ($r = 0.19$, $p < 0.05$). The HDL-C concentrations were related to the newborn size, i.e. large-for-gestation age newborns ($r = 0.17$, $p < 0.05$). The HbA1c levels were associated with the small-for-gestation age newborns ($r = 0.29$, $p < 0.05$). The BMI was related with the LGA ($r = 0.173$, $p < 0,01$), preeclampsia ($r = 0.228$, $p < 0,01$), triglycerides ($r = 0.137$, $p < 0,05$), and age ($r = 0.202$, $p < 0,01$).

The linear multiple regression analysis demonstrated that, triglycerides, LDL-C and total cholesterol concentrations were independent predictors of LGA ($p < 0.05$) (Table 2).

There were no independent relationships with the HDL-cholesterol concentrations and the HbA1c levels with the LGA. Surprisingly, the BMI was not independent predictor for LGA, as well as preeclampsia.

Table 2

Multivariate analyses of maternal LDL-C, triglycerides, and total cholesterol in the third trimester as independent predictors of neonate size (LGA)

	Unstandardized Coefficients		Standardized Coefficients	t value	p value
	B	Std. Error	Beta		
(Constant)	-0.072	1.019		-0.071	0.944
Triglycerides	0.253	0.119	0.608	2.133	0.043
Total cholesterol	-0.428	0.186	-1.161	-2.298	0.031
HDL-C	0.047	0.299	0.033	0.156	0.877
LDL-C	0.538	0.215	1.184	2.509	0.019
BMI	0.007	0.019	0.068	0.348	0.731
Hba1c I trimester	-0.073	0.059	-0.278	-1.238	0.228
Hba1c II trimester	0.118	0.156	0.200	0.754	0.458
Hba1c III trimester	0.007	0.137	0.013	0.053	0.958
Preeclampsia	-0.260	0.253	-0.197	-1.027	0.315

Dependent Variable: LGA; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; BMI: body mass index; HbA1c: glycosylated hemoglobin.

4. Discussion

In this retrospective study on pregnant women with DM2 and GDM, we found maternal triglyceride and cholesterol values in the third trimester of gestation to be important predictors for macrosomia, independent of the maternal pregnancy BMI and HbA1c values.

Macrosomia occurs in 30–56% of the pregnancies complicated by DM2 and 10–20% of the pregnancies complicated by GDM, according to the data from the literature. In this study, the frequency of macrosomia was similar in the DM2 and the GDM patients partly due to "good" glycemic control. HbA1c values were better than those found in the literature which emphasizes that in the glycemic control "almost good is not good enough". Further, in a recent study we found no correlation between HbA1c and fetal macrosomia. It means that HbA1c is not a sensitive marker for prediction of macrosomia, or that the strict glycemic control can fail to prevent macrosomia. The findings in our study showed that fetal growth, behind maternal glucose concentration is influenced also, by lipids. Many studies [12, 13] observed that maternal serum lipids are associated with baby birth weight, independent of maternal weight gain and glucose.

The normal pregnancy is a hyperlipidemic state mainly influenced by increments of estrogen [8]. The rising levels of estrogen with the progression of pregnancy reflects the changes in lipoproteins [9]. On the other hand, dyslipidemia increases with the insulin resistance found in obesity, GDM and preexisting diabetes, as it has been extensively researched in the studies of Kitajima et al. [10], Knopp et al. [14] and Gobi et al. [15]. Hyperlipidemia in diabetes might also affect the fetal growth. In this paper we focused on lipids in the third trimester in pregnant women with DM2 and GDM. Key points were macrosomia and preeclampsia. We found higher levels of total cholesterol (6.4 ± 1.4 mmol/l) in the GDM patients in comparison to the levels (5.5 ± 1.2 mmol/L) of the DM2 patients. Further, the multivariate analysis showed the positive correlation between the maternal cholesterol levels and the neonatal birth weight. Actually, the maternal cholesterol levels were independent predictor for macrosomia. This is in line with Schaefer et al. [16] who observed a positive correlation between the maternal cholesterol levels in the third trimester and the neonatal birth weight. Some studies have reported that lipids play an important role in baby weigh as compared to glucose [12, 13].

The most consistent lipid change in all studies is the level of triglycerides which shows progressive rise from the first, through the second, and to the third trimester [15]. The increase in the lipid metabolism is an adaptation of the increased fuel delivery to the fetus. When they are too high, macrosomic babies are more likely to be born. Mothers with GDM had higher triglycerides levels (3.4 ± 1.6 mmol/L) in comparison to the triglyceride levels in the DM2 patients (2.4 ± 1.4 mmol/L) ($p < 0.01$). In this study, the triglycerides in the third trimester were the strongest predictor of macrosomia. The increased triglycerides during the late pregnancy are due to the increased hepatic production of VLDL under the stimulation of estrogens and increased adipose tissue lipolysis.

The low density lipoprotein cholesterol and VLDL increase in parallel to the estrogen. HDL-C levels did not change dramatically during pregnancy, i.e. HDL-C increases slightly and remains high or in some patient goes down, but basically normal to delivery [8]. In the study that was conducted by Yang et al. [17], a negative correlation was found between the HDL-C levels and the neonatal birth weight. In the present study we stated that the negative correlation was found between HDL-C and LGA newborns ($r = -0.17$, $p < 0.05$), but without independent influence on LGA, when other lipid parameters were added. HDL-C values were in the normal range according to NCEP [18] in both groups. LDL-C levels were above the normal range in both diabetic pregnant women, and the linear multiple regression analysis revealed their predictive value for LGA newborns.

Lipids exhibit some adverse effects on the mother. Women with preeclampsia have high serum lipids in pregnancy. Hyperlipidemia might favor production of lipid peroxides and alter the balance of the vasoactive compounds leading to endothelial dysfunction i.e. vasoconstrictions. GDM patients are at increased risk for preeclampsia and it is equal to that seen in the insulin resistant pregnant women with DM2. Our findings are very supportive to this notation. The rate of preeclampsia was 11.5% and 5.2% in DM2 and GDM patients, respectively. Patients with DM2 were older and obese. Patients with GDM were overweight and had worst lipid profiles. The babies born by mothers with GDM were heavier in comparison to the babies of the DM2 patients. On the other side, not all GDM mothers were treated with insulin. Lower birth weight in DM2 than in GDM pregnancies might result from better

glycemic control throughout the pregnancy and insulin therapy starting before or in early pregnancy. In spite of that, mild hypertriglyceridemia and hypercholesterolemia were found, whereas the HDL-C levels were in the normal range according to NCEP [18]. The proportion of SGA in DM2 was higher than in GDM, due to the higher percentage of preterm delivery in DM2. Probably preeclampsia was the main reason for preterm delivery.

Obesity is a significant risk factor for both DM2 and GDM and may be related to the fetal size and adverse perinatal outcomes. In this study DM2 patients were obese and GDM patients were overweight. Almost all studies show the effects of obesity on newborn macrosomia [19, 20]. In this study the BMI was statistically significantly correlated with LGA, but without an independent effect on LGA. This finding can be explained by the lack of normal weight pregnant women in the observed cohort. Actually it is one limitation of this study.

5. Conclusion

This study pointed out the usefulness of measurement of the serum lipids in pregnancy. Triglycerides and LDL-C in the third trimester of pregnancy are independent predictors for fetal macrosomia in DM2 and GDM pregnancies. Maternal serum triglycerides and LDL-C levels determined in the maternal blood taken in the third trimester of the pregnancy may identify women who will give birth to LGA newborns. With proper regulation of the lipid profile we can avoid macrosomia in DM2 and GDM pregnancies.

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Р е з и м е

МАЈЧИНИТЕ ЛИПИДИ МОЖЕ ДА ГО ПРЕДВИДАТ ФЕТАЛНИОТ РАСТ КАЈ БРЕМЕНИ ЖЕНИ СО ДИЈАБЕТЕС МЕЛИТУС ТИП 2 И ГЕСТАЦИСКИ ДИЈАБЕТЕС МЕЛИТУС

Бранкица Крстевска[1], Саша Јовановска-Мишевска[1], Слаѓана Симеонова-Крстевска[2], Валентина Велкоска-Накова[3], Владимир Серафимоски[4]

[1] Клиника за ендокринологија, дијабетес и болести на метаболизмот, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[2] Клиника за гинекологија и акушерство, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[3] Универзитет за медицински науки, „Гоце Делчев“, Штип, Р. Македонија
[4] Македонска академија на науките и уметностите, Скопје, Р. Македонија

Цел: Комплексни метаболни промени во липидниот статус настануваат во тек на бременост кај жени со дијабетес. Целта на истражувањето беше да се открие предиктивната вредност на серумските липиди од третиот триместар на бременост кај жени со дијабетес тип 2 (DM2) и гестациски дијабетес (GDM) за раѓање на новородено големо за гестациската возраст (LGA).

Материјал и методи: Анализирани беа податоци за 43 бремени жени со DM2 и 200 жени со GDM. Анализите ги вклучуваа следниве параметри: возраст, индекс на телесна маса (BMI), липиден статус, HbA1c во првиот, вториот и третиот триместар од бременоста, преeклампспија и телесна тежина на новородено.

Резултати: DM2 и GDM-групите покажаа статистички значајни разлики во следниве параметри: вкупни липиди, триглицериди, вкупен холестерол, BMI, возраст, телесна тежина на новородено, инциденца на мало за гестациска возраст (SGA) и предвремено пораѓање: 9.4 ± 2.3 vs. 11.0 ± 2.3 mmol/L, 2.4 ± 1.4 vs. 3.4 ± 1.6 mmol/L, 5.5 ± 1.2 vs. 6.4 ± 1.4 mmol/L, 30.6 ± 5.4 vs. 26.9 ± 5.2 kg/m^2, 34 ± 7.8 vs. 31.5 ± 5.6 years, 3183 ± 972 vs. 3533 ± 699 g., 20% vs. 7.5%, 27.9 vs. 14%, соодветно, $p < 0.05$). Линеарната мултипна регресиона анализа покажа дека триглицеридите, LDL-C и вкупниот холестерол беа независни предиктори за LGA ($p < 0.05$).

Заклучок: Триглицеридите и LDL-C во третиот триместар од бременоста се независни предиктори за фетална макросомија во групите бремени жени со DM2 и GDM. Одовде, серумските триглицериди на мајката и нивото на LDL-C во крвта на мајката во третиот триместар од бременоста може да ги откријат жените кои раѓаат LGA-новородени.

Клучни зборови: липидни параметри, триглицериди, дијабетес тип 2, гестациски дијабетес, фетална макросомија

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DIAGNOSTIC AND SURGICAL APPROACH TO PRENATALLY DETECTED URINARY TRACT ANOMALIES

Mile Petrovski

University Clinic of Pediatric Surgery, Medical Faculty, Skopje, R. Macedonia

Corresponding Author: Mile Petrovski, University Clinic of Pediatric Surgery, Medical Faculty, Skopje, R. Macedonia; e-mail: milep22@yahoo.com

Abstract

Regular ultrasound examinations carried out in the second trimester of pregnancy help in detecting many anomalies in the fetal urinary tract. Their percentage ranges from 1% to 3% of all controlled pregnancies. There is a wide spectrum of anomalies that affect the urinary tract, but the most significant are: uretero/hydronephrosis (unilateral or bilateral), kidney agenesis, dysplastic kidney, polycystic and multicystic kidneys, anomalies of ascent, anomalies of kidney rotation or fusion, bladder exstrophy, posterior urethra valve etc.

Many of these anomalies do not have impact either on urine flow or on kidney function and hence they can be qualified rather as a condition than as a disease. At the same time, most of the hydronephroses that are seen prenatally are being resolved spontaneously, and they are not detected neither presented postnatally as uretero/hydronephroses of unobstructed type and do not require surgical treatment. Only one tenth of these anomalies are subject to active surgical treatment.

