IJBM

International Journal of

BIOMEDICINE

Available online at
www.ijbm.org

INTERNATIONAL JOURNAL OF BIOMEDICINE

Aims and Scope: *International Journal of Biomedicine (IJBM)* publishes peer-reviewed articles on the topics of basic, applied, and translational research on biology and medicine. Original research studies, reviews, hypotheses, editorial commentary, and special reports spanning the spectrum of human and experimental and tissue research will be considered. All research studies involving animals must have been conducted following animal welfare guidelines such as the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, or equivalent documents. Studies involving human subjects or tissues must adhere to the Declaration of Helsinki and Title 45, US Code of Federal Regulations, Part 46, Protection of Human Subjects, and must have received approval of the appropriate institutional committee charged with oversight of human studies. Informed consent must be obtained.

International Journal of BioMedicine endorses and behaves in accordance with the codes of conduct and international standards established by the Committee on Publication Ethics (COPE).

International Journal of Biomedicine (ISSN 2158-0510) is published four times a year by International Medical Research and Development Corp. (IMRDC), 6308, 12 Avenue, Brooklyn, NY 11219 USA

Customer Service: International Journal of Biomedicine, 6308, 12 Avenue, Brooklyn, NY 11219 USA; Tel: 1-917-740-3053; E-mail: editor@ijbm.org

Manuscript Submission: Original works will be accepted with the understanding that they are contributed solely to the Journal, are not under review by another publication, and have not previously been published except in abstract form. Accepted manuscripts become the sole property of the Journal and may not be published elsewhere without the consent of the Journal. A form stating that the authors transfer all copyright ownership to the Journal will be sent from the Publisher when the manuscript is accepted; this form must be signed by all authors of the article. All manuscripts must be submitted through the International Journal of Biomedicine`s online submission and review website. Authors who are unable to provide an electronic version or have other circumstances that prevent online submission must contact the Editorial Office prior to submission to discuss alternate options (editor@ijbm.org).

IJBM

INTERNATIONAL JOURNAL OF BIOMEDICINE

Azienda Ulss 12 Veneziana
Ospedale Civile di Venezia - Divisione di Cardiologia

VENICE INTERVENTIONAL CARDIOLOGY 2016

FOCUS ON HEART & BRAIN

VENICE
PALAZZO FRANCHETTI
MAY 5 - 7 2016

IJBM

INTERNATIONAL JOURNAL OF BIOMEDICINE

www.ijbm.org

Volume 6 Issue 1 March 2016

CONTENTS

CONTENTS

CONTINUED

IJBM

INTERNATIONAL JOURNAL OF BIOMEDICINE

Instructions for Authors

Editorial Policies

International Journal of Biomedicine (IJBM) publishes peer-reviewed articles on the topics of basic, applied, and translational research on biology and medicine. Original research studies, reviews, hypotheses, editorial commentary, and special reports spanning the spectrum of human and experimental animal and tissue research will be considered. All research studies involving animals must have been conducted following animal welfare guidelines such as *the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals*, or equivalent documents. Studies involving human subjects or tissues must adhere to the *Declaration of Helsinki and Title 45, US Code of Federal Regulations, Part 46, Protection of Human Subjects*, and must have received approval of the appropriate institutional committee charged with oversight of human studies. Informed consent must be obtained.

Manuscript Submission

Manuscript submissions should conform to the guidelines set forth in the ‘‘Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (ICMJE Recommendations),’’ available from http://www.ICMJE.org.

Original works will be accepted with the understanding that they are contributed solely to the Journal, are not under review by another publication, and have not previously been published except in abstract form.

Accepted manuscripts become the sole property of IJBM and may not be published elsewhere without the consent of IJBM A form stating that the authors transfer all copyright ownership to IJBM will be sent from the Publisher when the manuscript is accepted; this form must be signed by all authors of the article.

All manuscripts must be submitted through the International Journal of Biomedicine`s online submission and review website. Submission items include a cover letter (required), the manuscript (required), and any figures and tables. Revised manuscripts should be accompanied by a unique file (separate from the cover letter) that provides responses to the reviewers’ comments. The preferred order for uploading files is as follows: cover letter, response to reviewers (revised manuscripts only), manuscript file(s), table(s), figure(s). Files should be labeled with appropriate and descriptive file names (e.g., SmithText.doc, Fig1.eps, Table3.doc). Text, tables, and figures should be uploaded as separate files. (Multiple figure files can be compressed into a Zip file and uploaded in one step; the system will then unpack the files and prompt the naming of each figure. See www.WinZip.com for a free trial.)

Figures and tables should not be imported into the text document. Text and tables must be submitted as Word files. Complete instructions for electronic artwork submission, including acceptable file formats, can be found on the Author Gateway, accessible through the Journal home page (www.ijbm.org). Figures will be tested by an artwork quality check tool and authors asked to view the results before the submission can be completed. Figures can be forwarded for manuscript review if not up to production standards, but high-quality figures are required if the manuscript is accepted for publication.

Authors who are unable to provide an electronic version or have other circumstances that prevent online submission must contact the Editorial Office prior to submission to discuss alternate options (editor@ijbm.org).

Pre-submissions

Authors are welcome to send an abstract or draft manuscript to obtain a view from the Editor about the suitability of their paper. Our Editors will do a quick review of your paper and advise if they believe it is appropriate for submission to our journal. It will not be a full review of your manuscript.

Cover Letter

The cover letter should be saved as a separate file for upload. In it, the authors should (1) state that the manuscript, or parts of it, have not been and will not be submitted elsewhere for publication; (2) state that all authors have read and approved the manuscript; and (3) disclose any financial or other relations that could lead to a conflict of interest. If a potential conflict exists, its nature should be stated for each author. When there is a stated potential conflict of interest a footnote will be added indicating the author’s equity interest in or other affiliation with the identified commercial firms.

The corresponding author should be specified in the cover letter. All editorial communications will be sent to this author. A short paragraph telling the editors why the authors think their paper merits publication priority may be included in the cover letter.

Manuscript Preparation

Title Page

The title page should include (1) a brief and descriptive title of the article, (2) a short title of less than 65 characters with spaces, (3) the authors' names, academic degrees, and hospital and academic affiliations, (4) acknowledgment of grants and other support, (5) a word count, (6) the number of figures and tables, and (7) the name and address (including zip code), telephone, fax, and email address of the individual responsible for editorial correspondence and proofreading.

Disclaimers. An example of a disclaimer is an author's statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

All sources of financial support for the study should be cited on the title page, including federal or state agencies, nonprofit organizations, and pharmaceutical or other commercial sources.

Abstract

The article should include a brief abstract of no more than 200 words. The abstract should be structured with the following headings: Background, Methods and Results, and Conclusions. The Background section should describe the rationale for the study. Methods and Results should briefly describe the methods and present the significant results. Conclusions should succinctly state the interpretation of the data.

Key Words

Authors should supply a list of up to four key words not appearing in the title, which will be used for indexing. The key words should be listed immediately after the Abstract.

Text

The text of original research papers should be organized as follows: Introduction, Methods, Results, Discussion. The Introduction should describe the purpose of the study and its relation to previous work in the field; it should not include an extensive literature review. Methods should be concise but sufficiently detailed to permit repetition by other investigators. Previously published methods and modifications should be cited by reference. Results should present positive and relevant negative findings of the study, supported when necessary by reference to tables and figures. The Discussion should interpret the results of the study, with emphasis on their relation to the original hypotheses and to previous studies. The importance of the study and its limitations should also be discussed.

Reviews, Hypotheses, and State-of-the Art papers should be organized as follows: Introduction, other appropriate subject headings, Conclusion. The Editor invites brief Letters to the Editor commenting on papers appearing in the Journal and on other issues.

Authorship

Authorship credit should be based on the contribution of the individual authors to some combination of one or more of the following:

- ✓ conception or design
- ✓ data collection and processing
- ✓ analysis and interpretation of the data
- ✓ writing substantial sections of the paper.

Acknowledgments

All contributors who do not meet the criteria for authorship should be listed in an acknowledgments section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chairperson who provided only general support. Authors should declare whether they had assistance with study design, data collection, data analysis, or manuscript preparation. If such assistance was available, the authors should disclose the identity of the individuals who provided this assistance and the entity that supported it in the published article. Financial and material support should also be acknowledged.

References

References should be double-spaced in numerical sequence according to standard Vancouver/ICMJE style, using MEDLINE abbreviations for journal titles *(*www.ncbi.nlm.nih.gov/nlmcatalog/journals*)*. The first six authors should be listed in each reference citation (if there are more than six authors, "et al" should be used following the sixth). Periods are not used in authors' initials or journal abbreviations.

Journal Article: McClean D, Aragon J, Jamali A, Kar S, Ritzema-Carter J, Troughton R, et al. Noninvasive calibration of cardiac pressure transducers in patients with heart failure: an aid to implantable hemodynamic monitoring and therapeutic guidance. J Cardiac Fail. 2006; 12:568-76.

Book: Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. *Medical Microbiology*. 4th ed. St. Louis: Mosby; 2002.

Chapter in Edited Book: Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. *The Genetic Basis of Human Cancer*. New York: McGraw-Hill; 2002:93–113.

References should be typed in parentheses and cited in numerical order in the text and listed at the end of the article in citation order. References to unpublished materials or personal communications should be cited in the text in parentheses and include relevant researchers. Further information about Vancouver reference style is available at www.icmje.org.

Figures and Legends

All illustrations (line drawings and photographs) are classified as figures. All figures should be cited in the text and numbered in order of appearance. Figures should be provided in .tiff, .jpeg or .eps formats. Color images must be at least 300 dpi. Gray scale images should be at least 300 dpi. Line art (black and white or color) and combinations of gray scale images and line art should be at least 1,000 dpi. The optimal size of lettering is 12 points. Symbols should be of a similar size. Figures should be sized to fit within the column (86 mm) or the full text width (180 mm). Line figures must be sharp, black and white graphs or diagrams, drawn professionally or with a computer graphics package. Legends should be supplied for each figure and should be brief and not repetitive of the text. Any source notation for borrowed figures should appear at the end of the legend. Figures should be uploaded as individual files.

Tables

Tables should be comprehensible without reference to the text and should not be repetitive of descriptions in the text. Every table should consist of two or more columns; tables with only one column will be treated as lists and incorporated into the text. All tables must be cited in the text and numbered in order of appearance. Tables should include a short title. Each table submitted should be double-spaced, each on its own page. Each table should be saved as its own file as a Word Document. Explanatory matter and source notations for borrowed tables should be placed in the table footnote.

Units of Measurement

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples.

Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury. All measurements must be given in SI or SI-derived units.

Drug concentrations may be reported in either SI or mass units, but the alternative should be provided in parentheses where appropriate.

Abbreviations and Symbols

Use only standard abbreviations; use of nonstandard abbreviations can be confusing to readers. Avoid abbreviations in the title of the manuscript. The spelled-out abbreviation followed by the abbreviation in parenthesis should be used on first mention unless the abbreviation is a standard unit of measurement.

Drugs should be referred to by their generic names. If proprietary drugs have been used in the study, refer to these by their generic name, mentioning the proprietary name, and the name and location of the manufacturer, in parentheses.

Permissions

To use tables or figures borrowed from another source, permission must be obtained from the copyright holder, usually the publisher. Authors are responsible for applying for permission for both print and electronic rights for all borrowed materials and are responsible for paying any fees related to the applications of these permissions. This is necessary even if you are an author of the borrowed material. It is essential to begin the process of obtaining permission early, as a delay may require removing the copyrighted material from the article. The source of a borrowed table should be noted in a footnote and of a borrowed figure in the legend. It is essential to use the exact wording required by the copyright holder. A copy of the letter granting permission, identified by table or figure number, should be sent along with the manuscript. A permission request form is provided for the authors use in requesting permission from copyright holders.

Processing Fees

***Open Access Publication*:** all manuscripts submitted to IJBM will be submitted under the Open Access publishing model. In this publishing model, papers are peer-reviewed in the normal way under editorial control. When a paper is accepted for publication the author is issued an invoice for payment of a publication processing fee. Payment of this charge allows IJBM to partially recover its editorial process and production of the printed version, and development of online functionality, and provide our content at no cost to readers. IJBM charges a processing fee of $100 per printed black and white journal page and $150 per printed page of color illustrations.

IJBM charges a processing fee of $50 per page in the case of online-only publications. For online-only publications, all illustrations submitted in color will be published in color online, at no cost to the author.

Example 1 - Article length is 4 journal pages for online-only publication. Total Charge for article = $200.

Example 2 - Article length is 4 black and white journal pages.
°Total Charge for article = $400.

Example 3 - Article length is 5 journal pages, 5 color figures. Figures 1-3 fit on one journal page, figures 4-5 fit on one journal page.

- Page Charges = $100 *3 black and white journal pages = $300
- Color Cost= $150 * 2 color journal pages = $300

°Total Charge for article = $600

°Total Charge for article includes copyediting, typesetting, publishing (online and print), indexing of article in citation databases and shipping.

Surely, this processing fee does not cover the whole cost. For IJBM, the income might be from subscriptions for the printed journal, foundation and grant support, advertisements, and institutional support. Published papers appear electronically and are freely available from our website. Authors may also use their published .pdf's for any non-commercial use on their personal or non-commercial institution's website. A subscription to the printed version of IJBM remains available.

Under IJBM's existing policy certain categories of authors are eligible for a discount. The amount of discount depends on factors such as country of origin, position of the author in the institute and quality and originality of the work. Young researchers and first time authors may also qualify for a discount. There is also an author loyalty discount open to authors submitting more than one article within twelve months. To apply for a discount, please contact our office using the 'Contact Us' page or send email to the Publisher (editor@ijbm.org) with the following information:

- Your name and institution with full address details
- Reason for applying for a waiver
- Title of your paper
- Country of residence of any co-authors.

Commercial use: No articles from IJBM website may be reproduced, in any media or format, or linked to for any commercial purpose without the prior written consent of IJBM and payment to IJBM of an appropriate fee.

Page Proofs: Page proofs are sent from the Publisher electronically and must be returned within 72 hours to avoid delay of publication. All authors must sign and return the author approval and final page of Publication Agreement.
Generally peer review is complete within 3-4 weeks and the editor's decision within 7-10 days of this. It is therefore very rare to have to wait more than 6 weeks for a final decision.

AUTHOR'S CHECKLIST

When submitting manuscripts to the International Journal of Biomedicine please remember to include the following:

• Cover Letter

• The authors should (1) state that the manuscript, or parts of it, have not been and will not be submitted elsewhere for publication; (2) state that all authors have read and approved the manuscript; and (3) disclose any financial or other relations that could lead to a conflict of interest. If a potential conflict exists, its nature should be stated for each author.

• All sources of financial support for the study should be stated including federal or state agencies, nonprofit organizations, and pharmaceutical or other commercial sources.

• Manuscript, including:
− Title page
− Article title
− Short title (less than 65 characters w/ spaces)
− Authors' names, academic degrees, affiliations
− Acknowledgment of grants and other financial support
− Word count
− Number of figures and tables
− Name, address, telephone, fax, and email address of corresponding author
− All authors must disclose any financial or other relations that could lead to a conflict of interest. If a potential conflict exists, its nature should be stated for each author. When there is a stated potential conflict of interest a foot note will be added indicating the author's equity interest in or other affiliation with the identified commercial firms.
− All sources of financial support for the study should be stated including federal or state agencies, nonprofit organizations, and pharmaceutical or other commercial sources.

• Abstract

• Key words

• Text

• Acknowledgments

• References

• Table and Figure Legends

• Figures (individual tiff or eps file format)

• Tables (individual word documents)

• Permissions for the use of any previously published materials

• Disclosure Form (fax or e-mail to Editorial Office).

It is important to note that when citing an article from IJBM, the correct citation format is **Int J Biomed.**

Thank you for your interest in International Journal of Biomedicine. Please feel free to contact us with any questions. To ensure fast peer review and publication, manuscripts that do not adhere to this instructions will be returned to the corresponding author for technical revision before undergoing peer review. We are looking forward to your submission.


ACC.16
65th Annual Scientific Session & Expo
CHICAGO
APRIL
2 – 4
2016


ASPC
The American Society for
Preventive Cardiology
Making Waves in
Preventive Cardiology
September 16–18, 2016
2016 Congress on
Atherosclerotic Cardiovascular Disease Prevention
Boca Raton Resort and Club • Boca Raton, FL
A Waldorf Astoria Resort

International Journal of Biomedicine 6(1) (2016) 12-17
doi: 10.21103/Article6(1)_OA2

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Risk of Cardiovascular Death in the Remote Period after Myocardial Revascularization and in Association with Renal Dysfunction

Ekaterina S. Levitskaya, PhD[1]*; Mikhail M. Batiushin, PhD,ScD[1]; Aleksey V. Hripun, PhD[1]; Aleksandr A. Kastanajan, PhD,ScD[1]; Elena O. Golovinova[1]; Valentina V. Gul'chenko[1]; Dmitriy G. Pasechnik, PhD[1]; Vladimir A. Chistyakov, PhD, ScD[1, 2]; Igor V. Dudarev, PhD, ScD[1]; Galina V. Shavkuta, PhD, ScD[1]

[1]Rostov State Medical University, [2]Southern Federal University
Rostov-on-Don, Russia

Abstract

The aim of the present study was to assess the effectiveness of standard medical therapy in lowering the risk of cardiovascular death in the remote period after myocardial revascularization (MR), taking into account the presence of renal dysfunction.

Material and Methods: The study included 90 patients with coronary heart disease (CHD) and indications for revascularization. We evaluated a drug therapy obtained at different stages of revascularization, as well as the severity of patients' condition and the prevalence of renal dysfunction.

Results: In the remote period after MR (5.8±0.05 years), 71/78.9% patients participated in the study; death occurred in 10/12.3% patients. The duration of therapy for chronic myocardial ischemia before MR (*P=0.005*), as well as compliance with prescribed therapy during 6 months (*P=0.008*) after this procedure, affected cardiovascular death in the remote period after MR. Using statins before MR reduced the risk of cardiovascular death by 17.2% (*P=0.01*), beta-blockers -14.95% (*P=0.04*), and ACE inhibitors (ACEIs) - 15.75% (*P=0.03*). The lack of regular use of acetylsalicylic acid (ASA) for 6 months after RM was associated with an increase in the risk of cardiovascular death up to 36.2% (*P=0.005*). Statins and ACEIs are drugs that reduce the risk of cardiovascular death in the presence of renal dysfunction (*P<0.05*).

Conclusion: An efficient drug regimen for patients after MR is important in reducing a long-term prognosis of cardiovascular death and for an efficient correction of coronary artery patency. **(Int J Biomed. 2016;6(1):12-17.).**

Keywords: *risk of cardiovascular death; ischemic heart disease; revascularization; renal dysfunction.*

Abbreviations: **LVEDV,** left ventricular end-diastolic volume; **LVESV,** left ventricular end-systolic volume; **IVST,** interventricular septal thickness; **LVPWT,** left ventricular posterior wall thickness; **LVMI,** left ventricular mass index; **LVEF,** left ventricular ejection fraction; **GFR,** glomerular filtration rate; **MAU,** microalbuminuria.

Introduction

The effectiveness of various methods of treatment is one of the relevant aspects of research activity despite the development of modern medicine. The principle of evidence is the fundamental basis for the rational use and effectiveness of a therapy. Drugs with a broad evidence base are the means of first choice in the treatment of patients with any pathology. The right choice of drugs becomes a priority, especially in the treatment of socially significant diseases with a high risk of complications and death. Worldwide statistics indicate a primary role of the pathology of the cardiovascular system in the general structure of permanent disability and mortality from chronic non-communicable diseases [1,2], among which coronary heart disease (CHD) holds the leading position [3]. Patients with indications for surgical revascularization are the most disadvantaged group of patients regarding prognosis for cardiovascular death (CVD) and complications. Operations to restore coronary blood flow significantly improve the quality of life of patients and reduce the risk of developing acute myocardial infarction (AMI) and sudden CVD. The right selection of components of medical treatment for a

***Corresponding author:** Ekaterina S. Levitskaya, PhD. Rostov State Medical University, Rostov-on-Don, Russia. E-mail: es.med@mail.ru*

group of patients after MR is justified by the necessity to slow the progression of the pathogenic mechanisms of the atherosclerotic process, and, as a consequence, to significantly increase the effectiveness of interventional treatment.

The presence of cardiovascular disease is often accompanied by damage to target organs or the formation of clinical conditions, the development of which is caused by a single pathogenesis. Developing multimorbidity potentiates a poor prognosis with cardiovascular complications and requires the use of the most rational drug combinations, which minimize the risk of the progression of disease, death, and acute vascular catastrophes.

The existing evidence base for drug therapy in patients after restoration of coronary blood flow includes statins and low doses of acetylsalicylic acid (ASA), as well as ACE inhibitors (ACEIs) and beta-blockers in certain clinical situations [4]. Research and clinical activities seek to obtain information that complements the known data regarding the priority drug regimens. It is important to emphasize the special significance and the practical value of this information in the evaluation of long-term prognosis of cardiovascular events.

The aim of the present study was to assess the effectiveness of standard medical therapy in lowering the risk of cardiovascular death (rCVD) in the remote period after myocardial revascularization (MR), taking into account the presence of renal dysfunction (RD).

Materials and Methods

The present study included 90 CHD patients (80 men and 10 women; mean age 56.1±0.9 years) with indications for restoration of coronary blood flow. The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the Rostov State Medical University Ethics Committee. Written informed consent was obtained from all participants.

The inclusion criteria were indications for surgical revascularization of the myocardium by the method of coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI) with stent implantation. The need to restore coronary blood flow was determined by the results of coronary angiography (CAG). After completing the patient recruitment in the study group, we found that CABG was required in 64/57.6% patients and PCI with stent implantation in 26/42.4% patients.

At the beginning of the study, we assessed the prevalence of traditional risk factors (RFs): the presence and duration of smoking, arterial hypertension (AH), overweight and obesity, diabetes mellitus (DM), and the presence a prior MI and duration of CHD. We then estimated the biochemical markers (blood lipids and type of dyslipidemia), the main echocardiographic parameters (LVEDV, LVESV, IVST, LVPWT, LVMI, and LVEF), and the parameters of renal function – MAU and GFR. MAU (urinary albumin excretion of 30-300 mg/24 hours) was assessed by a semi-quantitative method using test strips for the determination of protein in the urine, in compliance with the rules for collecting morning urine. GFR was estimated by the Cockcroft-Gault formula. Stages of chronic kidney disease (CKD) were determined according to the KDOQI 2002 classification. Patients with CKD stages 4-5 were excluded from this study.

Clinical characteristics of the studied groups are presented in Table 1.

Table 1.

Cardiovascular risk factors and clinical characteristics of CHD patients

Criterion	Average value
Duration of therapy of CHD, years	6.1±0.6
Patients with excess body weight, abs (%)	34 (37.8)
Obesity, abs (%)	43 (47.8)
Smoking patients, abs (%)	32 (35.6)
Total cholesterol, mmol/l	5.65±0.15
High-density lipoprotein cholesterol, mmol/l	1.1±0.03
Low-density lipoprotein cholesterol, mmol/l	4.6±0.2
Triglycerides, mmol/l	1.95±0.1
Dyslipidemia, type IIa, abs (%)	47 (52.2)
Dyslipidemia, type IIb, abs (%)	29 (32.2)
LVEDV, ml	151.2±3.9
LVESV, ml	72.3±3.01
IVST, mm	12.53±0.17
LVPWT, mm	11.99±0.15
LVEF, %	53.0±0.7
Angina pectoris, class II, abs (%)	5 (5.6)
Angina pectoris class III, abs (%)	73 (81.1)
Angina pectoris, class IV, abs (%)	1 (1.1)
Unstable angina, abs (%)	8 (8.9)
Acute myocardial infarction, abs (%)	3 (3.3)
Myocardial infarction, abs (%)	66 (73.3)
Arterial hypertension, abs (%)	77 (85.6)
Diabetes, abs (%)	19 (21.1)
MAU, abs (%)	82 (91.1)
GFR, mL/min	90.2±2.2
CKD stage 1, abs (%)	48 (53.3)
CKD stage 2, abs (%)	33 (36.7)
CKD stage 3, abs (%)	7 (7.8)

Statistical analysis of the obtained data allowed us to establish the high prevalence of both traditional and renal risk factors in the studied group of patients. To achieve the study objective, we assessed CHD therapy duration before the revascularization, as well as groups of drugs, receipt of which was regular. The average duration of CHD medical treatment was 1.57±0.27 years.

Medical therapy aimed at slowing the progression of the atherosclerotic process was recommended to all patients after MR. We performed a statistical analysis of regular use of medicines also in the late period, 6.3±0.04 months after MR. Analysis of the frequency of regular medication intake before MR and in the late period after the intervention is presented in Table 2. On the second visit in the late period after coronary reperfusion, we also monitored the presence of MAU and GFR value.

The endpoint of the study was CVD in the remote period after MR (5.8±0.05 years). CVD occurred in 10/12.3% of 81 patients who continued participation in the study.

Table 2.
The frequency of regular medication intake (abs/%) before MR and in the late period after the intervention

Medications	Before MR	After MR (≈6.3 mth)
Statins	40/44.4	74 (84.1)
ASA	53/58.9	79 (89.8)
Beta-blockers	55/61.1	73 (83.0)
ACE inhibitors	48/53.3	69 (78.4)
Calcium channel blockers	7/7.8	10 (11.4)
Diuretics	5/5.6	12 (13.3)
Sartans	2/2.2	2 (2.2)
Nitrates	33/36.7	18 (20.0)
Trimetazidine	15/16.7	19 (21.1)

Statistical analysis of data was performed using the software Statistica 8.0. The mean (M) and standard error of mean (SEM) were calculated. Differences of continuous variables with a normal distribution between the two groups were calculated using the independent-sample *t*-test. Group comparisons with respect to categorical variables are performed using chi-square tests. Two-tailed *P* values <0.05 were considered statistically significant.

Results

rCVD in the remote period after MR decreased significantly with increased duration of regular CHD drug therapy prior to MR. Thus, regular treatment for 4 years was associated with a low risk of death amounting to 0.09%, but the absence of drug therapy led to an increased risk of death in the remote period after MR by 23.8% (*P=0.005*) (Figure 1).

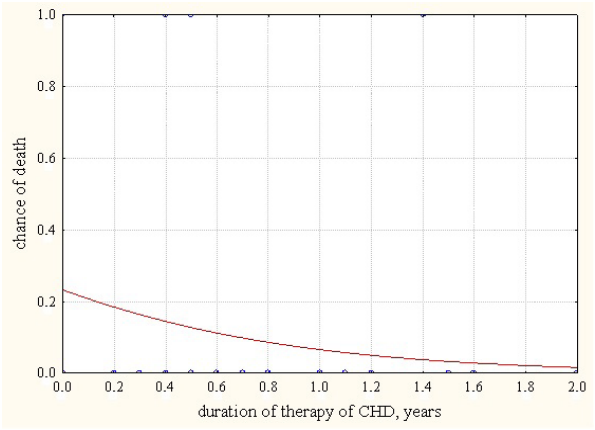


Fig. 1. *The probability of death in remote period after MR depending on the duration of CHD therapy*

Statistical data obtained when analyzing the frequency of use of drug classes before MR showed that statins, beta-blockers, and ACEIs significantly reduced rCVD. It should be noted that the degree of influence on cardiovascular prognosis was comparable for the presented classes. rCVD in the remote period after MR was 20.0% in the absence of continuous use of statins in the period before MR (*P= 0.01*); this rate was 21.2% for beta-blockers (P=0.04) and 20.51% for ACEIs (*P= 0.03*). Comparison of patient groups with the presence and absence of taking statins, beta-blockers and ACEIs before coronary reperfusion has allowed us to establish an increase in the probability of death among patients who did not take these drugs by 17.22%, 14.95% and 15.75%, respectively.

In patients with continuous ASA use before MR, the impact on rCVD reduction in the remote period after MR approached statistically significant values. The results of the regular medication intake in the later period (6.3±0.04 months) after MR showed that continuous use of the recommended regimens leads to significant rCVD reduction in the remote period after MR by 40.8% (*P=0.008*).

We have shown the high importance of ASA use in reducing rCVD by 36.2% (*P=0.009*). after 5.8±0.05 years following MR. When we analyzed the impact of ACEIs, beta-blockers and statins on rCVD in the remote period after MR, reliable results had not been established; at the same time, we identified a trend of statistical significance in the regular use of beta-blockers.

In the presence of MAU determined before revascularization, regular ACEI intake was accompanied by reduction of rCVD in the later period after MR by 10.22% (*P=0.03*), and statins by 5.67% (*P=0.02*).

In the analysis of GRF value calculated before MR, we found that in patients with normal filtration capacity of the kidneys, or a tendency to hyperfiltration combined with regular intake of ACEIs (*P=0.04*) or statins (*P=0.03*), rCVD was lower in comparison to patients with decreased GRF (Figures 2 and 3).

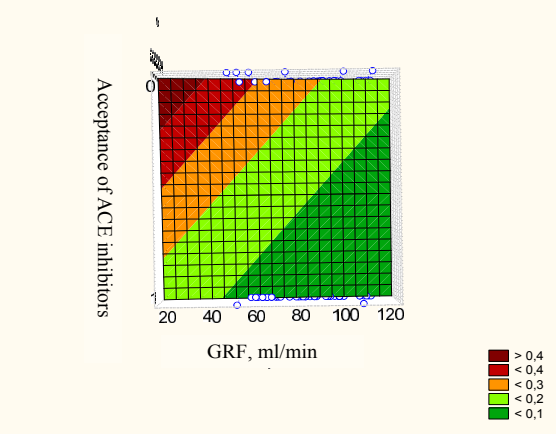


Fig. 2. *The probability of death in remote period after MR depending on the regular ACEIs intake and GFR level*

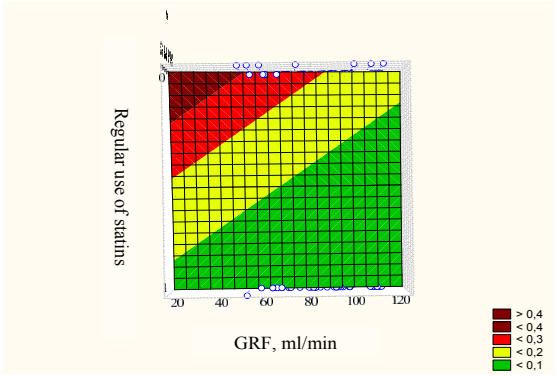


Fig. 3. *The probability of death in remote period after MR depending on the regular statin intake and GFR level*

We revealed rCVD reduction during regular intake of the prescribed regimen for 6 months after MR in patients with MAU and decreased GFR values. Continuous use of drugs for CHD treatment was associated with a decrease in rCVD by 11.9% in patients with MAU (*P=0.02*). We found a more significant decrease in the probability of death during medical therapy in patients with more impaired kidney filtration function. ASA intake was associated with rCVD reduction by 36.9% in patients with MAU (*P=0.03*). The risk of death amounted to 42.1% at GFR of 90 ml/min and ASA absence in the drug regime, while a regular ASA admission within 6 months after MR decreased the probability of death by 7.7%; these risks amounted to 64.1% and 17.7% (*P=0.02*), respectively in a GFR value of 30 ml/min.

Discussion

Evidence-based drug therapy is the basis for cardiovascular risk reduction. Surgical MR is the most effective method to treat coronary insufficiency that improves the quality of life of patients and predicts cardiovascular complications. At the same time, surgical restoration of intracoronary blood flow is not a tool that controls CVD pathogenesis. The further tactics for management of patients after MR should include a choice of the optimal medical treatment, which can effectively delay mechanisms of restenosis formation and reduce the risk of cardiovascular complications.

In the present study, we showed that the remote rCVD in patients receiving long-term anti-ischemic therapy was significantly lower in comparison to patients with short-term periods of drug therapy or the lack of it. It should be noted that the probability of death becomes minimal with an increase in the duration of therapy, whereas a fatal outcome can be predicted in almost one of four patient in the absence of the regular therapy.

Numerous studies have shown that statins, ACEIs and beta-blockers may reduce rCVD. Moreover, a significant decrease in rCVD in the remote period after MR was obtained under the condition of regular reception of these drug classes before surgical correction of coronary blood flow. Comparative analysis of the drug classes used within 6 months after coronary reperfusion has allowed us to establish the primary importance of ASA in reducing the risk of a fatal outcome in the long term. Based on these data, one can judge the priority of correction of the pathogenetic mechanisms of CHD progression depending on the treatment stage. Reducing the activity of neurohumoral systems, improving the endothelial function, and normalizing the lipid spectrum are the most important treatments in the period before MR, while an effective control of platelet activity is the priority in the near term after MR. Our results are consistent with numerous studies devoted to drug therapy in CHD patients.

Philip F. et al. [5] conducted a retrospective cohort study of 5,205 patients after first-time isolated CABG and assessed the impact of a discharge regimen including beta-blockers and statin therapy and their relationship to long-term all cause mortality and major adverse cardiovascular events. A discharge regimen with statin therapy was associated with and overall reduction in 30 day, 1 year and long-term mortality. In addition, statin and beta-blockers exerted synergistic effect on overall mortality outcomes short-term and in the long-term.

There are many studies confirming the protective role of statins in patients after MR, in which the probability of occlusion and rCVD was decreased by reducing the atherosclerotic process progression in a general coronary bed and arteries subjected to surgical correction [6,7].

The necessity of using beta-blockers in CHD patients is not in doubt and is dictated by the peculiarity of the mechanism of their action. Recent studies of beta-blockers used in patients after CABG demonstrate the need to include this drug class for continuous use.

The study conducted by Zhang H et al. [8] included 5926 consecutive patients who underwent CABG and were discharged alive. The prevalence and consistency of β-blocker use were determined in patients with and without a history of myocardial infarction (MI). In patients with or without previous MI undergoing CABG, the consistent use of β-blockers was associated with a lower risk of long-term mortality and adverse cardiovascular events.

Our earlier studies showed a need of beta-blocker use after MR for correction of a recurrent angina, which is a marker of coronary atherosclerosis progression and adverse cardiovascular events [9]. In the group of patients after 6 months following MR, we found that beta-blocker use before coronary reperfusion reduced the risk of recurrence of angina by 33.0% and a dose-dependent effect was demonstrated [9].

Suppression of the influence of angiotensin II by angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers may reduce or potentially reverse atherosclerosis and other inflammation-associated cardiovascular diseases [10].

L.Minuzzo et al [11] investigated the effect of previous use of angiotensin-converting enzyme inhibitors on cardiac troponin I measurement in patients with acute coronary syndrome without ST-segment elevation and clinical outcomes at 180 days. This study showed a correlation between prior use of ACEIs and reduction in the myocardial necrosis marker troponin I in patients admitted for acute coronary syndrome without ST-segment elevation. However, large-scale trials are still required to state that this reduction could lead to fewer severe clinical events such as death and re-infarction.

Current guidelines consider the validity of ACEI assignment for certain clinical situations in chronic CHD [4]. It is important to note that patients included in our study were characterized by a high prevalence of impaired kidney function. In this regard, the results obtained can be justified in view of the correction of cardio-renal syndrome with a significantly improved long-term cardiovascular prognosis.

The importance of optimal drug combinations increases significantly in the presence of multimorbidity, especially with the existence of conditions united by a common pathogenesis and risk factors. Development of secondary nephropathy of cardiac origin increases a poor prognosis and requires the most effective approaches in the pharmacological treatment. RD markers such as MAU and a decreased GFR are the most significant indicators in the formation of an

adverse cardiovascular prognosis. The strategy of medical management of such patients should be based on the concept of cardio-nephroprotection.

We have shown that a significant decrease in rCVD in patients with MAU/decreased GFR is associated with a regular application of ACEIs and statins prior to revascularization. Regular intake of these drugs allows not only reduction of rCVD, but also equalizes the magnitude of this risk in the presence and absence of RD. At the same time, the tendency towards hyperfiltration reduces the risk even in the absence of a permanent use of ACEIs or statins. The obtained information about the role of statins in cardiovascular complication risk correction in RD presence is consistent with results of other studies. According to R. Agarwal [12], subgroup analyses of major clinical studies and meta-analyses of smaller trials indicate that statin therapy slows the decline of the glomerular filtration rate. Additionally, statins appear to reduce proteinuria in patients with CKD. Statins are well recognized to reduce cardiovascular morbidity and mortality in patients with and without documented cardiovascular disease and in certain high-risk populations, such as persons with diabetes mellitus. Statins have also been shown to preserve glomerular filtration rate and reduce proteinuria in subjects with nondiabetic renal disease. However, conclusive evidence for improved cardiovascular outcomes with statin therapy for CKD is not yet available. Thus, atorvastatin had no statistically significant effect on the composite primary end point of cardiovascular death, nonfatal myocardial infarction, and stroke in patients with diabetes receiving hemodialysis [13]. According to B.Fellström et al. [14], in patients undergoing hemodialysis, the initiation of treatment with rosuvastatin lowered the LDL cholesterol level but had no significant effect on the composite primary end point of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke. At the same time, the SHARP trial showed that reduction of LDL cholesterol with simvastatin 20 mg plus ezetimibe 10 mg daily safely reduced the incidence of major atherosclerotic events (non-fatal myocardial infarction or coronary death, non-haemorrhagic stroke, or any arterial revascularisation procedure) in a wide range of patients with advanced chronic kidney disease [15].

Analysis of data in the literature and our results can be explained by the fact that the reduction of rCVD with statin therapy is slight in the presence of RD, whereas normal renal function or a tendency towards hyperfiltration has a protective effect. Long-term use of statins [12] and ACEIs [16,17] helps to slow down kidney remodeling, leading thereby to reduction of the progression of RD and rCVD.

Conclusion

The assignment of rational treatment regimens and RF management for patients at very high risk of cardiovascular complications is essential to maximum prognosis improvement of these patients. Patients after MR need an effective slowdown of the pathogenic mechanisms of disease progression with the definition of schemes and algorithms for drug therapy taking into account variations in clinical situations. The results of our study allow us to highlight the main points for the effective improvement of cardiovascular prognosis and rCVD reduction in the remote period after MR:

- A long-term treatment with drugs correcting processes of chronic ischemia is strongly recommended prior to planned surgical MR.
- Statins, beta-blockers, and ACEIs are most effective before MR.
- The use of ASA for at least 6 months in the postoperative period significantly improves the prognosis.
- Adherence to the prescribed CHD therapy after MR is an important condition for a favorable prognosis.
- The inclusion in the therapeutic regimens of statins and ACEIs before and ASC after MR is recommended in the presence of MAU and decreased GFR.
- An assessment of renal function is important for rCVD determination.

Competing interests

The authors declare that they have no competing interests.

Acknowledgment

V. Chistyakov was supported by grant 6.1202.2014/K from the Ministry of Education and Science of the Russian Federation.

References

1. World Health Organization. Global Status Report on Noncommunicable Diseases 2014. http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf
2. Castellano JM, Narula J, Castillo J, Fuster V. Promoting Cardiovascular Health Worldwide: Strategies, Challenges, and Opportunities. Rev Esp Cardiol (Engl Ed). 2014; 67(9): 724-30.
3. Tuso P, Stoll SR, Li WW. A Plant-based diet, atherogenesis, and coronary artery disease prevention. Perm J. 2015; 19(1): 62-7.
4. 2014 ESC/EACTS Guidelines on myocardial revascularization: The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS)Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). Authors/Task Force members, Windecker S, Kolh P, Alfonso F, Collet JP, Cremer J, Falk V, et al. Eur Heart J. 2014 Oct 1;35(37):2541-619.5.
5. Philip F, Blackstone E, Kapadia SR. Impact of statins and beta-blocker therapy on mortality after coronary artery bypass graft surgery. Cardiovasc Diagn Ther. 2015; 5(1): 8-16.
6. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med. 2005; 352(14):1425-35.
7. Kulik A, Brookhart MA, Levin R, Ruel M, Solomon DH, Choudhry NK. Impact of statin use on outcomes after coronary artery bypass graft surgery. Circulation 2008; 118(18):1785-92.
8. Zhang H, Yuan X, Zhang H, Chen S, Zhao Y, Hua K, et al. Efficacy of Long-Term β-Blocker Therapy for Secondary

Prevention of Long-Term Outcomes After Coronary Artery Bypass Grafting Surgery. Circulation. 2015; 131(25):2194-201.
9. Levitskaya ES, Chesnikova AI, Batiushin MM, Terent'ev V.P. Assessing the impact of renal risk factors on the probability of recurrence of angina pectoris in patients undergoing myocardial revascularization, optimization of drug therapy. Arch Inter Med. 2012;(5):45-50. [In Russian].
10. Ferrario CM, Strawn WB. Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease. Am J Cardiol. 2006; 98(1): 121-8.
11. Minuzzo L, Santos ES, Timerman A. Association between angiotensin-converting enzyme inhibitors and troponin in acute coronary syndrome. Arq Bras Cardiol. 2014; 103(6): 513-20.
12. Agarwal R. Effects of statins on renal function. Mayo Clin Proc. 2007; 82(11): 1381-90.
13. Wanner C, Krane V, März W, Olschewski M, Mann JF, Ruf G, et al. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. N Engl J Med. 2005; 353(3): 238-48.
14. Fellström BC, Jardine AG, Schmieder RE, Holdaas H, Bannister K, Beutler J, et al. Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. N Engl J Med. 2009; 360(14):1395-407.
15. Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. Lancet. 2011; 377(9784):2181-92.
16. Tan KS, Johnson DW. Managing the cardiovascular complications of chronic kidney disease. Aust Prescr 2008; 31: 154-8.
17. Bakris GL. Slowing nephropathy progression: focus on proteinuria reduction. Clin J Am Soc Nephrol. 2008; 3 Suppl 1:S3-10.

International Journal of Biomedicine 6(1) (2016) 18-21
doi: 10.21103/Article6(1)_OA3

ORIGINAL ARTICLE

Nephrology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Left Ventricular Structure during Antihypertensive Treatment in Patients with Chronic Kidney Disease

Batir T. Daminov[1], PhD, ScD; Sherzod S. Abdullaev[2]*

[1]Tashkent Pediatric Medical Institute; [2]Tashkent Medical Academy
Tashkent, Uzbekistan

Abstract

The aim of our study was to investigate the left ventricular (LV) echocardiographic parameters and estimate the antiremodeling efficacy of eprosartan and lercanidipine in patients with chronic kidney disease, depending on the presence or absence of diabetic nephropathy (DN).

Materials and Methods: The study included 121 patients (mean age 52.4±5.7 years) with CKD stage 3 (KDOQI, 2002). Patients were distributed in two groups according to the etiology of CKD. Group 1 consisted of 67 patients with non-diabetic CKD. Group 2 consisted of 54 CKD patients with DN. All patients had arterial hypertension grade 1 or 2 (ESH/ESC, 2013*)*. All patients underwent clinical examination, echocardiography; GFR was estimated by the Cockcroft-Gault formula. Stages of chronic kidney disease (CKD) were determined according to the KDOQI 2002 classification. Eprosartan and lercanidipine were prescribed to patients after one week of lavage from previous antihypertensive therapy. This 6-month follow-up study compared the effectiveness of two courses of treatment.

Results: Left ventricular hypertrophy (LVH) was observed in all CKD patients regardless of the presence or absence of DN. Eprosartan and lercanidipine showed the high antihypertensive efficacy expressing a reliable decrease in absolute values of SBP and DBP. In CKD patients with DN, on the background of a comparable antihypertensive effect, eprosartan, in comparison with lercanidipine, showed a more pronounced effect on the LV echocardiographic parameters associated with LVH regression. (**Int J Biomed. 2015;6(1):18-21.).**

Keywords: *chronic kidney disease; echo-geometric parameters; left ventricular hypertrophy; eprosartan; lercanidipine.*

Abbreviations

LVEDD, left ventricular end-diastolic dimension; **LVESD**, left ventricular end-systolic dimension; **LVEDV**, left ventricular end-diastolic volume; **LVESV**, left ventricular end-systolic volume; **LVSV**, left ventricular stroke volume; **IVST**, interventricular septal thickness; **LVPWT**, left ventricular posterior wall thickness; **RWT**, relative wall thickness; **LVM**, left ventricular mass; **LVMI**, left ventricular mass index; **LVH**, left ventricular hypertrophy; **GFR**, glomerular filtration rate; **CKD**, chronic kidney disease; **ESRD**, end-stage renal disease.

Introduction

Cardiovascular disease (CVD) remains the major cause of death in patients with chronic kidney disease (CKD). According to a well-established classification, cardiovascular involvement in CKD can be set in the context of cardiorenal syndrome type 4 [1]. The National Kidney Foundation Task Force about CVD in CKD has emphasized the high risk of CVD in patients with CKD, and has identified LVH and coronary artery disease as the major targets for intervention [2]. The prevalence of LVH is estimated to be between 16 and 31% in individuals with a GFR >30 ml/min; it increases to 60-75% prior to starting renal replacement therapy [3]. Foley et al. [4] followed 596 incident hemodialysis patients with no prior history of cardiac disease to investigate whether the incidence of LVH correlates with the duration of dialysis. After 18 months of dialysis, the author reported that 62% of the patients had an increased LV mass volume index and that 49% of them developed overt LV failure. These observations raise the question of whether dialysis therapy develops into LVH in ESRD patients [5]. The evaluation of LVH is a quite heterogeneous. Electrocardiography, 2D and 3D

***Corresponding author:** Sherzod S. Abdullaev. Tashkent Medical Academy. Tashkent, Uzbekistan. E-mail: dr.sherzod@rambler.ru*

echocardiography (ECHO) and cardiac magnetic resonance imaging (CMRI) represent three next steps to quantify and estimate the degree of LVH. Because of these clear limits of CMRI, ECHO is still established as the main device to evaluate LV mass in daily clinical practice although there are limitations in the determination and quantification of LVH [3].

