IJBM

International Journal of

BIOMEDICINE

ISSN 2158-0510

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 Accepted manuscripts become the sole property of the Journal and may not be published elsewhere without the consent of the Journal.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

INTERNATIONAL
MEDITERRANEAN
FAMILY MEDICINE CONGRESS

19 - 22 September 2019

Barcelona - Spain

www.imfmc.org

"Holistic Approach:
Innate Healing Powers of Family"

BARCELONA

IJBM

INTERNATIONAL JOURNAL OF BIOMEDICINE

www.ijbm.org

Volume 9 Issue 2 June 2019

CONTENTS

CONTENTS

CONTINUED

WORLD HEART CONGRESS 2019

AUGUST 19-20, 2019|VIENNA, AUSTRIA

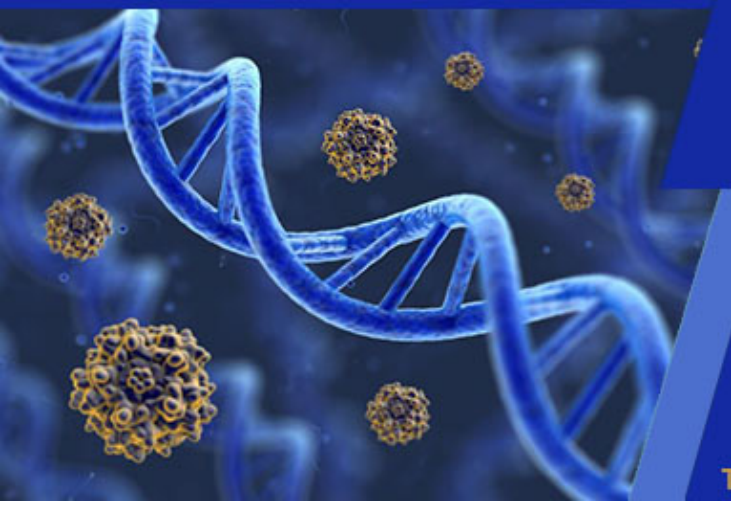
https://heart.insightconferences.com/

World Cardiology Meeting

SEPTEMBER 26-27, 2019

Florence | Italy

Florence - the city of tranquility made manifest



Genetics & Gene Therapy 2019

International Conference on

Genetic Disorders & Gene Therapy

August 26-27, 2019 | Bangkok, Thailand

Theme: Current research and futuristic challenges in Genetics

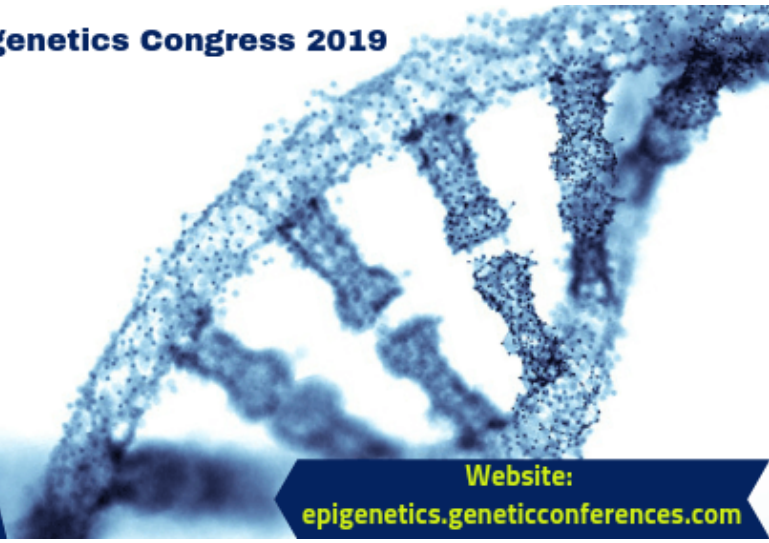
---

Epigenetics Congress 2019

6th World Congress on

Epigenetics and Chromosome

November 11-12, 2019 | Amsterdam, Netherlands



Mail us: epigenetics@memeetings.com

Website: epigenetics.geneticconferences.com

2nd World Congress on

Cell and Structural Biology

December 2-3, 2019
Manila, Philippines

Please visit:
https://cellbiology.conferenceseries.com

International Journal of Biomedicine 9(2) (2019) 87-90
http://dx.doi.org/10.21103/Article9(2)_RA1

REVIEW ARTICLE

Urology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Visceral Obesity and Urinary Stone Disease: A Systematic Review

Badr Alharbi, MD

College of Medicine, Qassim University
Buraidah, Saudi Arabia

Abstract

Background: Incidence of obesity is on rise worldwide due to several risk factors, including lifestyle changes and genetic causes. Obesity can be diagnosed with different anthropometric tools, such as body mass index, visceral fat, subcutaneous fat and waist circumference. The incidence of urinary stones disease has been linked to obesity as well as metabolic syndrome (MetS). The aim of the present study was to determine the relationship between obesity defined by visceral adipose tissue (VAT) and urolithiassis.