Therefore, the assessment of these conditions should be done by a specialized team, who will make adequate therapeutic decisions based on clinical guidelines, as well as will advise the parents on the future clinical implications of the detected anomaly.

Keywords: prenatally ultrasound examinations, urinary tract anomalies, treatment

Introduction

The basic function of the kidney is to produce protein free ultra-filtrate (urine) that contains adequate quantity of water, electrolytes and end products of the metabolism, by which homeostasis of the human organism is actively maintained. In addition, the kidney produces two hormones, renin and angiotensin, and at the same time it participates in the metabolism of vitamin D. The function of the urinary tract is to collect and eliminate the produced urine.

When there are structural disorders along the urinary tract that obstruct urine flow, there are preconditions for development of **obstructive uropathy.** Thus, the term *obstructive uropathy is defined as a damage of the kidney parenchyma when there is a presence of obstruction at any level of the urinary tract* [1].

The most significant consequence of the upper urinary tract obstruction is dilatation of the collecting system – hydronephrosis. Since dilatation of the renal pelvis and calyces may be present without obstruction, the term hydronephrosis should be used as a descriptive, anatomic entity, which defines only increasing – dilatation of the collecting system of the kidney [2].

The urinary system obstruction is one of the most common entities encountered by pediatric urologists. Despite the clear definition which provides easy recognition of the effects of **chronic obstruction** by the appearance of hydronephrosis, parenchymal atrophy and poor kidney function, these features also appear in conditions when morphologic disorders appeared during embryonic development, which have afterwards been resolved and hence the obser-

ved kidney does not have to be in the condition of obstruction [3].

Diagnostic procedures

Ultrasound Scan (US)

Prenatal ultrasound examinations

Fetal urine production begins between 10 and 12 weeks of gestation. The largest quantity of the urine participates in the volume of amniotic fluid and it is an essential factor for lung development in the fetus. (3) Normal kidney is visualized from 18 to 20 weeks of pregnancy, a period convenient for routine ultrasound examinations. In this period, dilatation of the urinary tract (as indirect sign of obstruction) can be detected in conjunction with some other structural anomalies and oligohydramnios, which suggest impaired kidney function [4, 5]. Griglin [6] and Blyth [7] have introduced classification of hydronephrosis grading. Coelho [8] applies classification that has a predictive value for postnatal hydronephrosis manifestation: in the second trimester – mild grade if pelvic anteroposterior diameter (APD) ranges from 4 to 7 mm, moderate if APD is from 7 to 10 mm and severe if APD is over 10 mm. In addition, structural anomalies (cystic formations, fusion of kidneys etc.) can be detected while if the bladder is not visualized on repeated controls, there is a doubt about its exstrophy. Cases with moderate and severe grade of hydronephrosis should also be evaluated in the third trimester of pregnancy (Fig. 1).

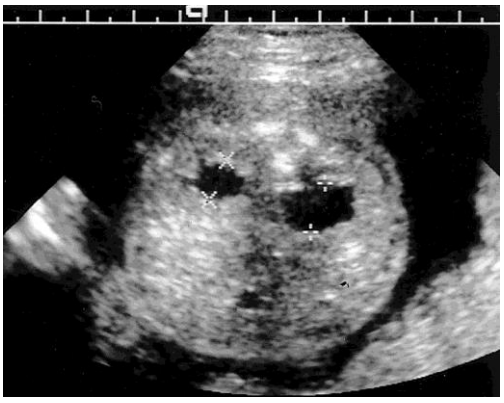

Fig. 1 – Prenatally diagnosed bilateral hydronephrosis

Postnatal ultrasound examinations

Although kidney function cannot be assessed by ultrasound, numerous and valuable pieces of information can be obtained, among which are: morphology, form, size and position of the kidney, then echogenicity; parenchymal thickness; uretero/hydronephrosis. Dilatation along the urinary tract is determined according to hydronephrosis grading system in five grades (from 0 to 4) according by the Society for Fetal Urology (SFU) [9].

Radiological investigations

Excretory urography

Excretory urography is the oldest method for diagnosis of hydronephrosis. This technique provides excellent data about morphology and partly, however not adequately quantified data about the function of the obstructed kidney. Its application is limited in current clinical practice.

Micturating cystourethrography (MCUG)

MCUG is performed in order to confirm or exclude subvesical obstruction as well as the presence of vesicoureteral reflux (VUR) (Fig. 2).

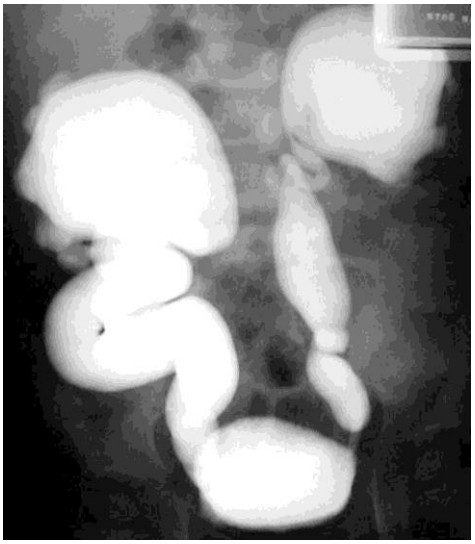

Fig. 2 – MCUG: Bilateral vesicoureretral reflux

Imaging techniques: computerized tomography and magnetic resonance

Computed tomography (CT) has a role in complementary explanation of more complex anomalies where hydronephrosis is present, such as duplex canal system, solitary kidneys, crossed ectopia etc.

By applying MRI urography as a non-ionizing technique which aids in visualization of the whole urinary tract morphology, the CT use has decreased. At the same time, the application of gadolinium, bound to DTPA through a software system, the functional ability of the kidney can be seen [10] (Fig. 3).

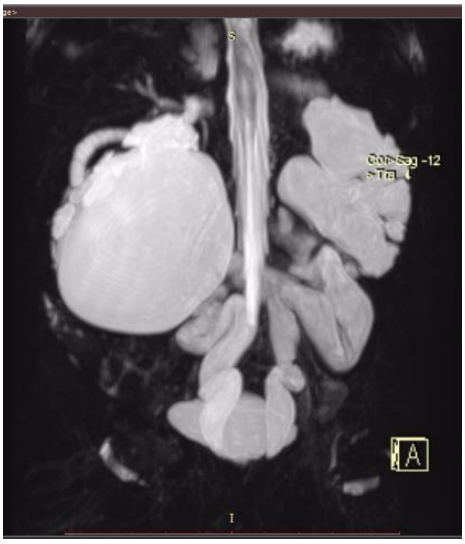

Fig. 3 – MRI urography (Bilateral UPJ stenosis and UPJ stenosis on right side)

Prenatal MR is indicated in precisely selected cases.

Radioisotopic investigations

Nuclear-medical methods are considered as techniques of primary importance for initial diagnosis and monitoring/screening of renal diseases in children. They are highly-sensitive techniques that enable early detection of diseases. Nuclear medicine provides unique functional and anatomical information by relatively low-dose radiation (Fig. 4).

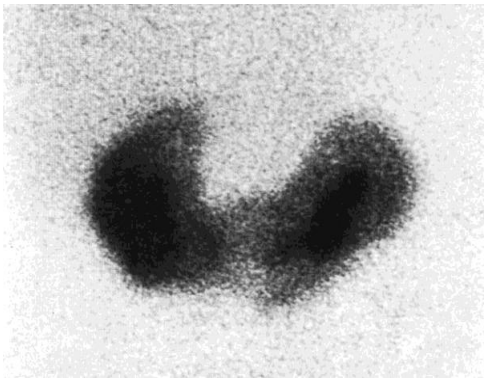

Fig. 4 – DMSA renal scan: Horseshoe kidney

Dynamic renography

Diuretic radioisotope renography (DRRG) has enormous role in diagnosing the obstructtive uropathies. DRRG is a physiologic study, which estimates the possibility of the kidney to respond to the diuretic-induced volume change. Analyzing the data obtained by DRRG the presence of obstruction is estimated by the model of radio-renogram curve by T/2 (the time when 50% of the tracer of the zone of interest is eliminated). In addition, the relative function (percentage of glomerular filtration rate – GFR) of the kidneys is determined [11].

Renal cortical scintigraphy is safe and practical imaging technique for initial evaluation and follow-up/monitoring of children with cortical renal impairment. This technique aids in determining parenchymal volume without interference of radiotracer from collecting system [12].

Classicifaction

The following classification of congenital ureteral anomalies is based more on structure rather than on functional disorders [13].

A. Kidney

- **Anomalies of number**
  - Agenesis (bilateral, unilateral)
  - Supernumerary kidney
- **Anomalies of volume and structure**
  - Hypoplasia
  - Multicystic kidney
  - Polycystic kidney (infantile, adult, medullary cystic disease, other cystic diseases)
- **Anomalies of ascent**
  - Ectopia: thoracic, proximal, pelvic, crossed (with or without fusion)
- **Anomalies of form and/or fusion**
  - Crossed ectopia with or without fusion
  - Unilateral fused kidney (Sigmoid or S-shaped kidney, L-shaped kidney, disc-like kidney, horseshoe kidney)
- **Anomalies of rotation**
  - Incomplete, excessive and reverse
- **Anomalies of renal vasculature**
  - Aberrant, accessory, or multiple vessels; renal artery aneurysm; arteriovenous fistula
- **Anomalies of the collecting system**
  - **Calyx and infundibulum**
    - ✓ Calyceal diverticulum, hydrocalyx, megacalyx, extrarenal calyx.
  - **Pelvis**
    - ✓ Exstrarenal pelvis, bifid pelvis, pyeloureteral stenosis.

B. Ureter

- **Hydroureter (megaureter)**
  - Obstructed
  - Vesicoureteral reflux (VUR)

- Obstruction + VUR
- Unobstructed, nonrefluxing

- **Anomalies in entering the urinary bladder**
- **Ureteral duplication**
- **Ureterocele**

C. Urinary bladder

- **Bladder exstrophy**
- **Neurogenic urinary bladder** (primary, secondary)

D. Urethra

- **Posterior urethral valve**
- **Posterior urethral diverticulum**
- **Hypospadias**
- **Epispadias**

In general, functional disorders as a result of congenital urinary tract anomalies affect the normal flow of urine. First of all, obstructions that appear lead to dilatation of the urinary system with subsequent loss of renal function.