Levi et al. [6] examined the relation of left ventricular mass to the incidence of cardiovascular disease, mortality from cardiovascular disease, and mortality from all causes in 3220 subjects enrolled in the Framingham Heart Study who were 40 years of age or older and free of clinically apparent cardiovascular disease, in whom left ventricular mass was determined echocardiographically. During a four-year follow-up period, there were 208 incident cardiovascular events, 37 deaths from cardiovascular disease, and 124 deaths from all causes. LVM, determined echocardiographically, was associated with all outcome events. This relation persisted after we adjusted for age, diastolic blood pressure, pulse pressure, treatment for hypertension, cigarette smoking, diabetes, obesity, the ratio of total cholesterol to high-density lipoprotein cholesterol, and electrocardiographic evidence of LVH. In men, the risk factor-adjusted relative risk of cardiovascular disease was 1.49 for each increment of 50g/m in LVM corrected for the subject's height (95% CI: 1.20 to 1.85); in women, it was 1.57 (95%CI: 1.20 to 2.04). LVM (corrected for height) was also associated with the incidence of death from cardiovascular disease (RR=1.73 [95% CI: 1.19 to 2.52] in men and 2.12 [95%CI: 1.28 to 3.49] in women). LVM (corrected for height) was associated with death from all causes (RR=1.49 [95% CI: 1.14 to 1.94] in men and 2.01 [95%CI:1.44 to 2.81] in women). Authors concluded that the estimation of LVM by echocardiography offers prognostic information beyond that provided by the evaluation of traditional cardiovascular risk factors. An increase in LVM predicts a higher incidence of clinical events, including death, attributable to cardiovascular disease.

Similar results were obtained in other studies. The aim of the study performed by E.Paoletti et al. [7] was to identify patient- and haemodialysis -related specific factors that might be associated with a higher risk of sudden cardiac death in subjects receiving renal replacement treatment (RRT) and observed over 10 year period.. The study included 123 patients (76 men; age 29-79 years) undergoing RRT for at least 6 months. During the 10 years, 85 patients died -16 from SCD, 30 from cardiac causes (CC) other than SCD, and 39 from other causes. Univariate Cox regression analysis demonstrated that the factors increasing the risk of SCD were CHD (*P=0.002*), the worsening of LVH (*P<0.0001*), and the presence of long-lasting arterial hypertension (*P=0.001*). An increase in LVH was the sole risk factor for SCD when comparing SCD with CC patients (*P=0.003*). By multivariate Cox regression analysis ΔLVMI was identified as the strongest predictor of SCD (*P<0.0001*).

The severity and persistence of LVH are strongly associated with mortality risk and cardiovascular events in CKD and ESRD patients as reported by Zoccali et al. [8] and London et al. [9] who observed how a 10% decrease in LVM was translated into a 28% decrease in cardiovascular mortality risk in a cohort of patients on hemodialysis. The predictors of LVH regression include better control of systolic blood pressure [10,11], a lower pulse wave velocity and higher hemoglobin levels [12]. It should be clear by now that more clinical trials are needed to assess guidelines for treating CKD-related LVH.

The aim of our study was to investigate the left ventricular echocardiographic parameters and estimate the antiremodeling efficacy of eprosartan and lercanidipine in CKD patients, depending on the presence or absence of DN.

Materials and Methods

The study included 121 patients (mean age 52.4±5.7 years) with CKD stage 3. The stage of CKD was identified based on the level of kidney function, irrespective of diagnosis, according to the KDOQI CKD classification (2002) [14].

Patients were distributed in two groups according to the etiology of CKD. Group 1 consisted of 67 patients with non-diabetic CKD (49 patients with chronic glomerulonephritis, 15 patients with chronic pyelonephritis, and 3 patients with polycystic kidney disease). Group 2 consisted of 54 CKD patients with DN according to the criteria of the Committee on Diabetic Nephropathy [15]. All patients had arterial hypertension grade 1 or 2 (ESH/ESC, 2013*)*. Exclusion criteria were CKD stages 4-5, coronary heart disease, atrial fibrillation and life-threatening ventricular arrhythmias, cancer, arterial hypertension (BP>159/99 mmHg), chronic heart failure (NYHA FC>II), and patients receiving renal replacement therapy.

The control group consisted of 25 healthy, age-matched, randomly selected persons without clinical and instrumental signs of CKD.

Before and during treatment all patients were checked on office BP using Korotkov's method and ambulatory blood pressure monitoring.

Two-dimensional and M-mode echocardiography were done in accordance with American Society of Echocardiography recommendations using Toshiba SSH-160A (Japan). The following parameters were measured and calculated: IVST, LVPWT, LVEDD, LVESD, LVEDV, LVESV, LVEF, and LVM. LVM was indexed to body surface area (LVMI). Left ventricular hypertrophy (LVH) was defined as LVMI of >110g/m^2 (women) and >134g/m^2 (men) [13].

GFR was estimated by the Cockcroft-Gault formula. Stages of chronic kidney disease (CKD) were determined according to the KDOQI 2002 classification.

Eprosartan and lercanidipine were prescribed to patients after one week of lavage from previous antihypertensive therapy. This 6-month follow-up study compared the effectiveness of two courses of treatment. Among Group 1 patients, 35 patients received eprosartan (600 mg/day) and 32 patients, lercanidipine (10 mg/day). Among Group 2 patients, 28 patients received eprosartan (600 mg/day) and 26 patients, lercanidipine (10 mg/day). Eprosartan and lercanidipine were prescribed in addition to standard treatment.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki. It was approved by

the Tashkent Medical Academy Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the statistical software «Statistica». (*v6.0*, *StatSoft*, *USA*). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. *S*tudent's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Group comparisons with respect to categorical variables are performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

Result and Discussion

LVH was observed in all CKD patients regardless of the presence or absence of DN. Eprosartan and lercanidipine showed the high antihypertensive efficacy expressing a reliable decrease in absolute values of SBP and DBP in both groups.

In Group 1 patients, compared to the control group, LVEDD, LVEDV, LVESD and LVESV, as well as LVPWT and IVST were significantly ($P<0.05$ in all cases) greater (Table 1). In Group 2 patients, we found a *more pronounced increase* in LVEDD, LVEDV, LVESD, LVESV, LVPWT, and IVST ($P<0.01$ in all cases). Consequently, LV dilation was more pronounced in Group 2 patients. Changes in LVEF and LVSV in both groups were not significantly different from the control values.

Table 1.

Left ventricular echocardiographic parameters in CKD patients before therapy

Parameters	CG	Group 1	Group 2
LVEDD, mm	43.1±1.18	48.1±3.44*	52.0±3.78*
LVESD, mm	31.2±1.14	38.8±3.26*	42.1±3.49**
LVEDV, ml	122.1±5.12	148.4±8.43***	156.3±11.24***
LVESV, ml	43.6±4.75	55.3±6.43	59.8±8.61*
LVSV, ml	78.5±5.16	92.1±6.05	95.2±8.47
LVEF, %	64.3±4.12	61.4±5.67	60.3±6.34
LVPWT, mm	8.6±0.73	11.5±1.25*	12.6±1.49**
IVST, mm	8.7±0.81	11.7±1.43*	13.0±1.52**
LVM, g	124.9±15.36	269.3±42.74***	283.4±48.36***
LVMI, g/m^2	70.2±11.21	162.4±21.62***	169.5±27.75***
RWT %	0.35±0.05	0.52±0.07*	0.55±0.09*

*CG - control group; * -P <0.05; ** - P <0.01; *** - P <0.001 versus the control group.*

At the same time, we found a significant increase in LVM, LVMI and RTW, especially in Group 2 patients. Thus, in Group 1 patients LVM, LVMI and RTW exceeded the normative values by 2.15 ($P<0.001$), 2.31 ($P<0.001$) and 1.48 ($P<0.05$) times, respectively, and in Group 2 patients by 2.27 ($P<0.001$), 2.41 ($P<0.001$) and 1.57 ($P<0.05$) times, respectively.

The changes in LV echocardiographic parameters during the 6-month therapy with eprosartan and lercanidipine are shown in Table 2. Eprosartan effects were more pronounced than lercanidipine effects in Group 1 patients with non-diabetic CKD. For example, we found a marked reduction of LVEDD, LVESD, LVEDV, LVESV, LVPWT, and IVST during treatment with eprosartan compared to lercanidipine; however, the values of these parameters were not fully normalized compared to the control group. LVSV tended to decrease and LVEF to increase compared to initial values. Initially high values of LVM, LVMI and RTW in these patients significantly decreased after the 6-month treatment with eprosartan ($P<0.05$ in all cases). In particular, in Group 1 patients, a 6-month therapy with eprosartan was associated with significant decrease in LVMI by 17.8% versus 11.6% during therapy with lercanidipine ($P<0.05$).

Table 2.

Left ventricular echocardiographic parameters in CKD patients after 6-month treatment with Eprosartan and lercanidipine

Parameters	Group 1		Group 2	
	LER	EPR	LER	EPR
LVEDD, mm	43.82±3.32	42.3±2.13*	48.7±3.27	47.3±2.75*
LVESD, mm	35.1±3.18	33,6±2.15*	38.8±3.09	37.4±2.41*
LVEDV, ml	133.1±8.55	127.3±8.22*	141.2±11.56	135.2±10.22*
LVESV, ml	46.5±6.35	41.5±6.28*	52.7±7.34	47.6±6.53*
LVSV, ml	86.6±6.32	85.7±6.17	88.5±7.45	86.6±7.23
LVEF, %	65.1±5.67	67.4±5.87	62.5±5.72	64.2±6.32
LVPWT, mm	10.7±1.23	10.3±1.12*	11.9±1.44	11.5±1.16*
IVST, mm	10.9±1.28	10.5±1.18*	12.2±1.35	11.9±1.12*
LVM, g	234.6±42.23	221.3±40.12*	248.2±44.63	234.3±42.28*
LVMI, g/m^2	143.6±21.76	133.5±20.45*	150.7±25.47	141.5±23.16*
RTW, %	0.50±0.06	0.47±0.05*	0.52±0.07	0.51±0,05*

*LER- Lercanidipine; EPR -Eprosartan; * -P <0.05 versus the indices before treatment.*

In Group 2 patients with DN and CKD, on the background of a comparable antihypertensive effect, lercanidipine, in comparison with eprosartan, did not show an appreciable effect on the LV parameters. All the studied parameters had only a tendency to normalization and were significantly different from the normative values.

In Group 2 patients, we found a reduction of LVEDD, LVESD, LVEDV, LVESV, LVPWT, and IVST during treatment with eprosartan compared to initial levels (P<0.05 in all cases); however, the values of these parameters were not fully normalized compared to the control group. LVSV tended to decrease and LV EF to increase compared to initial values. Initially high values of LVM, LVMI and RTW in these patients significantly decreased after the 6-month treatment with eprosartan ($P<0.05$ in all cases), but these changes were less pronounced than in Group 1 patients treated with eprosartan, and not fully normalized.

In conclusion, it can be noted that the LV echo-geometric parameters in CKD patients with and without DN during long-term treatment with eprosartan and lercanidipine improved slightly and that the reverse remodeling was less pronounced in CKD patients with diabetic nephropathy. More pronounced changes were observed during treatment with eprosartan. However, we did not find a full normalization of the studied

echo parameters, which indicates the preservation of LVH in the treated patients.

Findings:

1. LVH was observed in all CKD patients regardless of the presence or absence of DN.

2. In CKD patients with DN, on the background of a comparable antihypertensive effect, eprosartan, in comparison with lercanidipine, showed a more pronounced effect on the LV echocardiographic parameters associated with LVH regression.

Competing interests

The authors declare that they have no competing interests.

References

1. House AA, Anand I, Bellomo R, Cruz D, Bobek I, Anker SD, et al.; Acute Dialysis Quality Initiative Consensus Group. Definition and classification of Cardio-Renal Syndromes: workgroup statements from the 7th ADQI Consensus Conference. Nephrol Dial Transplant. 2010;25(5):1416-20.
2. Meyer KB, Levey AS. Controlling the epidemic of cardiovascular disease in chronic renal disease: Report from the National Kidney Foundation Task Force on Cardiovascular Disease. J Am Soc Nephrol. 1998; 9(12 Suppl):S31- S42.
3. Di Lullo L, Gorini A, Russo D, Santoboni A, Ronco C. Left Ventricular Hypertrophy in Chronic Kidney Disease Patients: From Pathophysiology to Treatment. Cardiorenal Med. 2015;5:254-266
4. Foley RN, Curtis BM, Randell EW, Parfrey PS. Left ventricular hypertrophy in new hemodialysis patients without symptomatic cardiac disease. Clin J Am Soc Nephrol. 2010;5(5):805-13.
5. Parikh SV, de Lemos JA. Biomarkers in cardiovascular disease: integrating pathophysiology into clinical practice. Am J Med Sci. 2006;332(4):186-97.
6. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med. 1990;322(22):1561-6.
7. Paoletti E, Specchia C, Di Maio G, Bellino D, Damasio B, Cassottana P, Cannella G. The worsening of left ventricular hypertrophy is the strongest predictor of sudden cardiac death in haemodialysis patients: a 10 year survey. Nephrol Dial Transplant. 2004;19(7):1829-34.
8. Zoccali C, Benedetto FA, Mallamaci F, Tripepi G, Giacone G, Stancanelli B, et al. Left ventricular mass monitoring in the follow-up of dialysis patients: prognostic value of left ventricular hypertrophy progression. Kidney Int. 2004;65(4):1492-8.
9. London GM, Pannier B, Guerin AP, Blacher J, Marchais SJ, Darne B, et al. Alterations of left ventricular hypertrophy in and survival of patients receiving hemodialysis: follow-up of an interventional study. J Am Soc Nephrol. 2001;12(12):2759 -67.
10. Mattioli AV, Zennaro M, Bonatti S, Bonetti L, Mattioli G. Regression of left ventricular hypertrophy and improvement of diastolic function in hypertensive patients treated with telmisartan. Int J Cardiol. 2004;97(3):383-8.
11. Devereux RB, Palmieri V, Liu JE, Wachtell K, Bella JN, Boman K, et al. Progressive hypertrophy regression with sustained pressure reduction in hypertension: the Losartan Intervention for Endpoint Reduction study. J Hypertens. 2002;20(7):1445-50.
12. Krane V, Winkler K, Drechsler C, Lilienthal J, Marz W, Wanner C; German Diabetes and Dialysis Study Investigators. Effect of atorvastatin on inflammation and outcome in patients with type 2 diabetes mellitus on hemodialysis. Kidney Int. 2008;74(11):1461-7.
13. Abergel E, Tase M, Bohlender J, Menard J, Chatellier G. Which definition for echocardiographic left ventricular hypertrophy? Am J Cardiol. 1995; 75(7):498-502.
14. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. National Kidney Foundation. Am J Kidney Dis. 2002;39(2 Suppl 1):S1-S266.
15. Haneda M, Utsunomiya K, Koya D, Babazono T, Moriya T, Makino H, et al.; Joint Committee on Diabetic Nephropathy. A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy. J Diabetes Investig. 2015;6(2):242-6.

International Journal of BioMedicine 6(1) (2016) 22-26
doi: 10.21103/Article6(1)_OA4

ORIGINAL ARTICLE

Nephrology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Complex Assessment of Risk Factors for the Development of Cardiovascular Calcification in Hemodialysis Patients

Liliya I. Rudenko, PhD*; Mikhail M. Batiushin, PhD, ScD;
Aleksandr A. Kastanayan, PhD, ScD; Dmitriy G. Pasechnik, PhD, ScD;
Igor V. Dudarev; Elena A. Kartashova, PhD; Ekaterina S. Lapina

Rostov State Medical University
Rostov-on-Don, the Russian Federation

Abstract

The aim of the present study was the integrated assessment of the role of non-traditional factors (inflammation, malnutrition, calcium-phosphorus disorder and imbalance in the concentration of inducers and inhibitors of calcification) in forming cardiovascular calcification (CVC) and the structural-functional rearrangement of LV myocardium in patients with chronic kidney disease (CKD) receiving hemodialysis (HD).

Materials and Methods: The present study included 84 HD patients with CKD 5D stage. We evaluated 3 components of the Dialysis Malnutrition Score (DMS), according to which body mass index (BMI), the level of serum albumin, and the percent saturation of transferrin with iron were determined. We also analyzed CRP, fibrinogen, and beta-2 microglobulin, and calculated the number of points (from zero to 2) according to the Glasgow Prognostic Score (GPS), which allowed us to combine indicators of inflammation and make a common prognostic assessment.

The serum levels of protein alpha-Klotho и FGF-23 were determined by enzyme immunoassay. Echocardiographic measurements were performed using B-mode, M-mode and Doppler-mode. Different patterns of LV geometry were identified according to Ganau et al. (1992). The severity of calcification was estimated by a semi-quantitative scale for assessing the degree of calcification of heart structures according to the National recommendations for CKD-MBD (2010).

Results: The increased risk for development of CVC, LVH, and diastolic dysfunction was associated with markers of malnutrition, anemia, and inflammation in HD patients. Reduced serum alpha-Klotho level, hypoalbuminemia and a high level of FGF-23 had a prognostic value in CVC formation. **(Int J Biomed. 2016;6(1):22-26.).**

Keywords: *chronic kidney disease; end-stage renal disease; renal replacement therapy; cardiovascular calcification.*

Abbreviations

CVD, cardiovascular disease; **CKD**, chronic kidney disease; **LVH**, left ventricular hypertrophy; **IVST**, interventricular septal thickness; **LVPWT**, left ventricular posterior wall thickness; **LVMI**, left ventricular mass index; **CRP**, C-reactive protein.

Introduction

In recent years, the number of patients with end-stage renal disease (ESRD) who require renal replacement therapy (RRT) is increasing dramatically worldwide (KDIGO, 2012). The main cause of mortality among dialysis patients with ESRD is CVD, which occurs more often in patients receiving hemodialysis (HD), than in the general population [1]. The frequency of deaths from CVD is high among young dialysis patients, despite the fact that in the population cardiovascular mortality progresses with age [2]. Thus, ESRD has not only medical but also socio-economic significance, and thus requires researching and clarifying mechanisms of calcification to find new diagnostic methods and make predictive algorithms of CVC development in dialysis patients.

The increased risk of adverse CVD outcomes is largely due to calcification of heart valves and vessel walls, which is an element of CKD- mineral bone disorder (CKD-MBD) along

***Corresponding author:** Liliya I. Rudenko, PhD. Rostov State Medical University, Rostov-on-Don, Russia. E-mail: rudenko.liliya@mail.ru*

with the imbalance in the metabolism of calcium, phosphorus, parathormone (PTH) and bone tissue. Previously, the calcium-phosphorus imbalance was estimated as renal osteodystrophy that was characterized by morpho-functional disorders of bone tissue, and then it was found that laboratory abnormalities in the metabolism of calcium, phosphorus and PTH levels are independent indicators of prognosis and are associated with the progression of CKD, cardiovascular complications and mortality [3,4].

Understanding the risk factors, etiology and pathogenesis of CKD–MBD has significantly improved due to data on known markers of mineral metabolism, and researchers have identified previously unknown factors that are components of renal failure. In addition to the previously known participants of the calcium-phosphorus homeostasis, new elements of metabolism are being studied, elements that could be involved in the process of CVC. In CKD patients, calcification is associated with both traditional risk factors, such as age, diabetes, hypertension, smoking, dyslipidemia, and non-traditional factors, including decreased GFR, anemia, hyperphosphatemia, secondary hyperparathyroidism (sHPT), inflammation, oxidative stress and imbalance in the concentration of inducers and inhibitors of calcification.

The aim of the present study was the integrated assessment of the role of non-traditional factors in forming CVC and the structural-functional rearrangement of LV myocardium in CKD patients receiving hemodialysis (HD).

Materials and Methods

The present study included 84 HD patients with CKD 5D stage (45 male and 39 female; mean age 53.5±14.9 years). The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the Rostov State Medical University Ethics Committee. Written informed consent was obtained from all participants.

All HD patients received online hemodiafiltration treatments three times per week for 4-5h, with ultra-pure bicarbonate-based dialysate using a high flux polysulfone membrane.

Exclusion criteria were age <18 or >80 years, pathology of the parathyroid glands, uncontrolled intake of vitamin D and/or calcimimetics, the history for drug and alcoholism, pathology of the bone tissue, mental disorders, kidney transplant.

We evaluated 3 components of the Dialysis Malnutrition Score (DMS), according to which body mass index (BMI), the level of serum albumin, and the percent saturation of transferrin with iron were determined. We also analyzed CRP, fibrinogen, and beta-2 microglobulin, and calculated the number of points (from zero to 2) according to the Glasgow Prognostic Score (GPS), which allowed us to combine indicators of inflammation and make a common prognostic assessment [5].

The serum levels of protein alpha-Klotho и FGF-23 were determined by enzyme immunoassay. The average level of serum FGF-23 was 69.3±23.5 pg/ml, alpha-Klotho – 460.4±141.3 pg/ml.

Serum levels of calcium, inorganic phosphorus, calcium-phosphorus compositions, intact PTH (iPTH) were investigated. sHPT was defined as PTH level >300 pg/ml; hyperphosphatemia at the phosphate concentration >1.45mmol/l (the National recommendations for CKD-MBD, 2010).

Echocardiographic measurements were performed using B-mode, M-mode and Doppler-mode. Different patterns of LV geometry were identified according to Ganau et al. (1992).

The severity of calcification was estimated by a semi-quantitative scale for assessing the degree of calcification of heart structures according to the National recommendations for CKD-MBD (2010). Patients underwent plain radiography of the abdominal cavity in lateral projection for the assessment of abdominal aortic calcification (AAC). Calcification was assessed using Kauppila's scale, which allowed us to assess the severity of calcification in the aorta at the level of the first through fourth lumbar vertebrae, and in the anterior and posterior walls of the aorta. Calcific densities were graded on a 0 to 3 scale at each lumbar vertebral segment. A score of 0 denoted no aortic calcific deposits; 1, small scattered calcific deposits filled less than one-third of the longitudinal wall of the aorta; 2, one-third or more but less than two-thirds of the longitudinal wall of the aorta was calcified; and 3, two-thirds or more of the longitudinal wall of the aorta was calcified. A separate score was determined for the anterior and posterior aorta, and the values were summed across the 4 vertebrae, resulting in an AAC index that could range from 0 to 24 points [6].

Statistical analysis of data was performed using the software Statistica 6.0. The mean (M) and standard deviation (SD) were calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Comparisons between three groups were performed with the one-way ANOVA with Tukey's post-hoc test. Group comparisons with respect to categorical variables are performed using $\chi 2$ tests. Linear and non-linear regression analysis, as well as logistic regression analysis was conducted. A probability value of $P<0.05$ was considered statistically significant.

Results

CVC was revealed in 42/50% of HD patients. Increased risk of calcification was detected in patients with low total protein *(P=0.003)*. A decrease in the level of total protein, even by 2 g/l, increased the risk of calcification by more than 5%. Low albumin levels significantly increased CVC risk *(P=0.01)*.The average albumin level in patients with calcification was detected in the lower range (37.2±4.4 g/l) compared with patients without diagnosed calcification (39.7±3.9 g/l, $p<0.01$); this can be explained by the role of malnutrition in the development of calcification. Thus, malnutrition, specifically low levels of albumin and total protein, is associated with a rising risk of CVC.

Patients with calcification of heart valves had a higher pulse pressure compared with patients without calcification before (66.2±16.9 vs 58.5±12.3, respectively, *P=0.02*), during (65.1±14.4 vs 57.3±13.0, respectively, *P=0.01*) and after hemodialysis (62.9±13.3 vs 56.6±14.3, respectively, *P<0.05*)

and in the interdialytic period (63.4±19.6 vs 51.5±14.4, respectively, *P<0.01*).

The echocardiography performed in the interdialytic period showed that the increasing degree of calcification in HD patients increased the risk of LVH (*P=0.03*), likely due to the hemodynamic changes because of existing areas of calcification. According to the degree of calcification of cardiac structures by a semi-quantitative scale, a 2-point score leads to an increase in the probability of LVH up to 71.9%, and in cases of maximum severity, up to 91.9%. We identified a positive correlation between the severity of heart valve calcification and LVMI (an index of myocardial mass) (r=0.27, *P=0.016*). Among patients with diagnosed CVC, LVMI was higher than in patients without changes in the valves (132.8±31.4 and 114.4±34.9 g/m^2, respectively, *P=0.02*). Characteristics are presented in the Table 1. Furthermore, IVST and LVPWT had a positive correlation with the severity of calcification (r=0.329, *P=0.01* and r=0.31, *P=0.02*, respectively).

Table 1.

Characteristics of clinical status of HD patients with or without CVC

Criterion	Patients with CVC- (n=42)	Patients with CVC+ (n=42)	*p*
Serum protein, g/l	71.05±4.26	67.3±4.49	*<0.01*
Serum albumin, g/l	39.7±3.9	37.2±4.4	*<0.01*
PP (before HD), mmHg	58.5±12.3	66.2±16.9	*<0.02*
PP (during HD), mmHg	57.3±13.0	65.1±14.4	*=0.01*
PP (after HD), mmHg	56.6±14.3	62.9±13.3	*<0.04*
PP (interdialytic period), mmHg	51.5±14.4	63.4±19.6	*<0.01*
LVMI, g/m^2	114.4±34.9	132.8±31.4	*<0.02*

PP - pulse pressure

LV remodeling was identified in 63/76.8% of HD patients. Concentric remodeling, concentric hypertrophy, and eccentric LVH were detected in 31/37.8%, 26/31.7% and 9/7.3% of patients, respectively. The diastolic dysfunction was identified in 70.4% of HD patients. An impaired relaxation pattern or grade 1 diastolic dysfunction was detected in 53/65,4% of patients and pseudonormal mitral inflow pattern or grade 2 diastolic dysfunction in 4/5,0% of patients. Restrictive filling dynamics (grade 3 and 4 DD) were not identified among the studied patients.

In the presence of cardiovascular calcification, the average DMS score was significantly higher (1.07±0.62 points), whereas in patients without calcification it was 0.40±0.57 points (*P<0.01*). One of the DMS components in HD patients is albumin; we found a significant negative correlation between the albumin level and LVMI (r=- 0.32, *P=0.003*).

Parameters of systemic inflammation were associated with LVH; the increasing GPS score significantly increased the manifestation of LVH and an increase in left atrium size. The presence of high phosphorus and two GPS points in HD patients increased the risk of CVC up to 92.2%, whereas in patients with normal phosphorus and zero GPS points, the risk was only 11.5% (*P<0.05*). In CKD patients, CRP is a marker of an inflammatory response. However, the role of CRP as a predictor of the development of CVC is ambiguous.

Logistic analysis showed that the increased CRP levels significantly increased the risk of CVC (*P=0.04*).

Patients with anemia had a larger LVPWT in comparison with patients with a normal range of hemoglobin (*P=0.01*). In patients with diagnosed CVC, hemoglobin level was lower than in the patients without calcification (*P<0.01*). Thus, the risk of CVC in patients with latent anemia amounted to 15.5%. In patients with the first degree of anemia, the probability of calcification was in the range of 15.5% to 36.6%; with the second degree, 37% to 64%; and with the third degree, more than 64.4% (*P=0.03*).

An increase in serum FGF-23 level and a decrease in serum alpha-Klotho level were identified in patients with progression of renal dysfunction. A negative correlation was established between the duration of dialysis and the alpha-Klotho level (r=-0.325, *P=0.006*). When the alpha-Klotho concentration was about 800 pg/ml, CVC risk was minimal and amounted only 4%; a reduction in the protein level by 50% increased the risk almost 6 times up to 23.3%, and a four-fold decrease, up to 43.3%. While the level of alpha-Klotho was below 100 pg/ml, the probability of the aortic calcification was more than 50% (*P=0.02*). The gain of CVC risk also was found in patients with high serum FGF-23 level and persistent high rates of urea (*P=0.048*). The CVC risk increased in patients with hypoalbuminemia and rising levels of FGF-23 (*P=0.01*). Thus, hypoalbuminemia with a high level of FGF-23 had a prognostic value in CVC formation.

Discussion

Given that CKD is considered a disease, manifested not only as a mineral bone disorder, components of the system's inflammatory response, malnutrition and anemia were analyzed to confirm the high risk of calcification in studied patients. It is known that protein-energy malnutrition is a predictor of mortality in patients with ESRD [7]. One of the most common markers of malnutrition is hypoalbuminemia [8]; low levels of this protein are involved in impeding the transport of calcium, phosphorus and other electrolytes, which can contribute to arterial calcification. We found that low serum albumin correlated with the severity of calcification. In addition, there was a negative correlation between the albumin level and the relationship between early diastolic mitral annular velocity (EA) and tissue Doppler diastolic velocity (Ea), which partially describes the participation of malnutrition in the development of DD in HD patients.

Inflammation is one of the fundamental pathophysiological phenomena associated with renal failure, particularly among patients receiving chronic HD [9,10]. L.Yan et al., in models on mice, found that an enhanced production of inflammatory cytokines induces the development of myocardial hypertrophy [11]. In addition, a few years earlier S.Barry described the role of proinflammatory chemokines in LVH formation [12].

In most patients (64.3%), we registered an increase in

CRP level and found that the higher CRP levels increased the probability of CVC. In addition, the likelihood of DD was increased with the growth of CRP level.

In the study [13] examining inflammation and LV diastolic dysfunction in peritoneal dialysis (PD) patients, the role of pro-inflammatorycytokines in a group of PD patients and controls was assessed. That study identified a significant correlation between LV diastolic dysfunction and serum TNF-a, and IL-6 levels in PD patients and an interaction between PD and inflammation. TNF-a was also shown to aggravate LV diastolic dysfunction.

Analyzing the parameters according to GPS, we found that GPS points in patients with CVC were higher than among people without calcification. The likelihood of developing calcification reached 60% in patients with maximum points. In addition, high GPS points increased the risk of LVH development, which is positively correlated with LVMI and LVPWT. Evidence is accumulating that chronic inflammation plays an important role in LVH. Cytokines are implicated in the development of cardiac hypertrophy, most likely via downstream activation of pro-inflammatory transcription factors such as nuclear factor-κB (NF-κB) [14].

The participation of albumin in LVH development is not completely known; however, there is an assumption that malnutrition in combination with chronic overload in dialysis patients contributes to an increase in the extracellular fluid volume, which is a risk factor for LVH development..

Persistent uremia and constant contact with the dialysis membrane, the release of pro-inflammatory cytokines, oxidative stress, and persistent activation of the inflammatory response [15] underlie the changes in morphological and functional properties of smooth muscle cells and development of calcification. The role of another acute-phase protein, β2-microglobulin, was statistically significant only in patients with an elevated pulse pressure estimated in the interdialytic period. Thus, it was found that the enlargement in pulse pressure and an increased level of β2-microglobulin contribute to the increasing probability of CVC development due to progressive vascular stiffness in CKD patients. According to M.Masuda et al. [16], serum β2-MG concentrations correlated significantly and positively with the echocardiographic parameters of left ventricular hypertrophy (LVH) in long-term HD patients; a deposition of β2-MG amyloid in the heart may be associated with LVH progression.

Thus, the obtained data on the relationship of systemic inflammation, cardiovascular calcification and anatomical and functional changes in the heart in patients receiving chronic HD, suggest that continuous activation of inflammatory responses in this category of patients is an important independent risk factor for cardiovascular disorders in dialysis patients.

Our data revealed that the ferritin level positively correlated with the degree of valve calcification, EA, and Ea in HD patients. In addition, the relationship between the fibrinogen level and LVM can be explained by the increased load on the myocardium under hyperfibrinogenemia.

Anemia contributes to the development of hemodynamic and non-hemodynamic mechanisms, which results in a compensatory increase in hemoglobin levels. Hemodynamic compensatory changes in existing chronic anemia include an increasing cardiac output because of reduced blood viscosity and vasodilation contributing to tissue perfusion. Anemia leads to stimulation of the sympathetic nervous system as well as to activation of the renin-angiotensin-aldosterone system, which is closely associated with increased oxidative stress [17] and defines the hyperdynamic state of the myocardium, causing hypertrophy. In our study, we also found that patients with severe anemia were characterized by a high risk of LVH development. The influence of anemia on the process of calcification may be due to the action of hypoxia leading to increased CVC risk.

Soft-tissue calcification is a prominent feature in both chronic kidney disease (CKD) and experimental Klotho deficiency. Wild-type mice with CKD had very low renal, plasma, and urinary levels of Klotho [18]. In humans, Hu et al. observed a graded reduction in urinary Klotho starting at an early stage of CKD and progressing with loss of renal function [18]. The beneficial effect of Klotho on vascular calcification was a result of more than its effect on renal function and phosphatemia, suggesting a direct effect of Klotho on the vasculature. Our study also found a direct association between reduced serum alpha-Klotho level and high CRP and the relationship between two studied morphogenetic proteins and β2-microglobulin. Based on these data it is possible to use serum FGF-23 and alpha-Klotho as diagnostic markers [19]. The results of the study allowed us to make the original predicting scheme for development of CVC, geometric remodeling, and functional alterations of LV, all of which indicates that the process of calcification is not only a calcium-phosphorus disorder.

M. Nasrallah defined that FGF-23 and ACI were significantly increased, and FGF-23 was independently associated with aortic calcification in haemodialysis patients [20]. Fibroblast growth factor 23 (FGF23) is the latest mineral metabolite to be linked to CVD and death. Like phosphate, PTH, and vitamin D before it, altered FGF23 levels were first recognized as an independent risk factor for mortality in dialysis patients [21] but later were shown to be a risk factor for CVD and death in the general population [22]. M. Kanbay et al. [23] provide the latest report on FGF23 and cardiovascular disease . In a cross-sectional study, the authors analyzed coronary angiograms from 177 patients who had “mild CKD” and for whom standard mineral metabolites were measured along with FGF23 and fetuin A levels. This study suggested that in a relatively young population with mild-to-moderate alteration of kidney function and with less traditional cardiovascular risk factors, anomalies of the serum FGF 23 and fetuin A levels appeared early in the course of disease and were independent major predictors for extent of coronary artery disease.

Conclusion

The study and identification of factors contributing to an increased risk for CCV is important for HD patients. The research into new CCV factors will help to improve the quality and length of life for those patients who receive RRT.

Competing interests

The authors declare that they have no competing interests.

References

1. Moody WE, Edwards NC, Chue CD, Ferro CJ, Townend JN. Arterial disease in chronic kidney disease. Heart. 2013; 99(6):365-72.
2. Foley RN, Collins AJ, Herzog CA, Ishani A, Kalra PA. Serum phosphorus levels associate with coronary atherosclerosis in young adults. J Am Soc Nephrol. 2009;20: 397–404.
3. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. J Am Soc Nephrol. 2004;15:2208–18.
4. Ganesh SK, Stack AG, Levin NW, Hulbert-Shearon T, Port FK. Association of elevated serum PO(4), Ca × PO(4) product, and parathyroid hormone with cardiac mortality risk in chronic hemodialysis patients. J Am Soc Nephrol. 2001; 12:2131–8.
5. Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dagg K, Scott HR. A prospective longitudinal study of performance status, an inflammation-based score (GPS) and survival in patients. Br J Cancer. 2005;92(10):1834–6.
6. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PW. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. Atherosclerosis. 1997;132(2):245–50.
7. Rambod M, Bross R, Zitterkoph J, Benner D, Pithia J, Colman S, et al. Association of Malnutrition-Inflammation Score with quality of life and mortality in hemodialysis patients: a 5-year prospective cohort study. Am J Kidney Dis. 2009;53(2):298-309.
8. Fouque D, McKenzie J, de Mutsert R, Azar R, Teta D, Plauth M, et al. Use of a renal-specific oral supplement by haemodialysis patients with low protein intake does not increase the need for phosphate binders and may prevent a decline in nutritional status and quality of life. Nephrol Dial Transplant 2008; 8 (9):2902–10.
9. Stenvinkel P, Alvestrand A. Inflammation in end-stage renal disease: sources, consequences, and therapy. Semin Dial. 2002;15(5):329-37.
10. Rudenko LI, Batjushin MM, Kastanajan AA, Vorob'ev BI. Cardiovascular calcification risk prognosis in patients receiving chronic hemodialysis. Nefrologiia. 2015;19(5):72-6. [Article in Russian].
11. Yan L, Mathew L, Chellan B, Gardner B, Earley J, Puri TS, et al. S100/Calgranulin-mediated inflammation accelerates left ventricular hypertrophy and aortic valve sclerosis in chronic kidney disease in a receptor for advanced glycation end products-dependent manner. Arterioscler Thromb Vasc Biol. 2014;34(7):1399-411.
12. Barry SP, Townsend PA. What causes a broken heart--molecular insights into heart failure. Int Rev Cell Mol Biol 2010; 284:113–79.
13. Lee JK, Lin HH, Tsai CT, Chen JJ, Kuo CC, Lien YC, et al. Differential association of proinflammatory cytokines with left ventricular diastolic dysfunction in subjects with and without continuous ambulatory peritoneal dialysis. Nutr Metab Cardiovasc Dis. 2012;22(11):974–80.
14. Smeets PJ, Teunissen BE, Planavila A, de Vogel-van den Bosch H, Willemsen PH, van der Vusse GJ, et al. Inflammatory pathways are activated during cardiomyocyte hypertrophy and attenuated by peroxisome proliferator-activated receptors PPARalpha and PPARdelta. J Biol Chem. 2008; 283(43):29109-18.
15. Wang AY, Wang M, Woo J, Lam CW, Lui SF, Li PK, et al. Inflammation, residual kidney function, and cardiac hypertrophy are interrelated and combine adversely to enhance mortality and cardiovascular death risk of peritoneal dialysis patients. J Am Soc Nephrol. 2004;15(8);2186-94.
16. Masuda M, Ishimura E, Ochi A, Tsujimoto Y, Tahahra H, Okuno S, et al. Serum β2-microglobulin correlates positively with left ventricular hypertrophy in long-term hemodialysis patients. Nephron Clin Pract. 2014;128(1-2):101-6.
17. Aronow WS, Ahn C, Kronzon I. Association of mitral annular calcium with symptomatic peripheral arterial disease in older persons. Am J Cardiol. 2001;88(3):333–4.
18. Hu MC, Shi M, Zhang J, Quiñones H, Griffith C, Kuro-o M, et al. Klotho deficiency causes vascular calcification in chronic kidney disease. J Am Soc Nephrol. 2011;22(1):124-36.
19. Rudenko LI, Batjushin MM, Kastanajan AA, Vorob'ev BI, Sarvilina IV. The serum alpha-Klotho, FGF-23 and their participation in cardiovascular calcification. Klinicheskaja nefrologija. 2015;1:23-6.[Article in Russian].
20. Nasrallah MM, El-Shehaby AR, Salem MM, Osman NA, El Sheikh E, Sharaf El, et al. Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification in haemodialysis patients. Nephrol Dial Transplant. 2010;25(8): 2679–85.
21. Gutierrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med. 2008;359:584–92.
22. Ix JH, Shlipak MG, Wassel CL, Whooley MA. Fibroblast growth factor-23 and early decrements in kidney function: The Heart and Soul Study. Nephrol Dial Transplant. 2010; 25:993–7.
23. Kanbay M, Nicoleta M, Selcoki Y, Ikizek M, Aydin M, Eryonucu B, et al. Fibroblast growth factor 23 and fetuin A are independent predictors for the coronary artery disease extent in mild chronic kidney disease. Clin J Am Soc Nephrol. 2010;5:1780–86.

International Journal of Biomedicine 6(1) (2016) 27-32
doi: 10.21103/Article6(1)_OA5

ORIGINAL ARTICLE

Gastroenterology & Hepatology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Adipocytokine Imbalance and Ghrelin in the Development of Insulin Resistance in Patients with Chronic Hepatitis C

Larisa Tkachenko, PhD*[1]; Viktor Maleev, PhD, ScD[2]; Ludmila Rtishcheva, PhD, ScD[1]

[1]*Stavropol State Medical University, Stavropol, Russia*
[2]*Central Research Institute of Epidemiology, the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, Moscow, Russia*

Abstract

The aim of this study was to determine the role of the most informative indicators of adipocytokine status and ghrelin in insulin resistance (IR) formation in chronic hepatitis C (CHC) patients at different stages of liver fibrosis.

Materials and Methods: This study included 205 CHC patients with HCV genotypes 1 and 3. A comparative analysis of the laboratory parameters was carried out in a group of patients with IR (n=110) and without IR (n=95) and in patients depending on the stage of liver fibrosis. The serum *levels* of adipocytokines and ghrelin were determined using Bachem Group (USA) and Immundiagnostik AG (Germany) test systems with microplate reader Elx800 (FinBio, Finland). Diagnosis and assessment of the degree of fibrosis was performed by liver biopsy, liver elastometry and calculation test FibroTest.

Results: Obtained data allow us to regard the decreased secretion of ghrelin, and increased production of leptin, resistin and TNF-α as a component involved in the formation of IR in CHC patients. **(Int J Biomed. 2016;6(1):27-32.).**

Keywords: *chronic hepatitis C; adipocytokines; ghrelin; insulin resistance.*

Introduction

Hepatitis C virus (HCV) infection affects hundreds of millions of people worldwide. Globally, the prevalence and number of people with anti-HCV has increased from 2.3% to 2.8% and from >122 million to >185 million between 1990 and 2005 [1].

Most acutely infected patients develop chronic hepatitis and become a potential source of virus transmission, and as many as 1 in 5 will develop cirrhosis and its complications [2]. HCV is also an increasingly recognized important cause of extrahepatic manifestations, including insulin resistance (IR) [3]. IR is of global importance since it is closely linked to the epidemic condition of obesity, precedes and predicts the development of type 2 diabetes mellitus (T2DM), and increases the risk of life-threatening complications such as cardiovascular diseases, renal failure, and infections [4]. However, these complications are not major causes of death in cirrhotic patients with IR. In contrast, the development of intrahepatic complications, including hepatocellular carcinoma (HCC), is known to be associated with IR [5]. IR is extremely common in patients with chronic HCV infection and has been associated with increased disease severity, extrahepatic manifestations and decreased response to antiviral therapy [6, 7]. Whereas the overall prevalence of IR is 10%-25% of the population [8], the prevalence IR in HCV infection reaches figures ranging from 30% to 70% [9,10]. Moreover, IR with HCV infection is increased at early stages of liver disease without liver fibrosis (LF), and is on average significantly higher than that found in patients with chronic hepatitis B, matched for age and body mass index (BMI) [11]. Understanding the basis of such associations is of paramount importance to inform treatment strategies for patients with HCV.

The main acquired factors of IR include abdominal obesity, aging, hyperglycemia, medications, and, recently, HCV infection. Obesity is associated with IR, hepatic steatosis and over-expression of TNF-α. All of these factors increase the risk of fibrosis and decreased antiviral efficacy. With obesity, the increased adipocytes produce both free fatty acids (FFAs), and adipocytokines. Adipocytokines, including leptin, resistin, TNF-α, and IL-6 cause liver injury associated with fat infiltration, causing steatosis, inflammation and LF. All these substances affect tissue sensitivity to insulin:

***Corresponding author*:** *Larisa Tkachenko, PhD. The Stavropol State Medical University,. Stavropol, Russia. E-mail: larisa308@mail.ru*

activation of TNF-α causes IR, the elevated level of leptin, which is also able to reduce the action of insulin in the liver, which was detected with obesity. Both TNF-α, and leptin have autocrine antilipolytic effect and inhibit the action of insulin in adipocytes themselves [12]. There are data that attest to the role of ghrelin, a peptide hormone produced by ghrelinergic cells, in the formation of IR. More than 80% of circulating ghrelin is synthesized and secreted into the blood by endocrine cells of the gastrointestinal tract [13]. It was found that ghrelin decreases the utilization of fat, promotes obesity (stimulates lipogenesis and inhibits lipolysis), and activates the synthesis of lipids by the liver. Ghrelin hypersecretion causes obesity. However, with the development of obesity, the ghrelin level in the blood decreases and persistent hypoghrelinemia occurs [14]. We have determined an inverse relationship between ghrelinemia and IR in adults and children: the lower the level, the more pronounced the IR [15]. In HCV infection, adipocytokines and ghrelin may be a link between viral infection, steatosis and metabolic disorders [16].

The aim of this study was to determine the role of the most informative indicators of adipocytokine status and ghrelin in IR formation in chronic hepatitis C (CHC) patients at different stages of LF.

Materials and Methods

We examined 205 CHC patients (mean age 44.2±10.3) with HCV genotypes 1 and 3. Males (128/62.4%) and the patients with HCV genotype 1 (123/60.0%) dominated. A comparative analysis of the laboratory parameters was carried out in a group of patients with IR (n=110) and without IR (n=95) and in patients depending on the stage of LF.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the Stavropol State Medical University Ethics Committee. Written informed consent was obtained from all participants.

The observation was conducted during 2011-2014 in the outpatient departments of Stavropol Regional Clinical Consultative and Diagnostic Center.