Methods: The search engines utilized for finding relevant studies were Medline, ScienceDirect, and Cochrane databases between January 2001 and January 2019. Published articles written in the English language and reporting an association between visceral obesity, urolithiasis and MetS were included.

Results: Obesity defined by visceral fat estimation is associated with increased risk of MetS and urinary stone formation. Insulin resistance, low urinary pH, hyperuricemia, hyperuricosuria and hyperoxaluria were the main observed metabolic derangements behind the pathogenesis and the increased risks of stone development in obese patients.

Conclusion: The role of VAT reduction in prevention of urinary stones disease is not yet established, and for this reason more studies are required in the future to clarify this sequence of events. (**International Journal of Biomedicine. 2019;9(2):87-90.**)

Key Words: visceral obesity • visceral adipose tissue • metabolic syndrome • urolithiassis

Abbreviations

BMI, body mass index; **IR**, insulin resistance; **MetS**, metabolic syndrome; **USD**, urinary stones disease; **VAT**, visceral adipose tissue; **VO**, visceral obesity; **WC**, waist circumference

Introduction

Nephrolithiasis is a morbid condition with increasing prevalence worldwide. Multifactorial causes, such as genetic factors and lifestyle, are thought to play significant roles in kidney stone formation.[1] Obesity and MetS have been shown to be significant risk factors for nephrolithiasis and various urinary biochemical abnormalities, including high excretion of calcium, oxalate, uric acid, and lower levels of citrate and pH.[2]

The relationship between obesity defined by BMI and nephrolithiasis has been reported by several studies. The higher fat mass and its abnormal distribution have a greater impact than total body weight on MetS.[3] Abnormal body fat with higher VAT distribution appears to have even a greater influence in MetS than does total body weight on cardiovascular disease and diabetes risk.[4] The National Cholesterol Education Program Adult Treatment Panel III considers WC, and thus VO, one of the major clinical criteria to diagnose MetS.[5] However, measurements of BMI and WC cannot distinguish VAT from subcutaneous fat, which is subject to inherent differences in fat distribution between subjects.[6] Measurement of visceral fat volume from a single axial CT slice at different levels has been developed and validated.[7]

Obesity has been linked in several studies to changes in serum and urine composition. Urinary excretion of oxalate and uric acid was higher in obese patients compared to non-obese. On the other hand, lower levels of stone inhibitors, such as citrate, pyrophosphate and magnesium (other causative factors for stone formation), are observed in these patients.[8]

Correspondence to Badr Alharbi, MD. *Ass. Prof., Chair of the department of Surgery. College of Medicine, Qassim University, Buraidah, Saudi Arabia. Mobile: +966555181799; E-mail: badralharbi@qumed.edu.sa*

Acidic urine is an important promoter for the most common types of stones, including calcium oxalate, calcium phosphate and uric acid stones.[9] Low urinary pH has been observed in individuals with obesity and MetS. The exact pathophysiology is not clear, but a possible cause is IR, which decreases excretion of renal ammonia and derangement of the hydrogen ion buffering system.[10] In addition, IR leads to lower insulin bioactivity in the proximal renal tubules, which affect ammonium metabolism and ultimately changes in urinary pH.[11] The aim of the present study was to determine the relationship between obesity defined by VAT and urolithiassis.

Materials and Methods

The prevalence, risk factor and morbidity of obesity defined by VAT and its association with urolithiasis and MetS are the main objectives of this systematic review. The search engines utilized for finding relevant studies were Medline, ScienceDirect, and Cochrane databases between January 2001 and January 2019. The Cochrane Collaboration and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were carried out for this review. All identified studies that met the inclusion criteria for this review were included and analyzed. The search for relevant studies was performed using the following keywords: "obesity," "visceral obesity," "visceral fat," "visceral adipose tissue," "insulin resistance," "nephrolithiasis," "uric acid," "hypercalciuria," "metabolic syndrome," "renal," "kidney," "calculi," "stone(s)," and "urolithiasis." The Boolean operator (OR, AND) combining the keywords was used to refine the search results. Published articles written in the English language and reporting an association between VO, urolithiasis and MetS were included. Exclusion criteria were case reports, brief notes, reviews, editorials, letters to editors and conference proceedings (Table 1).