The most common classification of functional dysfunction of the urinary tract is as follows:

A. Upper urinary tract
- **Obstruction of pyeloureteral segment (hydronephrosis)** (Fig. 5)
- **Obstruction of ureterovesical junction (ureterohydornephrosis)** (Fig. 6)

B. Lower urinary tract
- **Urinary bladder (unilateral or bilateral ureterohydronephrosis)**
- **Subvesical obstruction (bilateral ureterohydronephrosis)**

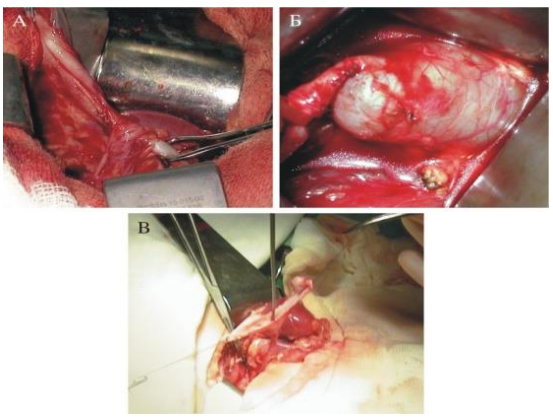

Fig. 5 – Ureteropelvic junction obstruction (A. Vas Aberans; Б. High insertion; B. Stenosis)

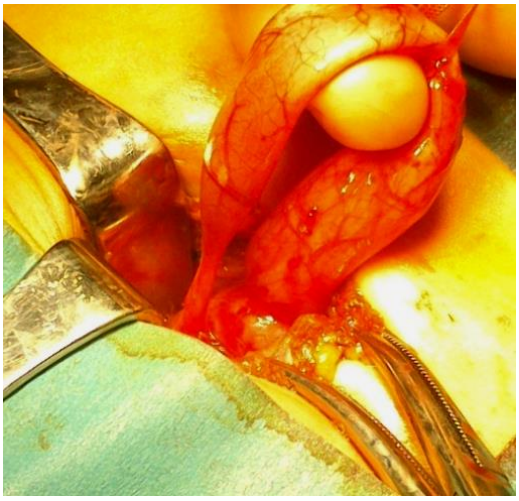

Fig. 6 – Prevesical ureteric stenosis

Hydornephrosis/ureterohydronephrosis

Hydronephrosis is a descriptive, anatomic entity defining dilatation of the renal collecting system. Chronic renal obstruction leads to onset of hydronephrosis, parenchymal atrophy and poor renal function. Obstruction causes hydronephrosis and hydronephrosis is not an equivalent to obstruction. Most often, nonobstructed uretero/hyronephrosis is a result of a spontaneously or surgically managed developmental anomaly or it is a diuretic phenomenon [14].

Distinction between obstructive and nonobstructive processes is based on the following facts:

- Unobstructed urinary tract transports urine throughout the entire physiologic range, without significant deformation changes and without increase in intraluminal pressures that would cause renal failure impairment.
- Obstructed system is more efficient in transporting small volumes than large ones [15].

To make a correct diagnosis a number of anatomic and functional investigations (US, MRI, MCUG, radioisotope methods) have to be conducted.

Pathophysiology of the obstructed kidney

Obstructive uropathy produces obstructive nephropathy via a hemodynamic cascade reaction that causes ischemic injury. Preglomerular arteriolar vasoconstriction is mediated by the renin-angiotensin system. Animal studies have shown that this system increases an-

giotensin-dependent vascular tonus of the obstructed kidney and decreases that of the opposite kidney, thus explaining the compensatory hypertrophy of the healthy kidney.

Partial ureteral obstruction causes activetion of other vasoactive substances: thromboxane-A_2, nitric monoxide and other eicosanoids. Other factors that cause kidney lesion are: 1. Mediators of inflammatory response; 2. Cellular apoptosis (programmed cellular death – modulated by the epidermal growth-factor); 3. Cytokine growth-factor β (initiates interstitial fibrosis); 4. Activation of the kallikrein-kinin system (impairs sodium metabolism); 5. Complexity in the pathophysiology [16–19].

Pathohistology of the obstructed kidney

In the beginning the dilatation of collecting tubules as well as deposition of fibroblasts and macrophages are predominating in the patho-anatomic substrate. Later, the collagen is transformed into fibrous tissue, manifested as interstitial and tubular fibrous degeneration, and in the end glomerular sclerosis develops, hence the entire functional kidney capacity is decreased [1, 20].

Incidence

Hydronephrosis is prenatally detected in 1%–3% of all controlled pregnancies, and 50% of them are verified postnatally. For comparison: the incidence of the most commonly surgically treated anomaly (pyelo-ureteral obstruction) is 1 : 1500 infants. About 10–20% of prenatally detected urinary anomalies require postnatal surgical treatment [21].

Postnatal approach to infants with prenatally detected anomalies of the urinary tract

By rule and due to the neonate's physiology the postnatal ultrasound is done the 3rd day after birth at the earliest. Early exams might underestimate or might not detect the degree of hydronephrosis [22, 23].

Treatment at tertiary level

In severe cases the neonate should be assessed immediately after birth by a nephrology/urology team of experts. Urgent series of US exams, determination of electrolytes, urea and blood creatinine are recommended in these infants.

This group comprises infants with the following anomalies:

- Abnormal urinary bladder and/or history of oligo/anhydramnios;
- Severe form of bilateral ureterohydronephrosis (APD > 20 mm);
- Solitary kidney with expressed dilatetion of the upper urinary tract;
- Bilateral hypoechogenic kidneys;
- Autosomal recessive polycystic kidney disease (large "bright/echogenic" kidneys).

Early consultation with a nephrologist/urologist

It is necessary to make US in the second week after birth.

Newborns with the following anomalies belong to this group:

- Severe unilateral hydronephrosis (APD > 20 mm), with or without dilated ureter;
- Bilateral hydronephrosis (APD>10 mm);
- Large multicystic kidney (> 6 cm);
- Multicystic kidney with contra-lateral ureteral dilation;
- Duplex kidney with ureterocele.

Antibiotic prophylaxis

Antibiotics (trimetroprim 2 mg/kg in the evening) are to be given to the newborns with these features:

- Abnormal urinary bladder and/or history of oligo/anhydramnios;
- Bilateral hydronephrosis (APD>10 mm);
- Duplex kidney with ureterocele;
- Multicystic kidney with anomaly in the opposite kidney and ureter.

The following cases do not require antibiotic prophylaxis.

- Moderate degree of hydronephrosis;
- Multicystic kidney with normal contralateral/opposite kidney.

Regular consultations with a pediatrician/nephrologist

It is recommended to make US the first month after birth, and control visits at 3 to 6 months in the following cases:

- Moderate hydronephrosis with these characteristics:

- Unilateral pelvic dilatation smaller than 15 mm;
- Bilateral pelvic dilatation smaller than 10 mm;
- Normal calyces;
- Normal echogenic kidney;
- Normal length of the kidney;
- Normal opposite kidney;
- Absence of ureteral dilation;
- Normal urinary bladder.

- Multicystic kidney with normal contralateral/opposite kidney (necessary verification at 6-month-age with DMSA radioisotope scan).

Regular consultations with a family pediatrician

Newborns who have been diagnosed with a minor anomalies of the urinary tract, including hydroneprhosis that has withdrawn during control prenatal scans, have to be taken to their family pediatricians for regular check-ups.

Additional investigations

In cases of suspicion of subvesical obstruction or vesicoureteral reflux micturating urethrocystography has to be conducted one month after birth. Diuretic radioreniogram is advised in order to define the existence of pyeloureteral or ureterovesical junction obstruction.

Discussion and conclusion

The incidence of urinary tract anomalies detected by prenatal screening is extremely high (up to 3%). This piece of information causes anxiety in parents, with suspicion of insecure future for the fetus. Due to insufficient knowledge medical professionals who are not very familiar with this problem can increase the suspicions in the future parents-to-be.

It is known that a large number of anomalies (ectopia, malrotation, fusion of the kidneys) do not affect urine drainage, and hence do not damage kidney function. Therefore, they are to be defined as a condition rather than a disease. In more than 50% of cases, prenatal hydronephrosis is not detected postnatally. Additionally, quite a large number of postnatally detected hydronephrosis are being self-resolved during the embryonic development.

On the other hand, there are conditions that require active surgical treatment (all obstructions at the level of the upper and lower urinary tract). Some anomalies (exstrophy of urinary bladder, posterior urethral valve) ask for a long-term and uncertain treatment, and even in some series they are a cause for abortion (in 25–50% of cases). Overall, less than 10% of prenatally detected anomalies require active surgical treatment (Fig. 7).

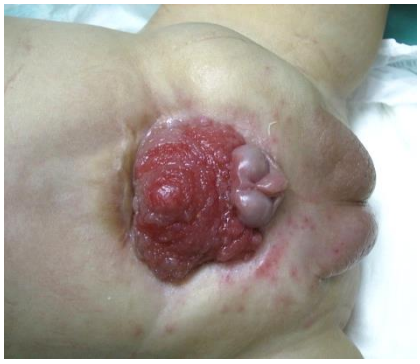

Fig. 7 – Vesical exstrophy

These conditions have to be subject of a team approach (gynecologist – perinatologist, pediatrician – neonatologist/nephrologist, pediatric surgeon/urologist). Thus, it is necessary to prepare a clinical guide (algorithm). This is the only way how we can reach the ideal: not a single unnecessary surgery or a single lost kidney.

Parents should consult and counsel with the members of the expert team in order to make quality-informed decisions about the future of their children.

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Резиме

ДИЈАГНОСТЧКИ И ХИРУРШКИ ПРИСТАП КОН ПРЕНАТАЛНО ДЕТЕКТИРАНИТЕ АНОМАЛИИ НА УРИНАРНИОТ ТРАКТ

Миле Петровски

Универзитетска клиника за детска хирургија, Медицински факултет, Скопје, Р. Македонија

Како резулатат на редовните ултразвучни контроли, во вториот триместар од бременоста се дијагностицираат голем број аномалии на уринарниот тракт на плодот. Нивниот процент се движи од 1% до 3% од сите контролирани болести. Постои широк спектар на аномалии што го засегаат уринарниот тракт, но најзначајни се следниве: уретеро/хидронефроза (едностарана или двострана), агенезија на бубрег, диспластичен бубрег, полицистични и мултицистични бубрези, аномалии во позиција, ротација или фузија на бубрезите, екстрофија на мочниот меур, валвула на задна уретра и др.

Голем број од овие аномалии не даваат реперкусии на уротекот, а со тоа ниту на функцијата на бубрегот, поради што можат да се квалификуваат како состојба, а не како болест. Воедно, поголемиот дел од хидронефрозите што се визуелизираат пренатално спонтано се разрешуваат, така што постнатално или не се детектираат или се презентираат како уретеро/хидронефрози од неопструктивен тип и не се предмет на хируршки третман. Само една десеттина од овие аномалии се предмет на активно оперативно лекување.

Затоа проценатa на овие состојби треба да е предмет на специјализиран тим, кој согласно со клиничко упатство ќе носи соодветни терапевтски решенија и квалитетно ќе ги советува родителите за идните клинички импликации на детектираната аномалија.

Клучни зборови: пренатални ултразвучни прегледи, аномалии на уринарниот тракт, третман

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CASE REPORT

SOLITARY FIBROUS TUMOR OF THE PANCREAS: A CASE REPORT AND REVIEW OF THE LITERATURE

Liljana Spasevska[1], Vesna Janevska[1], Vlado Janevski[2], Biljana Noveska[3], Julija Zhivadinovik[4]

[1] Institute of Pathology, Faculty of Medicine, Skopje, R. Macedonia
[2] University Clinic of Abdominal Surgery, Skopje, R. Macedonia
[3] General Hospital, 8-mi Septemvri, Skopje, R. Macedonia
[4] Institute of Anatomy, Faculty of Medicine, Skopje, R.Macedonia

Corresponding Author: Liljana Spasevska**,** Institute of Pathology, Faculty of Medicine, Skopje, R. Macedonia; e-mail: lspasevska@yahoo.com

Abstract

Pancreas is an extremely rare abdominal localization of the solitary fibrous tumor (SFT). It usually grows asymptomatically for a long time before a diagnosis can be made on the basis of symptoms and/or mechanical complications. Due to the rarity and nonspecific clinical presentation, this entity is diagnostically challenging.

We present a 47-year-old man with a history of progressive epigastric pain for the last two weeks, and jaundice, who was admitted to hospital for further investigation. Cystadenocarcinoma was suspected based on the radiologic findings, and a pancreatoduodenectomy was performed. The removed portion of the pancreas contained a 3.5 × 2 × 1.8 cm well-circumscribed, but not encapsulated white tumor mass with smooth cut surface, cystic component and duct dilatation within the tumor and within the adjacent pancreatic tissue. Based on the histology and immunostaining profile, a diagnosis of the solitary fibrous tumor was made. One week post-operatively, the patient died due to surgical complications.