The examination was conducted in accordance with EASL Clinical Practice Guidelines [17]. HCV infection was defined by the presence in serum of anti-HCV antibodies using the third generation ELISA. HCVs infection was confirmed by performing the COBAS TaqMan HCV-test. The RNA titter was expressed in international units (IU/mL). The detection limit is <40 IU/mL with a positive rate of 95%.

The serum concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein (VLDL-C), and high-density lipoprotein cholesterol (HDL-C) were evaluated using test systems of the company Thermo Fisher Sientific (Finland) with the automatic biochemical analyzer Konelab 30i (Finland).

The serum concentration of insulin (μIU/ml) was determined using the Human Insulin ELISA kit from DRG Diagnostics (Germany). For ELISA assays, a microplate reader Elx800 (FinBio, Finland) was used. IR status was calculated by using the homeostatic model assessment-insulin resistance (HOMA-IR); it was determined by the equation:

HOMA-IR=(fasting insulin [μIU/mL]×fasting glucose [mM/L])/22.5. IR was considered as HOMA-IR >2.77.

The serum levels of adipocytokines and ghrelin were determined using Bachem Group (USA) and Immundiagnostik AG (Germany) test systems with microplate reader Elx800 (FinBio, Finland).

Diagnosis and assessment of the degree of fibrosis was performed by liver biopsy, liver elastometry and calculation test FibroTest (BioPredictive, France). Knodell Histology Activity Index (HAI) was used to grade necro-inflammatory activity (Knodell et al., 1981). METAVIR group scoring system (F0-F4) was used for detecting the stage of fibrosis (French METAVIR, 1994). Hepatic steatosis was determined by ultrasound examination.

Metabolic syndrome (MetS) was defined according to IDF (2005) criteria [18]. Central (abdominal) obesity was determined as waist circumference (WC) ≥ 94 cm for men and ≥ 80 cm for women.

Exclusion criteria were antiviral therapy before liver biopsy, regular consumption of alcohol (>30g/day ethanol for men and >20g/day ethanol for women), and co-infection with other viruses (HBV, HAV, HDV, HIV), drug-induced and autoimmune hepatitis.

Thirty-five healthy volunteers, matched for sex, age and BMI, served as a control group.

The statistical analysis was performed using the statistical software «Primer of Biostat 4.0» and «STATISTICA 7». The mean (M) and standard deviation (SD) were calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution were tested by the Mann-Whitney U-test. Comparisons between three groups were performed with the one-way ANOVA with Tukey's post-hoc test. Spearman's rank correlation coefficient was calculated. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, 2-Tail Fisher's exact test when expected cell counts were less than 5.

Sensitivity, specificity, positive predictive value, and negative predictive value for each variable were calculated, including 95% confidence intervals (CIs) and relative risk (RR). A probability value of $P<0.05$ was considered statistically significant.

Results

HOMA-IR ≥2.77 was detected in 110/53.7% patients, hepatic steatosis in 61/29.8% patients, T2DM in 40/19.5% patients, and severe LF (METAVIR stage, F3 or F4) in 87/42.4% patients, including liver cirrhosis in 54/62.1% patients.

It was found that CHC patients with IR were older, they had a longer duration of disease, higher BMI and symptoms of abdominal obesity, compared to the patients with HOMA-IR<2.77 (Table 1). Moreover, in the IR group, there were more patients who were overweight and obese compared to CHC patients without IR, 89.0% and 40% ($P<0.001$), respectively.

In the HOMA-IR≥2.77 group, cholestatic syndrome was formed, evidenced by the significantly higher rate of total and conjugated bilirubin and alkaline phosphatase. This group of patients was characterized by a higher HAI and METAVIR F stage, with no difference in viral load.

Table 1.

Baseline characteristics for studied patients

Variable	HOMA-IR ≥2.77 n=110	HOMA-IR < 2.77 n=95	*P-value*
HCV Genotype 1, n/%	75/68.2	48/50.5	*0.01*
Steatosis, n/%	51/46.4	10/10.5	*0.000*
Age, yrs	46.0±8.5	40.8±11.1	*0.000*
Duration of the disease, yrs	13.3 ±7.8	10.2±6.6	*0.003*
Body mass index, kg/m^2	28.8±5.2	25.6±4.6	*0.000*
Waist, cm	94.1±18.5	82.0±14.3	*0.000*
WHR	0.92±0.14	0.8±0.1	*0.000*
Total bilirubin, μmol/l	22.9±16.4	16.4±8.2	*0.001*
Conjugated bilirubin, μmol/l	7.7±5.5	4.9±2.6	*0.000*
ALT/N	3.1±2.6	2.5±2.0	*0.069*
AST/N	2.4±1.9	1.6±1.2	*0.000*
γ-glutamyl transferase/N	1.9±1.6	1.9±2.1	*1.000*
Alkaline phosphatase/N	1.3±0.9	1.0±0.7	*0.009*
Fasting glucose, mmol/l	6.0±1.3	4.9±1.4	*0.000*
Fasting insulin, μIU/ml	19.2±9.7	6.8±3.4	*0.000*
HOMA-IR	5.1±2.7	1.5±0.7	*0.000*
Knodell HAI	11.2±2.6	8.9±3.0	*0.000*
METAVIR F0-F4	2.8±1.0	1.9±1.2	*0.000*
Viral load, log 10 IU/ml	5.2±1.3	5.2±1.4	*1.000*

TC level was higher in both groups compared with the control, but within the reference values (5.2 mmol/l). The decrease of HDL-D accompanied by increased VLDL-C in patients with IR contributed to the formation of secondary dyslipidemia of IV type by D. Fredrickson,1970 (Table 2).

Table 2.

Laboratory data of studied groups in relation to IR

Variable	Control group n=35	HOMA-IR ≥ 2.77 n=110 (a)	P_{a-b}	HOMA-IR < 2.77 n=95 (b)	one-way ANOVA *P-value*
TC, mmol/l	4.14±0.6	4.9±1.5*	*0.509*	4.7±1.2	*0.011*
TG, mmol/l	1.36±0.34	1.4±0.6	*0.000*	1.1±0.5*	*0.000*
HDL-C, mmol/l	1.3±0.32	0.9±0.3*	*0.000*	1.2±0.4	*0.000*
LDL-C, mmol/l	1.7±0.66	3.3±1.3*	*0.383*	3.1±0.9*	*0.000*
VLDL-C, mmol/l	0.49±0.14	0.6±0.3	*0.011*	0.5±0.2	*0.006*
Resistin, ng/ml	8.2±1.4	15.7±9.1*	*0.000*	10.4±5.9*	*0.000*
Leptin,ng/ml	5.8±1.1	23.0±16.2*	*0.000*	13.6±14.0*	*0.000*
Adiponectin,μg/ml	11.5±1.3	20.9±18.5*	*0.760*	22.4±13.5*	*0.001*
Ghrelin, ng/ml	0.4±0.1	0.3±0.2	*0.000*	0.8±0.5*	*0.000*
TNF-α, pg/ml	0.6±0.2	4.3±4.8*	*0.001*	2.4±2.6*	*0.000*

*- P <0.05 in comparison with control group

In the HOMA-IR≥2.77 group, a significant increase in indices both of pro-inflammatory adipocytokines (resistin, TNF-α, leptin) and of anti-inflammatory hormone adiponectin was revealed on the background of ghrelin decrease.

With the progression of fibrosis, the number of patients with IR has increased. IR was detected in 23.5% of patients with METAVIR F0-1 stage, in 56.7% of patients with METAVIR F2 stage, in 68.7% of patients with METAVIR F3 stage, and in 68.5% of patients with METAVIR F4 stage (*P<0.001*).

CHC patients with early signs of fibrosis (F0-1 METAVIR) and IR+ compared to IR- (Table 3) was characterized by metabolic changes and increased TNF-α blood level. Ghrelin level, in contrast, was lower on the background of IR. In the group of patients with IR, we found a high negative correlation of ghrelin with resistin (rs=-0.77), leptin (rs=-0.87), BMI (rs=-0.9), WC (rs=-0.77), waist-hip ratio (WHR) (rs=-0.67), and HOMA-IR (r=-0.79) (P<0.001 for each value). In patients with METAVIR F0-1 stage, IR was associated with a decrease in ghrelin level (Table 4).

Table 3.

Laboratory data of studied patients with METAVIR F0-1 in relation to IR

Variable	IR(+) n= 12	IR(-) n= 39	MW U-test *P-value*
BMI, kg/m^2	26.7±3.6	24.8±3.2	*>0.05*
Waist, cm	89.5±12.2	79.5±12.3	*0.007*
WHR	0.88±0.1	0.79±0.1	*0.01*
Total bilirubin, μmol/l	21.9±6.9	16.2±6.5	*0.006*
Conjugated bilirubin, μmol/l	4.4±1.4	5.0±1.7	*>0.05*
Triglycerides, mmol/l	1.2±0.4	0.9±0.4	*0.01*
HDL-C, mmol/l	1.1±0.1	1.2±0.2	*0.05*
LDL-C, mmol/l	2.8±1.1	3.2±0.7	*>0.05*
VLDL-C, mmol/l	0.6±0.2	0.4±0.2	*0.01*
Fasting glucose, mmol/l	5.9±1.3	5.2±2.3	*>0.05*
Fasting insulin, μIU/ml	15.4±2.8	7.4±3.3	*0.001*
Viral load, $\log_{10}$ IU/ml	5.4±0.9	5.5±1.4	*>0.05*
Resistin, ng/ml	14.4±8.3	12.3±5.1	*>0.05*
Leptin, ng/ml	20.9±15.8	13.3±12.3	*>0.05*
Adiponectin, μg/ml	22.4±14.0	27.9±15,.0	*>0.05*
Ghrelin, ng/ml	0.3±0.3	0.6±0.3	*0.001*
TNF-α, pg/ml	2.4±0.5	1.8±0,8	*0.004*

Table 4.

Laboratory data in an association with IR in CHC patients with METAVIR F0-1

Variable	F 0-1 (n=51)		Yates' χ2	*P*
	IR+(n=12)	IR-(n=39)		
TC > 5.2 mmol/l	5/41.7	11/28.2	0.274	*0.6007*
TG >1.7 mmol/l	3/25	3/7.7	FET	*0.13377*
HDL-C <1 mmol/l	1/8.3	5/12.8	FET	*1.0000*
Fasting glucose > 6 mmol/l	4/33.3	3/7.7	FET	*0.0445*
Ghrelin <0.4 ng/ml	8/66.6	10/25.6	5.086	*0.0241*
Resistin >8.2 ng/ml	8/66.6	22/56.4	0.088	*0.7667*
Leptin >5.8 ng/ml	10/83.3	27/69.2	0.345	*0.5569*
Adiponectin <11.5 μg/ml	3/25	7/17.9	FET	*0.6822*

Results are expressed as absolute numbers and percentages (n/%), FET - Fisher's Exact Test

The most significant marker of IR was a reduction in secretion of ghrelin lower than the norm. The test had a high degree of accuracy, specificity, positive and negative predictive value (Table 5). The high negative predictive value and accuracy of the tests showed an increased level of triglycerides > 1.7mmol/l, a decreased level of HDL-C < 1mmol/L, and elevated glucose > 6.1mmol/L.

Table 5.

Diagnostic Significance of IR markers in CHC patients (n=51) with METAVIR F0-1

Variable	PPV%	NPV%	Ac%	RR	95% CI	Se%	Sp%
TC > 5.2 mmol/l	41.7	71.8	64.7	1.6	1.1-2.4	60.0	57.4
TG >1.7 mmol/l	25.0	92.3	76.5	3.1	1.5-6.6	75.8	55.1
Glucose > 6.1 mmol/l	33.3	92.3	78.4	4.1	2.0-8.5	80.5	57.9
Ghrelin<0.4 ng/ml	66.6	74.4	72.5	2.6	1.8-3.7	72.0	69.2
Resistin >8.2 ng/ml	66.6	43.6	49.0	1.2	1.0-1.5	54.5	57.1
Leptin>5.8 ng/ml	83.3	30.8	43.1	1.2	1.0-1.4	54.6	64.6

Se – sensitive , Sp – specificity, Ac- accuracy; PPV – positive predictive value, NPV – negative predictive value; RR- relative risk

With an increase in the fibrosis stage on the background of IR, relative risk of disorders in lipid and carbohydrate metabolism increased, as well as the development of adipokine imbalance. Thus, in patients with METAVIR F0-1 stage on the background of IR, RR of hypertriglyceridemia increased by 3 times, hyperglycemia by 4 times, and hypoghrelinemia by 2.6 times. The most significant impairments were observed at METAVIR F3 and F4 stages (Table 6).

Table 6.

The risk of metabolic abnormalities and imbalance adipokines in CHC patients with IR according to METAVIR stage

Variable	METAVIR F0-F4							
	F0-1		F2		F3		F4	
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
TC>5.2mmol/l	1.6	1.1-2.4	1.0	0.1-0.8	0.9	0.7-1.3	1.6*	1.0-2.4
TG>1.7mmol/l	3.1*	1.5-6.6	1.4	1.0-2.1	1.1	0.8-1.5	3.7*	1.6-8.7
HDL<1mmol/l	0.6	0.3-1.4	2.1*	1.4-3.3	2.6*	1.7-3.9	2.0*	1.5-2.7
FG>6.1mmol/l	4.1*	2.0-8.5	10.7*	3.4-33.7	5.2*	2.8-9.6	2.5*	1.4-4.6
Ghr<0.4ng/ml	2.6*	1.8-3.7	1.0	0.8-1.2	4.2*	2.8-3.2	4.2*	2.7-6.4
Res>8.2ng/ml	1.2	1.0-1.5	0.9	0.8-1.1	2.3*	1.8-2.9	3.3*	2.6-4.7
Leptin>5.8ng/ml	1.2*	1.0-1.4	0.8*	0.6-0.9	2.2*	1.7-2.7	1.7*	1.4-2.0
AP<11.5µg/ml	1.4	0.8-2.4	1.7*	1.0-2.5	1.7*	1.2-2.5	1.3	0.8-1.9

**P < 0.05 in comparison with IR- patients, AP -adiponectin FG -fasting glucose, Res- resistin, Ghr- ghrelin*

In multivariate regression analysis, the most significant influence on the IR formation in HCV patients with F0-1 fibrosis was glucose, WHR, ghrelin, and cholesterol (Table 7). In the METAVIR F2 stage, IR significantly depended only on the basic parameters of glucose and insulin In the METAVIR F3 stage, in addition to BMI and WHR, IR significantly depended also on the duration of disease, serum levels of leptin and resistin. In patients with liver cirrhosis, the independent predictors of IR were BMI, triglycerides, C-reactive protein, leptin, and resistin.

Table 7.

Multiple linear regression analysis for factors associated with HOMA-IR in CHC patients with different METAVIR stages

Variable	Regression coefficient	SE of regression coefficient	95% CI	*P-value*
METAVIR F0-1				
Ghrelin	-1.183	0.259	-1.753 to -0.613	<0.001
WC	-0.087	0.024	-0.140 to -0.034	0.004
WHR	6.417	2.766	0.330 to 12.504	0.04
Fasting glucose	0.585	0.112	0.337 to 0.832	<0.001
TC	-2.020	0.426	-2.957 to -1.083	<0.001
LDL-C	1.815	0.410	0.912 to 2.718	<0.001
METAVIR F2				
Fasting glucose	0.566	0.047	0.473 to 0.659	<0.001
Fasting insulin	0.257	0.005	0.247 to 0.266	<0.001
METAVIR F3				
BMI	0.842	0.146	0.523 to 1.160	<0.001
Duration of Dis.	-0.231	0.039	-0.317 to -0,146	<0.001
Leptin	-0.297	0.045	-0.396 to -0.198	0.04
Resistin	0.106	0.05	-0.01 to 0.22	0.04
WHR	9.3	4.6	-0.737 to 19.34	0.045
METAVIR F4				
BMI	0.250	0.099	0.05 to 0.45	0.01
Triglycerides	-2.611	0.569	-1.47 to 3.753	<0.001
Leptin	-0.051	0.02	-0.102 to 0.001	0.045
Resistin	0.067	0.029	0.01 to 0.125	0.02
CRP	0.449	0.109	0.229 to 0.670	0.0001

Discussion

The causal relationship between HCV infection and IR development has been demonstrated by the increased prevalence of IR in chronic HCV infection even at early stages of liver disease without LF. Patients with IR were characterized by a higher histological activity and hepatic fibrosis index, which coincides with the data of JM Hui et al. and CO Zein et al. [19,20].

HCV-induced IR may be due to the HCV core protein inducing proteasomal degradation of IRSs 1 and 2 blocking intracellular insulin signaling. The latter is mediated by increased levels of both TNF-α and 3 SOC-3 [4]. A model of mice transgenic for the HCV core protein demonstrated insulin resistance, glucose intolerance, and elevated intrahepatic TNF-α mRNA; all of which were ameliorated by anti-TNF-α antibodies [21]. Currently, TNF-α is identified as a mediator of insulin resistance, which is induced by HCV [21,22]. TNF-α have deleterious effects on both glucose homeostasis and beta-cell function, and can disrupt insulin signalling pathways in both pancreatic beta cells and liver and adipose tissue [23]. Overflow of FFAs from adipose tissue to systemic circulation impairs insulin-mediated glucose uptake by the muscles resulting in hyperglycemia and peripheral IR. In contrast to the data of A.J. Sanyal [24] and S.A Harrison [10], we did not find an association between IR and viral load level.

IR, through different mechanisms, plays a role in the development of steatosis and its progression to steatohepatitis,

cirrhosis and even HCC [4,25-27]. The overall prevalence of steatosis in patients with HCV infection is approximately 55% ranging from 35% to 81% in various studies, which is approximately 2 to 3 fold higher than the prevalence of steatosis in other liver disease [28]. The current evidence suggests that HCV-associated hepatic steatosis is mainly virus-induced in genotype-3a infected patients [25], which seems to be mediated by an impaired VLDL-C secretion, most likely *via* an impaired activity of the liver microsomal triglyceride transfer protein (MTP) [29]. On the contrary, the host-factors (mainly IR) play a major role in steatosis in non-3 genotypes [25].

Peripheral IR increases adipose tissue lipolysis, leading to increased plasma and hepatic uptake of FFAs. Increased hepatic uptake of FFAs impairs β-oxidation in mitochondria, together with decreased excretion of VLDL, resulting in TG retention with subsequent development of hepatic steatosis [30].

The adipokine system was activated in our patients with CHC, which is reflected in higher levels of leptin, resistin and TNF-α, and more pronounced changes have taken place on the background of IR. A higher value of leptin according to E Tsochatzis can enhance IR, increase the cellular pool of fatty acids, and lead to the formation of liver steatosis [31]. B.Mattioli demonstrated that leptin may enhance the production of proinflammatory cytokines, including TNF-α [32]. In CHC patients with IR, resistin was higher than in the control group and in the group of patients without IR. According to M.Hayt [16], this hormone may be involved in the formation of obesity and IR. The adiponectin level did not depend on the presence of IR, but was higher than in the control group. Our study is consonant with data of V.D. Dixit on the inverse relationship of ghrelinemia and IR: the lower the level of ghrelin, the more pronounced is IR [33].

Thus, our data, as well as results of many authors [11,34,35], suggest that HCV is capable of producing an increase in IR, even before a minimal degree of hepatic fibrosis is present. Alternatively, several studies have reported that IR can adversely affect the course of chronic hepatitis C leading to enhanced steatosis and liver fibrosis [5,11,36-38] and even increase the risk of hepatocellular carcinoma [5,39]. At the same time, the presence of advanced liver disease is an even stronger diabetogenic factor than HCV infection itself. In other words, diabetes associated with HCV infection is less of a determinate than the effect of hepatic cirrhosis on glucose metabolism [40].

In conclusion, several mechanisms could explain the role of IR in the development of LF. Hyperinsulinemia per se stimulates the proliferation of stellate cells, thus enhancing the secretion of extracelular matrix. Moreover, both insulin and hyperglucemia are able to stimulate the expression of the connective tissue growth factor, a cytokine involved in the progression of fibrosis in the liver and other tissues [40,41]. Visceral adipose tissue is a causative risk factor for fatty liver and nonalcoholic steatohepatitis. [42,43] Visceral fat accumulation impairs adipocyte function and adipocytokine secretion. Our data demonstrates the potential role of adipocytokines in the development of IR, fatty liver and LF. The most informative indicator in predicting of IR was the reduced ghrelin secretion. Revealed correlations with BMI, WHR, steatosis, insulin and HOMA-IR allow us to regard the decreased secretion of ghrelin, and increased production of leptin, resistin and TNF-α as a component involved in the formation of IR and MetS in CHC patients.

Competing interests

The authors declare that they have no competing interests.

References

1. Mohd Hanafi ah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology, 2013, 57(4):1333–42.
2. Lavanchy D. The global burden of hepatitis C. Liver Int. 2009; 29:74–81.
3. Galossi A, Guarisco R, Bellis L, Puoti C. Extrahepatic Manifestations of Chronic HCV Infection. J Gastrointestin Liver Dis March. 2007;16:65–73.
4. El-Zayadi AR1, Anis M Hepatitis C virus induced insulin resistance impairs response to anti viral therapy. World J Gastroenterol. 2012;18(3):212-24. doi: 10.3748/wjg.v18.i3.212.
5. Machado MV, Cortez-Pinto H. Insulin resistance and steatosis in chronic hepatitis C. Ann Hepatol. 2009;8:S67–S75.
6. Alberti A, Vario A, Ferrari A, Pistis R. Review article: chronic hepatitis C--natural history and cofactors. Aliment Pharmacol Ther. 2005;22(Suppl 2):74–78
7. Ioannou GN, Ioannou GN, Bryson CL, Boyko EJ. Prevalence and trends of insulin resistance, impaired fasting glucose, and diabetes. J Diabetes Complications. 2007; 21:363–370
8. Ferrannini E, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Järvinen H. Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. European Group for the Study of Insulin Resistance (EGIR) Hypertension. 1997;30:1144–49.
9. Romero-Gómez M. Insulin resistance and hepatitis C. World J Gastroenterol. 2006;12:7075–80.
10. Harrison SA. Insulin resistance among patients with chronic hepatitis C: etiology and impact on treatment. Clin Gastroenterol Hepatol. 2008;6:864–76.
11. Moucari R, Asselah T, Cazals-Hatem D, Voitot H, Boyer N, Ripault MP, et al. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology. 2008;134:416–423
12. Jalil S, Mummad RR, Sood GK Chronic HCV infection causes insulin resistance—a meta-analysis (abstr). Gastroenterology. 2007; 132:(Suppl 2):A-784.
13. Kawaguchi T, Sata M. Importance of hepatitis C virus-associated insulin resistance: therapeutic strategies for insulin sensitization. World J Gastroenterol. 2010; 16: 1943-52.
14. Leclercq IA, Morais AS, Schroyen B, Hul NV, Geerts A. Insulin resistance in hepatocytes and sinusoidal liver cells: mechanisms and consequences. J Hepatol. 2007; 47:142–56.
15. Mori K, Yoshimoto A, Takaya K, Hosoda K, Ariyasu H, Yahata K, et al. Kidney produces a novel acylated peptide, ghrelin. FEBS Lett. 2000; 486: 213–6.
16. Marra F, Bertolani C. Adipokines in Liver Diseases.

Hepatology. 2009; 50 (3):957- 69.
17. EASL Clinical Practice Guidelines: Management of hepatitis C virus infection European Association for the Study of the Liver. J Hepatology. 2014; 60(2):392–420.
18. Alberti KG, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group. The metabolic syndrome--a new worldwide definition. Lancet. 2005; 366(9491):1059-62.
19. Hui J M, Sud A, Farrell GC, Bandara P, Byth K,. Kench J G, et al. Insulin resistance is associated with chronic hepatitis C and virus infection fibrosis progression Gastroenterology. 2003;125:1695–704.
20. Zein CO, Levy C, Basu A, Zein NN .Chronic hepatitis C and type II diabetes mellitus: a prospective cross-sectional study. Am J Gastroenterol. 2005;100:48–55.
21. Knobler H1, Schattner A.TNF-{alpha}, chronic hepatitis C and diabetes: a novel triad. QJM. 2005 Jan;98(1):1-6.
22. Knobler H1, Zhornicky T, Sandler A, Haran N, Ashur Y, Schattner A. Tumor necrosis factor-alpha-induced insulin resistance may mediate the hepatitis C virus-diabetes association. Am J Gastroenterol. 2003; 98(12):2751-6.
23. Greenberg AS1, McDaniel ML.Identifying the links between obesity, insulin resistance and beta-cell function: potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. Eur J Clin Invest. 2002;32 Suppl 3:24-34.
24. Sanyal AJ, Contos MJ, Sterling RK, Luketic VA, Shiffman M L, Stravitz RT et al. Hyperinsulinemia blocks the inhibition of hepatitis C virus (HCV) replication by interferon: a potential mechanisms for failure of interferon therapy in subjects with HCV and nonalcoholic fatty liver disease. Hepatology. 2004; 40:179A
25. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, UtiliR, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology. 2001; 33:1358-64.
26. Shaheen M, Echeverry D, Oblad MG, Montoya MI, Teklehaimanot S, Akhtar AJ: Hepatitis C, metabolic syndrome, and inflammatory markers: results from the Third National Health and Nutrition Examination Survey (NHANES III). Diabetes Res Clin Pract. 2007; 75:320–6.
27. Reaven G.M. Compensatory hyperinsulinemia and the development of the atherogenic lipoprotein profile. The price paid to maintain glucose homeostasis in insulin-resistant individuals. Endocrinol Metab Clin North Am. 2005; 34:49-62.
28. Lonardo A, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. Gastroenterology. 2004;126:586–97.
29. McGuinness PH, Painter D, Davies S, McCaughan GW. Increases in intrahepatic CD68 positive cells, MAC387 positive cells, and proinflammatory cytokines (particularly interleukin 18) in chronic hepatitis C infection. Gut. 2000;46:260–269.
30. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, et al. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology. 2002;35:373–79.
31. Tsochatzis E, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases Am. J. Gastroenterol. 2006; 101 (11): 2629-40.
32. Mattioli B, Straface E, Quaranta MG, Giordani L, Viora M. Leptin promotes differentiation and survival of human dendritic cells and licenses them for Th1 priming. J Immunol. 2005; 174:6820-8.
33. Dixit VD, Schaffer EM, Pyle RS , Collins G D, Sakthivel SK, Palaniappan R, et al. Ghrelin inhibits leptin and activation-induced proinflammatory cytokine expression by human monocytes and T-cells. J Clin Inv. 2004; 114:57-66.
34. Knobler H, Schihmanter R, Zifroni A, Fenakel G, Schattner A. Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection Mayo Clin Proc. 2000; 75:355-9.
35. Lecube A, Hernández C, Genescà J, Esteban JI, Jardí R, Simó R. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. Diabetes Care. 2004; 27:1171-5.
36. Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, et al. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol. 2001;35: 279–83.
37. Cammà C1, Bruno S, Di Marco V, Di Bona D, Rumi M, Vinci M, et al. Insulin resistance is associated with steatosis in nondiabetic patients with genotype 1 chronic hepatitis C. Hepatology. 2006;43(1):64-71.
38. Castéra L1, Hézode C, Roudot-Thoraval F, Bastie A, Zafrani ES, Pawlotsky JM, et al. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut. 2003;52(2):288-92.
39. Tazawa J, Maeda M, Nakagawa M, Ohbayashi H, Kusano F, Yamane M, et al. Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. Dig Dis Sci. 2002; 47:710 –15.
40. Lecube A1, Hernández C, Genescà J, Simó R. Glucose abnormalities in patients with hepatitis C virus infection: Epidemiology and pathogenesis. Diabetes Care. 2006;29(5):1140-9.
41. Paradis V1, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, et al.High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology. 2001;34(4 Pt 1):738-44.
42. Schäffler A1, Schölmerich J, Büchler C.Mechanisms of disease: adipocytokines and visceral adipose tissue--emerging role in nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol. 2005; 2(6):273-80.
43. Baranova A1, Jarrar MH, Stepanova M, Johnson A, Rafiq N, Gramlich T, et al. Association of serum adipocytokines with hepatic steatosis and fibrosis in patients with chronic hepatitis C. Digestion. 2011;83(1-2):32-40.

International Journal of Biomedicine 6(1) (2016) 33-37
doi: 10.21103/Article6(1)_OA6

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Uterine Artery Embolization and Pregnancy. Actual and Controversial Issues of Gestation Terms and Delivery

Julia E. Dobrokhotova, PhD, ScD*; Igor I. Grishin, PhD; Djamilya M. Ibragimova, PhD; Inessa G. Knysheva; Vera J. Ilchenko

N.I. Pirogov Russian National Research Medical University

Moscow, the Russian Federation

Abstract

Background: The aim of this study was to estimate the particular qualities of pregnancy and delivery among patients after uterine artery embolization (UAE) for uterine fibroids.

Materials and Methods: The study included 161 pregnant women. We performed a comparative analysis of pregnancy and delivery among patients after UAE, patients with uterine fibroids without UAE, and healthy patients with physiological pregnancy and childbirth.

Results: The frequency of complications during pregnancy and delivery among patients after UAE for uterine fibroids was not significantly different from the frequency of complications among patients without uterine fibroids and significantly lower than the complication rate among patients with uterine fibroids without UAE.

Conclusion: UAE use is a highly efficient alternative to surgical or medical treatments for uterine fibroids in patients of reproductive age, which plan pregnancy.**(Int J Biomed. 2016;6(1):33-37.).**

Keywords: *uterine fibroids; uterine artery embolization; pregnancy; childbirth.*

Introduction

Global trends in the reproductive health field are difficult to assess, but numerous studies have shown that many indicators of women's reproductive function have declined over the past half-century [1]. The effect of uterine artery embolization (UAE) on fertility remains unclear but is certainly relevant [2]. There is not enough data to evaluate the impact of UAE on fertility, pregnancy and its outcomes among women. Quite often, the presence of uterine fibroids occurs among women with infertility, although, according to some data, benign tumors are associated with infertility only in 5%-10% of cases, when all other causes of reproductive function disorders are excluded [3].

However, the exact role of fibroids in infertility and the periodic loss of pregnancy is uncertain. The complexity of assessing the impact of fibroids on fertility is primarily due to the patient's age, since the frequency of fibroids increases with age, and fertility decreases. Still, the absolute effects are physiological and anatomical factors, associated with the presence of uterine fibroids, which may contribute to the state of infertility in the population [4].

Among some researchers, there is a perception that the UAE is the reason for the decline of ovarian reserve, resulting in a greatly reduced possibility of pregnancy [5]. N. Berkane et al. suggested that one of the possible causes of failures with respect to infertility after the UAE may not be an unintended embolization of ovaries and endometrium, but the presence of confounding factors, such as age and the mere presence of uterine fibroids [6].

Other data show a decrease of reproductive potential after the procedure of UAE by perfusion violations in endometrium and the development of various pathological processes [7], which, according to G.Pron et al., may be the cause of failure to conceive and poor outcomes of pregnancies [8].

Thus, the data about pregnancy and childbirth after UAE, described in the literature, is very controversial, presented in separate reports on the fact of pregnancy and childbirth, and do not disclose the features of the gestation

***Corresponding author:** Professor Julia E. Dobrokhotova, PhD, ScD. Head of Department of Obstetrics and Gynecology, Medical Faculty, N.I. Pirogov Russian National Research Medical University, Moscow, the Russian Federation. E-mail: pr.dobrohotova@mail.ru*

period. At the Department of Obstetrics and Gynecology of Medical Faculty, UAE, including the treatment of uterine fibroids, has been applied since 2003, and, for the moment, we have the experience of more than 1,500 technically successful embolizations. With a decade of experience in the application of this intervention, as well as a sufficient number of successful pregnancies and births after UAE, we found it necessary and urgent to carry out this study, the aim of which was to study the particular qualities of pregnancy and childbirth in this category of patients.

Materials and Methods

We conducted clinical and laboratory analysis of the course of pregnancies, childbirths and the postpartum periods in 161 patients. All patients were divided into three groups. Group 1 included 59 pregnant women, who underwent UAE as a treatment for uterine fibroids; Group 2 included 67 pregnant women with uterine fibroids diagnosed before pregnancy and treated with medical therapy, or had no treatment at all; Group 3 included 35 pregnant women without uterine fibroids with physiological pregnancy and childbirths (control group).

Clinical examination included a thorough medical history, with the explanation of all diseases and surgical procedures, a physical examination, a special obstetric examination, and a laboratory examination in full accordance with the standards of healthcare with the involvement of all specialists according to relevant indications.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by N.I. Pirogov Russian National Research Medical University Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the statistical software «Primer of Biostat 4.0» and «STATISTICA 7». Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

Results

Spontaneous abortion occurred in 4/6.8% patients of Group 1 and in 12/17.9% patients of Group 2; the number of patients that remained under further observation was 55 women in each group. In Group 3, 3/8.6% pregnancies ended with a spontaneous abortion; an artificial abortion was carried out (according to the patients' requests) in 2/5.7% of cases, which eventually led to a reduction of the number of patients to 30.

Analyzing the complications of the first trimester of gestation, we revealed the following features. The threat of spontaneous abortion up to 13 weeks was noticed in 18.2% of patients in Group I and 16.6% of patients in Group 3, which was significantly different in comparison with 38.2% in Group 2 (*P=0.0197* and *P=0.0397*, respectively). It should be noted that hospitalization and in-patient treatment in Group 2 were required in 18/85.7% patients identified with the threat of termination of pregnancy (n=21). These data are significantly different from data in Groups 1 and 3.

We believe that a decrease in the incidence of threatened abortion among patients after UAE compared to patients without UAE is due to the shutdown of the pathological channel of fibroids from the main bloodstream, and the absence of a number of hormonal imbalances that a growing uterine fibroid provokes during pregnancy. The frequency of pregnancy complications are presented in Table 1.

Table 1.

Pregnancy complications among patients of the studied groups

Pregnancy complications	Group 1 (n=55)		Group 2 (n=55)		Group 3 (n=30)	
	abs	%	abs	%	abs	%
The 1st trimester of pregnancy						
Threatened abortion	10	18.2*	21	38.2**	5	16.6
The 2nd trimester of pregnancy						
Threatened abortion	2	3.6*	16	29.1**	-	-
The 3rd trimester of pregnancy						
Threatened preterm birth	1	1.8*	14	25.5**	-	-
Preeclampsia	5	9.1*	20	36.4**	-	-
Placental insufficiency	6	10.9*	28	50.9**	-	-

** - P <0.05 between Group 1 and Group 2; ** - P ≤0.05 between Group 2 and Group 3.*

An abnormal placenta location, before 13 weeks of pregnancy, was identified in 29.1% of cases in Group 2, which was significantly different compared to 5.5% in Group 1 (*P=0.0025*) and 0.0% in Group 3 *(P=0.0000)*. Differences between Group 1 and Group 3 were not significant. There were no statistically significant differences between patients of all three groups in other pregnancy complications in the first trimester.

The threat of spontaneous abortion in the second trimester was diagnosed in 3.6% of patients in Group 1, which was not significantly different from Group 3. In Group 2, this complication was diagnosed in 29.1% of cases. The differences between Group 2 and Groups 1 and 2 were reliable (*P=0.0008* and *P=0.0028*, respectively). Abnormal placenta location in the second trimester remained in 12/21.8% patients of Group 2. Pathological placenta location in the second trimester of pregnancy was not found in Groups 1 and 3.

Uterine fibroids node malnutrition up to 22 weeks of pregnancy was diagnosed in 4/7.3% patients of Group 2. Among these patients, the initial size of the myomatous node was more than 5 cm in diameter and had a tendency to growth in the second trimester. We noted no cases of myomatous node malnutrition among patients of Group 1. In this group, a significant increase of myomatous node (10% or more) is diagnosed in only 1/1.8% pregnant woman, that was significantly different from Group 2, in which the most pronounced growth of the nodes was identified among

9/16.4% patients (*P=0.0203*). According to the literature, the level of myometrium epidermal growth factor increases during pregnancy and in myomatous nodes which have an extensive vascular supply [9,10]. Massive changes occur in the receptor apparatus of the myometrium, characterized by increasing the number of receptors for progesterone and estradiol [11]. The totality of these factors can lead to a remarkable growth of myomatous nodes during pregnancy and may be accompanied by many complications. The application of UAE, as a method of treatment of uterine fibroids, makes it possible to avoid the development of these complications associated with the growth of fibroids in Group 1.

The threat of preterm labor in the third trimester among Group 1 patients was found in 1.8% of cases, which was significantly different from Group 2 (25.5%, *P=0.0009*) and did not differ from Group 3 (0.00%, *P>0.05*). In connection with the threat of premature birth, in-patient treatment was needed in 57.1% of cases in Group 2. In Group 1, the treatment was carried out on an outpatient basis. Preterm labor was not identified in Group 3.

The frequency of preeclampsia was significantly lower in Group 1 than in Group 2 (*P=0.0006*) and was not statistically different from Group 3. Thus, according to our data, the incidence of preeclampsia among patients who underwent UAE is not different from the frequency of preeclampsia cases during normal pregnancy. UAE has no pathological influence on the formation of placenta blood vessels and does not increase the risk of preeclampsia.

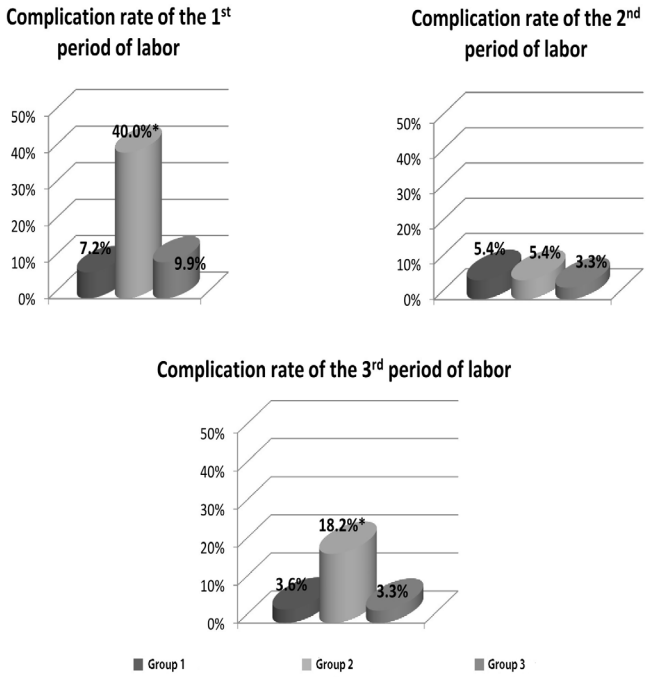
Rates of placental insufficiency formation (PI) in Group 1 (10.9%) and Group 3 (0.00%) were significantly lower compared with Group 2 (50.9%,) (*P=0.0000* and *P=0.0000*, respectively). We noticed an early PI development (up to 30 weeks of pregnancy) among patients of Group 2. At the same time, a severe course of PI, poorly treatable with medical correction, was also more frequent in Group 2. However, in contrast to the total number of patients with PI, the difference was not statistically significant.

Thus, the course of pregnancy in a group of patients after UAE had no statistically significant differences from the course of pregnancy in patients without uterine fibroids. Complications of pregnancy were significantly more frequent in Group 2. In general, the pregnancy course in Group 1 may be characterized as a physiological behaviour, comparable with the control group.

Labor at term occurred in 53/96.4% patients of Group 1, 47/85.5% patients of Group 2 and 30/100% patients of Group 3. Figure 1 shows the overall rate of birth complications (delay amniorrhexis, primary weakness of labor activity, secondary weakness of labor activity, intrauterine hypoxia, discoordination of labor activity, placental pathology) in all three groups according to periods.

The overall rate of complications in the 1st and 3rd periods of delivery was significantly higher in Group 2 compared to Group 1 and Group 3. Statistically significant differences during labor among pregnant women of Group 1 and Group 3 were not observed.

The frequency of cesarean section (C-section) was significantly higher in Group 2 (21/38.2%) compared to Group 1 (10/18.2%, *P=0.0341*) and Group 3 (5/16.6%, *P=0.0397*). Planned C-section was performed in 8/14.5% patients of Group 1, in 9/16.4% patients of Group 2, and in 4/13.35% patients of Group 3. An emergency C-section was performed in 2/3.6% patients of Group 1 and 1/3.35% patient of Group 3, that was significantly lower than in Group 2 (12/21.8% patients; *P=0.01* and *P=0.05*, respectively).



* - *p <0.05 between Group 1 and Group 2.*

Fig. 1. *The overall rate of birth complications among patients of studied groups*

Analysis of C-sections performed in three groups of patients revealed that UAE in anamnesis among Group 1 patients was not an indication for abdominal delivery in any case. The frequency of C-section, both in the planning and in the emergency order, was not different from that in the control group and coincided with the population-wide indicators, which apparently proves that pregnancies and childbirths among patients after UAE occur physiologically.

Discussion

Despite numerous reports of successful pregnancies after UAE, the feasibility of this method of treatment for uterine fibroids among patients of reproductive age is still very debatable.

According to J.P. Pelage et al. [11] and M.D. Levie [12], as well as some other authors, among patients of reproductive age who are planning to retain their reproductive function, UAE is not an optimal way to treat fibroids [13-15]. The authors substantiate their opinions by noting that there is a lack of sufficient data on the effect of embolization on pregnancy and childbirth, as well as possible further complications.

In some literature, we can find reports on the development of preterm menopause among patients after embolization, as well as the development of such complications as necrosis of embolized fibroids, fistula formation, pyometra and purulent endometritis, leading to the necessity of hysterectomy, intrauterine adhesions, endometrium atrophy, including the development of permanent amenorrhea with intact ovarian function [16-22].

According to another researchers, the frequency of pregnancies after embolization ranges from 23% to 61% [23-24]. Thus, in the study of Firouznia et al. [25], 14(61%) women of the 23 patients planning a pregnancy became pregnant, and 9 patients in the first time. McLucas et al. consider that the possibility of pregnancy after embolization is not less than after the conservative myomectomy [26].

We could not find enough evidential data in literature, which would allow us to allocate clear criteria under which the patient will not have any contraindications for pregnancy after UAE. Most sources indicate that this issue was solved purely individual [27,28]. We also found that the number of fibroids, uterine size and type of fibroid before embolization, affect on the onset of pregnancy after UAE, which is most likely, due to the successful outcome of UAE. However, pregnancies outcomes (labors, miscarriages, non-developing pregnancies, abortions) are not different from that among patients without uterine fibroids.

Conclusion

In sum, we can conclude that UAE has no adverse effect on pregnancy and childbirth. The frequency of complications during pregnancy, childbirth and the postpartum period among patients underwent, is not significantly different from patients without uterine fibroids. UAE is not a contraindication for pregnancy and childbirth, and not a reason for abortion. Thus, UAE use is a highly efficient alternative to surgical or medical treatments for uterine fibroids in patients of reproductive age, which plan pregnancy.

Competing interests

The authors declare that they have no competing interests.