Table 1.

Inclusion and exclusion criteria of the included records

Inclusion criteria	Exclusion criteria
Publication in the English language	Case reports
Original articles reporting the association between VAT, urolithiasis and MS	Brief notes
	Reviews
	Editorials
	Letters to editors and conference proceedings
	Languages other than English

Results

The initial search identified 1,811 related articles, from which, with a refined search, 1,759 records were excluded. Further filtration according to the inclusion and exclusion criteria determined 14 studies to be included for qualitative analysis in the present review (Fig.1).

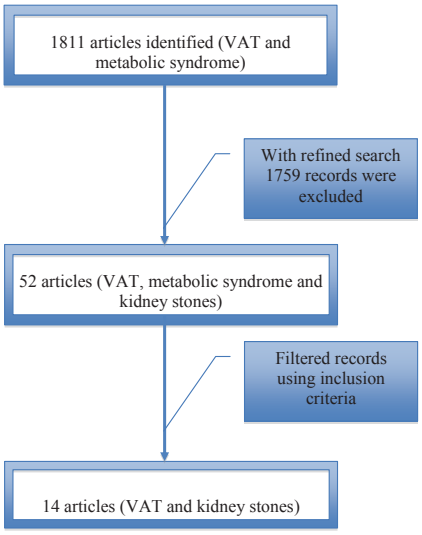


Fig. 1. *Flow diagram summarizing the literature search for this review.*

Measurement of visceral fat volume

With advances and revolutions in technologies, measurement of body fat distribution can be achieved utilizing different techniques. Examples of simple measures include waist-to-hip ratio and WC. These tests cannot distinguish between subcutaneous and visceral fat contents. CT and MRI have been considered the most accurate and reproducible techniques of abdominal fat assessment with the added advantage of distinguishing subcutaneous from visceral fat components.[7] The drawback of these techniques is the cost and radiation exposure, which can be minimized using a low dose CT scan or utilizing available images done for other purposes—most commonly CT being done for renal colic in patients with urolithiasis.[12,13]

CT-based fat delineation and calculation of visceral fat can be done either manually or with the help of automated image processing software to overcome the drawbacks of manual delineation.[14,15] To differentiate between visceral and subcutaneous fat, we used automatic fat analysis developed and evaluated by measures of accuracy and sensitivity in comparison to manual quantification. The differences between manual and automated techniques for estimating both subcutaneous and visceral fat were not significant.[16] The majority of the VAT and USD studies have been done by obtaining a single axial CT slice at the level of the fourth and fifth lumbar vertebrae (L4-5) (Fig.2), whereas some authors have suggested measurement of VAT at the level of the first and second lumbar vertebrae (L1-2) as a more accurate estimate (Fig.3). Kim et al. performed a study of VAT volume calculation by taking one slice of CT from the umbilicus level between fourth and fifth lumbar vertebrae and six additional CT slices from above and below the umbilicus level. Automated and manual evaluation methods were applied and found that more reliable results were obtained with assessments using a set of CT slices, compared to the use of a single CT slice.[17] VO was defined as a visceral fat volume more than 100 cm^2 and considered an important factor for MetS.[18]

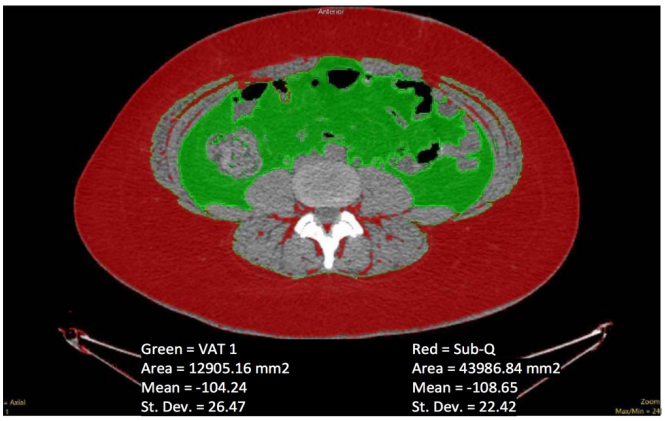


Fig. 2. *Axial CT slice at the level of the fourth and fifth lumbar vertebrae (L4–5).*