Microscopic and immunohistochemical examinations are necessary for accurate diagnosis of cystic SFT of the pancreas. Because there is limited data regarding the biological behavior of SFT with extra-pleural localization the authors recommend clinical follow-up for SFT treatment if the criteria of malignancy are not met.

Key words: pancreas, solitary fibrous tumors

Introduction

Pancreas is an extremely rare abdominal localization of the solitary fibrous tumor (SFT) with only 16 cases reported to date [1–16]. It usually grows asymptomatically for a long time before a diagnosis can be made on the basis of symptoms and/or mechanical complications. Due to the rarity and nonspecific clinical presentation, this entity is diagnostically challenging.

We describe a 47-year-old man with a solitary fibrous tumor arising in the head of the pancreas and review of the diagnostic literature.

Case report

A 47-year-old man with a history of progressive epigastric pain for the last two weeks, and jaundice, was admitted to hospital for further investigation. His past medical history revealed a conservative cholecystectomy performed 5 years ago. The family history including malignancy or inherited disease was unremarkable. The physical examination revealed

icteric sclera and yellowish skin. Laboratory investigations showed a normal hemogram: hemoglobin concentration 140 g/L (reference range: 140–180 g/L), hematocrit 40% (reference range: 37–54%), platelet 290 (reference range: 140–340), white blood cell 8000 (reference range: 4000-8000), glucose (ser) 6.1 mmol/L (reference range: 3.5–6.1 mmol/L) alpha amylase (ser) 119 U/L (reference range: 30-110 U/L). Abnormal laboratory findings included elevated LDH 605 U/L (reference range: 248 U/L), total bilirubin 133 umol/L (reference range: 6.8–20.5 umol/L), indir.bilirubin 14 umol/L (reference range: 5.1–13.6 uml/L), dir.bilirubin 119 umol/L (reference range: 1.5–6.8 umol/L), and alkaline phosphatase 608 U/L (reference range: 38–126 U/L). The serum tumor markers were within the normal limits for carcinoembryonic antigen 2. 4 ng/mL (reference range: 1–3.4 ng/mL) and increased for carbohydrate antigen 19–9, 198.0 U/ml (reference range: 1–37 U/ml). The abdominal ultrasonography showed a hypoechoic mass, 3.5 cm in cross diameter, located in the pancreatic head, without stones. The computed tomography (CT) imaging of the abdomen confirmed a 3.5 cm mass with enhanced contrast uptake in both arterial and venous phases on the pancreatic head. The tumor mass was well-delimited but not encapsulated, mainly solid but with a cystic component. The surrounding bile duct of the pancreaticoduodenal arcade was dilated. There was no sign of loco-regional invasion or metastasis. The diagnosis of cystadenocarcinoma was suspected and a pancreatoduodenectomy was performed.

The removed portion of the pancreas measured 10 × 4.5 × 3.5 cm and contained a 3.5 × 2 × 1.8 cm well-circumscribed, but not encapsulated white tumor mass with smooth cut surface, cystic component and duct dilatation within the tumor and within adjacent pancreatic tissue [Fig. 1]. The transection margin was tumor-free. The histological analysis showed that tumor had infiltrated the surrounding pancreatic parenchyma and consisted of spindle cells with eosinophilic cytoplasm and hyperchromatic nucleus with minimal cytological atypia, arranged in a fascicular pattern and with branched hemangiopericytoma-like vessels [Fig. 2, 3]. No necrosis was found and mitotic figures were very rare, 1–2 mitosis per 10 high-powered fields. Hyalinization and myxoid degeneration areas were seen in parts, which were hypocellular. The cystic component was related to retention cysts and duct dilatation. The tumor invaded the muscularis propria of the duodenum. The complete pathological examination revealed no vascular or nervous invasion. All seventeen lymph nodes were tumor-free.

Figure 1 – Solitary, circumscribed, neoplasm with white cut surfaces, in the head of the pancreas

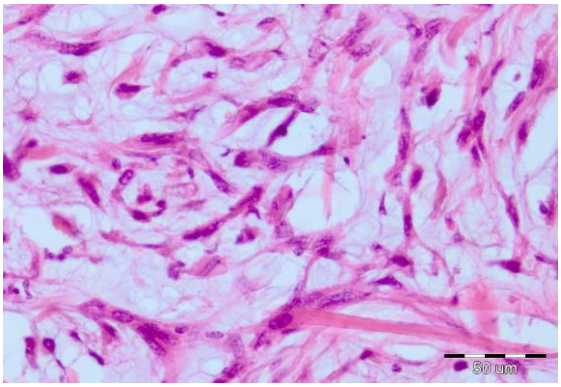

Figure 2 – Spindle cell with minimal nuclear pleomorphism (HE × 200)

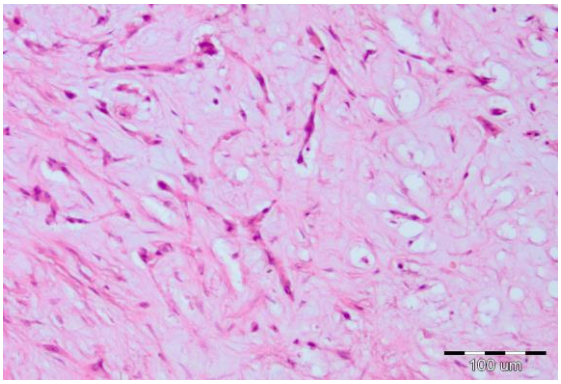

Figure 3 – Mixoid background and haemangiopericytoma-like vascular pattern (HE × 100)

Immunohistochemical analysis on the resected tumor revealed that the tumor cells express diffusely positive for CD34, vimentin and CD99, focally positive for bcl-2, nuclear beta-catenin and actin and were negative for CD117, EMA, Caldesmon, Desmin, S100 and Cytokeratins [Fig. 4–6]. Ki-67 proliferation index was observed below 1%.

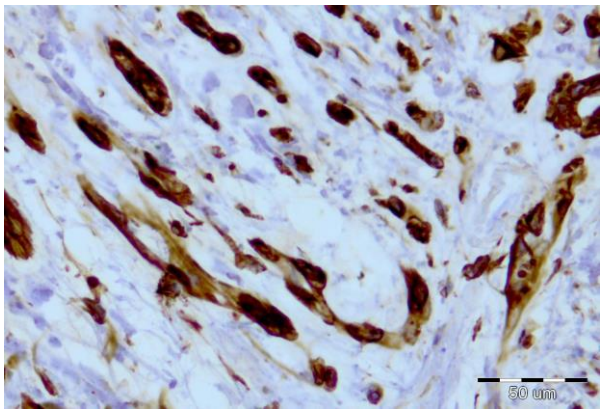

Figure 4 – Immunostaining for vimentin (× 200)

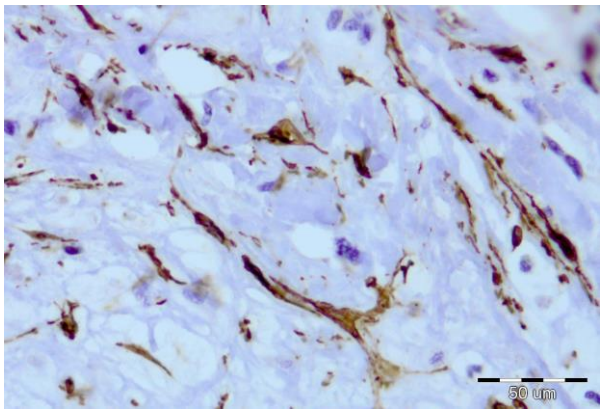

Figure 5 – Immunostaining for actin

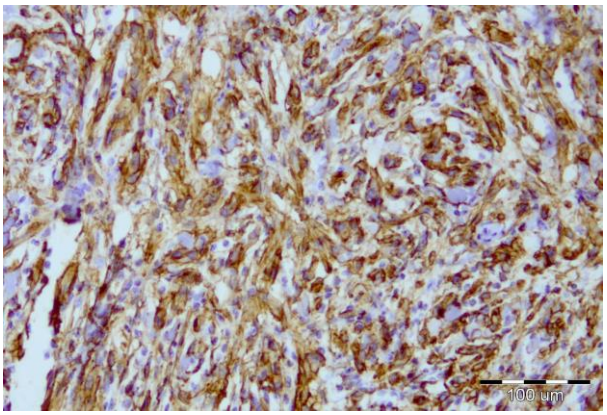

Figure 6 – Immunostaining for CD 34 (× 100)

Based on the histology and immunostaining profile, a diagnosis of solitary fibrous tumor was made. One week post-operatively, the patient died due to surgical complications.

Discussion

The 2010 World Health Organization classification defines SFT as a ubiquitous mesenchymal tumor of probable fibroblastic type with a prominent haemangiopericytoma-like branching vascular pattern [15]. SFT was first described as a distinct entity among primary neoplasm in the pleura by Klemperer and Rabin in 1931 [14]. SFT is quite a rare tumor; it accounts for 0.03% of all neoplasms and 3% of soft tissue tumors, and its incidence has been estimated to be 2–4 cases per million per year in the general population. SFT is typically found in the pleura (65%), fascial and musculo-aponeurotic tissues, but can occur in intra-abdominal localization. Intra-abdominal SFTs are most often associated with familial adenomatous polyposis or Gardner syndrome (familial adenomatous polyposis with multiple osteomas and mesenchymal tumors of the skin and soft tissues) in up to 70% of the cases while sporadic cases are uncommon. In contrast to the intra-abdominal forms, sporadic pancreatic fibrous tumors are more frequent than those associated with familial adenomatous polyposis. SFTs in extra-pleural localizations generally exhibit benign behavior [14]. However, it has been reported that several clinical and pathological features can predict more aggressive behavior and metastasis can be seen approximately in 10–15% of tumors [14].

In 65% of SFT cases, the tumor arises from the pleura, but they can also be found in other sites such as lung parenchyma, thyroid gland, liver, kidney, adrenal gland, salivary gland, soft tissue, head and neck. Pancreas is an extremely rare extrapleural location. Including our patient, only 16 cases of pancreatic SFTs have been reported with the clinical findings summarized in Table 1. Other mesenchymal tumors located in the pancreas include GIST, leiomyosarcoma, schwannoma, fibromyxoid sarcoma, perivascular epithelioid cell tumor (PECOoma), and vascular tumors. Pancreatic SFT is often asymptomatic because it is generally a benign and slow-growing tumor. Symptoms differ according to the location and size of the mass and include abdominal pain, constipation, jaundice, and weight loss. In our case, the patient presented with abdominal pain

and jaundice. The patients' age ranges from 24–78 years at diagnosis [1–15]. Although extrapleural SFT has no gender bias reported, the current pancreatic cases have female to male ratio of 12 : 3. Our patient was a 47–year-old man. In 10 cases, the radiologic impression favored an endocrine tumor, as these tumors can appear similarly well-circumscribed and hypervascular. In our case, the initial diagnosis was a cystadenocarcinoma. This was suggested because of the cystic component and female gender. In fact, the cystic component corresponded to retention cysts above the tumor. Only 4 published pancreatic SFT presented as cystic tumors. These cases confirm the difficulties of the radiologic diagnosis of the cystic pancreatic tumors. The size of the tumors ranged from 1.5 to 18.5 cm in diameter. SFTs were mostly localized in the head of pancreas (9/13) as in our case. Pancreatic SFT may show a wide range of histological patterns including palisading, diffuse sclerosing areas and storiform or hemangiopericytic patterns and can thus mimic other mesenchymal neoplastic and non-neoplastic proliferations [1, 4, 7, 8]. Mixed fibrotic, hyalinized and myxoid changes might be found in stroma. The diagnosis of SFT has been refined by the availability and the immunohistochemical markers such as CD34, vimentin, bcl-2, and CD99. Nuclear beta-catenin may occur in approximately one third of SFT.