References

1. Foster WG, Neal MS, Han MS, Dominguez MM. Environmental contaminants and human infertility: hypothesis or cause for concern. J Toxicol Environ Health B Crit Rev. 2008;11(3-4):162–76.
2. Mohan PP, Hamblin MH, Vogelzang RL. Uterine artery embolization and its effect on fertility. J Vasc Interv Radiol. 2013; 24(7): 925-30.
3. Khaund A, Lumsden MA. Impact of fibroids on reproductive function. Best Pract Rese Clin Obstet Gynaecol. 2008; 22(4):749-60.
4. Imaoka I, Wada A, Matsuo M, Yoshida M, Kitagaki H, Sugimura K. MR imaging of disorders associated with female infertility: use in diagnosis, treatment, and management. Radiographics. 2003; 23(6):1401-21.
5. ACOG Committee Opinion. Uterine artery embolization. Committee on Gynecologic Practice, American College of Obstetricians and Gynecologists. Obstet Gynecol. 2004;103(2):403–4.
6. Berkane N, Moutafoff-Borie C. Impact of previous uterine artery embolization on fertility. Curr Opin Obstet Gynecol. 2010;22(3):242–7.
7. Mara M, Horak P, Kubinova K, Dundr P, Belsan T, Kuzel D. Hysteroscopy after uterine fibroid embolization: evaluation of intrauterine findings in 127 patients. J Obstet Gynaecol Res. 2012; 38(5):823-31.
8. Pron G, Mocarski E, Bennett J, Vilos G, Common A, Vanderburgh L; Ontario UFE Collaborative Group. Pregnancy after uterine artery embolization for leiomyomata: the Ontario multicenter trial. Obstet Gynecol. 2005;105(1):67-76.
9. Mikhalevich SI, Kapusta AV. Pregnancy, childbirth and the postpartum period among women with uterine fibroids. Medical News. 2011;2:18-25.[Article in Russian].
10. Samoilova TE. Uterine fibroids. Justification of non-operative treatment (review). Reprod Problems. 2003;9(4):32-36. [Article in Russian].
11. Pelage JP,Walker WJ, Le Dref O, Rymer R. Ovarian artery: angiographic appearance, embolization and relevance to uterine fibroid embolization. Cardiovasc Intervent Radiol. 2003; 26(3):227-33.
12. Levie MD. Uterine artery embolization: Laparoscopic myomectomy. FIGO World Congress of Gynecology and Obstetrics (16th 2000 Washington DC). NY: Elsevier; 2000.
13. Hutchins FL Jr, Worthington-Kirsch R. Embolotherapy for myoma-induced menorrhagia. Obstet Gynecol Clin North Am. 2000; 27(2):397-405.
14. Goodwin SC, Bonilla SC, Sacks D, Reed RA, Spies JB, Landow WJ, et al. Reporting standards for uterine artery embolization for the treatment of uterine leiomyomata. J Vacs Interv Radiol 2003; 14(9 Pt2):S467-76.
15. Honda I, Sato T, Adachi H, Kobayashi Y, Shimada K, Watanabe H, Okada Y, Inoue M. Uterine artery embolization for leiomyoma: complications and effects on fertility. Nihon Igaku Hoshasen Gakkai Zasshi. 2003;63(6):294-302. [Article in Japanese].
16. Sakhautdinova IV. Uterine artery embolization is a uterine-conserving surgical method for fibroids treatment. Perm Med Zh. 2006;23(3):126-36. [Article in Russian].
17. Chitrit Y, Zafy S, Pelage JP, Ledref O, Khoury R, Caubel P. Amenorrhea due to partial uterine necrosis after uterine artery embolization for control of refractory postpartum hemorrhage. Eur J Obstet Gynecol Reprod Biol. 2006;127(1):140-2.
18. Gaia G, Chabrot P, Cassagnes L, Calcagno A, Gallot D, Botchorishvili R, et al. Menses recovery and fertility after artery embolization for PPH: a single-center retrospective observational study. Eur Radiol. 2009;19(2):481-7.
19. Guo WB, Yang JY, Chen W, Zhuang WQ. Amenorrhea after uterine fibroid embolization: a report of six cases. Ai Zheng. 2008;27(10):1094-9. [Article in Chinese].
20. Dewdney SB, Mani NB, Zuckerman DA, Thaker PH. Uteroenteric fistula after uterine artery embolization. Obstet Gynecol. 2011;118(2 Pt 2):434-6.
21. Maheux-Lacroix S, Lemyre M, Laberge PY, Lamarre A, Bujold E. Uterine artery embolization complicated by uterine perforation at the site of previous myomectomy. J Minim Invasive Gynecol. 2012;19(1):128-30.
22. Donnez O, Jadoul P, Squifflet J, Donnez J. Unusual

complication after uterine artery embolization and laparoscopic myomectomy in a woman wishing to preserve future fertility. Fertil Steril. 2008;90(5):2007.e5-9.
23. Kapranov SA, Breusenko VG, Dobrokhotova JE, Kurtser MA, Bobrov BJ, Krasnova IA. Uterine artery embolization: a modern view on the problem. Part 1. Diagn Interv Radiol. 2007;1:72-87. [Article in Russian].
24. Bradley LD. Uterine fibroid embolization: a viable alternative to hysterectomy. Am J Obstet Gynecol. 2009;201(2):127-35.
25. Firouznia K, Ghanaati H, Sanaati M, Jalali AH, Shakiba M. Pregnancy after uterine artery embolization for symptomatic fibroids: a series of 15 pregnancies. AJR Am J Roentgenol. 2009;192(6):1588-92.
26. McLucas B, Perrella R, Adler L. Embolization for the treatment of adenomyosis. AJR Am J Roentgenol 2002; 178(4):1028–9.
27. Murvatov KD, Obelchak IS, Myshenkova SA, Adamyan LV. Uterine artery embolization is minimally invasive method for uterine fibroid treatment (review). Probl Reprod. 2004; 6:43-50. [Article in Russian].
28. McLucas B. Pregnancy following uterine artery embolization: an update. Minim Invasive Ther Allied Technol. 2013;22(1):39-44.

International Journal of Biomedicine 6(1) (2016) 38-40
doi: 10.21103/Article6(1)_OA7

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Study of the Effects of the Age at Menopause and Duration of Menopause on Bone Mineral Density in Postmenopausal Women in Uzbekistan

Dilbar K. Najmutdinova, PhD, ScD; Lola S. Nurmukhamedova*;
Dilfuza A. Alieva PhD, ScD; Dilnoza S. Maksudova PhD; Zebiniso A. Nosirova

Republican Specialized Scientific Practical Medical Center of Obstetrics and Gynecology
Ministry of Health of the Republic of Uzbekistan
Tashkent, Uzbekistan

Abstract

The aim of the present study was to determine whether an association exists between the duration of menopause and the age of menopause onset, and the differences in bone mineral density (BMD) in postmenopausal women.

Materials and Methods: We have reviewed medical records of 112 postmenopausal women who had not taken any anti-osteoporosis treatment and/or hormone replacement therapy at the time of BMD measurement. The mean age of the postmenopausal women was 53.5±1.1 years, and the mean menopausal period was 4.5 years. The women were evaluated according to the duration of menopause at the time of BMD measurement and age at menopause onset. BMD was measured anteroposteriorly at the L1–L4 level by the dual-energy X-ray absorptiometry method.

Results: According to WHO criteria, osteoporosis and osteopenia were identified in 18(16.2%) and 44(39.2%) cases, respectively; overall, 50(44.6%) women had normal BMD.At the time of BMD measurement, osteoporosis was determined in 10.3% and 29.1% of the women with menopause duration of 0–3 years and >7 years, respectively (*P=0.047*). The percentages for osteopenia were similar among the three different menopause durations (36.2%, 43.3% and 41.6% for 0-3 years, 4-7 years and >7 years, respectively). No differences were determined in the prevalence of osteopenia and osteoporosis in women with menopause duration of >7 years. Thirty-three percent of women with the age of menopause onset of <40 years had osteoporosis; however, the percentages of women with osteoporosis among the other age groups were almost equal (18.7%, 14.29% and 15.0% for 40–46 years, 47–52 years and >52 years, respectively). The frequency of osteopenia did not differ between the groups according to the age of menopause onset.

Conclusion: According to our results, osteoporosis is related to the duration of menopause at the time of BMD measurement more than to the age of menopause onset among untreated postmenopausal women. **(Int J Biomed. 2016;6(1):38-40.).**

Keywords: *postmenopausal osteoporosis; age of menopause onset; risk factors; bone mineral density.*

Introduction

Osteoporosis: a multidisciplinary problem faced by doctors of different specialties — gynecologists, endocrinologists, rheumatologists, and orthopedic traumatologists [1]. Postmenopausal osteoporosis (PMO): the most common form of the disease, with a progressive decrease in BMD associated with menopause (spontaneous or surgical) [2]. Numerous studies show that the primary determinant of osteoporosis in postmenopausal women is estrogen deficiency caused by age-ovarian failure [3]. However, despite the decline in ovarian function, not all postmenopausal women develop impairments from mineralization of bone. Menopause is the most important risk factor for osteoporosis in adult women. Women lose about 2% of their cortical bone and 5% of their trabecular bone per year during the first 5-8 years [4].

For this study, it is important to take into account regional peculiarities of Uzbekistan: an early menopause, high birth rates at a low intergenetic range, a high prevalence of gynecological morbidity, and extragenital pathology.

As known, there are no typical clinical symptoms of osteoporosis, such as fractures, besides those already developed [5]. At the same time, carrying out a broad range of

***Corresponding author:** Lola S. Nurmukhamedova; E-mail: lola-ss@yandex.ru*

population BMD measurements is not possible due to limited access and economic expediency [6].

For these reasons, knowledge and evaluation of risk management in the diagnosis and prevention of osteoporosis are particularly important, as are a definition of risk factors and identification of women at risk for the development of this disease [6].

Materials and Methods

We have reviewed medical records of 112 postmenopausal women (the residents of Tashkent city and other regions of Uzbekistan) attending the menopause outpatient clinic of Tashkent Republican Specialized Scientific Practical Medical Center of Obstetrics and Gynecology because of various manifestations of the climacteric syndrome. The mean age of the postmenopausal women was 53.5±1.1 years (from 40 to 67 years), and the mean menopausal period was 4.5 years.

The women were evaluated according to the duration of menopause at the time of BMD measurement, age at menopause onset, presence of hypertension and/or diabetes mellitus, smoking habit (cigarette/day).

Exclusion criteria were anti-osteoporosis treatment and/or hormone replacement therapy at the time of BMD measurement.

BMD was measured anteroposteriorly at the L1–L4 level by the dual-energy X-ray absorptiometry method using Hologic "Delphi N". According to the WHO criteria, osteopenia is defined as a BMD T-score between -1 and -2.5 standard deviations (SDs) below the healthy young adult norm, while osteoporosis is defined as a BMD T-score of -2.5 SDs or lower [7,8].

The results were compared with the database densitometer designed for women of the Caucasus region, and compared with the results of BMD in women of the control group.

The prevalence of osteopenia and osteoporosis was investigated according to the duration of menopause at the time of BMD measurement in three different groups (0-3 years, 4-7 years, and >7 years) and the age of menopause onset in four different groups: <40 years, 40-46 years, 47-52 years, and >52 years.

Body mass index (BMI) was calculated as weight (kg)/ height (m^2). Main characteristics of the study population are presented in Table 1.

Table 1.

Main characteristics of the study population

Variables	The study population
Age	53.5±1.1
Height, sm	164.6±1.4
Body weight, kg	86.4±2.8
BMI, kg/m^2	31.5±1.0
Age at menarche onset, age	13.7±0.4
Age at menopause onset, age	47.8±1.5
The duration of menopause, age	14.7±2.1

Statistical analysis was performed using the SPSS for Windows. Baseline characteristics were summarized as frequencies and percentages for categorical variables. Group comparisons with respect to categorical variables are performed using $\chi 2$ tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Results and Discussion

According to the described criteria, osteoporosis and osteopenia were identified in 18(16.2%) and 44(39.2%) cases, respectively; overall, 50(44.6%) women had normal BMD.

At the time of BMD measurement, osteoporosis was determined in 10.3% and 29.1% of the women with menopause duration of 0–3 years and >7 years, respectively (p = 0.047) (Table 2). The percentages for osteopenia were similar among the three different menopause durations (36.2%, 43.3% and 41.6% for 0-3 years, 4-7 years and >7 years, respectively). No differences were determined in the prevalence of osteopenia and osteoporosis in women with menopause duration of >7 years.

Table 2.

Low BMD and menopause duration

Results of BMD	Menopause duration at time of BMD measurement (years)			Statistics
	0-3 (n=58)	4-7 (n=30)	>7 (n=24)	
Normal (n=50)	31/53.4%	12/40.0%	7/29.0%	$\chi 2 = 4.407$ *P = 0.110*
Osteopenia (n=44)	21/36.2%	13/43.3%	10/41.6%	$\chi 2 = 0.494$ *P = 0.781*
Osteoporosis (n=18)	6*/10.3%	5/16.6%	7*/29.1%	Yates' $\chi 2$=3.201 *P = 0.202* *Fisher's Exact Test *P = 0.047*

Thirty-three percent of women with the age of menopause onset of <40 years had osteoporosis; however, the percentages of women with osteoporosis among the other age groups were almost equal (Table 3). Differences among the four groups were not significant.

Table 3.

Low BMD and the age of menopause onset

Results of BMD	The age of menopause onset (years)				Statistics
	< 40 (n=11)	40-46 (n=32)	47-52 (n=49)	>52 (n=20)	
Normal (n=50)	5/45.4%	13/40.6%	23/46.9%	9/45.0%	$\chi 2$= 0.318 *P = 0.957*
Osteopenia (n=44)	4/36.4%	13/40.6%	19/38.8%	8/40.0%	$\chi 2$= 0.073 *P = 0.995*
Osteoporosis (n=18)	2/18.2%	6/18.8%	7/14.3%	3/15.0%	Yates' $\chi 2$=0.129 *P = 0.988*

According to S.V. Yureneva (2004), the prevalence of osteoporosis is reported as ranging from 7% to 30% [9]. On average, 40% of postmenopausal women had osteopenia (Table 3). The frequency of osteopenia did not differ between the groups according to the age of menopause onset.

Thus, women with >7 postmenopausal years at the time of the BMD test and age at menopause of <40 years are the most at risk for osteoporosis. However, there was no difference of BMI among the groups.

Peak bone mass is attained in the third decade of life. Age-related decline in bone mass probably begins around the age of 40. In women, bone loss accelerates around the time of menopause as it is related to estrogen deficiency [10,11]. J.S. Finkelstein et al. [12] reported that BMD changes begin substantially during late perimenopause, and BMD continues to decline rapidly during the early postmenopausal years. However, their study did not evaluate BMD changes in the late postmenopausal years. Consistent with our findings, H. Ahlborg (2003) reported that BMD had decreased significantly by 6 years after menopause [13]. It is important to determine when bone mass reaches the critical level since such information is helpful for the clinician in deciding the appropriate time to screen postmenopausal women for osteoporosis. According to our results, delay in the BMD measurement time of more than 7 years postmenopause in untreated postmenopausal women predicted an increased risk for low BMD.

Furthermore, duration of menopause was determined to be more important than age at menopause for osteoporosis. Pregnancy and lactation result in hormonal and physiological changes, which increase the serum calcium levels. Maternal adaptations include increased bone resorption, decreased bone formation, increased intestinal calcium absorption, and decreased urinary excretion.

According to a comprehensive survey of women, duration of menopause at the time of BMD measurement is positively correlated with both osteoporosis and osteopenia. An inverse correlation with age at menopause was found only for osteoporosis. When comparing the four groups of women, divided according to the age of menopause, women with a duration of more than 7 years of menopause showed no differences in the prevalence of osteoporosis or osteopenia.

In conclusion, determination of the risk factors for osteoporosis and identification of the candidate postmenopausal women is important for the management of this population. According to our results, osteoporosis is related to the duration of menopause at the time of BMD measurement more than to the age of menopause onset among untreated postmenopausal women.

Competing interests

The authors declare that they have no competing interests.

References

1. Poole KE, Compston JE. Osteoporosis and its management. BMJ 2006;333(7581):1251–6.
2. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 50, January 2003. Obstet Gynecol. 2004; 103(1):203–216.
3. Kanis JA, Johnell O, Oden A , Borgstrom F, Zethraeus N, De Laet C, et al. The risk and burden of vertebral fractures in Sweden. Osteoporos Int 2004;15(1):20-6.
4. Smetnik VP, Kulakov VI. *Guide for Menopause*. M: Med Inform Agentstvo; 2001. [in Russian].
5. Siris ES, Miller PD, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, et al. Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women: results from the National Osteoporosis Risk Assessment. JAMA. 2001;286(22):2815–22.
6. American Association of Clinical Endocrinologists (AACE) medical guidelines for the prevention and treatment of postmenopausal osteoporosis: 2001 edition, with selected updates for 2003. Endocr Pract. 2003; 9(6):544-64.
7. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. Geneva, World Health Organization, 1994 (WHO Technical Report Series, No. 843).
8. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9(8):1137-41.
9. Yureneva SV. Treatment and prevention of postmenopausal osteoporosis. Consilium Medicum. 2004; 6(9):702-7.
10. Smeets-Goevaers CG, Lesusink GL, Papapoulos SE, Maartens LW, Keyzer JS, Weedenburg JP. The prevalence of low bone mineral density in Dutch perimenopausal women: the Eindhoven perimenopausal osteoporosis study. Osteoporosis Int 1998; 8(5):404-9.
11. North American Menopause Society. Management of osteoporosis in postmenopausal women: 2006 position statement of the North American Menopause Society. Menopause. 2006;13(3):340-67.
12. Finkelstein JS, Brockwell SE, Mehta V, Greendale GA, Sowers MR, Ettinger B, et al. Bone mineral density changes during the menopause transition in a multi-ethnic cohort of women. J Clin Endocrin Metab 2008; 93(3):861–8.
13. Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK. Bone loss and bone size after menopause. N Engl J Med 2003; 349(4):327–34.

International Journal of Biomedicine 6(1) (2016) 41-45
doi: 10.21103/Article6(1)_OA8

ORIGINAL ARTICLE

Reconstructive Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

Biomedical Technologies in the Treatment of Skin and Soft Tissue Defects in Patients with Diabetic Foot Syndrome

Magomed D. Dibirov, MD, PhD, ScD[1]; Rasul U. Gadzhimuradov, MD, PhD, ScD[1]; Konstantin A. Koreiba, MD, PhD[2,3]*; Aidar R. Minabutdinov, MD[2,3]

[1]*Moscow State University of Medicine and Dentistry, Russia*
[2]*Kazan State Medical University, Russia*
[3]*«Diabetic Foot» Center, Kazan, Russia*

Abstract

The aim of our study was to investigate the effectiveness of collagen implants in the closure of tissue defects. We offer a method that enables us to avoid the drawbacks of autodermoplasty based on the free split-thickness skin graft.

Materials and Methods: This paper describes all steps of treatment of skin and soft tissue defects in patients with diabetic foot syndrome (DFS), including ultrasonic cavitation with hydrosurgical debridement to remove necrotic debris, purulent pellicle and bacterial biofilm, and an alternative technique for wound defect closure using high-tech biomaterials based on type I collagen ("Collost", "Salvecoll").

Results: Use of type I collagen ("Collost", "Salvecoll") as a component of combination treatment of tissue defects in DFS allowed us to increase the relative rate of wound healing, reduce the incidence of high-level amputations, and significantly reduce inpatient surgical coverage around the clock and speed up a patient's transfer to outpatient care.

Conclusion: Ultrasonic cavitation with hydrosurgical debridement is the most effective procedure for wound preparation for closure. The use of bioplastic collagen materials in patients with DFS is the most effective solution in the management of wound defects.**(Int J Biomed. 2016; 6(1):41-45.).**

Key words: *diabetic foot syndrome; ultrasonic cavitation with hydrosurgical debridement; wound defect closure; bioplastic collagen materials; autodermoplasty.*

Introduction

In 2014, worldwide, 387 million people had diabetes mellitus (DM), and its prevalence estimates tend to increase. According to statistical data, nearly 25% of DM patients suffer from DFS [1,2]. Based on analysis of our early data, we can confidently conclude that a rise in the number of DM patients is accompanied by a concomitant growth of patients with trophic skin lesions in the lower limbs. The number of patients with wound defects corresponding to wound depth stages W1, W2 and W3 (based on Wagner's classification) [3,4] was 43 in 2010, 117 in 2012, and 218 in 2014 (Figure 1). DFS is a condition with pathological changes of the peripheral nervous system, arterial bed and microvasculature, and osteoarticular structures representing a direct threat to the development of ulcerative and necrotic processes and gangrene of the foot [5], and leads to organ-resecting operations in 85% of cases [6].

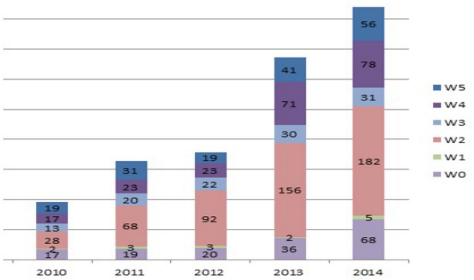


Fig. 1. *The number of patients with foot lesions (based on F.W. Wagner's lassification,1979) for 2010-2014 who received treatment at the "Diabetic foot" center in Kazan*

Autosympathectomy (loss of sympathetic nerve innervations), macroangiopathy (initial atherosclerotic lesions

***Corresponding author:** Konstantin A Koreiba, MD, PhD, Associate Professor. Department of General Surgery, Kazan State Medical University, Russia; "Diabetic Foot" Center, Kazan, Russia. diabetstopa5gb@mail.ru*

of peripheral vasculature, Monckeberg's calcific sclerosis and diffuse intimal fibrosis) and microangiopathy (qualitative and quantitative changes of the capillary basement membrane with excessive matrix production and excess permeability) lead to reduced tissue perfusion [7,8]. The balance between different protein fractions is disturbed in DFS patients. Of particular importance is the disorganization of collagen, which plays an exceptionally important role during the proliferative and remodeling phases of the wound healing process [9,10].

Collagen is a major component of the ECM and is a prominent target of non-enzymatic glycation with formation advanced glycation end products (AGEs) which play an important role in the pathogenesis of diabetic complications like a DFS [11,12].

Type 1 collagen undergoes a series of post-translational modifications that occur during non-enzymatic glycation. Electron microscopic investigation has revealed fine structural changes in the collagen fibrillar arrangement in diabetes [10]. These differences included increased packing density of collagen fibrils, decreases in fibrillar diameter, and abnormal fibril morphology showing collagen fibrils that appeared twisted, curved, overlapping, and otherwise highly disorganized.

Collagen deposition in acute wounds is impaired in type 1 diabetes, possibly due to a decreased fibroblast proliferation [13]. Overall, collagen enhances the wound contracture and cellular migration that are essential for wound healing. Dynamic interactions between growth factors and extracellular matrix (ECM) are integral to wound healing [14]. Thus, collagen disorganization leads to a disturbed physiological course of wound healing and chronic diabetic foot ulcers.

Upon admission to the Diabetic Foot Center in Kazan, each patient underwent a necessary diagnostic program that included the following:

1. Determination of the severity of diabetic polyneuropathy in accordance with the neuropathy disability score (NDS) developed by M.J. Young in 1986 and recommended by the Diabetes Neuropathy Study Group (NEURODIAB) of the European Association for the Study of Diabetes (EASD).
2. Transcutaneous measurement of tissue oxygen ($TcpO_2$).
3. Color-coded duplex ultrasonography (TCCS) of lower limb arteries.
4. X-ray examination of the affected foot.

Based on the examination data, all patients were assigned to two groups. Group 1 included patients with the neuroischemic form of DFS and stage III-IV chronic arterial insufficiency (CAI), who required an immediate arterial reconstruction. Group 2 included patients with the neuroischemic form of DFS and stage I-II CAI and trophic soft tissue lesions, who had no indications for revascularization.

Following the angiographic examination of lower limb arteries, we jointly selected the type of reconstructive surgery procedure according to TASC-II lesion classification and recommended interventions (2007).

The autodermoplastic reconstruction using a free split-thickness skin graft is one of the methods of wound defect closure. However, this treatment procedure in DFS patients is not valid for a number of reasons. The outcome of wound defect closure is not always successful, and is technically difficult to perform on certain areas of the foot. Besides, with the underlying diabetic polyneuropathy, micro- and macroangiopathy, the procedure triggers some pathomorphological mechanisms creating a high risk of an additional chronic wound defect occurring at the skin graft donor site. For that reason, in 2007 a clinical application of high-tech biomaterials based on type I collagen and an investigation of its effectiveness on the wound healing process was started.

Materials and Methods

The study was conducted in accordance with ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. The clinical data were obtained from 173 FDS patients (mean age 56.4±15.6 years; 64 men and 109 women). Ulcer defects had different anatomical locations over the lower extremities, namely, on the plantar and dorsal surfaces of the foot, the plantar surface of the fingers, inner or outer surface of the lower third of the tibia and in the area of postoperative wounds of the foot. The area of ulcer defect was, on average, 38.5±26.3 cm^2. According to Wagner Ulcer Grade Classification System, the ulcers were found to be in grade W1, W2 or W3.

The management of wound healing was performed with due consideration of general and local clinical manifestations, such as the general state of patients, intensity of pain, time course of wound bed detersion and granulation tissue formation, start of epithelialization, and changes in the rate of wound contraction. All patients received the same medicinal treatment against the backdrop of hypoglycemic therapy.

On the first day of patient hospitalization, we used ultrasonic cavitation with hydrosurgical debridement to make sure the wound was free of devitalized tissue. This exerts a favorable local effect on the wound healing process and helps to prepare the wound surface for biomaterial implantation. The procedure enabled us to selectively remove necrotic debris, purulent fibrinous pellicle, and devitalized tissue containing bacterial biofilm. The mechanical necrectomy was performed using an equipment for ultrasonic cavitation with hydrosurgical debridement. As a wound-rinsing fluid, we used a weak solution of local anesthetic instead of an antiseptic solution. Our empirical finding was that UC has a major impact on the bacterial biofilm and devitalized tissue whereas the anesthetic solution supplied through the nozzle serves to remove and wash off the ultrasonically cut tissues. The topical anesthesia has undeniable advantage and does not require using the infiltration anesthesia by paravulnar injection of anesthetic or general anesthesia.

After cleansing the wound defect from devitalized tissues, we used high-tech biomaterials based on type I collagen ("Collost", Russia / "Salvecoll", Europe) for optimal coating of skin and soft tissue defects [15]. Collost/ Salvecoll is a type I collagen-based material derived from the skin of cattle and processed in such way that the epidermis, fat tissue and all dermal cells are removed without destroying the collagen matrix. Collost/Salvecoll is devoid of foreign antigens and,

therefore, of immunogenic properties [16]. For implantation, we used collagen material in the form of membranes (60x50x1.5 mm), which were applied to the wound bed, and 7% or 15% gel, which was injected into the wound bed and walls and to the paravulnar tissues while the needle was being taken out. After the implantation of biomaterial, the wound surface was covered with a hydrocolloid dressing enabling us to maintain a physiological moist environment at the wound/dressing interface. A wound dressing was changed, on average, once in 3-5 days. From day 10-12 post-implantation, we switched to the atraumatic dressing in combination with a coating containing Ag^+ ions. The frequency of dressing changes depended on the amount of wound exudate and the reaction to the dressing adhesive. Patients were transferred to outpatient care on day 7-10 since the day of biomaterial implantation. Statistical analysis was performed using statistical package for the social sciences (SPSS 15.0 software).

Results

The epithelialization of wound defect began on 10.3±2.8 days. On days 14-20, we could see wound granulation tissue without signs of inflammation or rejection of the bioplastic material. Then, tissue regeneration occurred via formation of the body's own granulation tissues. In none of these cases did we observe hypertrophic scars. Application of Collost / Salvecoll in the treatment of DFS patients allowed us to reduce the average number of bed days by more than 20% (Table 1).

Table 1.

Comparative average number of bed days among DFS patients in "Diabetic Foot" Center and septic surgery departments in Kazan

"Diabetic Foot" Center					
Year	2010	2011	2012	2013	2014
Average number of bed days	18.21 ±1.2039	15.95 ±0.7801	15.29 ±1.1434	14.66 ±0.4533	12.13 ±0.3723
Septic surgery departments					
Year	2010	2011	2012	2013	2014
Average number of bed days	21,35 ±1.0420	20,74 ±1.1467	20,21 ±1.2039	19,52 ±1.2312	19,36 ±1.4221

We observed appropriate parallels (i.e. the higher number of clinical uses of bioplastic materials for closure of the non-healing chronic wounds, the fewer radically mutilating lower limb surgeries) (Figure 2).

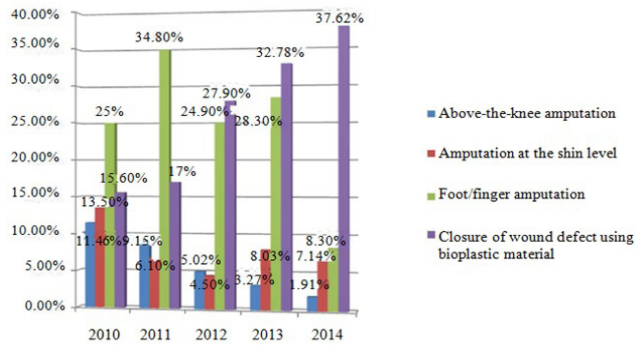


Fig. 2. *Dynamics of balance between lower limb amputations and organ preservation surgery at the "Diabetic foot" center in Kazan*

Case report 1.

Patient D., 78-year-old man admitted to the "Diabetic Foot" Center with diagnosis: Type 2 DM, insulin-dependent, severe course, decompensated. DFS, neuroischemic form, W5, diabetic gangrene of the left foot, diabetic angiopathy, CAI stage 4 of the left leg. *Photo 1:* The 4th day after amputation of the first finger on the left foot with resection at the middle-third of the first metatarsal bone, excision of purulent-necrotic foci of the left foot compartments and necrectomy. The 3rd day after X-ray endovascular angioplasty of the left lower limb. *Photo 2:* The 4th day after admission to the "Diabetic Foot" Center. The intra-operative implantation of collagen biomaterial Collost following ultrasonic cavitation with hydrosurgical debridement of the wound surface and exarticulation of the second finger of the left foot. *Photo 3:* The 35th day after admission to the "Diabetic Foot" Center. Diagnosis: DFS, neuroischemic form, W2, diabetic angiopathy, CAI stage 2a of the left lower limb. Wound epithelialization.

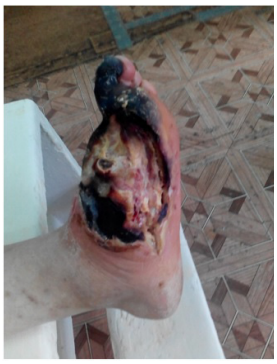


Photo 1.

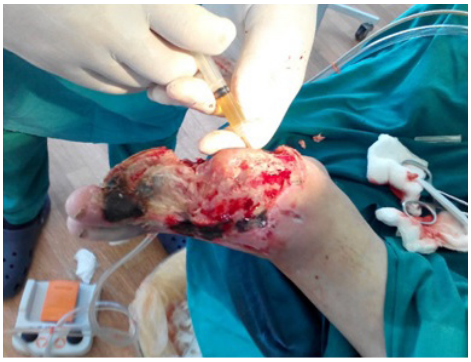


Photo 2.

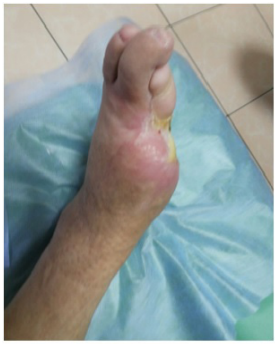


Photo 3.

Case report 2.

Patient M., 42-year-old man. Diagnosis: Type 2 diabetes mellitus, insulin-dependent, sub-compensated, severe course. DFS, W2. Trophic ulcers of the right foot. Diabetic angiopathy. CAI stage 3 of the right lower limb. Condition after amputation of the third finger of the right foot with resection of distal head of the third metatarsal bone. Diabetic neuropathy. Diabetic nephropathy. Diabetic retinopathy. *Photo 4:* View upon admission. *Photo 5:* Implantation of type I collagen in the form of 7% gel. *Photo 6*: The 27th day after complex treatment at the "Diabetic Foot" Center and implantation of biomaterial based on type I collagen in the form of membrane and 7% gel.

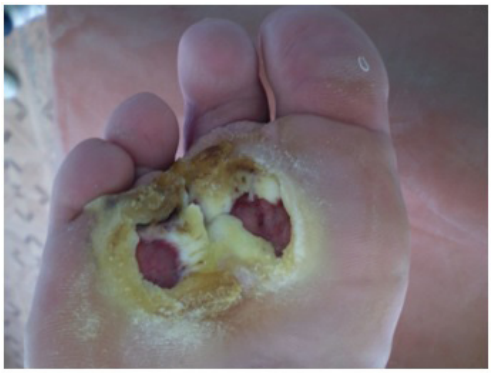


Photo 4.

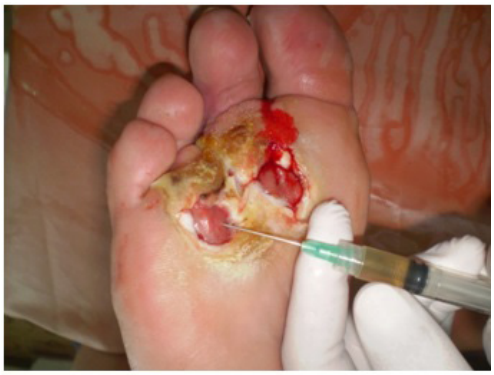


Photo 5.

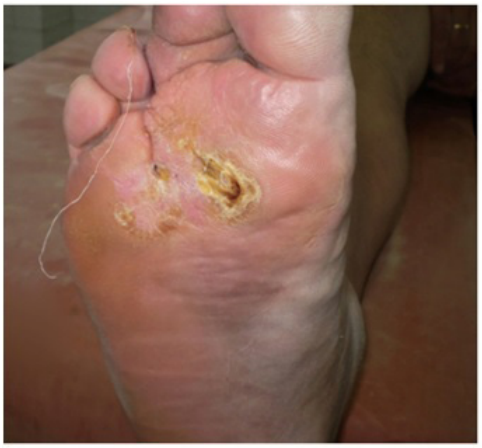


Photo 6.

In conclusion, the use of bioplastic collagen materials in DFS patients enabled us to (1) avoid creating two wound surfaces at the donor and recipient sites for wound defect closure as we do when using autodermoplasty by a free split-thickness skin graft, (2) stimulate tissue regeneration and epithelialization, and (3) reduce the inpatient stay and treatment period. This method can be used for both inpatient and outpatient treatment.

Competing interests

The authors declare that they have no competing interests.

References

1. Dedov II, Antsyferov MB, Galstyan GR, Tokmakova AY. *Diabetic foot syndrome.* Moscow; 1998. [in Russian].
2. Singh N., Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA. 2005; 293(2):217-28.
3. Wagner FW. A classification and treatment program for diabetic neuropathic and dysvascular foot problems. Instr Course Lect 28:143-65, 1979.
4. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1999;22(Suppl 1):S5–S19.
5. Standards of specialized diabetes care. Edited by Dedov II, Shestakova MV. 7th Edition. Diabetes mellitus. 2015;18(1S):1-112. [Article in Russian].
6. Lavery LA, Armstrong DG, Wunderlich RP, Tredwell J, Boulton AJ. Diabetic foot syndrome: evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. J Diabetes Care. 2003; 26(5):1435-8.
7. Bregovsky VB, Zaitsev AA, Zapevskaya AG. *Lower limb injuries in diabetes mellitus.* St. Petersburg: Dilya Publishers, 2004. [in Russian].
8. Chronopoulos A, Tang A, Beglova E, Trackman PC, Roy S. High glucose increases lysyl oxidase expression and activity in retinal endothelial cells: mechanism for compromised extracellular matrix barrier function. Diabetes. 2010;59(12):3159-66.
9. Mazurov VI. *Biochemistry of collagen proteins.* Moscow, 1974. [in Russian].
10. Ortolan EV, Spadella CT, Caramori C, Machado JL, Gregorio EA, Rabello K.: Microscopic, morphometric and ultrastructural analysis of anastomotic healing in the intestine of normal and diabetic rats. Exp Clin Endocrinol Diabetes 2008;116:198–202 [PubMed]
11. Singh VP, Bali A, Singh N, Jaggi AS. Advanced Glycation End Products and Diabetic Complications. Korean J Physiol Pharmacol. 2014; 18(1): 1–14.
12. El-Mesallamy HO, Hamdy NM, Ezzat OA, Reda AM. Levels of soluble advanced glycation end product-receptors and other soluble serum markers as indicators of diabetic neuropathy in the foot. J Investig Med. 2011;59:1233–1238.
13. Black E, Vibe-Petersen J, Jorgensen LN, Madsen SM, Agren MS, Holstein PE, et al. Decrease of collagen deposition in wound repair in type 1 diabetes independent of glycemic control. Arch Surg. 2003138(1):34-40.
14. Schultz GS1, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing.

Wound Repair Regen. 2009;17(2):153-62.
15. Briskin BS. *Use of bioplastic material Collost in the treatment of wound defects in patients with complicated forms of diabetic foot syndrome.* Moscow, 2014. [in Russian].
16. Safoian AA, Nesterenko VG, Nesterenko SV, Alekseeva NU. Bioresorbable collagen matrix, process of its preparation and use. Russian patent 2353397, A61L27/24, A61K35/32, A61K35/36, A61K38/39. 2009. [in Russian].

International Journal of BioMedicine 6(1) (2016) 46-47
doi: 10.21103/Article6(1)_OA9

ORIGINAL ARTICLE

Reconstructive Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

Augmentation-Mastopexy after Massive Weight Loss

ILya V. Sergeev, PhD[1]; Edward V. Shihirman, PhD[1]; Tagir R. Fayzullin, PhD[1,2]*

[1]*The clinic of plastic surgery "Dr.Plastic"*

[2]*The M. Vladimirsky Moscow Regional Research Clinical Institute (MONIKI)*

Moscow, Russia

Abstract

This paper presents an assessment of the results of mastopexy surgery carried out by using the technique of autologous breast enlargement axillary lateral flap, which the authors developed for patients who have suffered a decline in breast volume, as a result of massive weight loss after bariatric surgery. The mastopexy was carried out by the improved method with preliminary Doppler ultrasound of the perforating branches of intercostal arteries in women after a significant reduction in body weight. This method provides a good aesthetic result with the correct position of the nipple and a satisfactory volume of the breast and with a simultaneous removal of excessive skin flaps and excess fat in the anterolateral area of the chest. (**Int J Biomed. 2016; 6(1):46-47.).**

Keywords: *mammary glands; augmentation-mastopexy; weight loss; bariatric surgery.*

Introduction

A significant reduction in weight of overweight women leads to the appearance of areas with a large excess of skin and subcutaneous tissues. These involutional changes of the breast can be a source of aesthetic dissatisfaction in the patient [1,2]. Loss of breast volume with a significant reduction in body weight encourages ptosis and non-aesthetic changes in configuration of the entire surface of the upper half of the body [3]. Reducing the volume of the breast, performing only standard mastopexy, is generally insufficient to restore the breast to an aesthetically acceptable form, since such restoration requires a significant increase in the volume of the breast [4]. The results of mastopexy and reduction mammoplasty are largely determined by the presence or absence of breast asymmetry in the outcome [5]. In addition, patients are often worried about the decrease in volume of the tissues surrounding the implant, which leads to unfavorable relief of their contours [2,6,7].

The aim of this study was to evaluate the results of mastopexy carried out by an improved method using preliminary Doppler ultrasound of perforating branches of intercostal arteries in women after a significant reduction in body weight.

***Corresponding author:** Tagir R. Fayzullin, PhD, Associate Professor of the Oncology and Thoracic Surgery Department of the MONIKI; Moscow, Russia. E-mail: Tagir.Fayzullin@rambler.ru*

Materials and Methods

The study included 30 patients who underwent bariatric surgery to reduce body weight and applied for correction of the mammary glands. The mean age of women was 45.8 years. The average period after bariatric surgery was 2.4 years, average weight loss - 71 kg, mean BMI during the inspection - 30.6 kg/m^2.

Inclusion criteria were that patients had to meet the following criteria:

-Underwent bariatric surgery for weight loss;

- The preservation of sustainable weight control over the past 6 months;

-Indications for mastopexy surgery;

-The informed approval of patients for mastopexy surgery.

All patients underwent a vertical mastopexy with shearing and displacement of the additional lateral flap of the breast. The surgical field markup in a standard posture was performed before surgery in all cases. After the standard vertical mastopexy, the intervention was supplemented by shearing and sewing the lateral axillary flap. The base of the free flap was determined along the anterior axillary line, with a width of 6 to 8 cm.

Cutaneous perforating arteries were identified by using Doppler ultrasound of an anterior axillary area, in the preoperative period, with the location of blood vessels marked

on the skin. The length of the flap was generally from 15 to 20 cm, depending on the amount of excess skin and fatty tissue. The longitudinal axis of the flap was parallel to the upper edges of the slightly oblique deviation in the distal end.

The antibiotic prophylaxis was conducted in the preoperative period in all cases. The position of the patient was changed slightly after the anesthesia induction for better access to the lateral and posterolateral surface of the chest.

The musculocutaneous flap was isolated from its posterior edge toward the anterior edge. The perforating branches of intercostal arteries were carefully identified and preserved along the lateral chest wall. Primary closure of the lodge flap was performed with the use of the vicryl filaments 2-0 for deep layers and a monoacryla suture 3-0 for subcutaneous tissues and skin.

The subcutaneous pocket for the flap was formed above the fascia of the pectoral muscle in the direction of the clavicle. All perforating arteries supplying the flap were carefully preserved. After sufficient mobilization, the flap was rotated up to 90° around the lower leg, which was previously created, a desired image was set and then fixed to the chest.

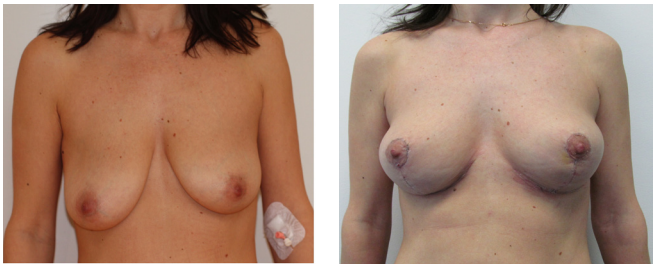
During the postoperative period, patients were invited for inspection after approximately one week, one month, three months, six months, and a year. The result of the operation was estimated based on the results of the flap engraftment, aesthetic results of operations, the number of re-operations and patients' subjective judgment of the operation results.

Results and Discussion

After the first week, in all cases, in the operated area the soft tissue swelling interstitial hematomas of various prevalence appeared, which were resolved usually by themselves after 1-2 months. Interstitial seromas with a volume of 100 ml were found in two patients. None of the cases showed signs of necrotic changes in adipose tissue or tissue flap, wound healing complications or skin necrosis. Complications from the donor area were not identified.

After one month and six months after surgery, we diagnosed a good position of the nipple and a satisfactory volume of the breast; thus the excessive amounts of "free" skin and subcutaneous fat in the anterolateral area of the chest were eliminated (Photo 1).

Patients were satisfied with the improvement of the forms and the increasing size of the breast, while reducing the skin excess and fatty tissue in 100% of cases. A vertical scar was cosmetically acceptable to all patients. During the first year after the surgery, we did not identify any complications of augmentation-mastopexy. In all cases, the displaced tissue flap remained unstrained and homogeneous in comparison with the surrounding breast tissue, and the border between them was practically not determined during the palpation.



A. Before surgery *B. After surgery (3rd week)*

Photo 1.

(A) Before and (B) after a vertical mastopexy with shearing and displacement of the additional lateral flap of the breast

Conclusion

Thus, preoperative identification of perforating vessels of the intercostal arteries provides a 100% engraftment of the lateral axillary flap at augmentation-mastopexy. This technique of augmentation-mammoplasty in conjunction with standard mastopexy makes it possible to achieve the satisfactory breast volume and the improved upper body contour after massive reduction in body weight.

Competing interests

The authors declare that they have no competing interests.

References

1. Adams WP Jr. The process of breast augmentation: four sequential steps for optimizing outcomes for patients. Plast Reconstr Surg. 2008; 122(6):1892-900.
2. Glicenstein J. [History of augmentation mammaplasty]. Ann Chir Plast Esthet. 2005; 50(5):337-49. [Article in French].
3. Schoeller T, Meier R, Otto-Schoeller A, Wechselberger G, Piza-Katzer H. Medial thigh lift free flap for autologous breast augmentation after bariatric surgery. Obes Surg. 2002;12(6):831-4.
4. Araco A, Gravante G, Araco F, Delogu D, Cervelli V, Walgenbach K. A retrospective analysis of 3,000 primary aesthetic breast augmentations: postoperative complications and associated factors. Aesthetic Plast Surg. 2007; 31(5):532-9.
5. Saratovtseva GY Secondary breast asymmetry after a mastopexy and reduction mammoplasty. Abstract of PhD Thesis. Moscow; 2011. [Abstract in Russian].
6. Milanov NO, Chausheva SI, Alyautdin SR, Kraskovsky FYa. Poland's syndrome: the choice of the surgical strategy Annals of Plastic, Reconstructive and Aesthetic Surgery. 2014; 2:35-41. [Article in Russian].
7. Lai YL, Yu YL, Centeno RF, Weng CJ. Breast augmentation with bilateral deepithelialized TRAM flaps: an alternative approach to breast augmentation with autologous tissue. Plast Reconstr Surg. 2003; 112(1):302-8.

International Journal of Biomedicine 6(1) (2016) 48-52
doi: 10.21103/Article6(1)_OA10

ORIGINAL ARTICLE

Child and Adolescent Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Multifactor Assessment of Metabolic Syndrome Risk in Uzbek Children and Adolescents with Obesity

Gulnara N. Rakhimova[1,2], PhD, ScD; Shakhnoza Sh. Azimova[1*]

[1] *Center for the Scientific and Clinical Study of Endocrinology*
[2] *Tashkent Institute of Post-Graduate Study, Department of Endocrinology*
Tashkent, Republic of Uzbekistan

Abstract

Metabolic syndrome (MetS) contributes to early atherosclerotic changes of blood vessels and type 2 diabetes mellitus not only among adults, but among children and adolescents, causing onset and progression of severe diseases resulting in early disablement and death. Multifactor analysis of MetS risk in Uzbek children and adolescents with exogenous-constitutional obesity (ECO) was the purpose of the study. The study included 100 Uzbek children and adolescents with ECO aged from 6 to 16 (mean age 11.7±0.25 years)—54(54.0%) boys and 46(46.0%) girls. Prognostic matrix was made up by means of a modification of Bayesian probability by E. Shigan (1986). Mathematical analysis confirmed a high degree of risk for MetS onset and progression in obese patients with disorders of lipid profile and hemodynamics. MetS risk is 8.2 times higher with levels of HDL-C <1.03 mmol/l, 3.6 times higher in patients with concentrations of TG ≥1.7 mmol/l, and 2 times higher in those with systolic arterial pressure (SAD) values ≥130 mmHg. The findings from our study confirm a high predictive value of HDL-C levels <1.03 mmol/l, TG ≥1.7 mmol/l and SAD for MetS onset and progression in Uzbek children and adolescents with obesity. Taking into account the integral assessment of MetS risk factors with waist circumference reference values established for the Uzbek pediatric population helped determine risk factors with very high disease dependence, such as atherogenic index >3.0, HbA1c >6.7%, and obesity onset before 5 years of age. **(Int J Biomed. 2016;6(1):48-52.)**.