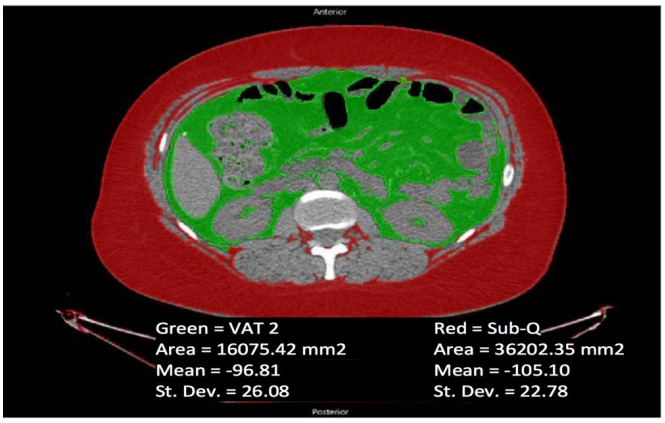


Fig. 3. *Axial CT slice at the level of the first and second lumbar vertebrae (L1–2).*

VAT and urolithiasis

In the literature, there is an inhomogeneous conclusion about the effect of obesity on urinary composition in patients with urolithiasis. Low urinary pH has been observed in obese patient as well as changes in urinary concentration of oxalate, phosphate, citrate, and uric acid.[19-21] Acidic urine is an important promoter of uric acid stone formation and has been found to have an inverse correlation with VAT.[22,23] Urinary concentration of uric acid was found to be higher when obesity defined by VAT, compared with calcium phosphate in obese patients, increases the risk for uric acid stone formation. Obesity defined by VAT was shown to have a strong association with the formation of calcium oxalate stones in comparison to calcium phosphate and mixed calcium oxalate phosphate stones.

Zhou and his colleagues analyzed 269 patients undergoing percutaneous nephrolithotomy and reported a significantly higher mean visceral fat volume in uric acid stone formers and found that the higher VAT level was an independent risk factor for the composition of uric acid stones after stone retrieval and chemical analysis.[24] Fujimura et al. looked at the association of VAT and the incidence of urolithiasis by measuring the VAT volume using a CT scan fat delineation. Increased VAT volume was associated with increased risk of urinary stone formation compared to BMI. They also noticed a strong association between VAT and IR and consequently an increase in the incidence of MetS.[25,26]

Ethan found that VAT was associated with increased quartile of creatinine, sodium, and urine volume in 24-h urine collection in men that was independent of BMI. Whereas in women, increased quartile phosphate urinary excretion was predicted only by increased VAT, while increased quartile of creatinine and oxalate were predicted by BMI. On multivariate regression analysis, VAT was found to be associated with uric acid stone formation, and none of the BMI or VAT estimation predicted the formation of other stone types.[27] Akarken demonstrated that the visceral abdominal area is a new and independent risk factor for urinary stone formation. The incidence of uric acid kidney stones was higher in patients with a high visceral abdominal area compared to those who do not have kidney stones.[28] A study of 98 kidney stone formers who had available a computerized tomography scan and twenty-four-hour urine for electrolytes showed that elevated VAT level was associated with low 24-hour urinary pH and higher urinary sodium excretion. As the level of VAT volume increases, urine pH becomes lower and the stone volume, greater. Formation of uric acid stones was associated with greater VAT volume and lower urinary pH.[29]

VAT, MetS and urolithiasis

MetS is a clinical diagnosis characterized by obesity, elevated blood pressure, dyslipidemia and elevated blood glucose.[30] IR and abdominal obesity are the principal risk factors for MetS development. People diagnosed with MetS are at higher risk of hyperuricemia, chronic kidney disease and cardiovascular disease. The risk of obesity-related disorders has been observed when VAT level exceeds 103.8 cm^2.[31] The incidence of urolithiasis has been observed to be on the rise in individuals with MetS.[32] Zhou et al.[24] found a significantly high level of VAT in patients with uric acid stones, and this proportion was not observed in patients who form non-uric acid stones. This finding was reinforced by a study done by Kim et al.[31] where obesity defined by VAT volume measured on a CT scan has a significant effect on the risk of forming uric acid stones compared with calcium phosphate and calcium oxalate stones. For obesity defined by BMI, no rules were found on the type of stones in adult patients who underwent surgical treatment for urinary stones. Daudon reported a strong association between type 2 diabetes and uric acid stones, with obesity being an additional risk factor in younger patients.[32,34] IR has been identified as a predisposing factor for uric acid stone formation and an important target to decrease the risk of nephrolithiasis.[35]

Conclusion

There is clear evidence for the increased prevalence and incidence of urinary stones in association with obesity. Obesity defined by visceral fat estimation is associated with increased risk of MetS and urinary stone formation. IR, low urinary pH, hyperuricemia, hyperuricosuria and hyperoxaluria were the main observed metabolic derangements behind the pathogenesis and the increased risks of stone development in obese patients. The role of VAT reduction in prevention of USD is not yet established, and for this reason more studies are required in the future to clarify this sequence of events.