The differential diagnosis of pancreatic SFTs includes several spindle cell neoplasms such as GIST, leiomyosarcoma, schwannoma, and fibromyxoid sarcoma [10, 11, 14]. Immunohistochemically SFT expresses CD34 and vimentin in 80–90% of cases and CD99 and bcl-2 in 70% of cases. They are usually negative for c-kit (CD117), smooth muscle actin, desmin, S-100 protein and cytokeratins which are markers of GIST, leiomyosarcoma, schwannoma, and fibromyxoid sarcoma, respectively [4, 9]. Diffusely positive staining for CD34, vimentin and CD99, focally positive for bcl-2, nuclear beta-catenin and actin and negative for CD117, EMA, Caldesmon, Desmin, S100 and Cytokeratins has been obtained in our case.

There is limited data regarding the biological behavior of SFTs with extra-pleural localization, because they are rare tumors. Approximately 10–15% of the extra-pleural SFTs are malignant. A study in literature showed local recurrence after 168 months; however, most of the metastasis or a local recurrence was seen within 2 years after treatment. Lung, liver, bone, mesentery, omentum, mediastinum and retroperitoneum were distant metastasis areas in this study [14]. The criteria for malignancy include large tumor size (> 50mm), disseminated disease at presentation, infiltrative margins, and histologic features consistent with high cellularity, nuclear pleomorphism, areas of tumor necrosis, and an increased mitotic index (> 4 mitoses/10 high powered fields) [15]. Malignant SFTs have reduced CD34 immunoreactivity. Relapse was seen in 80% of these cases. However, in our case no pleomorphism and necrosis was found and mitotic figures were very rare, less than 1–2 mitosis per 10 per high-powered fields. Ki-67 proliferation index has been observed below 1% and tumor was diagnosed as a benign.

In the cases of intra-abdominal SFTs, complete tumor resection is the treatment of choice [1, 3, 4, 6, and 7].

In conclusion, we report a rare case of pancreatic SFT. Cystic SFT of the pancreas is difficult to radiologically distinguish from other cystic pancreatic tumors. Microscopic and immunohistochemical studies are necessary for accurate diagnosis. Because there is limited data regarding biological behavior of SFT with extra-pleural localization the authors recommend clinical follow-up for SFT treatment if the criteria of malignancy are not met.

Table 1

Comparative data of patients with solitary fibrous tumors of the pancreas

Case	Author	Age	Gender	Clinical presentation	Tumor size (cm)	Location in the pancreas	Immunostaining (+)
1	Lüttges 1999	50	Female	Absent/incidental finding	5.5	Body	CD34, CD99, bcl-2, vimentin
2	Chatti 2006	41	Male	Abdominal pain	13	Body	CD34, CD99, bcl-2, vim
3	Gardini 2007	62	Female	Abdominal pain	3	Head	CD34, CD99, bcl-2, vim
4	Miyamoto 2007	41	Female	Right upper quadrant abdominal pain	2	Head/body junction	CD34, bcl-2
5	Kwon 2008	54	Male	Absent/incidental finding	4.56	Body	CD34, CD99, vim
6	Srinivasan 2008	78	Female	Back pain, weight loss	5	Body	CD34, CD99, bcl-2, vim
7	Amiot 2008	51	Female	Epigastric pain	6	Tail	Anti-beta-catenin
8	Chetty 2009	67	Female	Absent/incidental finding	2.6	Head	CD34, CD99, bcl-2
9	Ishiwatarii 2009	58	Female	Absent/incidental finding	3	Head	CD34, bcl-2
10	Sugawara 2010	55	Female	Absent/incidental finding	7	Body	CD34
11	Santos 2012	40	Female	Absent/incidental finding	3	Body	CD34, β-catenin
12	Tasdemir 2012	24	Female	Abdominal pain	18.5	Body	CD34, vim
13	Chen 2013	49	Female	Mild pain in the upper abdomen	13	Head	CD34, bcl-2, vim, muscle-specific actin (MSA), CD68, Ki67
14	Hwang 2014	53	Female	Absent/incidental finding	5.2 and 1.8	Head	CD34, bcl-2, muscle-specific actin MSA),CD10, ER, PR
15	Hee Han 2015	77	Female	Jaundice without other symptoms	1.5	Head	CD34, CD99
16	Baxter 2015	58	Female	Left lower quadrant abdominal pain	3.5	Head	CD34, bcl-2,
17	Current case	47	Male	Jaundice, abdominal pain	3.5	Head	CD34, CD99, bcl-2, vim, β-catenin, actin

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Резиме

СОЛИТАРЕН ФИБРОЗЕН ТУМОР НА ПАНКРЕАС: ПРИКАЗ НА СЛУЧАЈ И ПРЕГЛЕД НА ЛИТЕРАТУРА

Лилјана Спасевска[1], Весна Јаневска[1], Владо Јаневски[2], Билјана Новеска[3], Јулија Живадиновиќ[4]

[1] Институт за патологија, Медицински факултет, Скопје, Р. Македонија
[2] Универзитетска клиника за абдоминална хирургија, Скопје, Р. Македонија
[3] Општа болница, 8 Септември, Скопје, Р. Македонија
[4] Институт за анатомија, Медицински факултет, Скопје, Р. Македонија

Солитарните фиброзни тумори (СФТ) исклучително ретко се јавуваат во панкреасот. Тие обично се развиваат асимптоматски и тоа подолг временски период пред да може да се постави дијагноза врз основа на симптомите и/или механички предизвиканите компликации. Поради реткоста и неспецифичната клиничка слика, овој ентитет претставува вистински дијагностички предизвик.

Прикажуваме случај на 47-годишен маж со историја на прогресивна епигастрична болка во тек на две недели и појава на жолтица, кој беше хоспитализиран за понатамошно испитување. Врз основа на радиолошките наоди, поставена е дијагноза на цистаденокарцином, по што е направена панкреатодуоденектомија. Отстранетиот дел од панкреасот содржеше добро ограничена, неинкапсулирана, бела туморска маса со димензии 3.5 × 2 × 1.8 см. На пресек творбата беше мазна. Видливи беа цистични компоненти и дилатација на каналите во туморското ткиво и во ткиво на панкреасот во непосредната близина. Врз основа на хистолошката и имунохистохемиската анализа беше поставена дијагноза на солитарен фиброзен тумор. Една недела по операцијата, пациентот почина поради хируршки компликации.

За поставување точна дијагноза на цистичен СФТ на панкреас, неопходни се микроскопски и имунохистохемиски испитувања. Податоците за биолошкото однесување на СФТ со екстраплеврална локализација се ограничени, па затоа, и кога не се исполнети критериумите за малигнитет, авторите препорачуваат клиничко следење при третманот на СФТ.

Клучни зборови: панкреас, солитарен фиброзен тумор

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CASE REPORT

SUCCESSFUL KIDNEY TRANSPLANTATION IN A PATIENT WITH MULTIPLE PERIOPERATIVE RENAL TRANSPLANT COMPLICATIONS. CASE REPORT

Margarita Peneva[1], Elizabeta Zjogovska[1], Sasho Dohchev[2], Sotir Stavridis[2], Aleksandar Josifov[3], Marija Jovanovski Srceva[4], Anton Damevski[4], Josif Janchulev[2], Jelka Masin Spasovska[5], Goce Spasovski[5]

[1] University Clinic for Plastic and Reconstructive Surgery, Skopje, R. Macedonia
[2] University Clinic of Urology, Skopje, R. Macedonia
[3] University Clinic for Toracovascular Surgery, Skopje, R. Macedonia
[4]Intensive Care Unit, Skopje, R. Macedonia
[5]Department of Nephrology, Medical Faculty, University of Skopje, R. Macedonia

Corresponding Author: Margarita Peneva**,** University Clinic for Plastic and Reconstructive Surgery, Skopje, R. Macedonia; e-mail: mapeneva@yahoo.com

Abstract
Kidney transplantations have become common surgical procedures that are associated with high success rates. Nevertheless, the detection, accurate diagnosis and timely management of the perioperative surgical complications sometimes require multidisciplinary team approach for some of the complications may result in significant morbidity, risk of graft loss and/or mortality of the recipient.
A case of a 24-year old male patient that developed a number of different surgical complications is reported. The complications included venous graft thrombosis, urinary fistula, wound infection, wound dehiscence and a completely exteriorized transplanted kidney. Despite the various complications and, accordingly, a couple of revisions, finally the patient was discharged with a regular kidney function.

Key words: kidney transplantation, surgical complications, wound dehiscence, plastic surgery

Introduction

A successful kidney transplant offers enhanced quality and duration of life and is more effective regarding the medical outcome, patient satisfaction and cost-effectiveness than long-term dialysis treatment in patients with end stage chronic kidney disease (CKD) [1]. The first kidney transplantation in the Republic of Macedonia was performed in 1977 and since then more than 360 adult and around 10 pediatric kidney transplantations have been performed [2, 3]. In the last years there were major political, legislative, medical and educational initiatives adopted to improve the kidney transplant program as a routine procedure in our country [4].

Case report

The aim of this paper is to report a case of a 24-year old male patient with end stage renal disease due to a minimal change glomerulonephritis. He has been treated many times by pulses of methylprednisolone and maintenance corticosteroid therapy and was presented with constitution small for date (BMI of 18.3).

All pre-transplant investigations have been performed and after a couple of months on dialysis he had been transplanted a kidney from his father as a living donor. Although there was a slightly mismatched proportion between the donated kidney and the recipient's constitution, the graft was transplanted on the left-side performing end to end internal iliac artery anastomosis and end to side external iliac vein ana-

stomosis in the presence of short vein length. The uretero-vesical anastomosis was created according to the standard Lich-Gregoire procedure.

During the postoperative course the patient developed several complications. On the first day the postoperative diuresis was around 2000 ml. The ultrasound investigation showed daily worsening of the graft edema with a Doppler ultrasonography signal suggesting insufficient renal vein outflow. The renal CT angiography confirmed partial external iliac vein thrombosis. The thrombosis was due to the use of the left femoral vein for the dialysis catheter before transplantation. Venous thrombectomy was performed and an intensive course of anticoagulant therapy was administered. After a couple of days with an improved graft function a gradual increase in the proteinuria from 1.3 to 13.3 grams per day and diuresis reduction was noted. The ultrasonography examination showed again an edematous kidney with satisfying arterial perfusion. The transplant biopsy was contraindicated because of the enormous bleeding potential and this condition was considered as an acute rejection and/or recurrence of the baseline disease. A 3-day course of methylprednisolone 10 mg per body weight was administered and 3 plasmapheresis procedures were performed. Despite the slow improvement in the graft function and reduction of the proteinuria, the parenchymal edema (23–26 mm) persisted. At the 13th postoperative day a bleeding was noted around the transplanted kidney that required surgical revision. A small graft rupture near the renal hilum was detected. It was due to the external iliac vein stenosis that created a new thrombosis. Another venous thrombectomy associated with venoplasty was performed and the graft was enveloped in a vicryl mesh bag. In the following days the renal function completely recovered in the presence of insignificant proteinuria < 0.5 g/day.

One week later the patient had decreased diuresis again with a progressive and permanent graft pain. This time the CT scan confirmed ureteral necrosis with urinary fistula. The patient was operated and an anastomosis of the ureter of the graft with the contralateral native ureter of the recipient was performed.

Noteworthy, from the very beginning there was a mismatch between the proportions of the transplanted kidney and the size of the recipeent, thus making the soft tissue cover over the transplanted kidney insufficient. Namely, after the transplantation the skin was sutured under tension and was the only soft tissue coverage over the transplanted kidney. The skin was additionally damaged with every subsequent operation. Under such circumstances secondary soft tissue infection was highly expected. The microbiological examinations of the surgical wound at different time points revealed presence of various microorganisms including even Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* which were successfully treated according to the antibiogram. The soft tissue infection additionally damaged the soft tissue cover [5,6]. Hence, after the last surgery the transplanted kidney was functioning well but it was completely exposed with a soft tissue defect over it sizing about 20 × 10 cm. Then, plastic surgeons were included as part of the surgical team.