Key words: *obesity; metabolic syndrome; adolescents; children; prediction.*

Introduction

Metabolic syndrome (MetS) is a cluster of metabolic, hormonal, and clinical disorders closely associated with type 2 diabetes mellitus, and cardiovascular disease. In 2007, the International Diabetes Federation (IDF) established new international criteria for MS diagnosis in children and adolescents [1]. According to some authors, the incidence of MetS diagnosis in children with obesity in compliance with IDF criteria ranges from 16% to 34% [2-4]; others report on its diagnosis in half of obese adolescents [5]. Previously, we studied MS prevalence in obese children and adolescents in compliance with tables of waist circumference percentile regression in European-American children and adolescents [1]. The findings from our study demonstrated that MetS occurred in 19% of Uzbek obese children and adolescents [6]. Assessment of risk factors (RFs) governing onset and progression of hormonal-metabolic disorders in childhood, and their ranking by value, significance and controllability is a key task in any study on MetS. Since the search for new significant MetS-RF in obese children and adolescents is vital, multifactor analysis of MS risk in Uzbek children and adolescents with exogenous-constitutional obesity is ***the purpose*** of the study.

Material and Methods

All anthropometric measurements, such as height, body mass, body mass index (BMI), waist circumference (WC), hip circumference (HC) and waist to hip ratio (WHR), were made in 100 Uzbek children and adolescents with exogenous-constitutional obesity (ECO) aged from 6 to 16 (mean age 11.7±0.25 years) — 54/54.0% boys and 46/46.0% girls. BMI values within the range of 85th-97th percentiles were considered as overweight; those above the 95th were assessed as obese (2007 WHO Z-curves). WC values were assessed

***Corresponding author:** Shakhnoza Sh. Azimova. Center for the Scientific and Clinical Study of Endocrinology. Tashkent, Uzbekistan. E-mail: shahnoz74@yandex.com*

by means of percentile tables developed for the age and sex, according to reference values in the representative sample of Uzbek children and adolescents [7]. Arterial pressure was measured in the morning and in the evening with subsequent assessment of values by age, sex and height percentiles [8]. The glucose oxidase method was used to measure fasting glucose and a 2-hour oral glucose tolerance test (OGTT) in the capillary blood; all examinees underwent OGTT with per oral administration of 1.75g (max 75g) of glucose per 1kg of weight. The modified colorimetric procedure of Flückiger and Winterhalter was employed for estimating HbA1c levels. Serum concentrations of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured by enzymatic colorimetric method by means of reagent kits supplied by Human, GeselFürBiochemica Und DiagnosticambH (Wiesbaden, Germany). RIA was used to measure concentrations of sex-steroid binding globulin (SSBG), and fasting and 2 h IRI (GTT) (Immunotech, Czech Republic). The HOMA-IR index was used to assess insulin resistance (IR). "Micral-test-II" test-strips (Roche, Germany) were used to assess microalbuminuria (MAU).

A modification of the Bayesian probability method for standardization of intensive parameters [9] with calculation of prognosis and weighted indices, and with standardized intensive and integrated parameters underlies the development of a prognostic matrix. To make the matrix we obtained comparable parameters of the predicted phenomenon by gradations of the most significant factors. The significance of the factors and their gradations was established by means of a relative risk (RR) parameter, or so-called weighting factor. RR was considered low at 2, moderate at the range from 2 to 3, high at ≥3. In addition, we calculated etiological fraction (EF) and rank for each MetS-RF. The etiological dependence was considered practically complete with 1<RR<1.4 and EF<80; it was very high at 2<RR<3.2 and EF in the range between 56 and 66, moderate at 1.5<RR<2 and EF in the range between 33 and 55, and small at 1<RR<1.4 and EF<33 [10].

Results and Discussion

To assess the value of MetS-RFs in Uzbek children and adolescents with ECO we developed a prognostic risk matrix (Table 1). Mathematical analysis confirmed a high degree of risk for MS onset and progression in obese patients with disorders of lipid profile and hemodynamics. MetS risk is 8.2 times higher with levels of HDL-C <1.03 mmol/l (69.0±8.5), 3.6 times higher in patients with concentrations of TG ≥1.7 mmol/l (33.8±9.4), and 2 times higher in those with systolic arterial pressure (SAD) values ≥130 mmHg (50.0±24.5). Fasting glycemia demonstrated a rather low predictive value.

The atherogenic index (AI) >3.0(47.1±15.2), improving the prognosis by more than by 3 times, turned out to be the most significant MetS-RF in Uzbek obese children. HbA1c >6.7% (60.0±23.1 and obesity onset age over 10 years are newly established and significant MS risk factors in Uzbek children and adolescents, with obesity prognosis increasing by more than 2.5 times. It should be noted that SAD ≥139 mmHg increases MetS risk by 2 times (50.0 ± 24.5). Obesity duration more than 5-10 years (34.1±18.8), HOMA index >3<75th percentile (50.0±30.2), and total cholesterol level ≥5.2 mmol/l (34.3±21.5) increase MetS risk by almost 2 times.

RR and EF were used to determine a rank for each MetS-RF (Table 2). Analysis of relative risk and etiological fraction of MetS-RFs demonstrated that almost complete dependence is associated with HDL-C<1.03 mmol/l (RR=8.16; EF=87.75%). Very high dependence of the disease was found to be associated with TG ≥1.7 mmol/l (RR=3.6; EF=72.30%) and AI>3.0 (RR=3.11; EF=67.85%). MetS high dependence can be seen with HbA1c >6.7% (RR=2.60; EF=61.54%) and obesity onset age over 10 years (RR=2.56; EF=60.94%). MS moderate dependence is observed with SAD ≥130 mmHg, obesity duration 5-10 years, HOMA>3rd<75th percentile, and total cholesterol ≥5.2 mmol/l.

According to T. Bokova [2], biological factors, such as abdominal obesity (RR=4.77, EF=79.0%), insulin resistance (RR=4.04, EF=75.2%), hyperglycemia (RR=3.20, EF=68.0%), dislipidemia manifesting in the TG increase and decrease in HDL-C (RR=2.60, EF=61.5% and RR=2.40, EF=60.0, respectively), small weight at birth (less than 2,500g) (RR=1.90, EF=47.4%), hyperuricemia (RR=1.85, EF=45.9%), and thyroid enlargement (RR=1.75, EF=42.9%), prevailed among the hardly controllable risk factors for MS in children and adolescents with very high, high, and moderate dependence in the Russian population. A high degree of obesity (BMI>30kg/m^2) (RR=2.7, EF=63.0%), obesity duration more than 5 years (RR=1.74, EF=42.5%), eating behavior disorder(night eating syndrome) (RR=1.52, EF=34.3%), and teenage years (12-16 years of age) (RR=1.50, EF=3.3%) are the controllable risk factors for high and moderate dependence. Our findings are consistent with those obtained by T. Bokova [2] in many respects.

The findings from our study demonstrate that WC is a poor contributor to MS prediction in obesity both in calculation in accordance with the WC European percentile tables for children and adolescents [5] and in accordance with WC percentile tables for Uzbek children and adolescents [8]. This finding can be probably associated with the fact that initially most (97%) patients with ECO had WC>97th percentile. In addition, mean WC values in adolescents with ECO and MetS and with ECO but without MetS had no significant differences.

Thus, integral assessment of MetS-RFs for Uzbek obese children and adolescents demonstrated that those with low HDL-C, high TG, high AI, high glycated hemoglobin, obesity onset age over 10 years, and high SAD are at the highest risk.

Conclusion

The findings from our study confirm a high predictive value of HDL-C levels <1.03 mmol/l (RR=8.16), TG ≥1.7 mmol/l (RR=3.61) and SAD (RR=2.4) for MS onset and progression in Uzbek children and adolescents with obesity. Taking into account the integral assessment of MetS-RFs with WC reference values established for the Uzbek pediatric population helped determine RFs with very high disease dependence, such as AI>3.0 (RR=3.100), HbA1c>6.7% (RR=2.60), and obesity onset before 5 years of age (RR=2.56).

Table 1 .

Prognostic matrix for multifactor assessment of MetS risk in Uzbek children and adolescents with ECO

Factors	Factor gradation	M,%	SIP	RR	IRA	Min	Max
		29.0					
Sex	Boys	25.9	0.894	1.01	0.90	0.90	0.91
	Girls	26.1	0.900		0.91		
The Tanner stage	I	10.0	0.345	1.38		1.15	1.59
	II-III	24.1	0.832		1.15		
	IV-V	33.3	1.149		1.59		
BMI, kg/m^2	<85th percentile	0.0	0.000	1.41	0.00	0.00	2.18
	85th-97th percentile	40.0	1.379		1.94		
	>97th percentile	28.4	0.980		1.38		
WC, cm	>75th ≤90th percentile	0.0	0.000	1.29	0.00	0.0 0	1.49
	>90th≤97th percentile	25.8	1.149		1.15		
	>97th percentile	33.3			1.49		
WC/HC	Normal	22.6	0.779	1.40	1.09	0.09	1.52
	Pathology	31.6	1.089		1.52		
Capillary blood fasting glycemia, mmol/l	≥5.0	30.8	1.061	1.22	1.29	1.06	1.29
	< 5.0	25.3	0.872		1.06		
HbA1c,%	< 5,9	24.4	0.841	2.60	2.19	2.07	5.38
	≥5.9-6.7	23.1	0.796		2.07		
	> 6.7	60.0	2.069		5.38		
Total cholesterol, mmol/l	≥5.2	34.3	1.182	1.59	1.88	1.18	1.88
	< 5.2	21.5	0.743		1.18		
HDL-C, mmol/l	≥3.5	24.0	0.828	1.11	0.92	0.92	1.02
	< 3.5	26.7	0.920		1.02		
LDL-C, mmol/l	< 1.03	69.0	2.378	8.16	19.41	2.38	19.41
	≥1.03	8.5	0.291		2.38		
TG, mmol/l	≥ 1.7	33.8	1.166	3.61	4.21	1.17	4.21
	< 1.7	9.4	0.323		1.17		
Atherogenic index	>3.0	47.1	1.623	3.11	5.04	1.62	5.04
	≤3,0	15.2	0.522		1.62		
Systolic arterial pressure, mmHg	≥130	50.0	1.724	2.04	3.52	1.72	3.52
	< 130	24.5	0.844		1.72		
Diastolic arterial pressure, mmHg	≥85	33.3	1.149	1.32	1.52	1.15	1.52
	< 85	25.3	0.872		1.15		
Fasting IRI, mcU/ml	>25.0	38.5	1.326	1.32	1.76	1.33	1.76
	≤25.0	29.0	1.001		1.33		
2h IRI, mcU/ml	>25.0	35.0	1.207	1.23	1.48	1.21	1.48
	≤25.0	28.6	0.985		1.21		
HOMA-IR	<3rd percentile (0.47/0.45)	0.0	0.000	1.66	0.00	0.00	2.86
	>3rdpercentile<75th percentile (0.74/1.0)	50.0	1.724		2.86		
	>75th percentile <97th percentile (1.23/1.8)	35.7	1.232		2.04		
	>97th percentile (1.23/1.80)	30.2	1.041		1.72		
MAU, mg/l	< 30	25.0	0.862	1.20	1.03	1.03	1.24
	from 30 to 100	30.0	1.034		1.24		

M – a parameter per 100 children with obesity, SIP- standardized intensive parameter, RR – relative risk, IRA – integrated risk assessment.

Table 1.

Prognostic matrix for multifactor assessment of MetS risk in Uzbek children and adolescents with ECO (continued)

Factors	Factor gradation	M,%	SIP	RR	IRA	Min	Max
		29.0					
Heredity by diabetes mellitus	Yes	28.9	0.998	1.20	1.19	1.00	1.19
	No	24.2	0.834		1.00		
Heredity by arterial hypertension	Yes	28.6	0.985	1.13	1.11	0.99	1.11
	No	25.3	0.873		0.99		
Heredity by obesity	Yes	31.0	1.067	1.38	1.47	1.07	1.47
	No	22.4	0.773		1.07		
Obesity onset age	under 5 years	29.2	1.006	2.56	2.57	1.72	4.41
	5-10 years	19.6	0.675		1.72		
	over 10 years	50.0	1.724		14.41		
Obesity duration	under 5 years	1.88	0.647	1.82	1.18	1.18	2.14
	5-10 years	34.1	1.177		2.14		
	over 10 years	22.2	0.766		1.40		

M – a parameter per 100 children with obesity, SIP- standardized intensive parameter, RR – relative risk, IRA – integrated risk assessment.

Table 2.

Risk factors by value for MetS onset in Uzbek children and adolescents with ECO

Risk factors	RR	EF, %	Rank
HDL-C < 1.03 mmol/l	8.16	87.75	1
TG ≥ 1.7 mmol/l	3.61	72.30	2
AI >3.0	3.11	67.85	3
HbA1c > 6.7%	2.60	61.54	4
Obesity onset age over 5 years	2.56	60.94	6
Systolic arterial pressure	2.04	50.98	7
Obesity duration more than 10 years	1.82	45.05	8
HOMA-IR >3rd percentile <75th percentile	1.66	39.76	9
Total cholesterol ≥ 5.2 mmol/l	1.59	37.11	10
BMI 85th -97th percentile > 97th percentile	1.41	29.08	11

Competing interests

The authors declare that they have no competing interests.

References

1. Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents-an IDF consensus report. Pediatric Diabetes.2007; 8(5):299-306.
2. Bokova TA. Metabolic syndrome in children: peculiarities of formation and clinical course, approaches to diagnosis, prevention and treatment. Abstract of ScD Thesis. Moscow; 2014. [in Russian].
3. Leontieva IV. Diagnosis and treatment of metabolic syndrome in pediatric practice. Pediatrics. 2011; 2:13-23 [in Russian].
4. Sinitsyn P.A. Metabolic syndrome in children and adolescents. Clinical-pathogenetic parallels. Abstract of ScD Thesis. Moscow; 2011. [in Russian].
5. Rakhimova GN, Azimova ShSh. Integral assessment of risk factors for metabolic syndrome in children and adolescents with obesity. International Endocrinological Journal. 2012; (43):77-81 [in Russian].
6. Azimova ShSh, Rakhimova GN. Determination of metabolic syndrome risk by waist circumference in Uzbek children and adolescents. Patent No. DGU 02583 (2012).
7. Azimova Sh, Rakhimova G. Waist circumference

percentiles in a nationally representative sample of 7-18 years old Uzbek children and adolescents. Medical and Health Science Journal. 2013; 14 (3):123-27.
8. Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents. Pediatrics. 1996; 98 (4 Pt 1): 649–58.
9. Shigan EN. *Methods for prediction and modeling in social-hygienic studies*. M.: Meditsina; 1986. [in Russian].
10. Babanov S.A., Vorobyova E.V., Gailis P.V., Agarkova I.A. Epidemiology of non-infectious diseases: problems of this stage. Profilakt Meditsina 2011; (3):11-14 [in Russian].

International Journal of Biomedicine 6(1) (2016) 53-55
doi: 10.21103/Article6(1)_OA11

ORIGINAL ARTICLE

Child and Adolescent Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Sleep Patterns in Adolescents with Hypertension

Irina M. Madaeva, PhD, ScD; Olga N. Berdina, PhD*; Tamara Mandzyak, PhD; Sergey Kolesnikov, PhD, ScD; Liubov I. Kolesnikova, PhD, ScD

Scientific Centre for Family Health and Human Reproduction Problems
Irkutsk, the Russian Federation

Abstract

Background: There is growing evidence that psychological stress contributes to hypertension and leads to changes in sleep structure. Insufficient sleep may lead to cardiovascular disease. Thus, the aim of this study was to explore specific sleep patterns in adolescents with hypertension.

Methods: The study population consisted of 35 young patients (14–17 years old): 20 adolescents with hypertension and 15 healthy adolescents. Polysomnography (PSG) was performed on all patients.

Results: Statistical analysis showed significant changes of sleep patterns in the hypertensive adolescents compared to the normotensive adolescents. Hypertension was associated with significantly decreased slow wave sleep (16.86±0.3 vs. 22.7±0.3%; *P<0.05*) and increased rapid eye movement sleep (REM) (23.1±0.2 vs. 30.75±0.2%; *P<0.05*). At the same time, sleep latency increased compared to subjects without hypertension (27.2±0.3 vs. 11.2±0.4 min, *P<0.05*). Wake time after sleep onset (WASO) was also significantly (19.1±0.2 vs. 5.9±0.3 min, *P <0.05*) longer than in healthy adolescents, which led to reduced sleep duration.

Conclusion: These results suggest that these kinds of sleep deviations are closely associated with some adaptive reaction to prolonged exposure of psychogenic factors in the hypertensive adolescents. **(Int J Biomed. 2016;6(1):53-55.).**

Keywords: *sleep; polysomnography; adolescents; hypertension.*

Introduction

Hypertension is a leading public health challenge globally due to its high prevalence and related morbidity and mortality [1]. The roots of hypertension in adulthood extend back to childhood. That is to say, children and adolescents with elevated blood pressure (BP) are more likely to become hypertensive adults [2, 3]. There is growing evidence that psychological stress contributes to hypertension and cardiovascular disease [4]. A number of authors have shown that onset of hypertension corresponds to some level of insufficiency of psychophysiological regulation and could be driven by different neurogenic and psychogenic factors [5]. Blood pressure (BP) level correlates with the emotional status, level of anxiety, individual reactions to stressful situations, personal features, and mental status. Clinical data suggest raised reactivity of the cardiovascular system, which appears as an increasing heart rate and elevated BP in response to stress or other factors [6]. In addition, it is known that stress leads to changes in sleep structure [7,8]. However, sleep is a process that depends on conditions in the brain that integrate, differentiate and coordinate all personal psychosomatic activity [9]. Insufficient sleep impairs physical and psychological development, which may negatively affect concentration, performance, behavior, emotional well-being, and overall health.

The precise biological mechanisms are only partially understood, but the autonomic nervous system could be a potential pathway linking sleep problems with subsequent pathologies such as hypertension, diabetes, and cardiovascular disease [10-12]. It has been shown that rapid eye movement sleep (REM) is the main sleep pattern connected to psychic adaptation and emotional recovery [13].

Thus, analysis of sleep patterns in adolescents with hypertension can help either to detect how this pathogenic factor drives sleep structure to change and evaluate types of adaptive reactions in these patients.

The aim of this study was to explore specific sleep patterns in adolescents with hypertension.

***Corresponding author**: Olga Berdina, PhD. Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia. E-mail: goodnight_84@mail.ru*

Materials and Methods

The study included a cohort of 35 subjects (age range 14-17 years). All patients were divided into 2 groups. Group 1 consisted of 20 patients with hypertension, and Group 2 of 15 healthy adolescents. Hypertension in children and adolescents continues to be defined as systolic BP (SBP) and/or diastolic BP (DBP), that is, on three separate measurements, at or above the 95th percentile (The fourth report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, 2005)

Adolescents were invited to undergo a full night of PSG recording. This study was approved by the Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems Local Ethical committee and a written informed consent was obtained from all participants or their parents (if a child under 15 years old) at the assessment.

During a visit to the Somnological Center, trained technicians equipped the subjects with the Polysomnography (PSG) recorder (GRASS-TELEFACTOR Twin PSG-system with As 40 booster and integrated sleep-module SPM-1, USA) between 20:00 and 22:00. All sleep recordings took place in the special room and included a total of 18 channels: six electroencephalography, two electrooculography, three surface electromyography (one submental, two for right and left anterior tibialis muscles), one for electrocardiogram, nasal pressure, thoracic and abdominal belts, body position, oxygen saturation, and pulse rate. During the study, all patients were under observation by trained sleep technicians. We also used an infrared camera for night video monitoring. All PSG recordings were visually scored by a trained sleep physician. Sleep stages, leg movements, and arousals were scored according to the 2007 American Academy of Sleep Medicine (AASM) criteria [14].

The statistical analysis was performed using the statistical software Statistica v6.0 (StatSoft, USA). The mean *(M)* and standard error of the mean (SEM) were calculated. *F*or data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution were tested by the Mann-Whitney *U*-test. A probability value of $P<0.05$ was considered statistically significant.

Results

Table 1 presents the sleep characteristics according to the absence or presence of hypertension in adolescents. Statistical analysis showed significant differences in sleep structure between patients in the two groups.

In Group 1, in spite of relatively preserved cyclic sleep and balanced sleep phases, there was alteration in the normal duration of sleep phases (Figure 1). Hypertension was associated with significantly decreased slow wave sleep (16.86±0.3 vs. 22.7±0.3%; $P<0.05$) and increased REM (23.1±0.2 vs. 30.75±0.2%; $P<0.05$). At the same time, sleep latency increased compared to subjects without hypertension (27.2±0.3 vs. 11.2±0.4 min, $P<0.05$). Wake time after sleep onset (WASO) was also significantly (19.1±0.2 vs. 5.9±0.3 min, $P<0.05$) longer than in healthy adolescents, which led to reduced sleep duration. There were no significant differences between the two groups in the amount of arousals; however, there was a tendency to increased arousals in hypertensive subjects.

Table 1.

Sleep characteristics according to the absence or presence of hypertension in adolescents

Variable	Group 1 (n=20)	Group 2 (n=15)	*P*
Total sleep time (TST), min	402±3	400±2	*0.53*
Superficial sleep, % of TST	51.8±0.3	54.2±0.25	*0.21*
Slowly-wave sleep, % of TST	16.86±0.3	22.7±0.3	*<0.05*
REM, % of TST	30.75±0.2	23.1±0.2	*<0.05*
Sleep latency, min	27.2±0.3	11.2±04	*<0.05*
WASO, min	19.1±0.2	5.9±0.3	*<0.05*
Arousal index, n/h	18.45±0.4	15.2±0.3	*0.18*

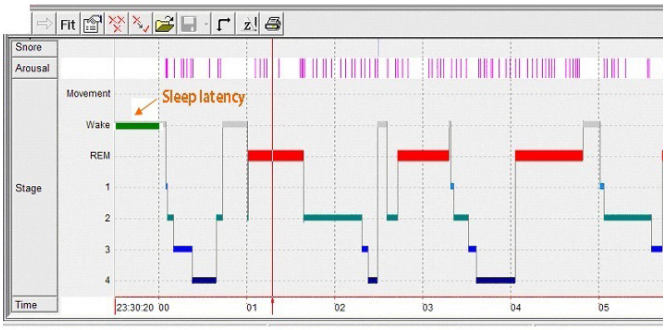


Fig. 1. The sleep histogram of 15-year-old patient with hypertension

Sleep latency from 11.30 PM until 12.05 AM is marked. Cyclicity of sleep is kept, however, insignificant reduction duration of slow wave sleep (SWS) (stages 3, 4) due to considerable increase of REM sleep is observed. In addition, it is marked increase of the period of wakefulness overnight (Wake).

Discussion

In the modern informational space with a high educational requirement, adolescents are exposed to daily life stress in the home or school environment. The initial deficiency of psychophysiological regulation and chronic stress can lead to activation of the neuroendocrine and immune systems (sympathetic nervous system, hypothalamus–pituitary–adrenal axis, and cytokines) and confer related cardiovascular risks [15,16]. We suppose that detected specific sleep patterns in adolescents with hypertension indicate some adaptive reorganization of the psychophysiological system. Difficulties falling asleep, an increasing WASO, and a tendency to an increase in the arousal index are determined by chronic stress. It possibly could be reflection of increased activity in the brain structures by ascending activation caused by different adverse factors. At the same time, a considerable increase in REM

stage fraction in the total sleep structure is an adaptive reaction to prolonged exposure to psychogenic factors. Thus, specific sleep features in adolescents with hypertension indicate the presence of psychophysiological adaptation. In this case, psychoemotional factors affect mainly the REM organization. This confirms the most widely accepted theories of sleep function, sleep mechanisms and brain organization [17,18].

Competing interests

The authors declare that they have no competing interests.

References

1. Danaei G, Finucane MM, Lin JK, Singh GM, Paciorek CJ, Cowan MJ, et al. National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants. Lancet 2011; 377(9765):568–577.
2. Bao W, Threefoot SA, Srinivasan SR, Berenson GS. Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: the Bogalusa Heart Study. Am J Hypertens 1995; 8(7):657–65.
3. Sun SS, Grave GD, Siervogel RM, Pickoff AA, Arslanian SS, Daniels SR. Systolic blood pressure in childhood predicts hypertension and metabolic syndrome later in life. Pediatrics. 2007; 119(2):237–46.
4. Dimsdale JE. Psychological stress and cardiovascular disease. J Am Coll Cardiol. 2008; 51(13):1237–46.
5. Esler M. Heart and mind: psychogenic cardiovascular disease. J Hypertens 2009; 27(4):692–5.
6. Claude J. Mental stress, hypertension and the baroreflex: what's new? J Hypertens 2009; 27(1): 31–3.
7. Palagini L, Drake CL, Gehrman P, Meerlo P, Riemann D. Early-life origin of adult insomnia: does prenatal–early-life stress play a role? Sleep med 2015; 16(4):446–56.
8. Winzeler K, Voellmin A, Schäfer V, Meyer AH, Cajochen C, Wilhelm FH, et al. Daily stress, presleep arousal, and sleep in healthy young women: a daily life computerized sleep diary and actigraphy study. Sleep med 2014; 15(3):359–66.
9. Raschke F, Fischer J. "Arousal" in der Schlafmedizin. Somnologie.1997; 2, 59-64 [Article in German]
10. Michels N, Clays E, De Buyzere M, Vanaelst B, De Henauw S, Sioen I. Children's sleep and autonomic function: low sleep quality has an impact on heart rate variability. Sleep 2013; 36(12):1939-46.
11. Knutson KL. Sleep duration and cardiometabolic risk: a review of the epidemiologic evidence. Best Pract Res Clin Endocrinol Metab 2010; 24(5):731-43.
12. Madaeva I, Kolesnikova L, Dolgikh V, Berdina O. Sleep-disordered breathing in 15-year-old boy with arterial hypertension. Respir Med Case Rep 2012; 8: 5–9.
13. Carr M, Nielsen T. Morning rapid eye movement sleep naps facilitate broad access to emotional semantic networks. Sleep 2015; 38(3):433–43.
14. Iber C, Ancoli-Israel S, Chesson A, Quan SF for the American Academy of Sleep Medicine. *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications*, 1st ed.: Westchester, IL: American Academy of Sleep Medicine, 2007.
15. Gerin W, Chaplin W, Schwartz JE, Holland J, Alter R, Wheeler R et al. Sustained blood pressure increase after an acute stressor: the effects of the 11 September 2001 attack on the New York City World Trade Center. J Hypertens 2005; 23(2):279–84.
16. Trudel-Fitzgerald C, Boehm J, Kivimaki M, Kubzansky L. Taking the tension out of hypertension: a prospective study of psychological well being and hypertension J Hypertens 2014; 32(6):1222–8.
17. Shneerson JM. *Sleep medicine: a guide to sleep and its disorders*. 2nd ed. Blackwell Publishing Ltd; 2005.
18. Kryger MH, Roth T, Dement WC. *Principles and Practice of Sleep Medicine*. 5th Ed. Saunders; 2011.

International Journal of Biomedicine 6(1) (2016) 56-59
doi: 10.21103/Article6(1)_OA12

ORIGINAL ARTICLE

Child and Adolescent Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Outcomes of Very Early Preterm Births in the Republic of Sakha (Yakutia)

Nyurguyana S. Baisheva*[1]; Natalia I. Douglas, PhD, ScD[1]; Tatiana Y. Pavlova, PhD[2]; Anisia I. Yakovleva[3]; Tatiana E. Burtseva, PhD, ScD[4]

[1]North-Eastern Federal University
[2]Ministry of Health of Republic of Sakha (Yakutia)
[3]Republican Hospital № 1 – National Center of Medicine, the Perinatal Center
[4]Yakutsk Research Center for Complex Medical Problems
Yakutsk, the Sakha Republic (Yakutia), Russia

Abstract

Protection of maternal and child health is a special health care industry. It largely determines the future of the nation. Therefore, it is an important matter of the state. This article presents an analysis of cases of very early preterm births (VEPB) and their outcomes in 2012-2014 in the Republic of Sakha (Yakutia). Retrospective analysis of the cases of VEPB and of the status of health of the babies born before 28 weeks of gestation was conducted during the research. The group at risk of VEPB consisted of women with burdened obstetric and gynecological history. The main causes of VEPB were premature amniorrhexis in pregnant women with carriage of infections and life-threatening severe pre-eclampsia. In the structure of morbidity of very preterm babies the first place among the main diseases belongs to perinatal lesion of central nervous system, the second place belongs to respiratory distress syndrome, and the third place to infectious and inflammatory diseases. In the structure of death, 57.1% of fatal cases are babies with a term of gestation less than 28 weeks. The causes of mortality were respiratory distress syndrome, congenital pneumonia, and intraventricular hemorrhage. **(Int J Biomed. 2016;6(1):56-59.)**

Keywords: *preterm birth; very early preterm birth; very low birth weight; extremely low birth weight.*

Introduction

In the current unfavorable demographic situation and with the health of women of childbearing age in Russia deteriorating, the problems of preservation of the life and health of each baby and reduction of infant mortality are especially relevant [1]. Preterm births (PB) are the leading cause of perinatal morbidity and mortality in the world and one of the most important problems of modern health care [2,3]. According to the WHO classification, very early preterm births (VEPBs) are deliveries which take place between 22 and 27 weeks of pregnancy; in this case the newborns have extremely low birth weight (ELBW) from 500 to 999.0 g. Deliveries in the term of 28-33 weeks are early preterm births (EPB), in 34-37 weeks are PB. Every year 20 million babies are born prematurely and 0.4%-0.5% of them have ELBW. In Russia, PBs range from 4% to 16%, and 0.3% of such newborns have ELBW [4]. With adoption of the order of the Russian Health Ministry №1687n [5], the problem of VEPB in the Republic of Sakha (Yakutia) (RS (Y)) became particularly acute.

Thus, in 2012, the perinatal mortality rate increased from 8.4 to 13.0 per 1000 born alive and dead in the region. In 2013 and 2014, this rate was respectively 10.8 and 10.0 per 1000 born alive and dead. There were 496 PBs in 2012; the proportion of VEPBs was 22.7%. In 2013, the proportion of VEPBs was 17.6%, and in 2014 it was 6.12%. Premature babies of Yakutia are being nursed on 40 beds of the Department of Pathology of Newborn and Premature Babies (DPNaPB) of the Perinatal Center (PC) at the State Budget Institution of RS(Y) "Republican Hospital №1 - National Center of Medicine (NCM)." Children from the entire region are transported there. Thus, the problem of VEPB and the

***Corresponding author*:** *Nyurguyana S. Baisheva, Senior lecturer at Department of Obstetrics and Gynecology. North-Eastern Federal University. Yakutsk, the Sakha Republic (Yakutia), Russia. E-mail: kosmos80-80@mail.ru*

outcome is actually not only for the health authorities but also for the social services and requires deep scanning for development of modern preventive measures.

The aim of this study was an assessment of the status of health of the women and their babies born before 28 weeks of gestation, and the identification of very early miscarriage risk factors. The research is based on the records of the marked PC taken in the period from 2012 to 2014.

Materials and Methods

We conducted a retrospective analysis of occurrence of very early miscarriage and study of health of extremely preterm infants born before 28 weeks of gestation who were hospitalized in the PC in 2012-2014.

Statistical analysis was performed using the statistical software «Statistica» (*v6.0*, *StatSoft*, *USA*). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Group comparisons with respect to categorical variables are performed using chi-square tests or, alternatively, Yates' $\chi 2$ when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the North-Eastern Federal University Ethics Committee.

Results and Discussion

In 2014, 16,948 childbirths were accepted in the medical institutions of RS (Y) and 1,172(6.9%) babies were born prematurely (Table 1).

Table 1.

The number of births in RS (Y) in 2013-2014

Variable	2012 (n/%)	2013 (n/%)	2014 (n/%)
Number of preterm births	1160/6.85	1078/6.5	1172/6.92
Number of other births	15762/93.14	15500/93.5	15776/93.1
Total number of births	16922/100	16578/100	16948/100

In the period from 2012 to 2014, 82 women had VEPB in the PC. Overall, 54.66% of the women were from the rural area and 46.34% were from the city. VEPB was common in the age group of 19-28 years (42.46%). The early turnout at the dispensary registration (before 12 weeks of the pregnancy period) was 60.97%. Overall, 68.3% of the women had chronic diseases of the female genital organs, 37.75% had medical abortions in the anamnesis, 28.1% – miscarriages, 4.87% – experienced fetal death, and 15.85% – PB. Extragenital diseases were found in all of the cases in the studied group: 75.6% of the women had urinary tract diseases, 54.87% – cardiovascular diseases, 36.58% – gastrointestinal diseases, 19.51% – anemia, 8.53% – respiratory system diseases, and 2.43% – endocrinopathies. Sexually transmitted infections were found in almost all the studied cases.

On average, 55.06% of the women entering an obstetric hospital had a severe condition; 87.33% had such a condition due to the severe pre-eclampsia. The causes of VEPB were premature amniorrhexis (42.6%), premature abruption of a normally located placenta (14.23%), threat of fetal asphyxia (9.13%), and isthmic-cervical incompetence (6.06%). On average, 18.74% of the women had independent childbirth, 81.26% - surgical delivery (cesarean section), and 13.41% - emergency childbirth. The main reasons for early deliveries (in 22-27 weeks) were severe pre-eclampsia (38.63%), premature abruption of a normally located placenta (9.1%), and premature amniorrhexis (16.23%).

The majority of mothers (42.2%) had a secondary education, 55.06% of the women were married, and 31 women (37.8%) smoked tobacco.

Due to the effective implementation of routing of the women in labor who have severe extragenital pathology and routing of women with a threat of PB in the RS (Y), there was an increase in preterm infants with ELBW born at the PC (Table 2).

Table 2.

The number of premature infants hospitalized to the DPNaPB (PC-NCM) from maternity wards of RS (Y)

Variable	2012 (n/%)	2013 (n/%)	2014 (n/%)
Maternity ward of PC NCM	89/41.8	141/62.7	145/84.3
Maternity ward of Yakutsk	86/40.4	59/26.2	4/2.3
Central hospital of Ulus (CHU)	31/14.6	22 /9.8	23/13.3
Other departments	6/2.8	3/1.3	-
Self-appeal	1/0.47	-	-
Total	213/100	225/100	172/100

The analysis of the health of newborns who had been admitted revealed that every year the number of babies in a very severe condition increased. In 2012, it was 39(18.3%) newborns, in 2013 – 68(30.2%) newborns, in 2014 – 67(38.9%) newborns. In 2012, a severe condition was diagnosed for 130(61.03%) newborns, in 2013 for 136 (60.4 %), in 2014 for 102(59.3%) newborns (Table 3).

Table 3.

The health of newborns at admission to DPNaPB of PC-NCM

Variable	2012 (n/%)	2013 (n/%)	2014 (n/%)	*P*
Very severe condition	39/18.3	68/30.2	67/38.9	*0.0000*
Severe condition	130/61.0	136/60.4	102/59.3	*0.9413*
Moderate condition	43/20.2	19/8.4	3/1.7	*0.0000*
Satisfactory condition	1/0.47	2/0.89	-	*0.7870*
Total	213/100	225/100	172/100	

Dynamics starting from 2012 revealed an increase in the number of extremely serious conditions of newborns. This tendency is associated with an increase of the number of babies with gestation less than 28 weeks: in 2012 – 37 (17.3%), in 2013 – 43 (19.1%) and in 2014 –38 (22.1%) (Table 4). The huge growth of the number of hospitalized babies with ELBW

and very low birth weight (VLBW) is revealed in annual dynamics. Thus, in 2012 the share of babies with ELBW was 31 (15% of all the cases of hospitalization), in 2013 – 30 (13.3 %), in 2014 – 41 (23.8%) (Table 5).

Table 4.

Distribution of hospitalized babies by gestation term

Weeks of gestation	2010 (n/%)	2011 (n/%)	2012 (n/%)	2013 (n/%)	2014 (n/%)	*P*
<28	20/8.2	16/7.0	37/17.4	43/19.1	38/22.1	*0.0000*
28-30	41/16.4	39/17.2	55/25.8	59/26.2	69/40.1	*0.0000*
31-33	107/43.7	102/44.9	85/39.9	93/41.3	55/32.0	*0.0887*
34-36	67/27.3	60/26.4	31/14.6	24/11	10/5.8	*0.0000*
> 36	10/4.1	10/4.4	5/2.3	6/2.6	-	*0.0734*
Total	245/100	227/100	213/100	225/100	172/100	

Table 5.

Distribution of hospitalized babies by weight at birth

Weight (g)	2010 (n/%)	2011 (n/%)	2012 (n/%)	2013 (n/%)	2014 (n/%)	*P*
>1000	10/4.1	22/9.7	31/14.6	30/13.3	41/23.8	*0.0000*
1000-1250	15/6.1	27/11.9	35/16.4	34/15.1	23/13.4	*0.0000*
1250-1500	33/13.5	36/15.8	37/17.4	45/20.0	51/29.6	*0.0006*
1500-2000	106/43.3	90/39.6	71/34.7	84/37.3	52/30.2	*0.0524*
2000-2500	56/22.8	46/20.3	27/12.7	18/8.0	5/2.9	*0.0000*
>2500	25/10.2	6/2.6	12/5.6	14/6.2	-	*0.0000*
Total	245/100	227/100	213/100	225/100	172/100	

In 2012, the majority of newborns (55.39%) in the Department of Pathology of Newborn and Premature Babies received artificial feeding, 28.63% received breastfeeding and 34 (15.96%) received mixed feeding. In 2013, 108 (50%) of newborns received artificial feeding, 64 (30%) received breastfeeding and 44 (20.3%) babies received mixed feeding. In 2014, 105 (61%) babies received artificial feeding, 42 (24.4%) received mixed feeding and only 25 (14.4%) babies received breast milk. Such a tendency is due to severe and very severe conditions of the health of very preterm babies and hypo- and agalactia of mothers (Table 6).

Table 6.

Distribution of hospitalized babies according to feeding type

Feeding type	2012 (n/%)	2013 (n/%)	2014 (n/%)	*P*
Breastfeeding	61/28.6	64/28.4	25/14.5	*0.0014*
Mixed feeding	34/16.0	44/19.6	42/24.4	*0.1160*
Artificial feeding	118/55.4	117/52.0	105/61.0	*0.1970*
Total	213/100	225/00	172/100	

In 2014, the most common disease in the structure of morbidity of hospitalized very preterm babies was perinatal lesion of central nervous system (95.9% of cases, 5.8% of them were intraventricular hemorrhage of the third degree); respiratory distress syndrome was in 80.8% of the cases of morbidity, infectious and inflammatory diseases in 14.5% of the cases. The following complications were observed: necrotizing enterocolitis (11.0%), conjugation hyperbilirubinemia (16.8%), and anemia of prematurity (39.5%).

According to the data obtained (Table 7), 57.1% of fatal outcomes belong to the babies with a term of gestation less than 28 weeks. On average, 75% of babies were born with ELBW.

Table 7.

The structure of hospital mortality among the premature infants

Variable	Fatal outcomes	2013 n=8 (n/%)	2014 n=14 (n/%)	*P*
Age	0-6 days	-	-	*0.9643*
	6-28 days	6/75	9/64.28	
	>28 days	2/25	5/35.71	
Gender	Boys	6/75	6/42.86	*0.3118*
	Girls	2/25	8/57.14	
Ethnicity	Ethnic Russians	--	2/14.28	*0.9650*
	Yakuts	7/87.5	11/78.57	
	Minority ethnicities	1/12.5	-	
	Others	-	1/7.14	
Residence	Rural	5/62.5	7/50.0	*0.9025*
	Urban	3/37.5	7/50.0	
Route of admission	Maternity ward of PC- NCM	7/87.5	13/92.85	*0.9175*
	City maternity ward	1/12.5	-	
	CHU	-	1/7.14	
Term of gestation	< 28 weeks	5/62.5	8/57.14	*0.8376*
	28-30 weeks	3/37.5	6/42.85	
	31-33 weeks	-	-	
	34-37 weeks	-	-	
Weight at birth (g)	<1000	4/50.0	12/85.71	*0.1678*
	1000-1250	1/12.5	2/14.28	
	1250-1500	3/37.5	-	
	1500-2500	-	-	

According to autopsies, the causes of mortality were the following: respiratory distress syndrome (37.5%), congenital pneumonia (12.5%), intraventricular hemorrhage of the third degree/noncommunicating hydrocephaly/coma (12.5%), respiratory syncytial virus infection (12.5%), a congenital heart defect (complete transposition of the great vessels) (12.5%), and neonatal necrotizing enterocolitis (12.5%).

Conclusion

The group at risk of VEPB consisted of women with burdened obstetric and gynecological history. The main causes of VEPB were premature amniorrhexis in pregnant women with carriage of infections and life-threatening severe pre-

eclampsia. In the structure of morbidity of very preterm babies the first place among the main diseases belongs to perinatal lesion of central nervous system, the second place belongs to respiratory distress syndrome, and the third place to infectious and inflammatory diseases. In the structure of death, 57.1 % of fatal cases are babies with a term of gestation less than 28 weeks.

Thus, implementation of routing of pregnant women with severe extragenital pathology who have a risk of PB has an opportunity to significantly reduce the medical and social effects of VEPB.

Competing interests

The authors declare that they have no competing interests.

References

1. Sidelnikova VM. Proceedings of the 8th Russian Forum "Mother and Child". M.: 2006: 241 [in Russian].
2. Sidelnikova VM, Antonov AG. Preterm birth. Premature baby. *Guide for physicians*. M.: GEOTAR-Media; 2008.
3. Kulakov VI, Antonov AG, Baibanna EN. Problems and perspectives of care for very low body-weight babies at the present stage. Ross Vest Perinat Ped. 2006;4:8-11.[in Russian].
4. Kryvkina NN, Akhmadeyeva EN, Valyulina AYa. The comparative characteristics of health premature infants depending on theirs birth weight. Vest Sovremen Klin Med. 2013; 6(1):26-30.[in Russian].
5. Order of the Health Ministry of Russia №1687n of December 27, 2011. "On the medical criteria of birth, the form of the birth order and its issuance". [in Russian].

International Journal of Biomedicine 6(1) (2016) 60-64
doi: 10.21103/Article6(1)_OA13

ORIGINAL ARTICLE

Molecular Markers and Diagnosis

INTERNATIONAL JOURNAL OF BIOMEDICINE

Molecular Mechanisms of Ischemic Preconditioning with Cardiovascular Aging in Elderly Patients with Arterial Hypertension

Elena A. Kartashova, PhD[1]; Irina V. Sarvilina, PhD, ScD*[2]

[1]*Rostov-on-Don State Medical University, Rostov-on-Don, Russian Federation*
[2]*Medical Centre "Novomeditsina", Rostov-on-Don, Russian Federation*

Abstract

The purpose of this study was to analyze molecular mechanisms of ischemic preconditioning (IPC) with cardiovascular aging in elderly patients with isolated systolic hypertension (ISH).

The study included 306 persons divided into two groups: Group 1 (the control group) included 150 elderly patients without AH, and Group 2 (the experimental group) included 156 elderly patients with ISH according to the inclusion/exclusion criteria. The duration of ISH was 13.5 years. All patients received a double combination of antihypertensive drugs. We applied standard methods for identification of ISH and secondary hypertension**.** Molecular phenotyping of blood plasma with the identification of molecules involved in IPC process were processed with methods of proteomics. The data of the molecular interactions and functional features of proteins were received with STRING 10.0 database. Bioinformatics analysis has revealed the presence of molecules that are the participants in the pathways of IPC, cardiovascular aging and the molecular interactions involved. **(Int J Biomed. 2016;6(1):60-64.).**

Keywords: *ischemic preconditioning; cardiovascular aging; isolated systolic hypertension; proteomics; molecular interactions.*

Introduction

Aging is an inevitable part of life and unfortunately poses the largest risk factor for cardiovascular disease. By 2030, approximately 20% of the population will be aged 65 or older. In this age group, cardiovascular diseases (CVD) will result in 40% of all deaths and rank as the leading cause [1]. Aging has a remarkable effect on the heart and arterial system, leading to an increase in cardiovascular diseases, including atherosclerosis, hypertension, myocardial infarction (MI), and stroke. Aging cardiovascular tissues are exemplified by pathological alterations, including hypertrophy, altered left ventricular (LV) diastolic function, and diminished LV systolic reverse capacity, increased arterial thickening and stiffness, and impaired endothelial function [2-6]. Isolated systolic hypertension (ISH) is the most common form of hypertension seen in the elderly [7,8] and the most interesting model of aging of the cardiovascular system. Age is a powerful predictor of mortality for patients with acute MI [9-12]. However, until recently, the fields of cardiovascular disease and molecular biology of aging have remained largely separate [1]. The higher mortality and morbidity associated with advancing age could be due to a reduction in some endogenous protective mechanism against myocardial ischemia, a classic example being "ischemic preconditioning" (IPC).

In 1986, Murry and colleagues revealed an intriguing paradox (ie, that one or more brief episodes of ischemia, too brief in themselves to cause myocyte death, render the heart resistant to a later, more prolonged period of coronary artery occlusion) [13]. This concept of "preconditioning with ischemia" has, since its first description, captured the interest of researchers worldwide. Cardiac IPC is represented as an anti-ischemic vaccination. In other words, IPC is a classical example of the hormetic effect of mild stress (i.e. brief and multiple ischemic episodes) which protects the heart against the more prolonged ischemic insult [14-16].