References

1. Daudon M, Bader CA, Jungers P. Urinary calculi: review of classification methods and correlations with etiology. Scanning Microsc. 1993;7(3):1081-104; discussion 1104-6.
2. Ramaswamy K, Shah O. Metabolic syndrome and nephrolithiasis. Transl Androl Urol. 2014;3(3):285-95. doi: 10.3978/j.issn.2223-4683.2014.06.03.
3. Liu P, Ma F, Lou H, Liu Y. The utility of fat mass index vs. body mass index and percentage of body fat in the screening of metabolic syndrome. BMC Public Health. 2013;13:629. doi: 10.1186/1471-2458-13-629.
4. Gruzdeva O, Borodkina D, Uchasova E, Dyleva Y, Barbarash O. Localization of fat depots and cardiovascular risk. Lipids Health Dis. 2018;17(1):218. doi: 10.1186/s12944-018-0856-8.
5. Lorenzo C, Williams K, Hunt KJ, Haffner SM. The National Cholesterol Education Program - Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions of the metabolic syndrome as predictors of incident cardiovascular disease and diabetes. Diabetes Care. 2007;30(1):8-13.
6. Camhi SM, Bray GA, Bouchard C, Greenway FL, Johnson WD, Newton RL, et al. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences. Obesity (Silver Spring). 2011;19(2):402-8. doi: 10.1038/oby.2010.248.
7. Tokunaga K, Matsuzawa Y, Ishikawa K, Tarui S. A novel technique for the determination of body fat by computed tomography. Int J Obes. 1983;7(5):437-45.
8. Aydogdu O. Urinary stone disease and obesity: Different pathologies sharing common biochemical mechanisms. World J Nephrol. 2012;1(1):12-5. doi: 10.5527/wjn.v1.i1.12.
9. Alelign T, Petros B. Kidney Stone Disease: An Update on Current Concepts. Adv Urol. 2018;2018:3068365. doi: 10.1155/2018/3068365. eCollection 2018.
10. Weiner ID, Verlander JW. Renal ammonia metabolism and transport. Compr Physiol. 2013;3(1):201-20. doi: 10.1002/cphy.c120010.
11. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19-39.
12. van der Kooy K, Seidell JC. Techniques for measurement of visceral fat: a practical guide. Int J Obes Relat Metab Disord. 1993;17(4):187-96.
13. Jebb SA, Elia M. Techniques for measurement of body composition: a practical guide. Int J Obes Relat Metab Disord 1993;17(11):611-21.
14. Shin SW, Choi YS, Lee YM, Yoon DK, Cho KH, Hong MH, Chang JA. The correlation between simple anthropometric indices and abdominal visceral fat accumulation by computed tomography. J Korean Acad Fam Med. 2001;22(3):316–323.
15. Kim JS, Yoo SM, Kim KN, Lee SY. Comparison of DEXA and CT for truncal obesity in adult women related to metabolic complications. J Korean Acad Fam Med. 2007;28(9):675–681.
16. Zhao B, Colville J, Kalaigian J, Curran S, Jiang L, Kijewski P, Schwartz LH. Automated quantification of body fat distribution on volumetric computed tomography. J Comput Assist Tomogr. 2006;30(5):777-83.
17. Kim YJ, Lee SH, Kim TY, Park JY, Choi SH, Kim KG. Body fat assessment method using CT images with separation mask algorithm. J Digit Imaging. 2013;26(2):155-62. doi: 10.1007/s10278-012-9488-0.
18. Examination Committee of Criteria for 'Obesity Disease' in Japan; Japan Society for the Study of Obesity. New criteria for 'obesity disease' in Japan. Circ J. 2002;66(11):987-92.
19. Zilberman DE, Yong D, Albala DM. The impact of societal changes on patterns of urolithiasis. Curr Opin Urol. 2010;20(2):148-53. doi: 10.1097/MOU.0b013e3283353b6d.
20. Maalouf NM, Cameron MA, Moe OW, Sakhaee K. Novel insights into the pathogenesis of uric acid nephrolithiasis. Curr Opin Nephrol Hypertens. 2004;13(2):181-9.
21. Powell CR, Stoller ML, Schwartz BF, Kane C, Gentle DL, Bruce JE, Leslie SW. Impact of body weight on urinary electrolytes in urinary stone formers. Urology. 2000;55(6):825-30.
22. Maalouf NM, Sakhaee K, Parks JH, Coe FL, Adams-Huet B, Pak CYC. Association of urinary pH with body weight in nephrolithiasis. Kidney Int. 2004;65(4):1422–5.
23. Kim JH, Doo SW, Yang WJ, Song YS, Hwang J, Hong SS, Kwon SS. The relationship between urinary stone components and visceral adipose tissue using computed tomography--based fat delineation. Urology. 2014;84(1):27-31. doi: 10.1016/j.urology.2014.01.026.
24. Zhou T, Watts K, Agalliu I, DiVito J, Hoenig DM. Effects of visceral fat area and other metabolic parameters on stone composition in patients undergoing percutaneous nephrolithotomy. J Urol. 2013;190(4):1416-20. doi: 10.1016/j.juro.2013.05.016.
25. Eguchi M, Tsuchihashi K, Saitoh S, Odawara Y, Hirano T, Nakata T, et al. Visceral obesity in Japanese patients with metabolic syndrome: reappraisal of diagnostic criteria by CT scan. Hypertens Res. 2007;30(4):315-23.
26. Fujimura M, Sakamoto S, Sekita N, Mikami K, Ichikawa T, Akakura K. Visceral fat accumulation is a risk factor for urinary stone. Int J Urol. 2014;21(11):1184-5. doi: 10.1111/iju.12556.
27. Fram EB, Agalliu I, DiVito J, Hoenig DM, Stern JM. The visceral fat compartment is independently associated with changes in urine constituent excretion in a stone forming population. Urolithiasis. 2015;43(3):213-20. doi: 10.1007/s00240-015-0770-8.
28. Akarken I, Tarhan H, Ekin RG, Çakmak Ö, Koç G, İlbey YÖ, Zorlu F. Visceral obesity: A new risk factor for stone disease. Can Urol Assoc J. 2015;9(11-12):E795-9. doi: 10.5489/cuaj.3145.
29. Patel ND, Ward RD, Calle J, Remer EM, Monga M. Computerized Tomography Based Diagnosis of Visceral Obesity and Hepatic Steatosis is Associated with Low Urine pH. J Urol. 2017;198(5):1085-1090. doi: 10.1016/j.juro.2017.05.079.
30. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al.; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112(17):2735-52.
31. Kim JA, Choi CJ, Yum KS. Cut-off values of visceral fat area and waist circumference: diagnostic criteria for abdominal obesity in a Korean population. J Korean Med Sci. 2006;21(6):1048-53.
32. Wong Y, Cook P, Roderick P, Somani BK. Metabolic Syndrome and Kidney Stone Disease: A Systematic Review of Literature. J Endourol. 2016;30(3):246-53. doi: 10.1089/end.2015.0567.
33. Daudon M, Lacour B, Jungers P: Influence of body size on urinary stone composition in men and women. Urol Res. 2006;34(3):193-9.
34. Daudon M, Traxer O, Conort P, Lacour B, Jungers P. Type 2 diabetes increases the risk for uric acid stones. J Am Soc Nephrol. 2006;17(7):2026-33.
35. Abate N, Chandalia M, Cabo-Chan AV Jr, Moe OW, Sakhaee K. The metabolic syndrome and uric acid nephrolithiasis: novel features of renal manifestation of insulin resistance. Kidney Int. 2004;65(2):386-92.