Because of the several operations, the local anatomical structures were severely displaced and dense adhesions were present, thus making the dissection very difficult and risky. In addition, the patient was having serious comorbiddities (malnutrition, hypertension, immunocompromised condition) and had a long history of CKD with a couple of months on dialysis. More over at that time point he was getting depressed because of the long time spent in hospital along with the plenty of procedures he underwent. That was the rationale why it was decided to cover the soft tissue defect in the simplest way.

After the necrotic tissue was debrided the overall soft tissue defect became larger (23 × 12 cm). A very long random skin flap from the upper third of the anterior abdomen was used in order to cover the defect (Figure 1). The flap was prepared as a transpositional one with the length to width ratio being 4,5 : 1. Starting at the first postoperative day there were signs of vascular insufficiency of the distal third of the flap that in the next days progressed to a complete necrosis (Figure 2). This problem was due to both the long flap and the infected recipient bed. At the 5th postoperative day a necrectomy was performed when the distal third of the flap was removed. Then the tensor fasciae latae (TFL) fasciomyocutaneous flap was used as a pedicled flap in order to cover the reminder of the defect. As this flap is considered a thicker and a more vascularized one it was a way to deal with the soft tissue infection [7–9]. Namely, myofasciocutaneous flaps are golden standard for management of complicated infected wound bed.

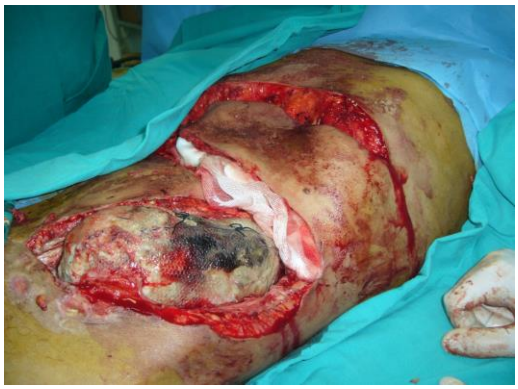

Figure 1 – Intraoperative view- completely exposed transplanted kidney after the necrotic tissue being debrided and the elevation of the abdominal random skin flap

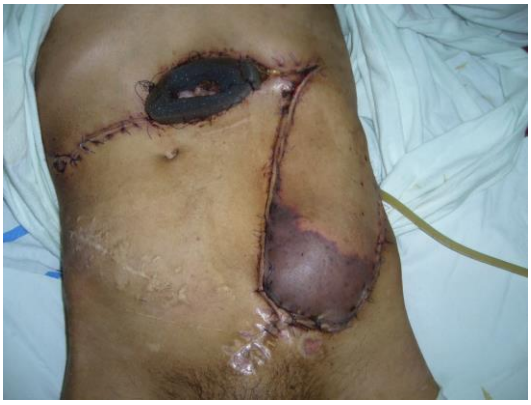

Figure 2 – Soft tissue necrosis of the distal part of the flap

Six weeks after the operation the flap was divided with a satisfying intraoperative result (Figure 3). Yet, two days later signs of vascular insufficiency on the distal part of the flap were noticed. Fortunately, this episode resolved only with partial dehiscence of the flap that healed spontaneously upon repeated dressings.

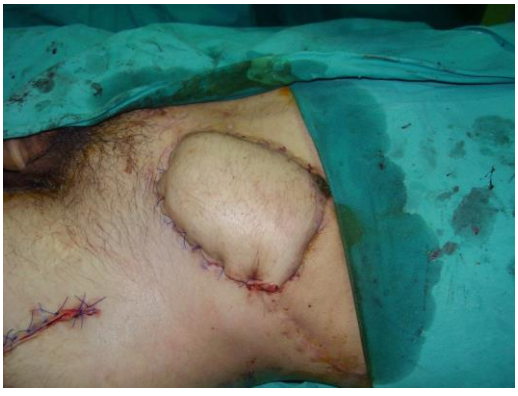

Figure 3 – Intraoperative view after the division of the TFL flap

The defect was successfully closed combining 2 different flaps at the same time avoiding the microsurgical anastomosis. As a result the graft was saved and the anterior abdominal wall restored in the manner of function and esthetics. Even now, three years after the transplantation the results remained (Figure 4).

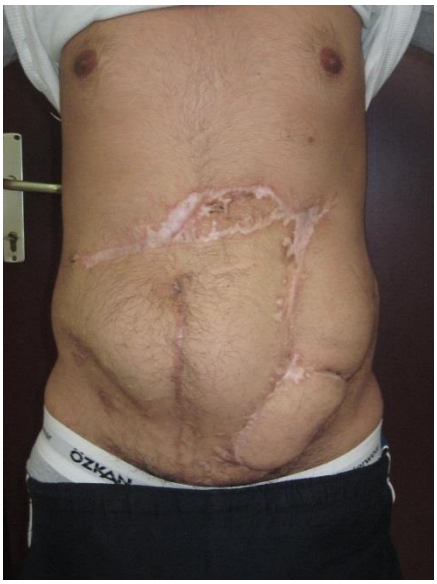

Figure 4 – Three years postoperative view of the flaps setting well over the defect

Discussion

Kidney transplantations have become common surgical procedures that are associated with high success rates. The complications associated with the procedure are low especially compared with other solid organ transplants. Nevertheless the detection, accurate diagnosis and timely management of surgical complications after kidney transplant are important tasks of the team managing these patients as some of the complications can result in significant morbidity of the recipient, risk of graft loss and mortality [10].

A case that developed a number of different surgical complications including venous thrombosis, wound infection, wound dehiscence and urinary fistula was presented.

Vascular complications despite being rare have devastating consequences particularly arterial and venous thrombosis [10]. Renal vein thrombosis (RVT) is usually seen during the first post transplantation week in 0.3–6.1% of patients. The prognosis of RVT is poor, and hence, its early recognition and treatment is crucial [11].

Approximately two thirds of the early urologic complications (urine leak or obstruction) occur in the first month after transplantation and are usually successfully treated by

the transplantation team. However, the urological complication rates are 4-8% with a very low patient mortality [12].

Surgical site infections have remained major complications in solid organ transplanttation [5, 6]. Even worse, in this case there was a surgical site infection along with a completely exteriorized allograft. The allograft was prone to mechanical injury and to infection both of which could have almost inevitably lead to its loss. Covering the wound with well vascularized tissue can facilitate wound healing, resist the soft tissue infection and preserve the graft in general [8]. The reconstructive options for extensive lower trunk defects include both free flaps and pedicled flaps each having its own advantages and disadvantages [7]. Using a free flap would have been very difficult because of the very bad local conditions in the presence of soft tissue infection and other patients' comorbidities.

In conclusion, kidney transplant may be associated with various nephrological and surgical complications whose treatment require a multidisciplinary team work. Although at one time point the kidney was supposed to be unsalvageable, the great effort and persistent care invested from all the multidisciplinary team physicians, nurses and with the support from the psychologist and patient's family all complications were successfully over-passed and after a 4 month hospitalization the patient was discharged with a good graft function.

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Резиме

УСПЕШНА ТРАНСПЛАНТАЦИЈА НА БУБРЕГ КАЈ ПАЦИЕНТ СО МУЛТИПЛИ ПЕРИОПЕРАТИВНИ КОМПЛИКАЦИИ. ПРИКАЗ НА СЛУЧАЈ

Маргарита Пенева[1], Елизабета Жоговска[1], Сашо Дохчев[2], Сотир Ставридис[2], Александар Јосифов[3], Марија Јовановски Срцева[4], Антон Дамевски[4], Јосиф Јанчулев[2], Јелка Масин-Спасовска[5], Гоце Спасовски[5]

[1] Универзитетска клиника за пластична и реконструктивна хирургија, Скопје, Р. Македонија
[2] Универзитетска клиника за урологија, Скопје, Р. Македонија
[3] Универзитетска клиника за торакална хирургија, Скопје, Р. Македонија
[4] Универзитетска клиника за анестезија, реанимација и интензивно лекување, Скопје, Р. Македонија
[5] Универзитетска клиника за нефрологија, Медицински факултет, Скопје, Р. Македонија

Бубрежните трансплантации претставуваат стандардни оперативни зафати кои имаат висок процент на успешност. Сепак, навремено откри-

вање, поставување точна дијагноза, како и навремен третман на периоперативните хируршки компликации бараат мултидисциплинарен пристап бидејќи некои од компликациите може да резултираат со сигнификантен морбидитет, ризик од губење на графтот и/или смрт на реципиентот.

Прикажуваме случај на 24-годишен пациент кај кого се јавија низа различни хируршки компликации. Компликациите вклучуваа венска тромбоза на графтот, уринарна фистула, инфекција на раната, дехисценција на раната и целосно екстериоризиран трансплантиран бубрег. И покрај бројните компликации, по неколкуте ревизии, пациентот беше отпуштен од болница со уредна бубрежна функција и реставриран мекоткивен дефект.

Клучни зборови: бубрежна трансплантација, хируршки компликации, дехисценција на рана, пластична хирургија

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CASE REPORT

TO ACCEPT OR REFUSE PATIENT'S GIFT IN MONEY? AND HOW? – CASE REPORT WITH REVIEW

Vesna Kos

Department of Family Medicine, University of Zagreb, Zagreb, Croatia

Corresponding Author: Vesna Kos, MD, MSc. Department of Family Medicine, University of Zagreb, 10 000 Zagreb, Rockfellerova 4, Croatia; e-mail: vesnakos.dr@zg.t-com.hr

Abstract

This report describes a (rare) situation when a patient's first gift to a young doctor was in money. This happened in very specific circumstances – in a refugee camp during the War in Croatia. The data are taken from a large study on gifts, conducted on a representative sample of Croatian general practitioners (GPs), N = 265, from 2358 in total.

Pro and contra factors are discussed, considering tradition and customs, but also a lack of knowledge of young doctors in handling gifts in general. The intention of this report is primarily educative, with review of (scarce) literature, and recommendations, where the generally accepted rules might have exceptions.

Keywords: Gift Giving, Physician-Patient Relation, Financial Gift, Human Dignity, Cultural Background

Introduction

Although doctors "are among the most gifted people on planet Earth" [1], little is known about appropriate conduct in these situations, because literature about it is scanty [2, 3], and because most medical schools do not have this covered in their education programs. Consequently, young doctors are mostly unprepared for something they will be facing almost daily.

There are some general recommendations for receiving patients' gifts, and these are as follows: small gifts (as token of appreciation) are not ethically problematic, and are certainly more acceptable than big and expensive ones, gifts in nature are more acceptable then those in money, gifts after intervention better then those given beforehand [2–8]. And as the most important guideline: the intention (and not the value!) of the gift is valued; meaning: only gifts without open or hidden intention for some beneficence are acceptable [2, 4, 5, 8, 9, 10].

Furthermore, there are only sporadic articles in the official medical literature about receiving gifts [2, 3, 8, 11, 12], and these are mostly based on single or hypothetical examples [1, 5, 7, 9, 13], without a serious and consistent research on gifts. There are only few studies on patients' gifts, and these studies are small and made only on hospital doctors [3, 8, 11, 12].

Aiming to get a real (and not only hypothetical) insight into what happens during the process of receiving gifts, a study was conducted on a representative sample of Croatian GPs. A part of the study deals with the very first present in a doctor's career, with the descriptions of the doctor's and patient's reaction – which both influenced the doctor-patient relationship, as a very important element in curing [2–5, 7, 8, 10–12].