Pre-infarction angina (PIA) is the most evident equivalent of IPC. A retrospective analysis of in-hospital outcomes in the well-known TIMI-4 trial revealed that patients with a history of angina at any time before acute MI had a lower incidence of in-hospital death, congestive heart failure and/or shock, as well as smaller infarct sizes sizes (determined by creatine kinase release) when compared to the cohort without PIA [17]. The age-related reduction of IPC has been successively confirmed in several studies [18-20]. Thus, PIA has been studied in adult and elderly patients in terms

***Corresponding author**: Irina V. Sarvilina, PhD, ScD. CEO of Medical Centre «Novomeditsina», Rostov-on-Don, Russia. E-mail: isarvilina@mail.ru*

of in-hospital primary and secondary events: in adult patients (<65 years), both in-hospital mortality and cardiogenic shock were more frequent in the absence than in the presence of PIA; CK-MB (creatine kinase myoglobin fraction) peak, transmural infarctions number, the incidence of ventricular tachycardia and fibrillation, and the ventricular dysfunction were significantly higher in the adult patients without than in those with PIA. In elderly patients (≥ 65 years), the protective effect of PIA angina seems to be lost: both in-hospital primary and secondary end-points were similar in elderly patients with and without PIA. Logistic regression, adjusted for several variables including the use of thrombolytic and anti-anginal therapy, demonstrated that PIA is a protective variable against mortality and cardiogenic shock in adult but not in elderly patients [21].

The molecular mechanism of ischemic preconditioning is very complex. From activation of G-protein-coupled receptors (GPCR) by adenosine, norepinephrine, bradykinin, opioids, ect., phosphoinositide-3-kinase (PI3K)/serine/threonine kinase (Akt) is activated with subsequent downstream activation of nitric oxide synthase (NOS) and nitric oxide (NO) formation, and guanylate cyclase, protein kinase G (PKG) and protein kinase C (PKC) activation. Mechanisms of the age-related reduction of IP require a thorough study.

Methods of molecular analysis of large interactomes (blood, urine) and human tissues (myocardium, vascular wall), including methods of genomics, transcriptomics, proteomics, and metabolomics, allow us to explore the age-related IPC mechanisms in elderly patients with ISH, as models of cardiovascular aging.

Material and Methods

The study was prospective comparative cohort with parallel design. The study conducted in accordance with WMA Declaration of Helsinki (1964-2013) and the permission of the Ethics Committee of the Rostov State Medical University. It included 306 persons divided into two groups: Group 1 (the control group) included 150 elderly patients without AH, and Group 2 (the experimental group) included 156 elderly patients (early old-age pensioners, between 65 and 74 years) with ISH according to the inclusion/exclusion criteria. Patients with ISH corresponded to the criteria for the classification of blood pressure levels (SBP >140 mmHg and DBP<90 mmHg) and the risk stratification – middle (n=87) and high (n=69) additional risk proposed by the WHO/ISH (1999), Guidelines for hypertension in Russia (2008), 2013 ESH/ESC Guidelines for the management of arterial hypertension [22]. The duration of ISH was 13.5 years. All patients of Group 2 received one of the combinations of antihypertensive drugs (calcium antagonist amlodipine+diuretic indapamide retard; angiotensin receptor blocker valsartan+diuretic indapamide retard; calcium antagonist amlodipine+angiotensin receptor blocker valsartan). The duration of therapy was 5,2 years.

At the stage of data collection and screening, we applied standard methods for identification of ISH and secondary hypertension: the assessment of the patient`s complaints, medical history, physical examination, 24-hour ABPM, ECG (ATES MEDICA, Italy-Russia), echocardiography (Samsung-Medison, South Korea), blood and urine tests, biochemical analysis of blood and urine, blood level of aldosterone and corticosteroids, plasma renin activity, urinary catecholamines and metabolites (ELISA, Siemens 2000, Germany), coagulogram («Instrumentation Laboratory», USA), and MRI of adrenal glands, kidney and brain (Philips Intera 1,5T, Japan). For estimating arterial distensibility, cardio-ankle vascular index (CAVI) and heart-brachial pulse wave velocity (B-PWV) were measured non-invasively using the VaSera VS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan). CAVI was measured at the following segments: CAVI1 (heart to ankle) and CAVI-2 (heart to iliac artery). All measurements were conducted in a room kept at a constant temperature with the subject resting in a supine position after resting for 5 min.

Molecular phenotyping of biosamples (blood plasma) with the identification of molecules involved in IPC process were processed with methods of proteomics: the prefractionation, the separation of proteins with standard sets (MB-HIC C8 Kit, MB-IMAC Cu, MB-Wax Kit, «Bruker», USA), matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS/MS, Ultraflex II, «Bruker», USA). The partially identified sequences were then submitted to "BLAST protein-protein" and screened against the *Homo sapiens* Swissprot database to check if this identification matched the MASCOT-identification (Matrix Science). The data of the molecular interactions and functional features of proteins were received with STRING 10.0 database. The analysis of functional activity of molecules in the mechanism of IPC was performed by the databases InterPro, Entrez, SWISS-PROT, NRDB, PDB, and KEGG.

Statistical analysis of the survey data was performed using the software "Statistica 12.0" (Statsoft, Russia). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

Results

Clinical and anamnestic characteristics of ISH patients are presented in Table 1. In Group 2, parameters of coagulogram, blood and urine tests, blood levels of glucose, uric acid, potassium, aldosterone and corticosteroids, plasma renin activity, urinary catecholamines and metabolites were in the range of reference values. MRI signs of leukoaraiosis were detected in all patients with ISH.

At the stage of data collection and screening, we noted a significant increase in indicators of 24-h daytime and nighttime SBP and DBP and heart rate in Group 2 patients compared to Group 1 patients (daytime SBP: Group 1-117.6±3.5mmHg, and Group 2 - 141.2±3.4mmHg, *P<0.001*; daytime DBP:

Group 1 – 69.4±2.4mmHg and Group 2 – 78.4±1.6mmHg, *P<0.001*; nighttime SBP: Group 1 - 117.6±2.9mmHg and Group 2 – 130.9±3.6mmHg, *P<0.001*; nighttime DBP: Group 1 – 67.6±1.8mmHg and Group 2 – 81.8±1.4mmHg, P<0,001; heart rate: Group 1- 72.3±1.2 bpm and Group 2 – 84.5±1.5 bpm, *P<0.001*) despite the use of antihypertensive drugs.

Table 1.

Clinical-anamnestic characteristics of the studied patients

Variable	Group 1 (n=150)	*P-value*	Group 2 (n=156)
Sex (male/female), n	84/66	*>0.05*	86/70
Age, years	69.2±2.9	*>0.05*	67.3±2.5
Weight, kg	66.7±1.3	*>0.05*	69.4±1.6
Height, cm	170.3±1.9	*>0.05*	167.5±1.3
BMI, kg/m^2	19.6±1.2	*>0.05*	20.8±1.3
Duration of disease, years	12.3±1.5	*>0.05*	15.7±1.9
Hypertensive crises, n	-		71
Risk factors: Heredity Arterial hypertension CVD Dyslipoproteinemia Anamnesis CVD Dyslipoproteinemia Smoking Poor nutrition Obesity Low physical activity	 24 24 24 25 38 21 32 - 45	 *0.000* *0.000* *0.000* *>0.05* *0.000* *0.000* *0.000* *0.000*	 74 74 74 27 124 68 83 - 93
Target organs and associated clinical conditions: Brain and eyes (headache, dizziness, impairment of view, speech, TIA, sensory and motor disorders) Heart (heartbeat, pain in the chest, shortness of breath, swelling) Kidneys (thirst, polyuria, nocturia, hematuria, swelling) Peripheral arteries (cold extremities, intermittent claudication)	 21 21 14 15	 *0.000* *0.000* *>0.05* *0.046*	 156 156 25 28
Physical examination: Vascular changes in the fundus Heart (offset heart borders, arrhythmia, CHF) Peripheral arteries (pulse weakening or disappearance, asymmetrical radial pulse, cold extremities, symptoms of skin ischemia) Carotid arteries (systolic murmur)	 47 47 18 15	 *0.000* *0.000* *0.000* *0.000*	 144 156 73 62
ECG data: Sokolov–Lyon index (SV1+RV$_{5-6}$) >3.5 mV, n Cornell voltage QRS duration product (>244mV*ms), n	 34 34	 0.000 0.000	 144 144

TIA – transient ischemic attack; CHF – chronic heart failure.

The concentric LVH was identified in all ISH patients compared to the control group (LVMI: Group 1 – 117.4±3.2g/m^2 and Group 2 – 134.3±4.3g/m^2, *P<0.001*; RWT: Group 1- 0.45±0.03 and Group 2 – 0.33±0.01, *P<0.001*).

We also noted significant differences in indicators of arterial stiffness between Groups 1 and 2 (B-PWV: Group 1- 6.6±1.2 m/sec and Group 2 – 10.3±1.6 m/sec, *P<0.01*; CAVI1: Group 1- 8.0±0.7 and Group 2 -11.2±1.5, *P<0.01*; CAVI2: Group 1- 6.7±0.7 and Group 2- 10.6±1.8, *P<0.01*).. Significant changes in lipid profile were identified in Group 2 patients compared to Group 1 patients (total cholesterol: Group 1 – 3.86±0.45mmol/l and Group 2 – 6.43±0.51mmol/l, *P<0.05*; LDL cholesterol: Group 1- 2.18±0.39mmol/l and Group 2 – 3.89±0.31mmol/l, P<0.05).

Proteomic analysis helps in the detection of differences in the component composition of blood plasma proteins involved in IPC in ISH patients compared with the control group (Table 2). Bioinformatics analysis has revealed the presence of molecules that are the participants in the pathways of IPC, cardiovascular aging and the molecular interactions involved.

Discussion

Proteomic analysis has revealed an increase in the absolute number of ISH patients with an abnormal profile of blood proteins performing certain biological functions and having various localizations in the intra- and extracellular spaces (Table 2). Molecules interact among themselves and with other molecules as participants of age-related IPC in ISH patients.

A significant reduction in the expression of dishevelled-associated activator of morphogenesis 1 (Daam1) in blood plasma indicates the remodeling process in the cardiovascular system through the pathological WNT–pathway. This protein promotes the incorporation of profilin in the cell membrane of cardiomyocytes, which that increase the rigidity and decrease the elasticity of the cardiovascular wall.

APOD is known to regulate smooth muscle cells and is found in abundance within atherosclerotic lesions. Paracrine secretion by endothelial cells causes partial downregulation of APOD expression. Additionally, cell contact-dependent Notch signaling plays a role. NOTCH3 contributes to the downregulation of APOD and by itself is sufficient to attenuate APOD transcript expression. Our data indicates the special role of APOD in the development of age-related IPC [23].

Myocardial ischemic preconditioning upregulated protein 1 (Mipu1) is a newly discovered upregulated gene produced in rats during the myocardial ischemic preconditioning process. Mipu1 is a nuclear factor with a variety of biological functions, such as participation in the process of myocardial ischemic preconditioning, protection of the myocardium from ischemic disease, and inflammation [24]. Analysis of the function of Mipu1, as well as vascular endothelial growth factor-A (an important neuroprotectant) and ACE (a central component of the renin-angiotensin system) in CVD is beneficial because it may provide new ideas for prevention of cardiovascular aging.

Each protein molecule in the functional group interacts with other protein molecules. For example, the molecular interactions of peroxisome proliferator-activated receptors D (PPARD) are presented in Fig.1. The concentration of PPARD decreases in the blood plasma of ISH patients compared to the control group. This fact with increasing age means there is a violation of the process of β-oxidation of fatty acids in

cardiomyocytes, and a lack of myocyte protection from apoptosis induced by oxidative stress. The decrease in the PPARD expression in the blood plasma of ISH patients can lead to the development of myocardial damage. In our study we found a significant decrease in the expression of endothelial nitric oxide synthase 3 (NOS3) in the blood plasma of ISH patients, leading to a reduction in compensatory vasodilation and angiogenesis, that is associated with cardiovascular aging and features of age-related IPC. We also registered a high expression of endothelin-1 in the blood plasma of ISH patients, which plays a key role in the holding of vascular endothelium homeostasis. This protein reveals a vasoconstrictive effect, induces the accumulation of collagen and stimulates the mitogenesis of fibroblasts (ie, cellular processes underlying in cardiovascular aging).

Conclusion

The dynamics in the proteome-map of blood serum in ISH patients revealed the molecular features of age-related IPC as the component of the universal pathway of cardiovascular aging.

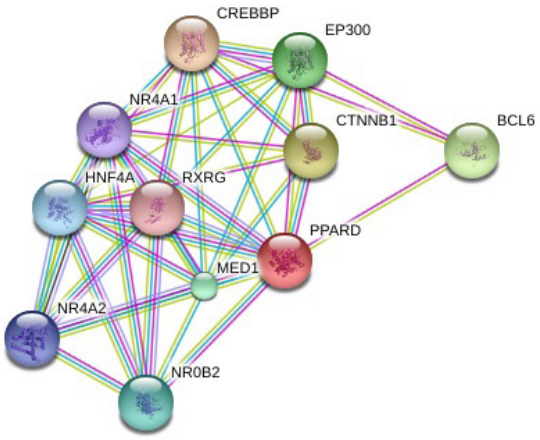


Fig. 1. Molecular interactions of PPARD (STRING 10.0 database)

***PPARD**, peroxisome proliferator-activated receptor delta; **CREBBP**, CREB binding protein; **CTNNB1**, catenin (cadherin-associated protein), beta 1; **BCL6**, B-cell CLL/lymphoma 6; **EP300**, E1A binding protein p300; **MED1**, mediator complex subunit 1; **NR0B2**, nuclear receptor subfamily 0, group B, member 2; **HNF4A**, hepatocyte nuclear factor 4, alpha; **NR4A2,** nuclear receptor subfamily 4, group A, member 2; **NR4A1**, nuclear receptor subfamily 4, group A, member 1; **RXRG**, retinoid X receptor, gamma.*

Table 2.

Qualitative profile of blood plasmain the studied patients

Protein name	Group 1 (n=150)	Goup 2 (n=156)	*P-value*	Molecular weight (Da)	Functional process (sources: InterPro, Entrez, SWISS-PROT, NRDB, , PDB, KEGG)
Disheveled-associated activator of morphogenesis 1	83	41	*0.000*	123396	Epidermal cell proliferation, and glucose and lipid metabolism
Apolipoprotein D	38	42	*0.7518*	21262	Fatty acid and steroid metabolism
Myocardial ischemic precon-ditioning upregulated protein 1	37	54	*0.0570*	56904	Nuclear factor, cell apoptosis, the gene expression of downstream inflammatory mediators
Gamma butyrobetaine hydroxylase	105	82	*0.0018*	44687	L-carnitine biosynthesis pathway, mitochondrial beta oxidation
Endothelial growth factor A	142	125	*0.0001*	27042	Vasculogenesis, neovascular age-related macular degeneration
Angiotensin-converting enzyme	54	68	*0.1753*	149715	The convertion of vasoactive peptides
Hypoxia inducible factor 1	54	23	*0.0000*	92670	Transcription factor, regulator the hypoxia in cells
Peroxisome proliferator-activated receptors D	65	38	*0.0004*	49903	Nuclear hormone receptor, integrator of transcription repression and nuclear receptor signaling
Nitric oxide synthase 3, endothelial	69	32	*0.0000*	133289	Vascular tone, cellular proliferation, leukocyte adhesion, platelet aggregation
Endothelin I	44	96	*0.0000*	24425	Vasoconstrictor, vascular homeostasis

Competing interests

The authors declare that they have no competing interests.

References

1. North BJ, Sinclair DA. The intersection between aging and cardiovascular disease. Circ Res. 2012;110(8):1097-108.
2. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises, part I: aging arteries: a “set up” for vascular disease. Circulation. 2003; 107: 139–146.
3. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises, part II: the aging heart in health: links to heart disease. Circulation. 2003; 107: 346–354.
4. Nielsen, W., Vestbo, J., Jensen, G. Isolated systolic hypertension (ISH): The most powerful risk factor of stroke and MI / W. Nielsen, J. Vestbo, G. Jensen // American Journal of Hypertension. – 1995. – Vol. 8. - №4. – P.41A.
5. Boutouyrie, P., Tropeano, A., Asmar, R., Gautier, I., Benetos, A., Lacolley, P., Laurent, S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study / P. Boutouyrie, A. Tropeano, R. Asmar, I. Gautier, A. Benetos, P. Lacolley, S. Laurent // Hypertension. - 2002. – Vol. 39. - № 1. – P.10-15.
6. McEniery, C., Hall, I., Qasem, A., Wilkinson, I.,

Cockcroft, J. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT) / C. McEniery, I. Hall, A. Qasem, I. Wilkinson, J. Cockcroft // J. Am. Coll. Cardiol. – 2005. – Vol.46. - № 9. – P. 1753-1760.
7. Basile, J. Hypertension in the elderly: a review of the importance of systolic blood pressure elevation / J. Basile // J. Clin. Hypertens. (Greenwich). 2002. Vol. 4. № 2. P. 108–112.
8. Kearney, P., Whelton, M., Reynolds, K., Muntner, P., Whelton, P., He, J. Global burden of hypertension: analysis of worldwide data / P. Kearney, M.Whelton, K.Reynolds, P.Muntner, P. Whelton, J. He // Lancet. 2005. Vol. 365. № 9455. P. 217–223.
9. Abete P, Testa G, Cacciatore F, Della-Morte D, Galizia G, Langellotto A,et al. Ischemic preconditioning in the younger and aged heart. Aging Dis. 2011 Apr;2(2):138-48.
10. Tresch DD, Brady WJ, Aufderheide TP, Lawrence SW, Williams KJ. Comparison of elderly and younger patients with out-of-hospital chest pain. Arch Intern Med. 1996;156:1089–93.
11. Berger AK, Radford MJ, Wang Y, Krumholz HM. Thrombolytic therapy in older patients. J Am Coll Cardiol. 2000;36:366–74.
12. Boersma E, Pieper KS, Steyerberg EW, Wilcox RG, Chang WC, Lee KL, et al. Predictors of outcome in patients with acute coronary syndromes without persistent ST-segment elevation. Results from an international trial of 9461 patients The PURSUIT Investigators. Circulation. 2000;101:2557–67.
13. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124-1136.
14. Kloner RA, Bolli R, Marban E, Reinlib L, Braunwald E. Medical and cellular implications of stunning, hibernation and preconditioning. An NHLBI workshop. Circulation. 1998;97:1848–67.
15. Napoli C, Pinto A, Cirino G. Pharmacological modulation, preclinical studies, and new clinical features of myocardial ischemic preconditioning. Pharmacol Ther. 2000;88:311–31.
16. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiol Rev. 2003;83:1113–51.
17. Kloner RA, Shook T, Przyklenk K et al. Previous angina alters in-hospital outcome in TIMI-4: a clinical correlate to preconditioning? Circulation 1995;91:37-47.
18. Fenton, R., Dickson, E., Meyer, T,. Dobson JG., Jr. Aging reduces the cardioprotective effect of ischemic preconditioning in rat heart J. Mol. Cell. Cardiol. – 2000. - №32. – P.1371-1375.
19. Bartling B, Friedrich I, Silber RE, Simm A. Ischemic preconditioning is not cardioprotective in senescent human myocardium. Ann Thorac Surg.2003;76:105–11. [PubMed]
20. Boengler K, Konietzka I, Buechert A, Heinen Y, Garcia-Dorado D, Heusch G, Schulz R, et al. Loss of ischemic preconditioning's cardioprotection in aged mouse hearts is associated with reduced gap junctional and mitochondrial levels of connexin 43. Am J Physiol Heart Circ Physiol. 2007;292:H1764–9.
21. Abete P, Ferrara N, Cacciatore F, Sagnelli E, Manzi M, Carnovale V, et al. Angina-induced protection against myocardial infarction in adult and senescent patients. A loss of preconditioning mechanism in aging heart. J Am Coll Cardiol. 1997;30:947–54.
22. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34(28):2159-219.
23. Pajaniappan M, Glober NK, Kennard S, Liu H, Zhao N, Lilly B.Endothelial cells downregulate apolipoprotein D expression in mural cells through paracrine secretion and Notch signaling. Am J Physiol Heart Circ Physiol. 2011; 301(3): H784–H793.
24. Han D, Zhang C, Fan WJ, Pan WJ, Feng DM, Qu SL, et al. Myocardial ischemic preconditioning upregulated protein 1(Mipu1):zinc finger protein 667 - a multifunctional KRAB/C2H2 zinc finger protein. Braz J Med Biol Res. 2015; 48(1): 1–5.

International Journal of Biomedicine 6(1) (2016) 65-69
doi: 10.21103/Article6(1)_OA14

ORIGINAL ARTICLE

Molecular Markers and Diagnosis

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Search for Molecular Prognostic Markers of Diabetic Nephropathy in Patients with Type 2 Diabetes Mellitus

V. M. Ibragimov, PhD[1]; I.V. Sarvilina, PhD, ScD[2*]; M.M. Batiushin, PhD, ScD[3]

[1]*Dagestan State Medical Academy, the Republic of Dagestan, Makhachkala, the Russian Federation*
[2]*Medical Centre "Novomeditsina", Rostov-on-Don, the Russian Federation*
[3]*Rostov-on-Don State Medical University, Rostov-on-Don, the Russian Federation*

Abstract

The purpose of this study was to search for molecular prognostic markers of diabetic nephropathy (DN) in patients with type 2 diabetes mellitus (T2DM).

The study included 205 patients with T2DM and DN (stages 1 to 4). All patients were stratified by the MDRD equation. The control group included 30 healthy individuals. All T2DM patients were divided into 4 groups depending on the DN stages. Group 1 included 42 patients with DN-Stage 1 (prenephropathy), Group 2 included 48 patients with DN-Stage 2 (incipient nephropathy); Group 3 included 65 patients with DN-Stage 3 (overt nephropathy), and Group 4 included 50 patients with DN-Stage 4 (kidney failure). Molecular phenotyping of urine was processed with methods of proteomics: the prefractionation, the separation of proteins with standard sets (MB-HIC C8 Kit, MB-IMAC Cu, MB-Wax Kit, «Bruker», USA), matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS/MS, Ultraflex II, «Bruker», USA). The data of the molecular interactions and functional features of proteins were received with STRING 10.0 database. Potentially new molecular markers of DN development were identified. ***(Int J Biomed. 2016; 6(1):65-69.).***

Keywords: *type 2 diabetes mellitus; diabetic nephropathy; proteomics; molecular markers.*

Introduction

Diabetic nephropathy DN is a chronic disease that affects 366 million people worldwide (6.4% of the adult population) and is expected to rise to 552 million by 2030 [1]. In Russia, the prevalence of DN among patients with type 2 diabetes mellitus (T2DM) is an average of 8% that below world values in 5 times. Active screening of patients with T2DM reveals that the true prevalence of DN exceeds that registered in various regions of Russia by 2 to 8 times. The epidemiology of DN in T2DM has been insufficiently investigated because it is extremely difficult to determine the time when DN begins. One of the main causes of mortality among T2DM patients is DN that leads to terminal renal failure (5% to 10% of cases of T2DM) [2-4]. Experimental and clinical trials performed from 1998 until 2014 showed that hyperglycemia [5,6,7], hyperglycemia [8], a high level of creatinine in the blood, glomerular hyperfiltration [9], proteinuria [10], arterial hypertension [11], and anemia [12] play an important role in the development of DN in T2DM patients. Genetic factors (genes of perlecan, N-deacetylase, IL-1, the receptor to IL-1, aldose reductase, catalase, SOD2, paraoxonase) can directly to define the development of DN together with the genes (genes of angiotensinogen, renin, ACE, AT2R1), defining human cardiovascular diseases [13].

The molecular pathogenesis of DN in T2DM patients cannot be described only on the basis of standard methods of clinical research. Modern methods and technologies of proteomic analysis allow us to search new prognostic markers and to explore pathways of DN in T2DM patients. Currently, we need progress in the development and clinical application of new molecular screening tests reflecting key genomic-proteomic interactions underlying DN in T2DM patients.

The purpose of this study was to search for molecular prognostic markers of DN in T2DM patients.

Materials and Methods

The study was prospective comparative cohort with parallel design. The study conducted in accordance with WMA Declaration of Helsinki (1964-2013) and the permission of the Ethics Committee of the Rostov State Medical University. It

***Corresponding author**: Irina V. Sarvilina, PhD, ScD. CEO of Medical Centre «Novomeditsina», Rostov-on-Don, Russia. E-mail: isarvilina@mail.ru*

included 205 patients with T2DM and DN (stages 1 to 4). The control group included 30 healthy individuals.

All patients were stratified by the MDRD equation. Patients corresponded to the criteria for the DN classification proposed by the Committee on Diabetic Nephropathy [14]. Clinical-anamnestic characteristics of T2DM patients with DN are presented in Table 1. All T2DM patients were divided into 4 groups depending on the DN stages. Group 1 included 42 patients with DN-Stage 1 (prenephropathy), Group 2 included 48 patients with DN-Stage 2 (incipient nephropathy); Group 3 included 65 patients with DN-Stage 3 (overt nephropathy), and Group 4 included 50 patients with DN-Stage 4 (kidney failure). The duration of DN was 10.5 years.

Table 1.

Clinical-anamnestic characteristics of the studied patients

Variable	Group 1 (n=42)	Group 2 (n=48)	Group 3 (n=65)	Group 4 (n=50)	Control (n=30)
Sex (M/F), n	24/18	23/25	27/38	24/26	14/16
Age, years	59.2±2.5	61.3±2.7	60.5±2.3	61.7±2.9	59.5±1.7$^{\&}$
Weight, kg	85.5±1.7	87.4±1.8	86.3±1.5	86.1±1.3	75.6±1.2
Height, cm	171.2±1.7	169.4±1.6	170.5±1.6	170.4±1.4	170.5±1.6
BMI, kg/m^2	31.4±1.2	30.2±1.1	31.0±1.2	30.9±1.2	26.1±0.8
T2DM duration, yrs	10.3±1.1	9.7±1.0	10.2±1.2	9.9±1.1	-
T2DM Total risk score:					
Low (< 7)	-	-	-	-	26
Slightly elevated (7–11)	12	6	-	-	4
Moderate (12-14)	19	24	35	22	-
High (15-20)	7	12	17	19*	-
Very high (>20)	4	6	13	9	-
Degree of metabolic compensation:					
HbA1c<9%	40	24**	2***	-	-
HbA1c: 9-10%	2^	24	42	32	-
HbA1c>10%	-	-	21	18	-
BP JNC7 category [15]					
-Normal	-	-	-	-	30
-Prehypertension	4	4	7	-	-
-Stage1 H	28***/^^	18^^	9^^^	15$^{\#}$/*	-
-Stage2 H	10^^/***	26	49$^{\#\#}$	35*	-
ECG data:					
-Sokolov–Lyon index ($SV1+RV_{5-6}$) >3.5mV, n	24	35	47	42^^^^	-
*-Cornell voltage QRS duration product (>244mV*ms), n*	24	35	47	42^^^^	-
Stages of retinopathy:					
-mild NPDR	12***	10	3$^{\#\#}$	-	-
-moderate NPDR	28	30	35	25	-
-severe NPDR	2	8	20”	12$^{\#\#\#}$	-
-PDR	-	-	7	13$^{\#}$	-

H – hypertension; NPDR - Nonproliferative diabetic retinopathy; PDR -proliferative diabetic retinopathy;

*P=0.000 for: *1 vs. 4; **2 vs. 3; ***3 vs. 1; ^1 vs. 2, 1 vs. 3, 1 vs. 4. P<0.01 for: ^^ vs. 2; ^^^ 2 vs. 3; ^^^^ 1 vs. 4;”1 vs. 3; $^{\&}$ 4 vs. control. P<0.05 for: $^{\#}$3 vs. 4; $^{\#\#}$2 vs. 3; $^{\#\#\#}$4 vs. 1.*

T2DM risks were evaluated with a special Type 2 Diabetes risk assessment form designed by Professor J.Tuomilehto (http://www.diabetes.fi/files/502/eRiskitestilomake.pdf).

At the stage of data collection and screening, we applied standard methods for identification of DN and DM: the assessment of the patient`s complaints, medical history, physical examination, 24-hour ABPM, ECG, the ultrasonography of kidney (Doppler spectrum of the intrarenal arteries in conjunction with evaluation of the renal cortical echogenicity, SonoAce R3, Samsung Medison, South Korea), blood and urine tests, biochemical analysis of blood and urine (ELISA, Siemens 2000, Germany), the estimation of T2DM compensation by HbA1c levels (Randox Laboratories Ltd., UK), coagulogram («Instrumentation Laboratory», USA), the measurement of albumin/creatinine in the urine. MAU (urinary albumin excretion of 30-300 mg/24 hours) was assessed by a semi-quantitative method using test strips for the determination of protein in the urine, in compliance with the rules for collecting morning urine. GFR was estimated by the Cockcroft-Gault formula.

Molecular phenotyping of biosamples (urine) was processed with methods of proteomics: the prefractionation, the separation of proteins with standard sets (MB-HIC C8 Kit, MB-IMAC Cu, MB-Wax Kit, «Bruker», USA), matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS/MS, Ultraflex II, «Bruker», USA). The partially identified sequences were then submitted to “BLAST protein-protein” and screened against the *Homo sapiens* Swissprot database to check if this identification matched the MASCOT-identification (Matrix Science). The data of the molecular interactions and functional features of proteins were received with STRING 10.0 database.

The duration of therapy (hypoglycemic drugs: glibenclamide, metformin, pioglitazone, insulin glargine; antihypertensive drugs: amlodipine, valsartan; hypolipidemic drug – fenofibrate; antiplatelet drug – acetylsalicylic acid) was 9.2 years.

Statistical analysis of the survey data was performed using the software “Statistica 12.0” (Statsoft, Russia). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student’s unpaired t-tests were used to compare two groups for data with normal distribution. Comparisons between three groups were performed with the one-way ANOVA with Tukey’s post-hoc test. Group comparisons with respect to categorical variables are performed using $\chi 2$ tests with Yates correction or, alternatively, Fisher’s exact test when expected cell counts were < 5. A probability value of $P<0.05$ was considered statistically significant.

Results

Parameters of coagulogram, blood test, uric acid, and potassium in the serum were in the range of reference values. Ultrasonographic signs of DN were detected in all patients: high resistive indices were obtained in the region of the arcuate or the interlobar arteries in patients with elevated serum creatinine levels.

We noted a significant increase in indicators of 24-h daytime and nighttime SBP and DBP and heart rate (daytime SBP: Group 1 – 115.2±3.1 mmHg, Group 2 – 134.7±3.9 mmHg, Group 3 – 145.4±4.1 mmHg, and Group 4 – 159.1±4.7 mmHg, *P1/2<0.001,P1/3<0.001,P1/4<0.,001*; daytime DBP: Group 1 – 66.7±2.1 mmHg, Group 2 – 72.8±2.4 mmHg, Group 3 – 82.0±2.8 mmHg, and Group 4 – 89.5±3.3 mmHg, *P1/2<0.001,P1/3<0.001,P1/4<0.001*; nighttime SBP: Group 1 - 115.4±1.8 mmHg, Group 2 – 120.5±2.3 mmHg, Group 3 – 129.4±2.6 mmHg, and Group 4 – 134.6±3.2 mmHg, *P1/2<0.001,P1/3<0.001,P1/4<0.001*; nighttime DBP: Group 1-66.8±1.4 mmHg, Group 2 – 75.2±1.6 mmHg, Group 3 – 81.5±1.7 mmHg, and Group 4 - 89.3±1.9 mmHg, *P1/2<0.001, P1/3<0.001,P1/4<0.001*; heart rate: Group 1 -72.3±1.2 bpm, Group 2 – 79.5±1.3 bpm, Group 3 – 85.3±1.5 bpm, and Group 4 - 88.2±1.8 bpm, *P1/2<0.001,P1/3<0.001,P1/4<0.001)* in Groups 2, 3, and 4 compared to Group 1.

All these changes are associated with a higher expression of urine proteins in the progression of epithelial-to-mesenchymal transition (EMT) and changes in the extracellular matrix (ECM) in kidneys in T2DM patients with DN. Proteomic analysis helps in the detection of differences in the component composition of the urine proteins in patients with DN of varying stages compared with the control group (Table 2). Molecules interact among themselves and with other molecules as participants in universal pathways in T2DM patients with DN, which are the key elements for EMT formation and changes in ECM: Smad, p38 MAPK, TLRs, Wnt, mTOR, Notch, small GTPase and Hedgehog, PI3K/AKT- signaling pathways.

Bioinformatics analysis has revealed the presence of molecules that are the participants of the universal pathways of DN and the molecular interactions involved.

Discussion

Proteomic analysis has revealed an increase in the absolute number of T2DM patients with DN with an expression of proteins performing certain biological functions and having various localizations in the intra - and extracellular spaces (Table 2). Major typical morphological changes are the result of EMT: an increase of the mesangial matrix, thickening of the glomerular basement membranes, and expansion of the tubulointerstitial space due to increased amounts of ECM. As mentioned above, all EMT events are regulated by multiple intracellular signaling pathways. We consider the functional activity of some proteins in this molecular process.

Fibronectin is found in the normal glomerular mesangial matrix; an enhanced fibronectin accumulation with high expression in urine is observed in DN. This fact could be associated with locally stimulated production of the insoluble or cellular form of fibronectin in mesangial and epithelial cells. Ceruloplasmin is more difficult to be filtered by the glomerulus than albumin. We observed higher urinary ceruloplasmin excretion in patients with DN compared to controls, even in the normoalbuminuric phase. Ceruloplasmin is a promising marker of damaged glomerulus in DN, but further studies are necessary to characterize its functional role in the progression of the disease.

Table 2.

Qualitative profile of urine proteins in T2DM patients with DN

Protein name	Group 1 (n=42)	Group 2 (n=48)	Group 3 (n=65)	Group 4 (n=50)	CG (n=30)	MW (Da)	Functional process (sources: InterPro, Entrez, SWISS-PROT, NRDB, PDB, KEGG
TGF- β1	18 $P_{CG-1=0.001}$	27 $P_{CG-2=0.000}$	52 $P_{CG-3=0.000}$	48 $P_{CG-4=0.000}$	2	44341	Pro-fibrotic and anti-inflammatory activities, the regulation of tubular EMT
E-cadherin	22 $P_{CG-1=0.000}$	38 $P_{CG-2=0.000}$	62 $P_{CG-3=0.000}$	49 $P_{CG-4=0.000}$	1	97456	The regulation of tubular EMT; the maintenance of epithelial integrity, cell phenotype; the progression of renal fibrosis.
Cystatin C	15 $P_{CG-1=0.001}$	40 $P_{CG-2=0.000}$	60 $P_{CG-3=0.000}$	45 $P_{CG-4=0.000}$	1	15799	Cysteine proteinase inhibitor, tubular damage marker.
Collagen IV	4 $P_{CG-1>0.05}$	32 $P_{CG-2=0.000}$	48 $P_{CG-3=0.000}$	42 $P_{CG-4=0.000}$	1	164038	Constituent of mesangial matrix, marker of the phase of compromised renal filtration function.
MMP 9	8 $P_{CG-1>0.05}$	32 $P_{CG-2=0.000}$	60 $P_{CG-3=0.000}$	49 $P_{CG-4=0.000}$	1	78458	Potent modulator of ECM[7] turnover and also of shedding of syndecans.
Fibronectin	4 $P_{CG-1>0.05}$	35 $P_{CG-2=0.000}$	52 $P_{CG-3=0.000}$	43 $P_{CG-4=0.000}$	1	262625	Adhesive glycoprotein, locally stimulated mesangial and epithelial cell production.
NGAL	10 $P_{CG-1=0.043}$	42 $P_{CG-2=0.000}$	62 $P_{CG-3=0.000}$	48 $P_{CG-4=0.000}$	1	22588	Kidney development; it loses through the damaged glomerulus, injured tubular cells produce NGAL as a compensatory mechanism against intracellular oxidative stress and complement- induced apoptosis.
Ceruloplasmin	12 $P_{CG-1=0.006}$	37 $P_{CG-2=0.000}$	42 $P_{CG-3=0.000}$	35 $P_{CG-4=0.000}$	1	122205	Marker of damaged glomerulus.
β2-microglobulin	6 $P_{CG-1>0.05}$	37 $P_{CG-2=0.000}$	62 $P_{CG-3=0.000}$	49 $P_{CG-4=0.000}$	1	11774	The indicator of incipient DN; detecting injured epithelial cells in the proximal tubules.
Podocine	23 $P_{CG-1=0.000}$	45 $P_{CG-2=0.000}$	63 $P_{CG-3=0.000}$	49 $P_{CG-4=0.000}$	1	42201	Podocyte-specific protein, interact with the PI3K/AKT-signaling pathway for maintenance of functional integrity.
MCP-1	11 $P_{CG-1=0.011}$	39 $P_{CG-2=0.000}$	48 $P_{CG-3=0.000}$	46 $P_{CG-4=0.000}$	1	2583	Chemotactic factor for monocytes; regulates the memory T lymphocytes, NK cells; increases with TNFα and IL-6 in damaged kidneys.

CG - control group; MW- molecular weight; P-value between groups based on Fisher's Exact Test

E-cadherin is expressed in the membrane and cytoplasm of renal tubular epithelial cells, and its expression is decreased in DN compared with healthy controls. E-cadherin is identified as DN-related biomarker, which is specifically increased in urine of DN patients.

We revealed a high expression of urinary cystatin C, a tubular damage marker, which is associated with the progression of type 2 DN. In healthy subjects, cystatin C is freely filtered by the renal glomeruli and entirely reabsorbed in the proximal tubule. Increased urinary cystatin C has been recognized as the marker of renal tubular dysfunction [16-18].

The data suggest that high urinary expression of autocrine factors including TGFβ, MCP-1 and NGAL is associated with DN progression. Obviously, the autocrine signaling network stimulated the hypertrophy, expansion of the mesangial matrix and atrophy of proximal tubules. Bioinformatic analysis suggested that elevated ET-1 secretion may evoke autocrine cytokine- and chemokine-based signaling.

Transforming growth factor-β TGF-β has a broad spectrum of biological functions in a variety of cell types. It is widely accepted that TGF-β and its downstream Smad cascade is a key mediator in the pathogenesis of renal fibrosis both in experimental models and in human kidney diseases [19-20].

TGF-β mediates progressive renal fibrosis by stimulating extracellular matrix production, while inhibiting its degradation. TGF-β is also considered to induce EMT of the injured tubule epithelial cells, whereas the in vivo relevance of EMT remains controversial. In diabetic nephropathy, TGF-β also mediates mesangial matrix accumulation [21].

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein belonging to the lipocalin superfamily initially found in activated neutrophils, in accordance with its role as an innate antibacterial factor. However, it subsequently was shown that many other types of cells, including in the kidney tubule, may produce NGAL in response to various injuries. In DN, an increased quantity of circulating NGAL could be lost through the damaged glomerulus. Injured tubular cells may actively produce NGAL as a compensatory mechanism against oxidative stress and complement-induced apoptosis in cells. NGAL may become one of the most promising next-generation biomarkers in clinical nephrology and beyond [22].

Monocyte chemoattractant protein-1 (MCP-1/CCL2) is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages,memory T lymphocytes, and natural killer (NK) cells.

Podocyte specific proteins are early biomarkers of DN, especially podocin. The podocyte is a specialized visceral epithelial cell that helps to establish the glomerular filtration barrier and prevents protein loss, along with the glomerular basement membrane and the endothelial cell layer. Occurrence of podocytopenia (decreased number) and podocyturia (podocytes in urine) in DN are well established [23-25] Podocyte loss initiates the process of glomerulosclerosis by accelerating synechiae between podocytes and the glomerular basement membrane. In DN, altered expression of podocyte specific proteins such as synaptopodin , podocin and nephrin have been described [26].

We observed increased renal production of type IV collagen, a prominent constituent of the thickened basement membrane and expanded mesangium. The excretion of collagen IV has been found in DN (stages of microalbuminuria or overt proteinuria), which demonstrates the progression of glomerular injury with additional structural damage induced by increased synthesis of type IV collagen in extramesangial sites.

We found also the activation of urinary MMP9. This fact could be associated with excessive accumulation of ECM, which is thought to contribute to the development of DN. MMP9 may remodel ECM in DN.

Each protein molecule in the functional group interacts with other protein molecules. For example, the molecular interactions of NGAL are presented in Fig. 1. The concentration of NGAL increases in the urine of T2DM patients with DN that was most pronounced in the groups with incipient and overt nephropathy.

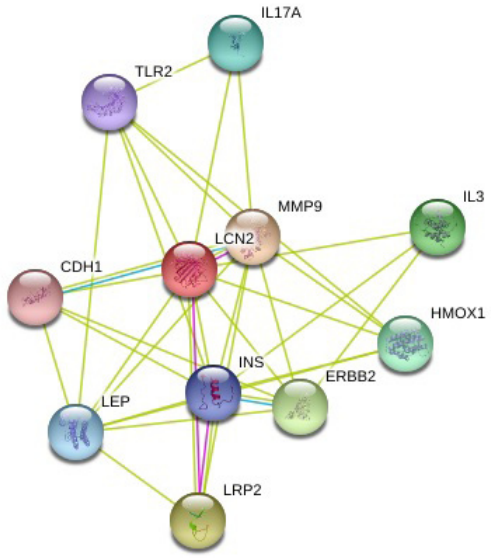


Fig. 1. Molecular interactions of NGAL (STRING 10.0 database)

LCN2*, lipocalin-2;* ***MMP-9****, matrix metallopeptidase 9;* ***LRP2****, low density lipoprotein-related protein 2;* ***ERBB2****, erythroblastic leukemia viral oncogene homolog 2 (neuro/glioblastoma derived oncogene homolog);* ***IL3****, interleukin 3 (colony-stimulating factor, multiple);* ***HMOX1****, heme oxygenase (decycling) 1;* ***IL-17A****, interleukin 17A;* ***LEP****, leptin;* ***INS****, insulin;* ***TLR2****, toll-like receptor 2;* ***CDH1****, cadherin 1, type 1, E-cadherin (epithelial cadherin).*

Conclusion

The study identified the biomarkers of tubular damage that have a key role in the development and progression of DN. The research into signaling pathways and the molecules that are involved in ECM formation may help in developing strategies to prevent DN.

Competing interests

The authors declare that they have no competing interests.

References

1. IDF Diabetes Atlas, Fifth Edition. International Diabets Federation, Brussels, Belgium; 2011.
2. Shestakova M, Shamalova M. Diabetic nephropathy: clinic, diagnostic, therapy. Dedov II, editor. M: Medicine; 2009. [in Russian].
3. Shlipak M. Diabetic nephropathy: preventing progression. BMJ Clin Evid. 2010;2010. pii: 0606.
4. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 Update. Am J Kidney Dis. 2012;60(5):850-86.
5. King, P, Peacock I, Donnelly R. The UK Prospective Diabetes Study (UKPDS): clinical and therapeutic implications for type 2 diabetes / P. King, I.Peacock, R. Donnelly. Br J Clin Pharmacol. 1999; 48(5):643–8.
6. Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes. ADVANCE Collaborative Group, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, et al. N Engl J Med. 2008;358(24):2560-72.
7. Wood AJ, Churilov L, Perera N, Thomas D, Poon A, MacIsaac RJ, et al. Estimating glomerular filtration rate: Performance of the CKD-EPI equation over time in patients with type 2 diabetes. J Diabetes Complications. 2016;30(1):49-54.
8. Hall J, Brands M, Dixon W, Smith MJJr. Obesity-induced hypertension. Renal function and systemic hemodynamics. Hypertension. 1993;22(3):292–9.
9. Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. Cell Metab. 2013;17(1):20-33.
10. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al.; RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med. 2001;345(12):861-9.
11. Van Buren PN, Toto R.Hypertension in diabetic nephropathy: epidemiology, mechanisms, and management. Adv Chronic Kidney Dis. 2011;18(1):28-41.
12. Stauffer ME, Fan T. Prevalence of Anemia in Chronic Kidney Disease in the United States. PLoS One. 2014;9(1): e84943.
13. Ha SK, Seo JK. Insertion/deletion polymorphism in ACE gene as a predictor for progression of diabetic nephropathy. Kidney Int Suppl.1997;60:S28-32.
14. Haneda M, Utsunomiya K, Koya D, Babazono T, Moriya T, Makino H, et al.; Joint Committee on Diabetic Nephropathy. A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy. J Diabetes Investig. 2015;6(2):242-6.
15. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al.; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. JAMA. 200321;289(19):2560-72
16. Kim SS, Song SH, Kim IJ, Jeon YK, Kim BH, Kwak IS, et al. Urinary cystatin C and tubular proteinuria predict progression of diabetic nephropathy. Diabetes Care. 2013;36(3):656-61.
17. Conti M, Moutereau S, Zater M, Lallali K, Durrbach A, Manivet P, et al. Urinary cystatin C as a specific marker of tubular dysfunction. Clin Chem Lab Med. 2006;44(3):288–91.
18. Lan HY. Diverse roles of TGF-beta/Smads in renal fibrosis and inflammation. Int J Biol Sci 2011;7:1056-67.
19. Bottinger EP. TGF-beta in renal injury and disease. Semin Nephrol 2007;27:309-20.
20. Lan HY. Tubular epithelial-myofibroblast transdifferentiation mechanisms in proximal tubule cells. Curr Opin Nephrol Hypertens 2003;12:25-29.
21. Yanagita M. Inhibitors/antagonists of TGF-β system in kidney fibrosis. Nephrol Dial Transplant. 2012;27(10):3686-91.
22. Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, Buemi M. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. Am J Kidney Dis. 2008;52(3):595-605.
23. Jim B, Ghanta M, Qipo A, Fan Y, Chuang PY, Cohen HW, et al. Dysregulated nephrin in diabetic nephropathy of type 2 diabetes: a cross sectional study. PLoS One. 2012;7(5):e36041.
24. Nakamura T, Ushiyama C, Suzuki S, Hara M, Shimada N, Ebihara I, et al. Urinary excretion of podocytes in patients with diabetic nephropathy. Nephrol Dial Transplant. 2000;15(9):1379-83.
25. White KE, Bilous RW, Marshall SM, El Nahas M, Remuzzi G, Piras G, et al. Podocyte number in normotensive type 1 diabetic patients with albuminuria. Diabetes. 2002;51(10):3083-9.
26. Doublier S, Salvidio G, Lupia E, Ruotsalainen V, Verzola D, Deferrari G, et al. Nephrin expression is reduced in human diabetic nephropathy: evidence for a distinct role for glycated albumin and angiotensin II. Diabetes. 2003; 52(4):1023–30.