International Journal of Biomedicine 9(2) (2019) 91-96
http://dx.doi.org/10.21103/Article9(2)_OA1

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Characteristics of Humoral Regulation of Differentiation of Bone Marrow Monocyte Subpopulations in Patients with Ischemic Cardiomyopathy

Olga I. Urazova, PhD, ScD[1,3]; Svetlana P. Chumakova, PhD, ScD[1*]; Maria V. Vins, PGS[1]; Elena S. Maynagasheva, PGS[1]; Vladimir M. Shipulin, PhD, ScD[1,2]; Andrey S. Pryahin, PGS[2]; Vadim S. Poletika, PGS[1]; Tatyana E. Kononova, PhD[1]; Yulia V. Kolobovnikova, PhD, ScD[1]; Vyacheslav V. Novitskiy, PhD, ScD[1,3]

[1]*The Siberian State Medical University*
[2]*Cardiology Research Institute*
[3]*Tomsk State University of Control Systems and Radioelectronics*
Tomsk, the Russian Federation

Abstract

Background: Monocytes and macrophages play an important role in atherogenesis and myocardial remodeling. Impaired differentiation of monocyte subpopulations may contribute to ischemic cardiomyopathy (ICMP). The aim of the present research was to study the features of the humoral cytokine-dependent regulation of differentiation of classical, intermediate, non-classical and transitional monocytes in bone marrow (BM) of CHD patients with or without ICMP.