The refusal of a gift in an improper manage might hurt patient (or his dignity) [5, 7, 10, 11, 14, 16, 17], which is often overlooked in healthcare. Thus, the damage for the patient can by far exceed the value of the gift (because of long-term negative curing effects, based on the disturbed doctor-patient relation) [2, 4, 5, 7, 10].

The aim is to show that it might be some specific situations in reality, regarding gifts,

where the lack of knowledge is an obstacle, since an individual approach should be applied, regardless sometimes "obvious" and generally accepted recommendations [2, 3, 7, 10, 14, 16, 17].

Methods and examinees

The survey, aimed to explore GPs' experiences concerning gifts, was conducted in 2006 on Croatian GPs and approved by the Ethical Committee of the Medical School of the University in Zagreb, approval number 380-59/11-500-77/178.

The target population were all active GPs in Croatia, N = 2358. The sample was collected randomly as proportionally stratified, with the next stratum criteria: region (21 counties), gender, the number of patient in care (all visible from the List of Family Medicine Teams). The first participants were chosen as purposive sample and they formed the sample for pilot study. The GPs with less then two years of practice were excluded before giving them the oportunity to participate. Final N = 265, response rate 95.7%.

The survey was originally designed in a form of a large questionnaire, to explore GPs' experiences concerning gifts, both those given to and those received from patients.

The first page aimed to describe the experience of the first patients' gift.

The questions on that page are as follows:

– (describe) What was your first gift (including time of study and internship)?

– At that time you were: student or intern or physician? If student, then write the year of study, if intern or physician write how many months.

– (describe) How did you react? Meaning: How did you feel?

– Did you say something and what was it? (write)

– How the patient did react? (describe)

The survey was conducted as an open-ended, led questionnaire, under supervision and with the presence of the same researcher. Questions were allowed during the survey, but the respondents were not permitted to agree among themselves about their responses, and no discussion with the researcher was allowed.

The oral and written instructions were clearly defined beforehand, and given immediately before the questionnaire was filled in. The survey respondents were pre-announced, but not the contents, only the theme.

Results

The data collected from the whole study show that Croatian physicians receive their first gift very early: 5.7% in their student days, 41.5% during internship, and 50.2% as young doctors, with a peak in the 1st month of being a doctor.

Table 1

The structure of the first gifts

SORTING GIFTS BY TYPE

Type of gift	Number	%
coffee and/or sweets (standard gifts)	161	60.8%
food (mostly in rural practices)	24	9.1%
flowers	19	7.2%
drinks	7	2.6%
handmade products (embroidery)	5	1.9%
cosmetics	5	1.9%
money	3	1.1%
others (books, cigarettes, souvenirs)	13	4.9%
do not remember or left blank	13	4.9%
combined gifts	15	5.7%
TOTAL	265	100%
Combinations include all types of gifts. Coffee and/or sweets are present in 14 combined gifts = 5.3%, money in two (five cases in total).		

Comment:

Most of the first gifts are symbolic and common (coffee and/or sweets in 66.1%).

Only in three cases the first gift was in money, or partially in money (1.9% in total).

Description of the case wrote by the participant

"I was an intern with about three months of practice. You wouldn't believe, but my first present was MONEY! It happened in a war-refugee camp. I was shocked and refused the gift. The patient was offended." (Author's translation)

Discussion

What can we see behind this short description by a doctor about refusing a monetary gift from a patient in a refugee camp?

At first glance the young physician was completely right: the patient was clearly poor. He might be rich "once upon a time", but not in the described situation: he was homeless, without any source of income, unemployed and without possibility to earn anything, without possibility to rear or cultivate something appropriate as a gift to the doctor from his own farm or garden. His only material possession was money, probably taken in a hurry while running away from his home. So, who would ever take anything from a man in such a bad life-situation? The mere idea sounds horrible. According to the official recommendations, it was almost normal to refuse a gift: A) in money, [2–7, 11, 12], B) of a relatively big value regarding the circumstances [2–8, 11, 12, 18], C) from a poor patient [2, 4, 5, 7, 14], i.e. from whom taking away the only value he had (i.e. money) would certainly diminish his already low property.

Yet, the next question is: why did this obviously poor patient offer a gift to the doctor? Why he tried to do it at all? The answer to this question is not so simple, and the right answer casts a very different light on the situation.

Refugees in the camp were mostly people from remote villages or provincial towns; they did not run away from metropolis like Zagreb. Customs are not the same in big cities as in villages, and local tradition might be very different [4, 5, 7, 8, 14–17], thus should be considered [4, 5, 7, 14, 15, 17]. In very remote places, people might have become accustomed over the centuries to the fact that there is no government or king to take care of them. Therefore, they appreciate help which is "here-and-now", not connecting it with "some far-off thing such as government", but only directly with the person who gives it. The doctor is the very one who helped, not the system, not state, not government [14].

=> "Me, as a patient, as a man given the help, I have to thank directly to doctor."

Furthermore, in the patient's mind, the healing is traditionally a kind of a gift. "Therefore, as a decent man I have to give another gift in return – because it is polite, and it is wrong not to say thanks, to oversee such a gift as healing, and not to give something in return. I don't want to become an indecent, dishonourable and dishonest man, this is about my dignity [14, 15, 17]."

On the other hand, the young doctor studied and lived in a big city for many years, knowing the customs of a big city. Even if she might come from a provincial area, she forgot the village tradition and customs over the years. Most importantly, she had heard something about "inappropriate gifts in money" [2–4, 6, 11], but nobody in her medical school ever taught her the meaning of patients' gift and the behaviour in these situations. The sad fact is that gifts to doctors are much more discussed in daily papers than they are in competent and professional venues – by skilled teachers in medical schools. So, the young doctor could not see that this gift had no intention to any benefit, what is the key in distinction what gift is to be accepted vs. refused [2, 4, 5, 8, 9, 10].

What should this young doctor do?

The first rule is that the doctor must not hurt the patient. This is one of the basic principles regarding gifts from patients [2, 5, 7, 10, 13, 17]. Yet, she did hurt him, she humiliated him and insulted his dignity (she wrote "he was offended").

Furthermore, the way the young doctor refused the gift disturbed the doctor-patient relationship. This relation is very important in curing process [2, 4, 5, 7, 8, 10–13, 19], since disturbed physician-patient relation influence negatively on curing outcome. It is known that even just once disturbed relationship might

diminish the curing effect forever [2, 5, 7], especially in family medicine [5, 7].

Which would have been the right choices for this young doctor?

1. She could have taken this "inopportune" gift, keeping in mind its value, and after some time give to this patient "occasionally" something of the similar value in return, like a gesture of favour, something that he really needed – and she "just happened to have".

2. She could have refused the gift, but in a very polite and careful manner [2– 5, 7, 10].

Just saying "no, thanks, I wouldn't take this" was not polite in this situation. This kind of refusal couldn't be polite to a man who had nothing to offer, nothing but his human dignity embodied in some money. Actually, she was embarrassed by the fact that a completely impoverished man offered her money. In such a situation the young doctor could not recognise the "love embodied in gift", as Stein described patients' gifts [18], as signs of gratitude and appreciation [1, 2, 5, 7, 10]. Overwhelmed by her own discomfort, as it often might happen [3, 5–7, 8, 11, 12], she couldn't react appropriately and put the patient's best interest in the first place, as it is recommended [2, 4–6, 10, 13], and as sometimes suggested to be done against the own discomfort [7,10].

She had to respect the only thing he had, his human dignity, and say for example:

"Oh, how nice and polite from you! I appreciate that, I see you are a good man. I promise you, we will have a coffee or lunch together the first day you leave this camp. We will celebrate this, and I'll be your guest. So, keep this money till this day, it will be so soon."

The patient would not see this as refusal, but rather as acceptance in some other and delayed way.

By making one of these choices she would not have offended the patient or hurt his dignity. Moreover, she would not break professional and her own rules, and she would not disturb the already well-established doctor-patient relationship [2, 4, 7, 10].

But, is it expected the doctor with three months of practice, and without any training on how to accept or politely refuse patients' gifts, to conduct this in such a fine way? Many authors agree this is not to be expected without proper education [2, 5–7, 15].

Conclusion

1. There might be exceptions in avoiding monetary gifts, contrary to the general recommendation about accepting gifts in nature rather than those in money, because "there is nevertheless general consensus on what constitutes acceptable versus nonacceptable behavior" [2].

2. The general rule about not hurting the patient should be at the first place when receiving patients' gifts.

3. The local tradition and customs should be taken in consideration in these situations.

4. A doctor should recognise what is very important for a particular patient; for example: the high need of human dignity – even in certain extreme circumstances.

It seems that medical students need an adequate education about patients' gifts [1, 2, 5–7, 15], to develop appropriate and professsional conduct in the future.

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Резиме

ДА СЕ ЗЕМЕ ИЛИ ДА СЕ ОДБИЕ ПАРИЧЕН ПОДАРОК ОД ПАЦИЕНТ? И КАКО? – ПРИКАЗ НА СЛУЧАЈ СО ПРЕГЛЕД

Весна Кос

Оддел за семејна медицина,
Универзитет во Загреб, Загреб, Хрватска

Се опишува (ретка) ситуација кога прв подарок во кариерата на млад лекар биле пари. Тоа се случило во многу специфична ситуација: во кампот за бегалци за време на Татковинската војна во Хрватска. Податоците се одбрани од голема студија за подароци, спроведена на општите лекари во Хрватска, N = 265, од вкупно 2.358.

Се дискутираат причините за и против, земајќи ги предвид традицијата и обичаите, но, исто така, и неупатеноста на младите лекари во правилното професионално однесување при примањето подароци, генерално. Целта на овој извештај е првенствено едукативна, со преглед на (скудна) литература и препораки, при што може да има исклучоци од општите правила.

Клучни зборови: давање подароци, однос лекар – пациент, паричен подарок, човечко достоинство, културно потекло

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LETTER TO THE EDITOR

WHAT SHOULD BE THE APPROPRIATE REFERRAL TO THE NEPHROLOGISTS – DO WE HAVE THE DATA?

Goce Spasovski

University Department of Nephrology, Medical Faculty, University of Skopje, R. Macedonia

Corresponding Author: Prof. Goce Spasovski University Department of Nephrology, Medical Faculty, University of Skopje, R. Macedonia, Vodnjanska 17, 1000 Skopje; тel.: +389 (0)2 070 26 82 32; fax: +389 (0)2 3 17 81 02; e-mail: spasovski.goce@gmail.com

Dear Sir,

The overall prevalence of chronic kidney disease (CKD) with estimated glomerular filtration rate (eGFR) < 60 mL/min per 1.73 m^2 (stage 3) has substantially increased over the last decades and is further growing. On the other hand, it's still a debatable issue whether such population should be referred to the nephrologists given the professional burden of care versus the potential of the preventable complications in advancing CKD stages.

Despite the numerous recommendations proposed none of the early referral practice patterns has been universally adopted. At present, only the eGFR < 30 mL/min per 1.73 m^2 (GFR stage 4) has been accepted with a need for nephrology care. Such patients are prone to a progressive CKD deterioration towards end stage renal disease (ESRD) and associated with higher mortality before or after the commencement of the renal replacement therapy (RRT). Again, the question remains, whether such consequences may be prevented or RRT postponed if nephrology care should have been instituted earlier at CKD stage 3, i.e. about the referral in patients with higher eGFR. According to the current KDIGO guidelines [1], general nephrology care should be provided for CKD patients with urine albumin-to-creatinine ratio (ACR) ≥ 300 mg/g (34 mg/mmol), hematuria, rapid eGFR decline, anemia requiring erythropoietin therapy, abnormalities of bone and mineral metabolism, increased potassium, young patients < 18 years, resistant hypertension, nephrolithiasis and hereditary diseases. In diagnosed patients, nephrologists should recommend specific and conventional therapies to slow progression of CKD and prevent adverse outcomes (kidney failure – ESRD, CVD mortality – death). However, it is still debatable whether early nephrology referral for CKD complications such as anemia, hyperphosphatemia, hypertension and CVD, and the associated outcomes of ESRD or mortality would be beneficial for patients in stage 3b–4 given the increased burden of care for nephrologists.