International Journal of Biomedicine 6(1) (2016) 70-73
doi: 10.21103/Article6(1)_OA15

ORIGINAL ARTICLE

Molecular Markers and Diagnosis

INTERNATIONAL JOURNAL OF BIOMEDICINE

New Features of Molecular Diagnostics of Ulcerative Colitis

A.S. Volkov, PhD[1]; I.G. Stolyarova, PhD[1]; I.V. Sarvilina, PhD, ScD[2*]

[1]*Rostov-on-Don State Medical University, Rostov-on-Don, Russian Federation*
[2]*Medical Centre "Novomeditsina", Rostov-on-Don, Russian Federation*

Abstract

The purpose of this study was to search for new molecular markers for the diagnosis of ulcerative colitis (UC). The study included 65 patients (range from 22 to 35 years, 24 men and 41 women) with left-sided UC (Montréal classification), mild and moderate activity, infrequent (≤1/year) relapses according to the inclusion/exclusion criteria in the research. Criteria of the diagnosis of UC corresponded to ECCO Consensus. The duration of UC was 5.3 years. The control group included 30 healthy individuals. Molecular phenotyping of colon mucosa was processed with methods of proteomics. The data of the molecular interactions were received with STRING 10.0 database. Potentially new molecular markers of the development of UC were identified. **(Int J Biomed. 2016; 6(1):70-73.).**

Keywords: *ulcerative colitis; colon mucosa; proteomics; diagnostic markers.*

Introduction

The worldwide incidence rate of ulcerative colitis (UC) varies between 0.5 and 24.5 cases per 100,000 persons. Currently, the incidence of UC in Russia is 5–30 cases per 100,000 per year. The peak age of onset for UC is most common between 15 and 30 years, although it may occur at any age. About 10% of the cases occur in individuals under the age of 18. UC is slightly more common in males [1,2].

UC is characterized by an even and continuous distribution of the inflammatory infiltrate that only affects the lamina propria. Furthermore, the disruption of normal crypt architecture and the presence of crypt abscesses are the main histological characteristics of UC. UC patients have a well-known risk of colorectal cancer [3].

So far, mechanisms of UC remain to be fully understood and require a detailed multidisciplinary approach [4-6]. In 1990, an international consortium revealed universal genetic sequences which enable a genetic map of UC [7]. According to Medscape, methods of cell and molecular biology have shown the role of thrombocytosis (1966), antibodies to E.coli (1969), key components of the inflammation with UC using granulocytes labeled Indium-111 (1985), interleukin-6 (IL-6), tumor necrosis factor (TNF)-α, pANCA, C-reactive protein (CRP) (1990), thrombocytes, sedimentation rate of erythrocytes in differential diagnosis between UC and infectious diarrhea (1991), IL-12 (1995), fecal lactoferrin (1996), the level of α4β7-integrin in T-lymphocytes (1997), pANCA and ASCA (1998), bacterial antibodies and fecal calprotectin (1999), serum protein S100A12 (2003,2006), and IL-23 in colon mucosa (2004).

Modern achievements of proteomic methods of analysis are ideal for research that is free from hypotheses and allows us to define molecular characteristics of inflammation in colon mucosa of UC patients.

Material and Methods

The study conducted in accordance with WMA Declaration of Helsinki (1964-2013) and the permission of the Ethics Committee of the Rostov-on-Don State Medical University (Rostov-on-Don, Russia).

The study was prospective comparative cohort with parallel design and included 65 patients (range from 22 to 35 years, 24 men and 41 women) with left-sided UC (Montréal classification) [8], mild and moderate activity (Truelove - Witts' criteria, Mayo score) [9,10], infrequent (≤1/year) relapses according to the inclusion/exclusion criteria in the research. Criteria of the diagnosis of UC corresponded to ECCO Consensus [11]. The duration of UC was 5.3 years. The induction of UC remission assumed the acceptance of mesalazine 3 to 4 g/day p.o. and rectally 1 to 2 g/day; the maintenance of UC remission included the acceptance of mesalazine 1.5 g/day p.o. for 3 years by patients with intolerant UC. The duration of therapy was 4.6 years. The control group included 30 healthy individuals.

***Corresponding author**: Irina V. Sarvilina, PhD, ScD. CEO of Medical Centre «Novomeditsina», Rostov-on-Don, Russia.E-mail: isarvilina@mail.ru*

At the stage of data collection and screening, we applied standard methods for identification of UC: the assessment of the patient`s symptoms, risk factors, medical history, physical examination, complete blood count, erythrocyte sedimentation rate (ESR) (Advia 120, Bayer Diagnostics, Germany), biochemical analysis of blood and urine, serological markers - perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA) (ELISA, Siemens 2000, Germany), faecal calprotectin (ELISA, Buhlmann, Швейцария), the stool culture (culture-dependent methods, DNA-PCR and FISH analysis), patient's immunization status to various viral diseases and tuberculosis status.

Biosamples of colon mucosa (3-10mg) in patients with UC in the active stage and in healthy persons were received by ileocolonoscopy (Olympus, Japan) with colon mucosa biopsy (Rachmilewitz index). We used endoscopic scoring by the Schroeder classification for UC and Endoscopic Index of Severity (UCEIS) [12,13]. Histological characteristics of colon mucosa in UC in the active stage were performed by light-microscopy (architectural features, epithelial abnormalities, and inflammatory features).

The storage of biosamples before proteomic analysis was carried out at -80ºC. Sample preparation was conducted as follows: biosamples were homogenized and processed by lysis buffer (1mg bioptate/10µl lysis buffer, pH 3–10, GE Healthcare, Sweden), CHAPS (Applichem, Germany), and 1% DTE (Sigma-Aldrich D8255) in water. After the incubation during 2h at room temperature, lysed cells were centrifuged at 10000 RPM for 20 min at 4 ºC.

The separation of individual proteins of colon mucosa was based on technologies of IEF, SDS-PAGE, 2DPAGE, by standard sets (MB-HIC C8 Kit, MB-IMAC Cu, MB-Wax Kit, «Bruker», USA). Automated mass spectrometry imaging was performed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS/MS, Ultraflex II, «Bruker», USA). The partially identified sequences were then submitted to "BLAST protein-protein" and screened against the *Homo sapiens* Swissprot database to check if this identification matched the MASCOT-identification (Matrix Science, UK). The data of the molecular interactions and functional features of proteins were received with STRING 10.0 database.

Based on the data of standard methods of identification of UC and molecular phenotyping of colon mucosa we conducted new prognostic markers, molecular pathways of UC and diagnostic tests in patients with UC.

Statistical analysis of the survey data was performed using the software "Statistica 12.0" (Statsoft, Russia). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student's unpaired paired t-tests were used to compare two groups for data with normal distribution. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

Results

Clinical-anamnestic and laboratory characteristics of UC patients are presented in Table 1.

Table 1.

Clinical-anamnestic and laboratory characteristics of UC patients

Variable	Group patients with UC (n=65)			Control group (d)
	Active stage (a)	Remission (1st year) (b)	Remission (3rd year) (c)	
Sex (male/female), n	39/26	39/26	39/26	17/13
Age, yrs	29.2±2.5	30.3±2.7	32.1±2.9	30.1±3.3
Weight, kg	58.3±1.7*	60.4±1.8**	64.3±2.2	69.7±2.3
Height, cm	171.2±1.7	169.4±1.6	170.5±1.6	170.2±1.7
BMI, kg/m^2	19.9±1.3	21.2±1.6	22.3±1.9	25.8±2.1
UC duration,yrs	4.2±1.1	4.0±1.0	4.4±1.1	-
UC activity: mild moderate	 39 26	 52 13	 65 -	-
Mayo score: 1 2	 39 26	 52 13	 65 -	-
Weight loss, n	65	57	43	-
Anemia, n	65	52	33	
Arthralgia, n	29	9	9	-
Erythema nodosum, n	14	9	9	-
Laboratory parameters				
pANCA (>1:40), n	65	57	45	-
ASCA, RU/ml	35.4±3.2	18.9±2.6	19.2±2.7	-
Faecal calprotectin, µg/g	234.5±9.3^	186.8±8.4^	145.3±7.1^	32.4±3.1

** - P = 0.004 between (a) - (d); ** - P = 0.029 between (b) - (d);*
^ - P = 0.000 between (a) - (d), (b) - (d), and (c) - (d).

Results of ileocolonoscopy with biopsy and histological characteristics of UC are shown in Table 2. Parameters of complete blood count, ESR, serum urea, creatinine, electrolytes, liver enzymes, serum iron levels, CRP were changed in active stage of UC: high platelet count (n=32), elevated ESR (n=65), hypokalemia (n=35), hypomagnesemia (n=32), elevated level of CRP (n=65), elevated level of serum urea and creatinine (n=8), alaninaminotrasferase (n=37), a decreased level of serum iron (n=65).

All laboratory tests were in the range of reference values for patients in the stage of induction and the maintenance of UC remission.

We found a dysbiotic relationship between protective and aggressive bacterial species in patients with UC in the active stage and in the stage of induction of UC remission: the increase of *Escherichia coli, lactose-negative strains* (n=57; 10^5/g), *Proteus spp.* (n=45;10^5/g), *Enterococcus spp.* (n=42;10^{4-6}/g), *Staphylococcus spp.* (n=50,10^{5-6}/g), *Streptococcus spp.* (n=42;10^{5-6}/g), *Bacteroides spp.* (n=55;10^{4-6}/g), *Clostridium spp.* (n=38;10^4/g) and the decrease of *Bifidobacterium spp.* (n=63;10^5/g), *Lactobacterium spp.* (n=63;10^{4-6}/g) in stool culture.

Table 2.

Histological characteristics of ulcerative colitis

The characteristic findings at ileocolonoscopy (Truelove - Witts' criteria, Schroeder classification, histological characteristics)		n
Active stage		
Mild activity of UC[2] (grade 1)	Erythema, decreased vascular pattern, mild friability Basal plasmacytosis, the inflammatory infiltrate in the lamina propria, absent crypt architectural distortion	39
Moderate activity of UC (grade 2)	Marked erythema, absent vascular pattern, friability, erosions Basal plasmacytosis or subcryptal, heavy, diffuse transmucosal lamina propria cell increase and widespread crypt architectural distortion	26
Induction of remission		
Mild activity of UC (grade 1)	Erythema, decreased vascular pattern, mild friability Basal plasmacytosis, the inflammatory infiltrate in the lamina propria, absent crypt architectural distortion	52
Moderate activity of UC (grade 2)	Marked erythema, absent vascular pattern, friability, erosions Basal plasmacytosis or subcryptal, heavy, diffuse transmucosal lamina propria cell increase and widespread crypt architectural distortion	13
Maintenance of remission		65
Mild activity of UC (grade 1)	-	-
Moderate activity of UC (grade 2)	-	-

All these changes correlate with different expressions of peptides and proteins in damaged and undamaged colon mucosa in patients with UC in the active stage and in healthy persons (Table 3). Molecules of peptides and proteins were seen to interact among themselves and with other molecules as participants in universal pathways in patients with UC in the active stage: cytokine, oxidative stress, Klotho, STAT-JAK signaling pathway, PPAR γ, TLR, NF-kB, β-defensin, INK4 tumor suppressor proteins pathway, MUC1-mediated signaling pathways. Bioinformatics analysis revealed the presence of molecules that are the participants in the universal pathways of UC in the active stage, and the molecular interactions involved.

Table 3.

Qualitative profile of peptides and proteins in colon mucosa

Protein name	MW (Da)	pI	CG (n=30)	UC (active stage) (n=65)	*P-level*
IL-2	17628	7.6	2	22	*0.005*
RBP4	23010	5.4	1	7	*0.428*
SMAD2	48081	6.1	2	11	*0.096*
HSP47	70052	9.0	2	13	*0.058*
HSP27	27000	6.12	1	9	*0.162*
HSP2	90000	5.1	1	9	*0,162*
TNF-α	25644	6.4	2	24	*0.002*
KNG1	71957	4.8	1	10	*0.164*
APOC3	10852	4.6	1	15	*0.018*
NF-kB	105356	5.5	2	25	*0.001*
RTKs	104000	6.96	1	13	*0.058*
PPARγ	57620	5.78	12	9	*0.007*
IL-6	23718	6.17	1	12	*0.028*
IL-8	11098	9.1	2	13	*0.133*
IL-12A	24874	8.4	1	7	*0.428*
IL-1β	30748	6.1	1	6	*0.426*
CASP8	55391	5.12	2	9	*0.493*
CASP10	58951	5.97	2	10	*0.328*
HβD-1	7420	4.1	16	3	*0.000*
CFTR	168142	8.91	13	1	*0.000*
PHB	29804	9.8	12	2	*0.000*

pI – isoelectric point; P-value between groups based on Fisher's Exact Test

Discussion

We identified following functional groups of peptides and proteins in molecular patterns of bioptates of colon mucosa in UC patients: peptides and proteins regulating the barrier function of colon mucosa; proteins-participants of specific metabolism in epitheliocytes and endocrinocytes; proteins of the fibrosis in colon mucosa; proteins regulating cell cycle, oncogenesis, proteolysis in cell, hormones processing, angiogenesis, coagulation factors; proteins of free radical oxidation and antioxidant system; proteins regulating the receptor activity of epitheliocytes and immune cells; structural proteins of colon mucosa; transcription and translation factors regulating the activity of cell nucleus, regulators of protein folding; transport proteins; proteins-enzyms of detoxification. Below we have provided the functional activity of some of them.

SMAD family member 2 (SMAD2) activates the transcription of TFG1β, which increases the activity of Rho/ROCK signaling pathway in fibroblasts of colon submucosa that leads to specific regulation of the CCN2 gene in cells and the development of fibrosis in colon submucosa in UC patients. The stimulation of the expression of apoC-III in affected colon mucosa in UC is associated with the activation of the FOXO1 signaling pathway that supports inflammatory processes in colon mucosa.

The second small heat shock protein (HSP2) controls the apoptosis of colonocytes and immune response in damaged colon mucosa through expression of Bcl-2 and IL-17; HSP2 is also responsible for the mucosa resistance to therapeutic strategies. Anti-apoptotic functions of HSP27 are possible through the interaction with DAXX7, the activation of Akt and the inhibition of the apoptosis. HSP47 interacts with collagen I, II, III, IV and V types, which contributes to the launch of autoimmune process in UC.

Caspase 8 protects colonpcytes from TNFα-induced cell death through a necroptosis mechanism via the blockade of the RIP3 expression. The expression of prohibitin maintains

optimal activity of the electronic transport chain through the activity of transcription factor STAT3 and the decrease in the TNFα expression.

Significant decrease of the PPARγ expression promotes the activation of STAT and AP-1 signaling pathways, which promotes an increase in the synthesis of IL-2,6,8,12, TNFα, matrix metalloproteinases, the activity of immune and inflamation processes in colon mucosa. A significant increase in the NF-kB expression in colon mucosa is associated with the activation of TNFα and IL-1, which promotes the increase of immune processes in colon mucosa.

The molecular interactions of β-defensin-1 are presented in Figure 1. The reduction of the β-defensin-1 expression in cells of colon mucosa is accompanied by increased expression of CCR6, which promotes the formation of inflammatory infiltrates in coloc submucosa in UC.

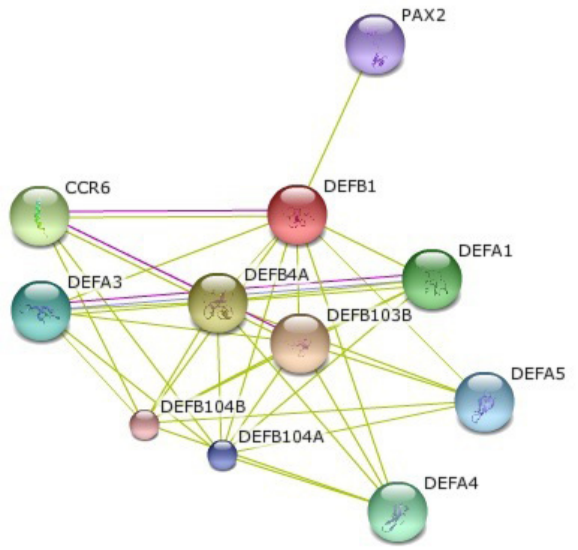


Figure 1. Molecular interactions of β-defensin -1 (STRING 10.0 database)

DEFB1*, defensin beta 1;* ***DEFA4****, defensin alpha 4, corticostatin;* ***DEFB4A****, defensin, beta 4A;* ***DEFA3****, defensin alpha 3, neutrophil-specific;* **DEFA5,** *defensin alpha 5, Paneth cell-specific;* ***DEFA6****, defensin alpha 6, Paneth cell-specific;* ***CCR6,*** *chemokine (C-C motif) receptor 6;* ***ALB****, albumin; MYC, v-myc avian myelocytomatosis viral oncogene homolog;* ***CAMP****, cathelicidin antimicrobial peptide;* ***PAX2****, paired box 2.*

In conclusion, we identified potentially new molecular markers of the development of UC. This information may provide new avenues for the development of novel diagnostic tests for UC.

Competing interests

The authors declare that they have no competing interests.

References

1. Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? World J Gastroenterol. 2006;12(38):6102-8.
2. Büsch K, Ludvigsson JF, Ekström-Smedby K, Ekbom A, Askling J, Neovius M. Nationwide prevalence of inflammatory bowel disease in Sweden: a population-based register study. Aliment Pharmacol Ther. 2014;39(1):57–68.
3. Garrett WS, Gordon JJ, Glimcher LH. Homeostasis and inflammation in the intestine. Cell. 2010;140(6):859-70.
4. Andersen V, Christensen J, Ernst A, Jacobsen BA, Tjønneland A, Krarup HB, et al. Polymorphisms in NF-kB, PXR, LXR, PPARγ and risk of inflammatory bowel disease. World J. Gastroenterol. 2011;17(2):197–206.
5. Andersen V, Nimmo E, Krarup HB, Drummond H, Christensen J, Ho GT, et al. Cyclooxygenase-2 (COX-2) polymorphisms and risk of inflammatory bowel disease in a Scottish and Danish case–control study. Inflamm Bowel Dis. 2011;17(4):937–46.
6. Comelli EM, Lariani S, Zwahlen MC, Fotopoulos G, Holzwarth JA, Cherbut C, et al. Biomarkers of human gastrointestinal tract regions. Mamm Genome. 2009;20(8):516–27.
7. Li X, Conklin L, Alex P. New serological biomarkers of inflammatory bowel disease. World J. Gastroenterol. 2008;14(33):5115–24.
8. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006;55(6):749–53.
9. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. Br Med J. 1955;2(4947):1041–8.
10. D'Haens G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology. 2007;132(2):763–86.
11. Dignass A, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis. Part 1: Definitions and diagnosis. J Crohns Colitis. 2012; 6(10):965–90.
12. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med. 1987;317(26):1625–9.
13. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF, et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Gut. 2012;61(4):535–42.

International Journal of Biomedicine 6(1) (2016) 74-77
doi: 10.21103/Article6(1)_OA16

ORIGINAL ARTICLE

Dentistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Oral Lichen Planus and Features in the Short Chain Fatty Acid Pattern Produced by Colonic Fermentation

Umida A. Shukurova*, PhD; Olga E. Bekjanova, PhD, ScD

Tashkent State Dental Institute
Tashkent, Uzbekistan

Abstract

The aim of the study was to assess the content of short chain fatty acids (SCFAs) in feces of patients with different clinical forms of oral lichen planus **(**OLP).

Materials and Methods: The study included 139 patients with different clinical forms of OLP in the acute stage. The diagnosis of OLP was performed according to both clinical and histopathological criteria. Patients were distributed in four groups according to the clinical form of OLP. Group 1 included 36 patients with the reticular form of OLP; Group 2 included 34 patients with the exudative-hyperemic form of OLP; Group 3 included 27 patients with the erosive-ulcerative form of OLP; Group 4 included 42 patients with the bullous form of OLP. The four groups to be compared were randomized by sex and age. The control group consisted of 40 healthy, age-matched, randomly selected persons without clinical and instrumental signs of OLP and other diseases of the oral cavity. The concentration of SCFAs in feces was evaluated by gas-liquid chromatography. The profiles (specific concentration) of C2, C3, and C4 acids, the ratio of iso-acids to straight-chain acids (iso-Cn/Cn) and iso-C5/C5 were also calculated.

Results: Three types of SCFA changes reflecting the activity of certain groups of microorganisms were found. For all types of the SCFA disorders, we found a marked increase in the iso-C5/C5 ratio. The severity of dysbiosis increased with the severity of clinical forms of OLP. Changes in the qualitative and quantitative contents of SCFAs reflect the disturbances in gut microbiocenosis in LPO patients, which may be one cause for aggravation of the pathological process. (***Int J Biomed. 2016;6(1):74-77.).***

Keywords: *oral lichen planus; short chain fatty acids; microbiota; dysbiosis.*

Introduction

Lichen planus (LP) is a chronic systemic inflammatory disease that affects the skin and mucous membranes. LP occurs in 0.1 to 4% of the general population, most often in perimenopausal women. Oral LP (OLP) is a mucosal subtype of LP that most commonly occurs in middle-aged adults; the population frequency of OLP is 0.5% to 2.2%. The etiology of this disorder remains uncertain. Several immunological mechanisms of its pathogenesis have been proposed, including antigen-specific cell-mediated immune response, nonspecific immunological mechanisms, autoimmune response, and humoural immunity [1]. OLP is the target of much controversy, mainly in relation to its pathogenesis and possible potential for malignancy. OLP is usually regarded as a multifactorial disease in which endogenous and exogenous factors, in addition to genetic defects, can play a role in the formation and nature of the pathological process.

As is known, disorders of oral mucosa and the gastrointestinal tract may occur simultaneously as a manifestation of a generalized disorder. Human mucosal sites are colonized by an astonishing number of microorganisms of different genera (e.g., Bifidobacterium, Eubacterium, Fusobacterium, Escherichia and Candida). Most of these non-human cells are located in the gastrointestinal tract (GIT) where they exert protective (i.e., natural defense barrier and production of anti-microbial factors), structural (i.e., development of immune system and induction of IgA), and metabolic (i.e., fermentation of non-digestible dietary residues, synthesis of vitamins and ion absorption) functions [2].

Important effects of these microorganisms and their products have been demonstrated not only in the GI tract but also in adipose tissue, immune and nervous systems [3-5]. Short chain fatty acids (SCFAs), which are the major metabolic products of anaerobic bacteria fermentation, have been suggested to be the link between microbiota and host

***Corresponding author:** Umida A. Shukurova, PhD. Tashkent State Dental Institute. Tashkent, Uzbekistan. E-mail: shua1981@mail.ru*

tissues. SCFAs, also called volatile fatty acids because of their relatively more volatile nature compared to longer fatty acids, have been studied for more than a century. The concentration of these fatty acids in the GIT may predispose to or prevent a large number of pathological conditions. Modifications in the concentrations or the ability of host tissues to use SCFAs have been described in these conditions [6-11].

Acetate, propionate, and butyrate are found in the human intestine at concentrations of approximately 13 mM in the terminal ileum, ~130 mM in the caecum, and ~80 mM in the descending colon [12]. They are produced by anaerobic fermentation of non-digestible dietary residues and endogenous epithelial-derived mucus in the gut. SCFAs released in the intestinal lumen are readily absorbed and used as an energy source by colonocytes (5 to 10% of human basal energy requirements are provided by SCFAs) and also by other tissues, including liver and muscle [13]. SCFAs, of which butyrate is the most studied, modulate different processes, including cell proliferation and differentiation, hormone secretion (e.g., leptin and peptide YY) [14,15], and activation of immune/inflammatory responses [5,16]. Therefore, in addition to energy supply, these fatty acids have other important functions [18].

The aim of the study was to assess the content of SCFAs in feces of patients with different clinical forms of OLP.

Materials and Methods

The study included 139 patients (mean age with different clinical forms of OLP in the acute stage. Inspection of the oral cavity was carried out by the usual method (Borovsky EV et al., 2001). The diagnosis of OLP was performed according to both clinical and histopathological criteria [19]. Patients were distributed in four groups according to the clinical form of OLP. Group 1 included 36 patients with the reticular form of OLP; Group 2 included 34 patients with the exudative-hyperemic form of OLP; Group 3 included 27 patients with the erosive-ulcerative form of OLP; Group 4 included 42 patients with the bullous form of OLP. The four groups to be compared were randomized by sex and age. The reticular form manifested as bilateral, asymptomatic Wickham striae on the oral mucosa or other parts of the mouth, such as the gingiva, tongue, palate, and lips. The bullous form manifested as fluid-filled vesicles. The erosive form was characterized by ulcerated, painful, erythematous areas.

The control group consisted of 40 healthy, age-matched, randomly selected persons without clinical and instrumental signs of OLP and other diseases of the oral cavity.

A 4-mm punch biopsy was use. The histopathological criteria were a characteristic “saw-tooth” pattern of epidermal hyperplasia; hyperparakeratosis with thickening of the granular cell layer; and vacuolar alteration of the basal layer of the epidermis, with an intense infiltration (mainly T cells) at the dermal-epidermal junction.

The concentration of SCFAs in feces was evaluated by gas-liquid chromatography. We determined: 1) the total concentration of volatile fatty acids; 2) the absolute contents of acetic acid (C2), propionic acid (C3), butyric acid (C4); and valeric acid (C5); and 3) the anaerobic index (AI) – the ratio of propionic and butyric acids to acetic acid. The profiles (specific concentration) of C2, C3, and C4 acids were calculated by the formula pCn= Cn/(C2+C3+C4); the ratio of iso-acids to straight-chain acids (iso-Cn/Cn) and iso-C5/C5 were also calculated.

The study was conducted in accordance with the requirements of the WMA Declaration of Helsinki (2008). The study was approved by the Tashkent State Dental Institute Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the statistical software STATISTICA *v. 6.0*. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. *S*tudent’s unpaired t-test was used to compare two groups for data with normal distribution. A probability value of $P<0.05$ was considered statistically significant.

Results

According to the obtained data, the total absolute content of the studied SCFAs was significantly different from control values in all patients, regardless of the clinical form of OLP; these results indicate changes in the activity of obligate microflora and an increase in the activity of pathogenic and/or opportunistic pathogens.

Three types of SCFA changes reflecting the activity of certain groups of microorganisms were found (Table 1).

Type 1 was characterized by slight changes in the total content, composition, and spectrum of SCFAs, indicating moderate changes in the activity of obligate and facultative anaerobic microflora. Type 2 was characterized by a change in the total number and profiles of C2-C4, indicating the predominant activity of the anaerobic microorganisms, mainly genera Bacteroides, Clostridium, Eubacterium, Fusobacterium, Coprococcus and the strains of the anaerobic microorganisms with proteolytic activity. Type 3 was characterized by changes in the SCFA spectrum, which were associated with the increased activity of the aerobic microorganisms having hemolytic activity (E. Coli, enterococci, streptococci, staphylococci, hemolytic strains of E. Coli, Klebsiella, Proteus, etc.).

For all types of the SCFA disorders, we found a marked increase in the iso-C5/C5 ratio, indicating an increase in proteolytic activity of facultative, aerobic, and anaerobic microorganisms. The pronounced negative values of the AI ratio, compared with reference values, reflect the changes in the intestinal microflora which contributed to the growth of anaerobic microorganisms.

It should be noted that the severity of dysbiosis increases with the severity of clinical forms of OLP. For example, Type 1 was identified in patients of Groups 1 and 2 with a marked increase in the iso-C5/C5 ratio by 20.67% and 40.66%, respectively. Type 2 was identified in patients of all groups, with a more marked increase in the iso-C5/C5 ratio (from 74.7% for Group 1 to 250% for Group 4). Type 3 was also identified in patients of all groups with an increase in the iso-C5/C5 ratio by 128% to 183.3% (Table 1).

Table 1.

Fecal short-chain fatty acids in patients with different clinical forms of OLP

Group	Type Δ	∑ mg/l	C2 (U)	C3 (U)	C4 (U)	AI	iso-Cn/Cn	Iso-C5/C5
The control group		10.28±0.42	0.638±0.02	0.188±0.008	0.174±0.005	-0.567±0.02	0.428±0.02	1.50±0.07
Group 1	I	12.32±0.55^	0.602±0.02^	0.202±0.008^	0.196±0.004^	- 0.661±0.03^	0.471±0.02^	1.81±0.08^
	II	14.32±0.63^	0.562±0.01^,*	0.249±0.01^,*	0.211±0.008^,*	-0.821±0.04^	0.503±0.03^	2.62±0.11^
	III	8.1±0.31^	0.728±0.03^	0.123±0.004^	0,149±0,006•	-0.374±0.008^	0,542±0,02•	3.42±0.12^
Group 2	I	13.21±0.65^	0.582±0.02^	0.224±0.01^	0,194±0,008^	-0.718±0.03•	0.495±0.02^	2.1±0.10^
	II	14.25±0.69^	0.546±0.02^	0.292±0.01^	0.162±0.007^,*	-0.832±0.03^	0.616±0.02^,*	2.92±0.12^
	III	8.66±0.38^	0.756±0.03^	0.118±0.04^	0.126±0.004^	-0.323±0.01^,*	0.719±0.03^,*	4.09±0.18^,*
Group 3	II	7.26±0.28^	0.773±0.03^,#	0.117±0.005	0.110±0.003^,#	-0.293±0.01^	0.920±0.03^	4.61±0.11^
	III	16.23±0.77^	0.534±0.02^	0.252±0.01^	0.214±0.01^	- 0.873±0.04^	0.760±0.02^	3.81±0.08^
Group 4	II	6.81±0.26^	0.780±0.03^	0.110±0.003^	0.110±0.003^	-0.282±0.01^	0.854±0.03^,#	5.25±0.25^,#
	III	16.46±0.81^	0.520±0.02^	0.250±0.05^	0.230±0.01^	-0.923±0.03^	0.732±0.02^	4.25±0.20^

*Δ - Type of SCFA changes; ^- P<0.05 vs. control; *- P<0.05 between Groups 1 and 2; # - P <0.05 between Groups 3 and 4.*

Changes in SCFA were obviously due to the increase in activity of anaerobes and activity of aerobic microflora with a strong protolithic and hemolytic activity.

There was a progressive increase in the frequency of the Type 3 SCFA disorders with the increase of the LPO severity (Table 2). In particular, the Type 1 SCFA disorders occurred only in patients with the reticular and exudative-hyperemic forms of OLP. In patients with the reticular form of OLP, the frequency of Type 3 SCFA disorders was 44.44±8.28% in comparison with Type I (22.22±6.93%, *P<0.05*). In patients with the exudative-hyperemic form of OLP, the frequency of Type 3 SCFA disorders was 52.94±8.56% in comparison with Type I (11.76±5.52%, *P<0.05*). The frequency of Type 3 and Type 2 disorders was 70.37±8.8% vs. 29.63±8.79% (*P<0.05*) for the erosive-ulcerative form and 61.90±7.50% vs. 38.10±7.56% (*P<0.05*) for the bullous form of OLP (Table 2).

Table 2.

Changes in the SCFA spectrum in association with different clinical forms of OLP

Type Δ	Clinical form of OLP			
	Group 1 (n=36)	Group 2 (n=34)	Group 3 (n=27)	Group 4 (n=42)
I	8/(22.2±6.93)*	4/(11.8±5.52)*#	-	-
II	12/(33.3±7.85)	12/(35.3±8.19)	8/(29.6±8.79)^	16(38.1±7.56)^
III	16/(44.4±8.28)	18(52.9±8.56)	19(70.4±8.80)“	26(61.9±7.50)“
Total	36/(100)	34/(100)	27/(100)	42/(100)

Δ - Type of SCFA changes;values are presented as n/(%);- P<0.05 between Types 1 and 3; # - P<0.05 between Types 1 and 2; ^ - P<0.05 between Types 2 and 3; “- P<0.05 versus Group 1.*

Discussion

Manifestations of OLP are rarely isolated. Patients with OLP often have the comorbid diseases, including disorders in the GIT, which are found between 15% and 80% of patients, according to the data of different authors [20-23].

The presence of co-morbidity of different parts of the digestive tract is inevitably accompanied by dysbiosis in the mouth and intestines. Several authors have identified the expressed disbiotic disorders in the oral cavity with OLP [24-27]. Breakdown of the normal microbial community increases the risk of pathogen infection, the overgrowth of harmful pathobionts, and immuno-inflammatory disease. Understanding the interaction of the microbiota with pathogens and the host might provide new insights into the pathogenesis of systemic disorders.

In general terms, patients with LPO exhibit abnormal microbiota with instability of dominant species, which is higher than in healthy controls. High fecal concentrations of total or individual SCFAs might also be the result of increased microbial production, shifts in microbial cross-feeding patterns, and low mucosal absorption. Nevertheless, it is known that changes in the concentration and proportion of individual SCFAs are concurrent with changes in bacterial groups.

SCFAs present multiple effects in different cells involved in the inflammatory and immune responses. In general, SCFAs, such as propionate and butyrate, inhibit stimuli-induced expression of adhesion molecules and chemokine production, and consequently suppress monocyte/macrophage and neutrophil recruitment, suggesting an anti-inflammatory action. However, there is also evidence in favor of a pro-inflammatory action of SCFAs in some conditions [17,28]. This discrepancy may be in part explained by the ability of SCFAs to induce neutrophil migration. In sites of anaerobic bacteria infection or after loss of intestinal epithelial integrity, high concentrations of SCFAs may lead to neutrophil accumulation and amplification of the inflammatory process [18]. Another possible explanation is that these fatty acids may present divergent effects depending on the cell type (e.g., anti- and pro-inflammatory effects of SCFAs on macrophage and microglial cells have been demonstrated [29-31]. Therefore, although SCFAs modulate the function of immune cells, more studies are necessary in order to understand the precise role of SCFAs on the interaction between bacteria and host

immune cells in vivo, particularly in the GI tract and in sites of anaerobic infections, including the skin and oral cavity [18].

In conclusion, changes in the qualitative and quantitative contents of SCFAs reflect the disturbances in gut microbiocenosis in LPO patients, which may be one cause for aggravation of the pathological process.

Competing interests

The authors declare that they have no competing interests.

References

1. Roopashree MR, Gondhalekar RV, Shashikanth MC, George J, Thippeswamy SH, Shukla A. Pathogenesis of oral lichen planus: a review. J Oral Pathol Med. 2010;39(10):729–34.
2. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep. 2006;7(7):688–93
3. Diaz Heijtz R1, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA. 2011;108(7):3047–52.
4. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 2010;328(5975):228–31.
5. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, YuD, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461(7268):1282–6.
6. Huda-Faujan N, Abdulamir AS, Fatimah AB, Anas OM, Shuhaimi M, Yazid AM, Loong YY. The impact of the level of the intestinal short chain Fatty acids in inflammatory bowel disease patients versus healthy subjects. Open Biochem J. 2010;4:53–58.
7. Vernia P, Caprilli R, Latella G, Barbetti F, Magliocca FM, Cittadini M. Fecal lactate and ulcerative colitis. Gastroenterology. 1988;95(6):1564–8.
8. Murphy EF, Cotter PD, Healy S, Marques TM, O'Sullivan O, Fouhy F, et al. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. Gut. 2010;59(12):1635–42.
9. Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, Hardt PD. Microbiota and SCFA in lean and overweight healthy subjects. Obesity. 2010;18(1):190–5.
10. Simark-Mattsson C, Eklund S. Reduced immune responses to purified protein derivative and Candida albicans in oral lichen planus. J Oral Pathol Med. 2013;42(9):691-7.
11. Petrova LV. Clinical features of lichen planus of the oral mucosa. Ross *Zh* Kozh Vener Bolezney. 2002;3:28-31.[Article in Russian].
12. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut. 1987;28(10):1221–7.
13. McNeil NI. The contribution of the large intestine to energy supplies in man. Am J Clin Nutr.1984;39:338–42.
14. Zaibi MS, Stocker CJ, O'Dowd J, Davies A, Bellahcene M, Cawthorne MA, et al. Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett. 2010; 584(11):2381–6.
15. Plaisancie P, Dumoulin V, Chayvialle JA, Cuber JC. Luminal peptide YY-releasing factors in the isolated vascularly perfused rat colon. J Endocrinol. 1996;151(3):421–9.
16. Vinolo MA, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC, Curi R. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. J Nutr Biochem. 2011; 22(9):849–55.
17. Vinolo MA, Rodrigues HG, Hatanaka E, Hebeda CB, Farsky SH, Curi R. Short-chain fatty acids stimulate the migration of neutrophils to inflammatory sites. Clin Sci. 2009;117(9):331–8.
18. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of Inflammation by Short Chain Fatty Acids. Nutrients. 2011; 3(10): 858–76.
19. Kraft R, Usatine RP. Lichen planus. In: *The Color Atlas of Family Medicine*. Usatine RP, Smith MA, Chumley H, Mayeaux EJ Jr., Tysinger J, eds. New York, NY: McGraw-Hill; 2009:634–639.
20. Beleva NS, Libik TV. Factors systemic risk in patients with manifestations of lichen planus of the oral mucosa. Proceedings of the All-Russian scientific-practical conference of students, graduate students and young scientists. Perm, 2009:191-4. [Article in Russian].
21. Surdina ED. Modern conceptions of the leading factors in development and treatment of lichen planus with manifestations in the oral mucosa. Vestnik St. Petersburg University. Ser.11. 2011;4:112-118. [Article in Russian].
22. Oskolsky GI, Zagorodnaya EB. Role of dysbiotic shifts in patients with lichen planus of the oral mucosa. In: *Actual problems and prospects of development of dentistry in the North*. Ushnitskii ID, ed. Yakutsk, NEFU; 2011:170-174.
23. Ron GI, Epishova AA. Modern ideas about the etiology and pathogenesis of lichen planus of the oral mucosa (review). Problemi Stomatol. 2011;4:15-17. [Article in Russian].
24. Slujaev IF, Oskolsky GI, Zagorodnaya EB. Oral lichen planus of the oral mucosa: clinical features, treatment. Dal'ne-Vostochniy Med Zh. 2010;2:132-136. [Article in Russian].
25. Pourshahidi S, Fakhri F, Ebrahimi H, Fakhraei B, Alipour A, Ghapanchi J, Farjadian S. Lack of association between Helicobacter pylori infection and oral lichen planus. Asian Pac J Cancer Prev.2012;13(5):1745-7.
26. Taghavi Zenouz A, Mehdipour M, Jafari Heydarlou M, Gholizadeh N. Relationship between lichen planus and Helicobacter pylori infection. J Dent Res Dent Clin Dent Prospect. 2010;4(1):17-20.
27. Ardatskaya MD, Dmitrieva LA, Khubutia BN, Georgieva OA, Osipov TL. The study of oral microflora metabolites (short chain fatty acids) in patients with lichen planus; diagnostic and tactical importance. Estestv Tekhnich Nauki. 2012;6(62):194-204. [Article in Russian].
28. Niederman R, Buyle-Bodin Y, Lu BY, Robinson P, Naleway C. Short-chain carboxylic acid concentration in human gingival crevicular fluid. J Dent Res. 1997;76(1):575–9.
29. Halili MA, Andrews MR, Labzin LI, Schroder K, Matthias G, Cao C, et al. Differential effects of selective HDAC inhibitors on macrophage inflammatory responses to the Toll-like receptor 4 agonist LPS. J Leukoc Biol. 2010;87(6):1103–14.
30. Bailon E, Cueto-Sola M, Utrilla P, Rodriguez-Cabezas ME, Garrido-Mesa N, Zarzuelo A, et al. Butyrate in vitro immune-modulatory effects might be mediated through a proliferation-related induction of apoptosis. Immunobiology. 2010;215(11):863–73.
31. Huuskonen J, Suuronen T, Nuutinen T, Kyrylenko S, Salminen A. Regulation of microglial inflammatory response by sodium butyrate and short-chain fatty acids. Br J Pharmacol. 2004;141:874–80.

International Journal of Biomedicine 6(1) (2016) 78-81
doi: 10.21103/Article6(1)_OA17

ORIGINAL ARTICLE

Sports Medicine

INTERNATIONAL JOURNAL OF BIOMEDICINE

Influence of Electrophoresis of Antler Mass on Restorative Processes in Young Athletes during the Preparatory Period of a One-Year Training Cycle

Kristina S. Gavril'eva[1,2]; Maria V. Handi[1]; Marianna I. Solovieva[1]; Sargilana S. Kuzmina[1]; Konstantin M. Stepanov[1]; Sardana V. Markova[1]; Natalya V. Makharova[2]; Ekaterina N. Mestnikova[2]; Tatiana E. Burtseva[3]

[1]North-East Federal University; [2] School of the Highest Sports Skill
[3]Yakutsk Research Center for Complex Medical Problems
Yakutsk, the Sakha Republic, Russia

Abstract

We investigated the influence of electrophoresis of antler mass (according to Vermel's method) on the peripheral blood and indicators of cardiac function in elite athletes during intensive exercise during the preparatory period. This study included 27 male athletes, aged 16-17 years old. Application of electrophoresis of antler mass led to improvement of hemoglobin level and hematocrit, mean corpuscular hemoglobin concentration, normalization of hormonal status and myocardial metabolism, and promoted increased fitness and adaptability to physical stress. **(Int J Biomed. 2016;6(1):78-81.).**

Keywords: *athletes; intensive exercise; blood test; myocardial metabolism; antler mass; general electrophoresis.*

Introduction

The human body is designed to thrive under conditions of regular physical exertion because the process of adaptation arising from different training stimuli ensures an optimal function of various systems of the organism. On the one hand, its backup capabilities increase at the cellular, system and intersystem levels. On the other hand, response to a load decreases over time, which leads to stopping the improvement in sporting performance. Intensive physical exercise in athletes leads to overstraining the musculoskeletal system, hypoxia, and the formation of an excess of free radicals [1]. Given this circumstance, many experts believe that all qualified athletes need to be in functional rehabilitation during training and competition [2].

Currently, rehabilitation and treatment measures in sports are mostly of pharmacological orientation. However, the restrictive list of approved pharmaceuticals narrows the possibilities of sports physicians to ensure effective recovery and treatment of athletes. To prevent fatigue and mitigate its consequences, new, largely alternative, pharmacological and non-pharmacological measures of influence on key mechanisms of athletes' performance are being required today [3,4].

To solve tactical and strategic objectives in the training process and competitive activities without fear of sanctions in connection with the use of anabolic doping in elite sports, the use of antler products is a priority choice for remediation after intense exercise.

In extreme conditions of the Far North in the struggle for survival in the short polar days, a deer generates the greatest amount of biologically active substances. This unique phenomenon explains the burst of vitality among the indigenous peoples, who use deer blood in food. At the same time, the vital biological substance in the body helps a person to survive in the harsh conditions of the north. Products from reindeer antlers combine centuries-old traditions and the latest scientific developments. The indigenous people of the north for many centuries used the antlers of reindeer to overcome the most difficult climatic conditions and the highest exercise stress [5]. It is generally known that extracts from deer antlers are primarily tonic medicines. More than two thousand years

***Corresponding author:** Kristina S. Gavrileva. North-East Federal University, Yakutsk, the Republic of Sakha (Yakutia), Russia. E-mail: gks.79@mail.ru*

in the traditional medicine of East Asia, the antlers have being used as a means of relieving fatigue and increasing the efficiency of the organism. Currently, the domestic industry produces the following products from deer antlers: Pantocrinum, Rantarin, Velkornin [5], Epsorin, and Cigapan [6-9]. Numerous clinical studies have identified three main properties of antlers: tonic effect on the body, stimulation of sexual function, and acceleration of tissue regeneration [8].

In the literature, there are sporadic reports on the effect of drugs from deer antlers during intensive physical exercise in athletes. In the I.M. Sechenov Moscow Medical Academy, an extract from deer antlers, Velkornin, was clinically tested in athletes. As a result, it was found that Velkornin is an effective means of enhancing physical performance and stimulation of humoral immunity for athletes in sports that require endurance in the preparatory period of training. Velkornin does not cause side effects and allergic reactions and can be recommended for use in practice [5].

The effect of intravenous administration of the powder of deer antlers, Pantovital, stimulates erythropoiesis and helps to improve the oxygen-transport function of blood, thereby increasing the adaptive capacities of the athletes' organisms and tolerance to training loads [10].