Materials and Methods: Forty-five patients with coronary heart disease (CHD), with and without ICMP (19 and 26 male patients, respectively), were examined. Subpopulations of classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), non-classical ($CD14^{+}CD16^{+}$), and transitional ($CD14^{+}CD16^{-}$) monocytes in bone marrow (BM) samples were quantified by flow cytometry. Concentrations of IL-1β, IL-13, TNF-α, IFN-γ, and M-CSF in BM supernatants and blood plasma were evaluated by ELISA.

Results: Concentrations of all cytokines in the blood and IL-1β, IL-13, TNF-α, M-CSF in BM supernatants as well as the capacity of M-CSF to activate, and IL-13 to inhibit, differentiation of classical monocytes from intermediate forms—were not dependent on the clinical form of CHD. Monocytopoiesis in ICMP was characterized by elevated BM concentration of IFN-γ, low M-CSF/IL-13 ratio, and a decreased percentage of classical and intermediate monocytes, accompanied by an increased number of transitional cells in BM, as compared to patients without ICMP.

Conclusion: Excess of IFN-γ and low M-CSF/IL-13 ratio in BM were associated with inhibition of differentiation of mature monocyte forms and development of ICMP. (**International Journal of Biomedicine. 2019;9(2):91-96.**)

Key Words: monocytopoiesis • coronary heart disease • cytokines

Introduction

Ischemic cardiomyopathy (ICMP) remains a major issue in modern cardiology, as pharmacological therapy for this disease is largely ineffective, while surgical treatment often results in a progressive cardiac remodeling. The mortality of patients with ICMP within 5 years after hospitalization for heart failure reaches 42.3%.[1,2] ICMP is characterized by diffuse myocardial ischemia (in contrast to focal ischemia found in CHD), which results in the dilatation of the heart chambers and cardiomegaly.[3] A key mechanism underlying development of ICMP is the lesion of cardiac microvasculature accompanied by endothelial dysfunction. It is speculated that this process can be also associated with atherosclerotic lesions of small arteries, since patients with ICMP often demonstrate atherosclerosis of the coronary vessels, similar to patients with CHD.[4]

***Corresponding author:** Prof. Svetlana P. Chumakova, PhD, ScD. Siberian State Medical University, Tomsk, Russia. E-mail:Chumakova_S@mail.ru*

Pathogenesis of ICMP is not fully understood, and multiple factors are considered to be involved in the underlying cardiac dilatation, including cellular infiltration of the myocardium, apoptotic death of cardyomyocytes, destruction of the interstitial matrix, proliferation of fibroblasts and associated collagen synthesis, and viral infections.[3-6] Among cells present in the cardiac tissue, macrophages are considered to be critically involved in the above mechanisms, as well as in the processes of atherogenesis.[7,8] Macrophages are represented by several subpopulations that induce various processes in damaged tissues. Their precursors, monocytes, also show considerable functional heterogeneity. Thus, there are classic $CD14^{++}CD16^{-}$ cells specialized for phagocytosis, intermediate $CD14^{++}CD16^{+}$ monocytes that are involved in the immune interaction with T-lymphocytes, and non-classical $CD14^{+}CD16^{+}$ cells with high affinity for the endothelium, sometimes referred to as patrolling cells.[7,9] The role and origin of another monocyte subpopulation, transitional ($CD14^{+}CD16^{-}$) monocytes, remains to be fully elucidated. They appear to participate in the initiation of immune reactions, and either differentiate from classical monocytes or represent their precursors.[10]

A number of studies have demonstrated that in atherosclerosis and heart failure, subpopulation ratios of blood monocytes and tissue macrophages undergo substantial changes.[11-13] In patients with CHD, atherosclerosis of coronary arteries is associated with an increased content of intermediate monocytes and a decreased number of classical subset.[7,11] In contrast, ICMP is characterized by a deficit of non-classical monocytes in the blood, while the number of other monocyte subtypes remains unchanged.[14] This suggests that in ICMP patients, the process of monocyte differentiation in BM might have considerable differences compared to CHD patients without ICMP. Identification of patterns of humoral regulation of monocytopoiesis in ICMP will allow for further elucidation of the pathogenesis of this disease and, possibly, discovery of new treatment strategies. Thus, the aim of the present research was to study the features of the humoral cytokine-dependent regulation of differentiation of classical, intermediate, non-classical and transitional monocytes in BM of CHD patients with or without ICMP.