The results from a recent, retrospective, observational study confirmed gradually increased ESRD and mortality risk in a referred cohort CKD stage 3–5 with available follow up (FU) data from the nephrology hospital records. In total, for the median FU of 3 years 14% of patients reached ESRD and 16% died, with most similar incidence of ESRD and death prior to ESRD being in CKD stage 4, around 8% [2].

The baseline data analysis from another observational, prospective multicenter study of patients referred to nephrology with stages 3 and 4 CKD confirmed gradual increase in the morbidity from all CVD with the declining GFR (stage 3a < stage 3b < stage 4). The same pattern was observed for all other complications despite the fact that care has been delivered mainly by nephrologists [3].

The data obtained in a large cohort of prospectively followed CKD patients stage 1–5 exclusively by general practitioners showed

ESRD and the mortality risk was higher only in stages 3b to 5 compared to stage 1–2, but not the CKD stage 3a. However, during the median 7.2 years FU mortality (22.9%) prevailed in comparison with the development of ESRD (1%) in all CKD stages. Anemia and albuminuria as modifiable risk factors significantly predicted either outcome, and the hypertension predicted only the mortality [4].

In addition to the increasing prevalence of CVD events with advanced CKD stages, ESRD and mortality data from the above mentioned studies are in favor of early nephrology referral in order to increase the number of patients reaching ESRD (1 vs. 14%), and reduction of the possibly preventable deaths (16 vs 22%), respectively. Thus, management is to be guided by the CKD stage, but also according to the underlying etiology, the risk of progression and complications of CKD, and the presence of albuminuria with an appropriate treatment, intensity of monitoring and patient's education.

An early referral of CKD patients to nephrology is still underutilized option and mainly in CKD stages 4 + 5, although even in a referred cohort of CKD patients there is an increased risk of dying prior to ESRD development over the stages 3–4.

In conclusion, nephrology referral should be considered for CKD patients at stage 3b because of the significant increase in ESRD and mortality risks observed in majority of the reported studies. On the other hand, the workload for nephrologists seems justifiable because the majority of CKD 3a patients are referred to the primary care physicians. Nevertheless, they should be aware of the specific CKD risk factors (hypertension, anemia, albuminuria, CKD-MBD) associated with greater risk of ESRD and deaths in the advanced CKD stages.

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ERRATUM

XXXVI/2

VISUALISATION OF MICROGLIA WITH THE USE OF IMMUNOHISTOCHEMICAL DOUBLE STAINING METHOD FOR CD-68 AND Iba-1 OF CEREBRAL TISSUE SAMPLES IN CASES OF BRAIN CONTUSIONS

Aleksandar Stankov, Viktorija Belakaposka-Srpanova, Natasa Bitoljanu, Ljupco Cakar, Zdravko Cakar, Gorazd Rosoklija

Additional contact information about the following author:

Gorazd B. Rosoklija, MD, PhD, Associate Professor of Psychiatry,
Department of Psychiatry
College of Physicians & Surgeons, Columbia University, 1051 Riverside Drive
New York, NY 10032, Unit#42

Tel. 646-774-7541 Lab.
Tel. 646-774-7601 Office
Fax. 646-774-7589 Office

УПАТСТВО ЗА АВТОРИТЕ

Списанието „Прилози“ на Одделението за медицински науки на Македонската академија на науките и уметностите излегува трипати годишно и е цитирано во Index Medicus и во Medline и е достапно на www.manu.edu.mk/prilozi. Во него се објавуваат едиторијали, изворни научни трудови, научни соопштенија и прегледни статии (клинички, лабораториски и епидемиолошки искуства, прикази на случаи, куси соопштенија, писма до уредникот, историски записи и др.) од областа на медицинските науки. Трудовите не треба да ги содржат резултатите што авторите веќе ги објавиле во други публикации или списанија.

Трудовите предложени за објавување во „Прилози“ ги рецензираат двајца стручњаци од соодветната научна област, кои за авторите остануваат анонимни.

Трудовите се објавуваат на англиски јазик со резиме на англиски и на македонски јазик.

Трудот треба да биде отчукан со новинарски проред (28‡30 реда), на бела хартија од формат А4, со маргини најмалку 3 cm или на компјутер ‡ програма Word for Windows, со приложена дискета или ЦД.

Обемот на оригинален научен труд, вклучувајќи ги и прилозите (илустрации, графикони, табели) не смее да биде поголем од еден авторски табак (30.000 знаци, односно 16 страници од 28 реда). Обемот на кратките соопштенија не треба да биде поголем од седум страници.

На трудот, покрај името и презимето на авторот/авторите, треба да биде наведена установата или организацијата во која е изработен трудот.

Трудот треба да содржи апстракт од најмногу 250 збора на англиски јазик, како и апстракт на македонски јазик. Апстрактот треба да содржи краток приказ на целта на трудот, методите на работа, битните резултати (со нумерички податоци) и основни заклучоци. Заедно со апстрактот треба да бидат доставени најбитните клучни зборови. Клучните зборови за медицинските науки се наведуваат во согласност со M.E.S.H.

Трудот по правило треба да содржи: вовед, материјал и методи, резултати, дискусија и заклучок. Воведот мора да биде краток, со јасно дефинирана цел и со досегашно познавање на проблемот. Материјалот и методите треба да содржат доволно податоци од кои читателот ќе биде во состојба да ги повтори испитувањата без дополнителна информација. Резултатите треба да бидат напишани кратко и јасно, а дискусијата да ги објасни резултатите.

Мерните единици и другите технички податоци мораат да бидат усогласени со Sl-системот.

Илустрациите се приложуваат посебно. Графиконите и цртежите треба да бидат на паус или на бела хартија, контрастни, а ознаките и бројките во графиконите сразмерни на големината на цртежот, за да останат читливи по редуцирањето на големината на цртежот. По правило не треба да бидат повеќе од четири. Нивното место во текстот да биде означено. Сите илустрации треба да имаат легенди на англиски јазик.

Табелите можат да бидат приложени посебно, но нивното место во текстот да биде означено. Насловите на табелите треба да бидат напишани на англиски јазик.

За трудовите од областа на медицинските науки, во принцип, важат упатствата објавени во „Brit. Med. Journal“, Vol. 296, 1988, p. 101–105 („Ванкуверските правила“), односно N. Engl. J. Med., Vol. 324, 1991, p. 424–428.

Литературата се цитира во оригинал, и тоа по следниов редослед: презиме и почетна буква од името на авторот, наслов на трудот, назив на списанието, година на објавување на цитираниот труд, годиште и број, страници (од‡до). Доколку се цитира книга или зборник на трудови, се наведува и издавачот и местото на издавање (пред страниците). Ако се цитира труд од повеќе од три/шест автори, по третиот/шестиот се додава „и сор.“, односно *„et al.“*. Во текстот на трудот се наведува првиот автор и годината ставени во загради [ ], односно бројка во загради, доколку библиографијата е нумерирана. При прва употреба на кратенка, во заграда да се даде нејзиниот полн назив.

Кратките соопштенија кои се објавуваат во сп. „Прилози“ (до 7 чукани страници) не мора да ги содржат поглавјата вовед, материјал и методи, дискусија и заклучок, но тие мораат да бидат содржани во текстот.

Трудот треба да е придружен со писмо од авторот за контакт, со изјава дека трудот не бил објавен или поднесен/прифатен за печатење во друго списание или научна публикација, и со потврда дека трудот е разгледан и одобрен од сите коавтори, т.е. со придружна декларација за можен конфликт на интереси од авторите. Треба да се објасни придонесот на секој автор во трудот.

Ракописите се доставуваат до Уредувачкиот одбор на сп. „Прилози“ на Одделението за медицински науки на МАНУ во два примерока (оригинал и копија) и електронска верзија на трудот.

Авторите на трудовите треба да ги покријат трошоците за печатење и за интернет-објавување на своите трудови во износ од 250 ЕУР во денарска противвредност.

Авторите на трудовите треба да ги потпишат двата документа што го придружуваат трудот: Формуларот за конфликт на интереси и Формуларот за етичка одговорност.

Формуларите се достапни на: manu.edu.mk/prilozi

INSTRUCTIONS FOR AUTHORS

The journal *Prilozi, Oddelenie za medicinski nauki (Contributions, Section of Medical Sciences of the Macedonian Academy of Sciences and Arts)* is published three times a year and is cited in Index Medicus and Medline and available on www.manu.edu.mk/prilozi. The journal publishes editorials, original research works, research reports and reviews (clinical, laboratory and epidemiological experiences, case studies, short communications, letters to the editor, historical notes, etc.) from the area of medical sciences. The manuscripts should not represent research results which the authors have already published in other books or journals.

The papers submitted for publication in *Prilozi* are peer-reviewed by two experts from the respective scientific field who remain anonymous to the authors.

The papers are published in English, accompanied by a summary written in Macedonian.

The paper should be typed with double-spacing (28–30 lines), on a white paper in A4 format, with margins of 3 cm, or on a computer using Word for Windows programme enclosing the CD, or USB.

The length of the original research paper, including the annexes (illustrations, graphs, tables, etc.) should not exceed a signature or printer's sheet (30,000 signs, that is, 16 pages with 28 lines each). The length of shorter reports should not exceed seven pages.

The submitted manuscript must contain the name/s and surname/s of the author/s, the name and address of the institution and or organisation where it was prepared.

The abstract should not exceed 250 words, written in English, and a summary in Macedonian. The abstract should represent briefly the goals, methods, main results (with numerical data) and basic conclusions of the research. The most essential key words must be added to the abstract. The key words for the medical sciences are given according to M.E.S.H.

The manuscript contains an introduction, materials and methods, results, discussion and a conclusion. The introduction must be concise with a clearly defined goal and with previous knowledge of the problem. The materials and methods ought to contain sufficient data to enable the reader to repeat the investigation without seeking additional information.

The results should be presented briefly and clearly, and the discussion should explain the results.

The measuring units and other technical data should be given according to the Sl-system.

Illustrations are submitted separately. Graphs and drawings should be prepared on tracing paper or on a white sheet of paper, in contrast, while the markers and figures in the graphs must be proportional to the size of the drawing in order to remain readable after the reduction of the size of the drawing. There should not be more than four drawings and their places in the text should be clearly indicated. All illustrations must be accompanied by legends in English, and the abstract in English and in Macedonian.

Tables can be enclosed separately, but their places in the text must be indicated. The titles of the tables should be written in English.

The references used are cited in the original and as follows: surname and the first letter of the author's name, the title of the work, the title of the journal, year of publication of the work cited, publishing year and number, pages (from-to). If a book or a collection of works is cited, then the publisher and the place of publication should be given (before the pages). If the manuscript cited has more than three authors, then *et al.* is added after the third author. In the text of the paper the first author and the year are cited in brackets [ ], i.e. a number in brackets when the bibliography is numerated. When using acronym for the first time, provide the full phrase in brackets. An example: Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet Transplantation in Seven Patients with Type 1 Diabetes Mellitus Using a Glucocorticoid-Free Immunosuppressive Regimen. N Engl J Med 2000; 343(4): 230–8.

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www.manu.edu.mk
email: manu@manu.edu.mk
tel: +389 2 32 35 400 fax: +389 2 32 35 500

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www.manu.edu.mk
email: manu@manu.edu.mk
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