In the Republic of Sakha (Yakutia), the dietary supplement Epsorin from deer antlers is widely used. Using Epsorin can significantly improve the functional state of athletes, ensure fast adaptation when moving across time zones and in different climatic zones in precompetitive and competitive periods, and achieve a significant increase in the functionality of the organism. It should be noted that Epsorin increases only the positive reactions of the organism to physical stress and thus enhances adaptation to the increasing intensity of training loads in the precompetitive period, maintains the optimal fitness during the competition, and accelerates the process of recovery during rehabilitation [9].

Deer antlers have been used in traditional *medicine* for a long time, but, unfortunately, we could not find any information on the non-invasive method of the introduction of antler mass by general electrophoresis in athletes during intense exertion.

The aim of this study was to investigate the effect of electrophoresis of antler mass by Vermel's method on the condition of the peripheral blood, antioxidant protection, hormone status, and indicators of cardiac performance in athletes at the preparatory stage of the training cycle.

Materials and Methods

The study included 27 elite athletes in wrestling, males aged 16-17 years old, having qualifications from the first sports category to the candidates for Master of Sports. Sports experience was 5-7 years. Young athletes have been involved in the study on a voluntary basis. The studies were conducted in the period of intensive training. Athletes were randomly divided into 2 groups. Group 1 (n = 13) received antler mass by electrophoresis for 20-30 minutes during 5-7 days. The procedures were carried out at the preparatory stage of sports cycle. Group 2 included 14 athletes without intervention.

All athletes underwent a physical examination, ECG, general blood test (HGB, RBC, MCV, HCT, MCH, MCHC), and determination of blood testosterone and cortisol, malonic dialdehyde (MDA), low molecular weight antioxidants (LMAO) and catalase. MDA content in serum was determined by spectrophotometric method by reaction with thiobarbituric acid at λ=532 nm [11]. The content LMAO was determined by by ortho-phenanthroline colour method [12]. o-phenantroline quantitatively forms complex with Fe^{2+}, which get disrupted in the presence of chelating agents. The antioxidant interfered with the formation of ferrous-ophenantroline complex which is spectrophotometrically read at 510 nm.

Testosterone and cortisol levels were determined in the blood serum using automated EIA and the chemistry analyzer ChemWell (Awareness Technology, Inc.). The collection of blood specimens was performed from 8:00 a.m. to 9:00 a.m.

To determine the influence of electrophoresis of antler mass on athletes' organisms, we used an antioxidant protection coefficient (C_{AOP}) [6]. The activity of the antioxidant blood systems of healthy people served as the normalizing parameters (n=50). The activity of blood antioxidant systems (C_{AOP}) and coefficient of antioxidant-prooxidant balance (C_{AOP}/C_{LPO}) were calculated according to the formulas (1) and (2):

$(C_{AOP})N= \Sigma$ (parameters AO systems)$_N$ /quantity studied AO systems (1),

where: AO system $_N$ - parameters of LMAO, superoxide dismutase (SOD), and peroxidase, normalized to the control values;

$(C_{AOP}/ C_{LPO})_N=(C_{AOP})_N$ /[activity LPO]$_N$ (2),

where activity LPO$_N$ - level of MDA, normalized to the control vakues.

ECG was recorded on an electrocardiograph, Shiller AT-101, in the morning the day before the training event.

Electrophoresis method for antler mass application according to Vermel's procedure

For galvano-mud therapy [13], antler mass was heated on a steam bath to 38°-40°C and placed in gauze baggies (300 cm^2 in size); the thickness of an antler mass layer was 2cm-2.5cm. The baggies were placed on the interscapular region. The current-carrying electrodes were placed on the baggies and connected to the anode. Bifurcated electrodes were used as the cathodes that were placed on the rear surface of the calves of both legs using pads of 150 cm^2.

Electrophoresis was performed by using the apparatus for galvanization, Potok-1. The current density with galvano-mud therapy was 0.05 mA/cm^2 for 20-30 min. Electrophoresis was scheduled for 5 to 8 sessions per course on alternate days. After each procedure, the athlete rested for 30-40 minutes. Antler mass has normative and technical documentation (Specifications TU 9219-003-00549163-06).

Statistical analysis was performed using SPSS Statistics v.19.0. The mean (M) and the standard error of the mean (SEM) were calculated. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability

value of *P<0.05* was considered statistically significant.

Young athletes have been involved in the study on a voluntary basis. Written informed consent was obtained from all participants.

Results

Despite the fact that the red blood parameters in both groups conformed to generally accepted standards, as a result of the analysis of the dynamics of the studied parameters during the course of electrophoresis of antler mass, we found statistically significant differences between groups. The most pronounced changes were found in oxygen provision mechanisms. The increase in hemoglobin in the blood reflects the body's adaptation to physical stress in hypoxic conditions [14].

During intense workouts, differences in red blood parameters were detected in the studied groups (Table 1). The number of erythrocytes decreased in Group 2 an average of 2.33% (from $4.72*10^{12}$/l to $4.61*10^{12}$/l; *P<0.05*), whereas in Group 1 this parameter increased by 1.96% (from $4.59*10^{12}$/l to $4.68*10^{12}$/l; *P<0.05*). More significant changes were obtained in the study of hemoglobin and hematocrit. In Group 1, the hemoglobin level and hematocrit significantly increased by 2.88% (from 147.9 g/l to 152.2 g/l; *P=0.001* and 2.74% (from 41.97 to 43.12; *P=0.001*), respectively, after a course of electrophoresis with antler mass. The opposite pattern was obtained in Group 2, where we observed a statistically significant reduction in hemoglobin level by 3.99% (from 150.44 g/l to 144.44 g/l; *P=0.005)* and hematocrit by 2.43% (from 42.30 to 41.27; *P<0.05*).

Table 1.

Red blood parameters of athletes during intense workouts preparatory stage of sports cycle

Variable	Group 1			Group 2		
	Initial data	*P*	10th day AT	Initial data	*P*	10th day AT
HGB, g/l	147.87±5.96	*0.001*	152.25+6.98	150.44+6.69	*0.005*	144.44+8.39
RBC, mln/mm^3	4.59±0.17	*0.032*	4.68+0.22	4.72+0.22	*0.033*	4.61+0.24
MCV	91.38±2.49	*0.001*	92.15+2.21	89.73+1.69	*0.408*	89.62+1.72
HCT,%	41.97±2.09	*0.011*	43.12+2.10	42.30+2.00	*0.035*	41.27+2.32
MCH, pg	32.12±0.78	*0.006*	32.56+1.02	31.82+0.79	*0.001*	31.27+0.59
MCHC, g/l	351.87+5.08	*0.429*	353.00+5.34	355.11+4.98	*0.001*	349.33+3.74

AT - after training

During intense exercise, there was a destruction of RBCs and a decrease in hemoglobin concentration. Increasing the concentration of hemoglobin in Group 1 was accompanied by an increase in RBC concentration by 1.92% (from $4.59*10^{12}$/l to $4.68*10^{12}$/l; *P<0.05*) and hematocrit by 2.67% (from 41.97 to 43.12; *P=0.011*).

We also noted that in Group 2, a decrease in RBC concentration was accompanied by a significant decrease in MCHC by 1.63% (from 355.1 g/l to 349.3 g/l; *P=0.001*). In Group 1, on the contrary, there was a statistically insignificant increase in MCHC by 0.32% (from 351.9 g/l to 353.0 g/l; *P>0.05*).

The training loads were accompanied by increasing hormonal activity [15]. An increase in blood cortisol level in response to physical exercise reflects the activation of a stress-realizing system due to the need to mobilize energy reserves [16].

We detected a high cortisol level in athletes of Group1 in the preparatory phase of the training cycle. After electrophoresis, blood cortisol level in that group significantly decreased by 41.17% *(P=0.001)*, whereas in Group 2 the level increased by 22.95% *(P=0.001)*. Blood testosterone level significantly increased by 18.5% (P=0.001) in Group 1, but decreased by 9.5% *(P=0.008)* in Group 2 (Table 2).

Table 2.

Blood levels of cortisol and testosterone in athletes during intense workouts preparatory stage of sports cycle

Variable	Group 1			Group 2		
	Initial data	*P*	10th day AT	Initial data	*P*	10th day AT
Cortisol, ng/ml	769.9+230.2	*0.001*	452.9+96.7	318.8+106.0	*0.001*	392+93.2
Tes, ng/ml	23.45+3.18	*0.001*	27.80+3.85	16.23+6.33	*0.008*	14,68+5.1

AT - after training; Tes- testosterone

According to the criterion of binomial distribution, a trend to improvement of ECG parameters was identified in Group 1: a decrease in the incidence of early repolarization syndrome (ERS) by 15.4%, incomplete RBBB by 30.77%, and full correction of metabolic changes (Table 3). In Group 2, we observed an increase in metabolic changes in the myocardium by 7.14% and incidences of sinus arrhythmia by 21.43% on the background of intense exercise.

Table 3.

Dynamics of ECG in athletes during intense workouts preparatory stage of sports cycle

ECG data	Group 1		Group 2	
	Initial data	10th day AT	Initial data	10th day AT
ERS	5 (38.5%)	3 (23.1%)	7 (50%)	7 (50%)
IRBBB	5 (38.5%)	1 (7.7)	5 (35.7%)	4 (28.6%)
Metabolic changes in the myocardium	3 (23.1%)	-	1 (7.14%)	2 (14.3%)
Sinus arrhythmia	3 (23.1%)	1 (7.7)	1 (7.1%)	4 (28.6%)

AT - after training;

A course of electrophoresis had a positive impact on antioxidant status. We identified an improvement of antioxidant–pro-oxidant balance with an increasing C_{AOP}/C_{LPO} ratio only in Group 1 (Table 4).

Table 4.

Antioxidant status in athletes during intense workouts preparatory stage of sports cycle

Vaiable	Indicators	Group 1		Group 2	
		Initial data	10th day after training	Initial data	10th day after training
∑ LMAO	Indicators (mgeq/ml Erith)	0.419±0.09	0.832±0.01*	0.718±0.03	0.374±0.01*
	The ratio normalized to control	0.58	1.15	0.92	0.52
Catalase activity	Indicators (mcat/l)	1.921±0.18	2.77±0.19*	2.775±0.18*	2.347±0.11*
	The ratio normalized to control	0.69	1	1.0	1.1
LPO	Indicators (MDA, nmol/l)	0.131±0.05	0.108±0.04*	0.180±0.02	0.165±0.05*
	The ratio normalized to control	0.79	0.65	1.0	0.84
C_{AOP}		0.92	1.65	0.96	1.07
C_{AOP}/C_{LPO}		1.45	2.68	1.0	1.8

**- $p \leq 0.05$, statistically significant differences relative to initial data*

Conclusion

The results obtained indicate that after a period of intense exercise on the background of electrophoresis with antler mass, the recovery processes aim at maintaining the oxygen-providing system, hormonal status, and normalization of metabolic processes.

Competing interests

The authors declare that they have no competing interests.

References

1. Blinov TV, Troshin VV, Kuznetsova LV. Assessment of antioxidant status in athletes involved in rowing, in training - competition period. Medicine for Sports, 2013. Proceedings of III All-Russia Congress with international participation. Moscow, April 9-10, 2013. M.: Meditsina; 2013:46-47. [in Russian].
2. Jordanskaya FA. Monitoring of functional preparedness of young athletes, the provision of high performance sport (stages of in-depth training and sports perfection). M .: Soviet Sport; 2011. [in Russian].
3. Ponomarenko GN, Ulashchik VS, Zubovsky DK. Sports physiotherapy. SPb., 2009. [in Russian].
4. Uskov GV, Voznitskaya OE. Physiotherapy methods to improve the physical condition of athletes. Actual problems of diagnosis, treatment and prevention in the system of medical rehabilitation and sports medicine. Proceedings of the regional scientific-practical conference. Chelyabinsk: ChelGMA; 2013:73-84**.** [in Russian].
5. Osintsev NS, Osintsev SN. The healing power of antlers. Kaluga: "Manuscript"; 2004. [in Russian].
6. Ahremenko YaN. Mechanism of colonization resistance disorders in children in the North. Abstract of PhD Thesis. Yakutsk; 2004. [in Russian].
7. Burnasheva ZhM. Physical health status of children with pathology of vision and ways of their rehabilitation in the conditions of preschool educational institution in the Republic of Sakha (Yakutia). Abstract of PhD Thesis. Moscow; 2013. [in Russian].
8. Kuleshov YuV, Kuleshov RS, Kuleshov SM. Biologically active preparations from deer antlers and wound-healing effect in animals. KubGAU Nauch Zh. 2007;27(3):12-22. [in Russian].
9. Platonova RI, Haliyev SD, Pavlova AD, Neverkovich SD, Kershengolts BM. The study of mental and emotional state of athletes involved in shooting, during the testing of dietary supplements «Epsorin». Uchennie zapiski universiteta imeni PF Lesgafta. 2013; 10(104):128-32. [in Russian].
10. Smirnova IN, Barabash LV, Kremenko SV, Zaitsev AA, Khlusov IA, Naumov AO, et al. Clinical and experimental study of the use of antlers of Siberian stag to stimulate a hematopoiesis in athletes. Proceedings of scientific and practical Conference "Modern technologies sanatorium treatment and medical rehabilitation". 2013:368-73. [in Russian].
11. Stal'naya ID, Garshvili TG. Method for determination of malonic dialdehyde using thiobarbituric acid. Modern methods in biochemistry. M.: Meditsina; 1977. [in Russian].
12. Rogozhin VV, Kuryliuk TT. Methods of biochemical analysis. Yakutsk: YaGU; 1997. [in Russian].
13. Ulashchik VS. Physiotherapy. Universal Medical Encyclopedia. Mn.: Knizhnii Dom; 2008. [in Russian].
14. Zaitsev AA, Barabash LV, Smirnova IN, Abdulkina NG. Impact of the antler products on the blood oxygen-transport system parameters in athletes in the competitive period. Issues of balneology, physiotherapy and medical physical culture. M.: Meditsina; 2012; 6:17-9. [in Russian].
15. Kremer WJ, Rogol AD. The endocrine system, sport and physical activity. Translated from English. Moscow: Olympic Literature; 2008. [in Russian].
16. Pogodina SV, Filippov MM YuferevVS. Content of steroid hormones in the athletes and untrained persons of the first and second periods of adulthood. Bulletin of the Northern (Arctic) Federal University. A series of 'Life Sciences'. 2015; 2: 81-91.

International Journal of Biomedicine 6(1) (2016) 82-84
doi: 10.21103/Article6(1)_ShC1

Short Communication

INTERNATIONAL JOURNAL OF BIOMEDICINE

Regional Lymphotropic Therapy in Combination with Low Level Laser Therapy for Treating Multi-Drug-Resistant Tuberculosis

Oksana Dogorova; Ekaterina Pavlova, PhD*; Maria Vinokurova, PhD, ScD

Republic Research-and-Practice Center for Tuberculosis
Yakutsk, the Republic of Sakha, Russia

Abstract

With the growing incidence of Multi-Drug-Resistant Tuberculosis (MDR-TB) in newly identified patients, novel multimodality treatment methods are needed, aimed at reducing the time to sputum conversion and cavity healing, which would be applicable in MDR cases.

Our experimental treatment consisted of the following: 1) chemotherapy based on the drug sensitivity profile, 2) local laser irradiation therapy for 25 days, and lymphotropic administration of isoniazid (to subcutaneous tissue in alternating locations: underarm area; fifth intercostal space along the sterna border; subclavian area where the first rib meets the sternum) in a daily dose of 10mg/kg 5 times a week. This treatment was significantly more effective in newly detected destructive MDR-TB versus the standard Category IV regimen for MDR-TB in terms of reduced time for sputum culture conversion and cavity healing, estimated to be 6 months after initiation of treatment. **(Int J Biomed. 2016; 6(1):82-84.).**

Keywords: *multi-drug-resistant tuberculosis; laser irradiation therapy; lymphotropic therapy; isoniazid.*

Introduction

WHO estimated 9 million new tuberculosis (TB) cases and 1.5 million TB deaths in 2013. Globally, 480,000 MDR-TB cases were noted [1]. Despite the introduction of combination regimens throughout the world many years ago, the presence of drug resistance has been progressively documented in an ever wider geographical area [2]. Recent estimates by WHO suggest that nearly half a million cases of MDR-TB (defined as tuberculosis caused by strains of Mycobacterium tuberculosis that are resistant to at least isoniazid and rifampicin, the 2 most powerful first-line anti-TB drugs) emerged globally in 2008 [3]. MDR-TB cases are difficult to treat and have high mortality. M.K.Vimokurova et al. [4] have observed in the Sakha Republic (Yakutia) a growth in the incidence of TB cases presenting with destructions in lung tissue and a bacillary-positive state, a persistent trend towards an increase in TB cases caused by MDR MTB, and a high proportion of deaths during the first year of outpatient follow-up for TB. Many experts feel that high-dose isoniazid can be used against strains resistant to low concentrations of isoniazid but susceptible to higher doses [5] (>1% of bacilli resistant to 0.2 mcg/ml but susceptible to 1 mcg/ml of isoniazid), whereas, isoniazid is not recommended for high-dose resistance (>1% of bacilli resistant to 1 mcg/ ml of isoniazid). Some experts give 900 mg three times a week [6] in adults while others use as high as 16–20 mg/kg/day [7]. Good data are not available on the safety of high-dose isoniazid, and there may be possible associated higher rates of peripheral neuropathy, hepatitis, and other unforeseen adverse effects [8].

Isoniazid metabolism is known to be impacted by genetic factors [9]. This in turn could have important clinical implications in terms of therapeutic efficacy and occurrence of adverse events. Isoniazid is metabolized by acetylation; this conjugation reaction is catalyzed by NAT2. Several studies over the years have shown that human subjects show a wide degree of variation in their capacity to acetylate or inactivate isoniazid to acetyl isoniazid. NAT2 genotyping prior to isoniazid administration would help clinicians in predicting pharmacokinetic variability, and adjusting the isoniazid dose.

The relationship between ethnicity, genetic background, and response to tuberculosis treatment has not been well studied. In the studies by S.S. Gavriliev et al. [10], it was found that slow acetylators of isoniazid prevail among the population of Yakutia.

The method of regional lymphotropic therapy (RLTT),

***Corresponding author**: Ekaterina Pavlova, PhD. Republic Research-and-Practice Center for Tuberculosis, Yakutsk, the Russian Federation. E-mail: esp71@mail.ru*

which is characterized by drug administration into the lymphatic system, has been known for more than 30 years. Laser irradiation (low-level laser therapy) has been used as an adjuvant to anti-tubercular drugs in cases of chronic drug resistant pulmonary tuberculosis for several years [1,10-12].

The aim of this study was to develop a treatment method for MDR-TB based on laser-assisted lymphotropic administration of isoniazid in combination with the standard Category IV regimen.

Material and Methods

Ninety-one patients with newly diagnosed destructive infiltrative pulmonary MDR-TB were included in the study, all HIV-negative. Resistance to isoniazid was assessed as low (1 mkg/mg) in all patients; isoniazid acetylation type was either slow or moderate. All patients (except pregnant women) received hospital-based treatment at the Department for Pulmonary MDR-TB of the Research-and-Practice Center for TB, from 2011 to 2013.

We employed conventional examination methods, which included physical examination, clinical laboratory tests, X-ray and tomographic exams, sputum culture test with simultaneous drug sensitivity determination by absolute concentration method, using solid Lowenstein-Jensen medium; isoniazid activity was assessed by Wollenberg-Grebennik method (1961), and prescribed anti-tuberculosis treatment based on drug sensitivity profiles and in compliance with the RF MOH Order no.109 [13].

The control group included 44 patients who received an intensive phase of standard Category IV chemotherapy regimen for MDR-TB based on drug sensitivity profiles.

The experimental group included 47 patients who received the experimental treatment method, which included a lymphotropic isoniazid administration coupled with laser irradiation on the background of standard Category IV chemotherapy regimen for MDR-TB. Contraindications to the experimental treatment were: 1) individual intolerance to substances, pyoinflammatory diseases of the skin and subcutaneous tissue, severe pain syndrome developing in response to administration of medicinal substances; and 2) pulmonary hemorrhages and hemoptysis, pronounced cardiopulmonary insufficiency, neoplasms, organic brain lesions, II-III degree thyrotoxicosis, DM decompensation, pregnancy, and diseases of the blood.

Isoniazid, known as a highly lymphotropic agent, possesses great potential to enter and reversibly bind with the cell structures of the lymphatic system. To ensure the entry of isoniazid into the lymphatic stream, its administration is preceded by the introduction of a drug carrier and lymph-stimulating agent, heparin (1 ml = 5000 IU), diluted in 4mL of Novocaine solution 0.5%. The daily dose of isoniazid solution (10 mg/kg) is administered to subcutaneous tissue, right after the injection of heparin, using the same needle. It is important, that the injection locations must alternate each day. The injection site is immediately exposed to laser irradiation. Treatment is administered 5 times a week, for 25 days. The procedure is performed in the procedure room, in strict compliance with the aseptic and antiseptic regulations. On weekends and after the end of the laser-assisted RLTT course, isoniazid was administered in the morning (10 a.m.), intramuscularly, a daily dose of 10 mg/kg. Twenty-one days after the end of laser-assisted RLTT course, X-ray and CT checks were performed. Based on the findings, a doctor can assign a repeated treatment course.

Statistical analysis was performed using the SPSS for Windows. Baseline characteristics were summarized as frequencies and percentages for categorical variables. Group comparisons with respect to categorical variables are performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the Republic Research-and-Practice Center for Tuberculosis Ethics Committee. Written informed consent was obtained from all participants.

Results and Discussion

Patients of both groups were matched by age and sex, pulmonary disease duration and predominance of cases with disseminated TB involving 2 or more lobes, sizes of destructions in lung tissue, and a primary MDR profile (Table 1).

Table 1.

Mycobacterium tuberculosis the primary MDR profiles

Drug combinations	EG n=47		CG n=44		Total n=91	
	Abs. number	%	Abs. number	%	Abs. number	%
HR	2	4.3	1	2.3	3	3.3
HR/S/E	23	48.9	26	59.1	49	53.9
HRSKm/Cm/E/PAS/Et/Cs	17	36.2	14	31.8	31	34
HRSOf/E/Et	5	10.6	3	6.8	8	8.8

H – isoniazid; R – rifampicin; S – streptomycin; Km – kanamycin; E – ethambutol; Cm – capreomycin; Et – ethionamide; PAS – para-amynosalicilic acid; Of – ofloxacine; EG - Experimental group; CG - Control group

After 6 months of chemotherapy, sputum culture conversion was observed in 41/87.3% patients of the experimental group versus 25/56.2% patients in the control group ($P=0.02$). X-ray and tomographic examinations after 6 months of chemotherapy showed cavity closures in 29/62.9% patients in the experimental group and in 17/38.7% patients in the control group ($P=0.007$). Differences between the experimental and control groups, in terms of sputum culture conversion and radiological cavity closure, were statistically significant (Table 2).

The developed method of regional lymphotropic isoniazid administration in combination with laser irradiation on top of an intensive phase of regimen IV for MDR-TB significantly improved treatment effectiveness in newly

detected destructive TB patients with low resistance to isoniazid and a slow or moderate isoniazid acetylation type, resulting in a 1.5 times faster sputum culture conversion and 1.6 times faster cavity healing.

Table 2.

Effectiveness of regional lymphotropic isoniazid administration in combination with laser irradiation in newly detected destructive MDR-TB patients

Study groups	Sputum culture conversion after 6 months of therapy		Radiological cavity closure after 6 months of therapy	
	Abs. number	%	Abs. number	%
Experimental group	41	87.2	29	61.7
Control group	25	56.8	17	38.6
	χ^2=10.551 *P=0.0026*		χ^2=4.837 *P=0.0466*	

The developed method can be adopted by specialized tuberculosis clinics and requires only conventional equipment commonly used in procedure and laser therapy rooms.

Competing interests

The authors declare that they have no competing interests.

References

1. Bhagwanani NS1, Bhatia CC1, Sharma N1, Hemvani N1, Chitnis DS1.Low level nitrogen laser therapy in pulmonary tuberculosis.Laser Ther. 2015;24(3):209-14.
2. Wright A, Zignol M, Van Deun A, Falzon D, Gerdes SR, Feldman K,, et al.; Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Epidemiology of antituberculosis drug resistance 2002-07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Lancet 2009; 373(9678):1861–73.
3. World Health Organization (WHO). Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. Geneva, Switzerland: WHO, 2010. (WHO/HTM/TB/2010.3).
4. Vinokurova MK, Alexandrov VL, Yakovleva LP, Oshchepkova NM. Trends in the development of the epidemiological situation of tuberculosis in the Republic Sakha (Yakutia) in 2004-2011. YAKUT MEDICAL JOURNAL. 2013;(1):58-63. [Article in Russian].
5. Katiyar SK, Bihari S, Prakash S, Mamtani M, Kulkarni H. A randomised controlled trial of high-dose isoniazid adjuvant therapy for multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. 2008;12(2):139–45. [PubMed]
6. PIH guide to medical management of multidrug-resistant tuberculosis. Boston: Partners in Health; 2003. [15 March 2014]. http://www.pih.org/publications/entry/pih-guide-to-the-medical-management-of-multidrug-resistant-tuberculosis/
7. Rom WN, Garay SM, editors. Tuberculosis. Philadelphia: Lippincott Williams & Wilkins; 2004.
8. Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis. WHO Guidelines Approved by the Guidelines Review Committee. Geneva: World Health Organization; 2014.
9. Ramachandran G, Swaminathan S. Role of pharmacogenomics in the treatment of tuberculosis: a review. Pharmgenomics Pers Med. 2012; 5:89–98.
10. Gavriliev SS, Vinokurova MK, Illarionov TS. *Individualized chemotherapy for pulmonary tuberculosis*. Yakutsk; 2003. [in Russian].
11. Gavriliev SS, Vinokurova MK, Mordovskaya LI. *Semiconductor lasers for phthisiatry. New treatment technology*. Novosibirsk; 2004. [in Russian].
12. Puri MM, Arora VK. Role of gallium arsenide laser irradiation at 890 nm as an adjunctive to anti-tuberculosis drugs in the treatment of pulmonary tuberculosis.Indian J Chest Dis Allied Sci. 2003; 45(1):19-23.
13. MOH. Ministry of Health and Social Development of the Russian Federation. Order No 109 "On the improvement of TB control activities in the Russian Federation"; 2003. Available from: www.consultant.ru/document/cons_doc_LAW_100873/

International Journal of Biomedicine 6(1) (2016) 85-86
doi: 10.21103/Article6(1)_ShC2

Short Communication

INTERNATIONAL JOURNAL OF BIOMEDICINE

Determination of the Elemental Composition of Lichens by Atomic Emission Spectrometry

Albina V. Stepanova*; Semen M. Timofeev; Aigerim Sh. Smagulova; Dmitry M. Uvarov

North-Eastern Federal University named after M.K. Ammosov
Yakutsk, the Republic of Sakha, Russia

Abstract

This work represents the results of a determination of the elemental composition of lichen thallus as a promising, ecologically pure, and renewable raw material for biotechnological processing. The content of toxic elements and heavy metals in the samples analyzed does not exceed their maximum allowable concentrations and therefore does not preclude the possibility of use of these lichens as a renewable raw material for biotechnology. Indeed, the samples contained 20 macro- and microelements and 9 trace elements that would be valuable in a food source. These include the elements Fe, Mg, and Ca, required for prevention of deficiency diseases. **(Int J Biomed. 2015;6(1):85-86.).**

Keywords: *lichen; elemental analysis; biotechnology; emission spectromenry.*

Introduction

In recent years the lichen blastema has been widely used as a valuable raw material for obtaining a series of biopreparations used in medicine, and in the veterinary and food industries [1-2].

The objective of this work was to study the elemental composition of two types of lichen thallus: *Cladoniarangiferina (reindeer lichen)* and *Cetrariacuculata* as a prospective raw material for use in biotechnology.

Materials and Methods

The lichens were collected in the summer from an area of their typical habitat, 30 km to the north of Yakutsk and 1.5km away from any highway. The collected lichenoid raw material was dried in accordance with GOST-13727-68.

Solutions of the samples were prepared [3] and analyzed by inductively coupled plasma mass-spectrometry on an iCAP6300 Duo (Thermo Scientific, USA) optical emission spectrometer with inductively coupled plasma, set to an axial view of the plasma flame.

***Corresponding authors:** Albina V. Stepanova, North-Eastern Federal University, the Republic of Sakha, Yakutsk, Russia.*
E-mail: anshakova_v@mail.ru

Results and Discussion

The results obtained from this elemental analysis of the two types of lichen are presented in Table (1). The content of most micro- and macroelements in the studied samples didnot differ much between the two species. In Cetrariacuculata, there are trace amounts of lead and cadmium, but at this level they would not cause any toxic effect in the human body. Their content is considerably lower than the amounts stated in the Sanitary Regulations and Standards (SanPiN).3.2.560-02. Maximum Allowable Concentrations.

Conclusion

The content of toxic elements and heavy metals in the samples analyzed does not exceed their maximum allowable concentrations and therefore does not preclude the possibility of use of these lichens as a renewable raw material for biotechnology. Indeed, the samples contained 20 macro- and microelements and 9 trace elements that would be valuable in a food source. These include the elements Fe, Mg, and Ca, required for prevention of deficiency diseases.

Competing interests

The authors declare that they have no competing interests.

Table 1.

The micro- and macro element content of thalli of Cladoniarangiferina and Cetrariacuculata

Elements	Mineral content as percentage of dry weight		Elements	Mineral content as percentage of dry weight	
	Cladoniarangiferina	Cetrariacuculata		Cladoniarangiferina	Cetrariacuculata
Al	0.07090	0.17630	Pb	0.00040	0.00140
Ba	0.00350	0.01420	Rb	0.00330	0.01000
Be	0.00020	0.00020	Sc	0.00004	0.00009
Ca	3.54440	17.7840	Si	0.29140	0.68530
Cd	0	0.00004	Sr	0.00830	0.03990
Ce	0.00102	0.00043	Ti	0.00360	0.00770
Co	0.00050	0.00080	V	0.00008	0.00027
Cr	0.00110	0.00250	Y	0.00006	0.00003
Cu	0.03900	0.03680	Yb	0.00001	0
Fe	0.07000	0.19750	Zn	0.06740	0.11600
K	3.94900	12.8360	Mn	0.02680	0.03970
La	0.00007	0.00043	Na	2.68240	22.7980
Li	0.00020	0.00130	Nd	0.00280	0.00020
Mg	2.15820	13.7170	Ni	0.00570	0.01000

References

1. Anshkova VV, Stepanova AV, Uvarov DM, Smagulova ASh, Naumova KN, Vasilyev PP, et al. Actoprotective activity of complex biologics based on Lichen thalli and Rhodiola Rosea. Ekologiia Cheloveka. 2015: 5:46–51. [in Russian].

2. Anshakova VV. The mechanochemical technology for producing of biocomplexes based on lichen material. Int J Biomed. 2012; 3:232-236. [in Russian].

3. Vershinina SE, Vershinin KE, Kravchenko OYu, Chebykin EP, Vodneva EN. Elemental composition of lichen p. Cetraria ACH from the different regions of Russia. Chemistry of Plant Raw Materials. 2009;1:141–146. [in Russian].

International Journal of Biomedicine 6(1) (2016) 87-89
doi: 10.21103/Article6(1)_CC1

CURRENT CONCEPTS
Health Services Research

INTERNATIONAL JOURNAL OF BIOMEDICINE

Health Relationship Management Services (HRMS) A New Healthcare Paradigm Using the 5Rs

Nik Tehrani, PhD

DynoSense Academy, CA, USA

Abstract

Health Relationship Management Services (HRMS) is a new paradigm comprised of the 5Rs which define a comprehensive patient healthcare system in the digital health age. This service uses a health monitoring system with sensors and a patient application portal that can *Read* data and securely send it to the cloud with amended data from Electronic Health Records (EHR). The read data is *Restructured* by analytics software for evaluation and personalization in an aggregated form represented by an individualized health scoring and demographic grading system. The *Results* are previously restructured data that must become actionable to lead to an escalation path to the Communication Center for a *Response*. On a periodic basis, *Repeat* of the cycle will improve patient health outcomes through an interconnected link of services for optimal continuous health relationship management. Providers can leverage the HRMS platform to generate data-driven approaches fostering a positive and lasting impact on their patient's health. Payers can track data to ensure a more healthy patient population and review meaningful outcomes. **(Int J Biomed. 2016;6(1):87-89.).**

Keywords: *Health Relationship Management Services (HRMS); new health paradigm; 5Rs; Patient Health Narratives (PHN); Patient Engagement***.**

Health Relationship Management Services (HRMS)

Digital health has been quite successful in monitoring patients' health and, according to a latest finding, U.S. investments in digital health have reached $2.3 billion for the first six months of this year, which has broken all past records [1]. Healthcare, today, is one of the sectors that is most influenced by advanced technology. Developments in medical technology have progressed from digital wristbands that measure heart rates and other health data to a new generation of products that are improving healthcare delivery and outcomes [2] by enabling providers to remain continuously connected with their patients no matter where they are. These innovative systems can indicate a patient's adherence to protocols and, in some cases, may even act as an early-warning system for "degenerative illnesses such as Alzheimer's and Parkinson's disease" [2]. High-tech sensors can now "monitor the at-home cardiac patient's heart every minute of every day" without being hooked up to a monitor [2]. Similar to something out of science fiction, new technological devices now can remotely scan a human body to detect a myriad of symptoms, with the results available to patients, physicians, care providers and relatives. The patient data that is read by the sensors is more valuable than an intermittent "snapshot" [2] [3] because it is uninterrupted and it is sent instantaneously to the cloud for restructuring by analytics. For instance, Steinhubl is working on a "continuous blood pressure measurement watch" to monitor blood pressure over time, not just when a patient is at the doctor's office. According to Steinhubl, there is a lack of "synchronicity—there are asynchronous appointments for doctor visits, and so on; so most of healthcare hasn't really been designed around the patient to make sure their blood pressure, lipids, diabetes, are well-controlled." "Patient care has become a primary focus in the development of new concepts and knowledge in healthcare technology" [4]. To improve patient care, the health sector is using data and technology [5]. The core idea is that digital technology ought to be planned to help manage patients more efficiently and that manufacturers should provide, if need be through strategic alliances, more than just a device by also offering patient healthcare services and solutions in one coordinated platform.

The current paradigm of medical care relies on the "autonomous and highly trained doctor" [6] to gather and manage information required for every patient's care. This paradigm is confronted by (i) the accumulation of obligations

***Corresponding author:** Nik Tehrani, PhD. DynoSense Academy, San Jose, CA, USA. E-mail: ntehrani@dynosense.com*

for knowledge by patients and physicians; (ii) by the demand to evaluate patients not part of a physician's practice; (iii) "by consistently unmet quality of care expectations; (iv) by the expense of outmoded, fragmented, and suboptimal care; (v) and by a seemingly insurmountable demand for chronic disease care" [6]. Refinements within the old paradigm of medical care may not solve such challenges, suggesting the need for a new paradigm [6]. There is a need for a unified definition of digital healthcare, or connected health, in which everyone can speak the same language.

Health Relationship Management Services (HRMS) is this new paradigm which defines comprehensive patient healthcare in the digital health age for individualized patient care, engagement, and managed outcomes. An obvious benefit of this technology paradigm is the generation of quality clinical data, since up until recently, was raw data that may not have been transferred to a care provider in a way it could be actionable [4] [7]. HRMS is a revolutionary new definition to place all digital health activities and technologies within one cohesive cyclical system where patient data is collected from variety of sources, analyzed, made actionable, acted upon, and repeated. HRMS is a complete health ecosystem that produces positive outcomes as a comprehensive service, much as having a physician at home monitoring the patient on a daily basis to minimize health risk while reducing cost of care.

An example of one part of HRMS is a health monitoring system that can measure physiological parameters such as ECG, blood pressure, respiration, heart rate, and other vitals to safeguard a patient's good health. This digital healthcare comes in the forms of small devices or limited smartphone applications. HRMS works on the principle of five services known as the 5Rs, which are *Read*, *Restructure*, *Results*, *Response*, and *Repeat*. Metaphorically speaking, these services are "turning gears," with the first one turning, causing the next one to turn, and so on, as one related collaborative routine (Figure 1). Read turns the *Restructure*, which turns *Results,* which turns Response, and Repeat completes and enhances the overall service.

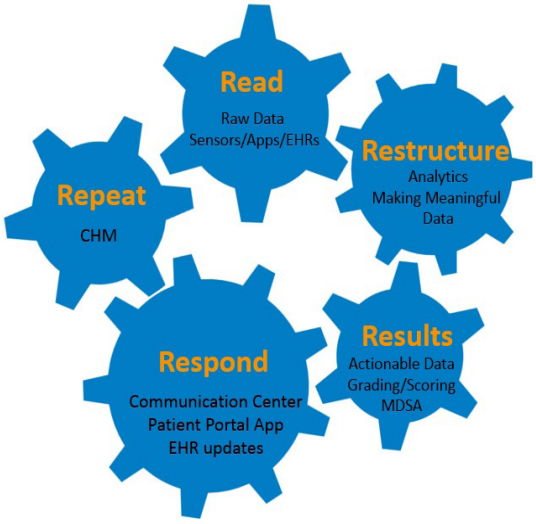


Fig. 1. *Health Relationship Management Services represented as "gears", with each turning the other in sequence for a continuous cycle.*

Read

Read is defined as compilation of raw captured data from a variety of sources such as: (i) a health monitoring system with sensors, (ii) an interactive patient application portal, (iii) Electronic Health Records (EHR), (iv) care specialist feedback, and (v) care friends responses. *Read* data is securely sent to the cloud to ensure patients' health data privacy.

An example of a health monitoring system is the "Dyno™" from DynoSense Corp., [8] which is able to measure, at home, various health metrics such as ECG, heart rate, blood O_2, respiration rate, breathing efficiency, blood pressure change, body temperature, breath gases, and body compositions. Taking readings efficiently depends on the quality of the sensors and simplicity of usage. Furthermore, the patient portal, DynoLife™, interactively engages the patient for psychological and behavioral feedback among a community of care experts and care friends. *Read* data are stored in the cloud and are ready to be restructured as required [9].

Restructure

Read patient data stored in the cloud is a raw format where it is required to be *Restructured* by analytics software for evaluation and personalization. This analyzed data in an aggregated form is represented by an individualized health scoring and demographic grading system. *Restructure* of the data is a vital step as it involves the data analysis and interpretation of the measurement obtained. There can be many ways to interpret the readings, but the best systems are based on proven medical standards identified by sources, such as the AMA (American Medical Association). By a solid interpretation, HRMS is able to convert the acquired reading into valuable and meaningful data, not just an accumulation of meaningless Big Data, which can be used for an overall evaluation of the patient.

Results

Once the restructuring has been completed, actionable Results must be generated for proper response through an escalation path which indicates all of the individual's significant health data. This is exhibited by examples such as (i) individualized health scoring and demographic grading; (ii) Health Indexing (HI), which captures health data over a period of time to create better understanding of the true health condition of the individual; (iii) Medical Decision Support Algorithm (MDSA™), which filters unnecessary data for the physicians and care providers; and (iv) medication effectiveness. The validity of this system can be cross-checked to confirm if the results indicate a health risk that the care specialist and/or care provider can verify.

Response

From the results, a series of actions are identified that are required to be further qualified by the *Response* system. Such a quantifying process takes many forms, such as (i)

interactive questionnaires or surveys through the patient portal application platform, (ii) text or email messaging, (iii) automated robocalls, and (iv) live video or voice interactions with a health specialist from the Communication Center. Following the quantifying process, an appropriate response is developed. Examples of appropriate responses are (i) escalation to a care specialist for further intervention, (ii) scheduling a clinic or hospital visit, (iii) Emergency room visit, (iv) entry into EHR/EMR records of the individual, or (v) updated individualized status for further personalized activities. Activities may include (i) news feeds with up-to-the minute health information, (ii) gamification, which is health recognition games with a reward system to encourage [10] (iii) and an interactive online magazine encouraging individuals to share their Patient Health Narratives (PHN) and become a member of a healthcare community [11]. Patient Health Narratives (PHN) is an emergent trend of providing a platform for patients to share their health related stories and dialogues. This delivers a voice to the experiences of illness, to avoid feelings of isolation, and alleviate concerns and fears of chronic conditions and medical procedures. This interactive patient application portal may create a sense of community and support for the patient.

Repeat

Repeat is the Continuous Health Management (CHM) of an individual's status passing through the entire cycle. On a periodic basis, *Repeat* of the cycle will improve patient health outcomes through an interconnected link of services for optimal continuous health relationship management. Individuals are empowered by this continuous process not only by the up-to-the minute availability of their health information, but also, in a meaningful and actionable process, they become part of the customized social health community of care experts and care friends. The HRMS platform can be leveraged to produce data-driven approaches to advance a positive and long-term influence on their patient's health. Data can be tracked by payers to assure a more healthy patient population and to review meaningful outcomes.

Conclusion

Health Relationship Management Services (HRMS) works on the principle of five services known as the 5Rs, which are *Read, Restructure, Results, Response,* and *Repeat*. The combined 5Rs complete the cycle of HRMS through a continuous cyclic HRMS process in that one element is dependent upon the others. Through patient engagement, the individual becomes part of the solution with continuous personalized feedback, encouragement, empowerment, inspiration, and inclusion in a health community that ultimately may lead to lifestyle changes.

HRMS was conceived to define and standardize the entire health ecosystem with continuous follow-up and feedback to the patient, providers, and payers. As companies adopt the entire patient experience of HRMS, patients will receive health services as a product. Health related costs will decrease, especially for providers visits while improving quality of care.

References

1. Health. (2015). Digital Health Funding Soars To Record $2.3 Billion, But Crowdfunding Tumbles - See more at: http://nuviun.com/content/digital-health-funding-soars-to-record-23-billion-but-crowdfunding-tumbles#sthash.3vxkm3LZ.dpuf. Retrieved from http://nuviun.com/: http://nuviun.com/content/digital-health-funding-soars-to-record-23-billion-but-crowdfunding-tumbles
2. Morrisey J. (2015). The medical technologies that are changing health care. New, eye-popping medical technology provides earlier diagnoses, personalized treatments and a breathtaking range of other benefits for both patients and health care professionals. Retrieved from HYPERLINK "http://www.hhnmag.com/articles/3580-the-medical-technologies-that-are-changing-health-care" http://www.hhnmag.com/articles/3580-the-medical-technologies-that-are-changing-health-care
3. Steinhubl S. (2016). Steven Steinhubl, M.D., director of digital medicine at the Scripps Translational Science Institute, shares his perspectives on the current opportunities in mobile health. IHT2. Institute for health technology transformation. Retrieved from http://ihealthtran.com/wordpress/2014/01/steven-steinhubl-md-director-of-digital-medicine-scripps-translational-science-institute-clinical-cardiologist-scripps-health/
4. Cassano C. (2014). The right balance –technology and patient care. OJNI Volume 18, Number 3. Retrieved from http://www.himss.org/ResourceLibrary/GenResourceDetail.aspx?ItemNumber=33541
5. PWC. (2015). Healthcare and innovation. Retrieved from http://www.pwc.com/us/en/health-industries/top-health-industry-issues/healthcare-technology-and-innovation.html
6. White. (2008). Health information technology will shift the medical care paradigm. J Gen Intern Med. 2008 Apr;23(4):495-9. doi: 10.1007/s11606-007-0394-y.
7. Schmittdiel J. (2015). Using technology to improve health. research scientists study the intersection of technology and health. Kaiser permanente. Retrieved from https://www.dor.kaiser.org/external/DORExternal/research_report/research_technology.aspx
8. Tehrani N, Meckl-Sloan C. Remote health monitoring in action. International Journal of Business Management and Economic Research (IJBMER). 2015;6(1):111-4.
9. Lupton D. (2013). Donna Haraway: The digital cyborg assemblage and the new digital health technologies. The Palgrave handbook of social theory in health, illness and medicine, 567-581.
10. Merriam-Webster. (2015). Gamification. Retrieved from http://www.merriam-webster.com/dictionary/gamification
11. Meckl-Sloan C. (2015). A new healthcare paradigm: patient health narratives: enhancing patient engagement through sharing stories. Retrieved from http://www.gjar.org/publishpaper/vol2issue9/d288r70.pdf

e s c

c s ə

european stroke conference

ESC 2016

25th EUROPEAN STROKE CONFERENCE

13–15 APRIL 2016

VENICE (ITALY)

www.eurostroke.eu

Join us for the

2016 7th Annual ABPP Conference and Workshops

May 11-14, 2016

CHICAGO

Details coming soon on our website. Visit **www.abpp.org** for more information.

ABPP
SPECIALTY CERTIFICATION IN PSYCHOLOGY SINCE 1947

THE GWEN
A LUXURY COLLECTION HOTEL
Chicago

Mark your calendars! 2016

XXII.
CONGRESS OF THE
EUROPEAN SOCIETY FOR
STEREOTACTIC AND FUNCTIONAL
NEUROSURGERY
28 September-01 October 2016
Conrad Hotel, Istanbul

XXII.

CONGRESS OF THE
EUROPEAN SOCIETY FOR STEREOTACTIC AND FUNCTIONAL NEUROSURGERY

28 September-01 October 2016
Conrad Hotel, Istanbul

www.essfn2016.org