Materials and Methods

The study included 45 male CHD patients with stable angina (FC II-IV) and NYHA class II-III.. All patients had a history of acute myocardial infarction and underwent coronary bypass surgery in combination with reconstruction of the left ventricular cavity under artificial blood circulation. CHD patients were divided into two groups: Group 1 included 19 patients (mean age of 52.23±4.09) with ICMP (group ICMP+: LVEF ≤40%, acute myocardial infarction or revascularization, ≥75% stenosis of left main coronary artery or proximal left anterior descending artery, or ≥75% stenosis of two or more epicardial vessels)[15]; Group 2 included 26 patients (mean age of 59.12±3.86) without ICMP (group ICMP-: LVEF>40%, acute myocardial infarction or revascularization, ≥75% stenosis of coronary vessels of any localization). At the preoperative stage, both groups received similar pharmacological treatment: antianginal therapy with long-acting nitrates, β1-adrenergic receptor blockers, and calcium channel blockers; hemostasis correction using antiaggregants, and correction of lipid metabolism using statins. Premedication and induction of anesthesia in patients from both groups was conducted using sedative and anesthetic agents, narcotic analgesics, and muscle relaxants (diazepam, ketamine, fentanyl, promedola, pipecuronium) in comparable doses.

Exclusion criteria were as follows: a) autoimmune diseases, acute phases of allergic diseases, malignancies, hypoplastic, B12-deficient and folate-deficient anemia, chronic infections (viral hepatitis, syphilis, HIV infection); b) pre-operative treatment with iron-containing drugs, erythropoietin or immunosuppressive therapy; c) the occurrence of acute infectious diseases less than 3 weeks before surgery; d) patients' refusal to participate in the study.

For all patients (in ICMP+ and ICMP– groups), peripheral venous blood samples were collected from the median cubital vein immediately before the operation. During the operation, after accessing the heart by median sternotomy and prior to establishing cardiopulmonary bypass, red BM was collected from the sternum incision. In BM samples, the percentages of different monocyte subsets, including classical (CD14++CD16-), intermediate (CD14++CD16+), non-classical (CD14+CD16+), and transitional (CD14+CD16-) monocytes, were determined by flow cytometry using monoclonal CD14-FITC and CD16-PE antibodies (BD Biosciences, USA), according to the manufacturer's recommendations. The number of all cells positive for CD14 was considered as 100%.

Blood plasma and BM supernatants were obtained via centrifugation of collected samples at 200g and then stored at -80°C. Subsequently, concentrations of cytokines, including IL-1β, IL-13, TNF-α, IFN-γ, and M-CSF were evaluated by ELISA according to manufacturer's recommendations (for IL-1β, TNF-α and IFN-γ - Vector-BEST, Novosibirsk, Russia; for IL-13 - eBioscience, Austria; for M-CSF - RayBiotech, US).

The study was approved by the local ethics committee at the Siberian State Medical University (protocol №5046 dated November 28, 2016).

Statistical analysis was performed using the Statistica 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Non-normal variables were reported as median (interquartile range [IQR]). Mann-Whitney U test was used to compare means of 2 variables not normally distributed. Spearman's rank correlation coefficient was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The content of transitional monocytes in BM of patients with ICMP was higher compared to patients without ICMP, while classical and intermediate subpopulations were observed less frequently (Fig.1). The number of non-classical monocytes showed a tendency to decrease in ICMP-

and ICMP+ groups, although the difference did not reach statistical significance. The results obtained were in line with the previously described deficit of this monocyte subtype in CHD, and could indicate an impairment of non-classical monocyte generation in patients with CHD accompanied by ICMP.[14] Non-classical monocytes were shown to perform a scavenging function via elimination of dead cells, pathogens and oxidized lipids from vessel walls.[16] Therefore, it can be speculated that insufficient generation of these monocytes in atherosclerosis patients can predispose to an enhanced fixation of lipids to the endothelium of coronary microvessels and thereby contribute to the development of diffuse myocardial ischemia and ICMP.[14]

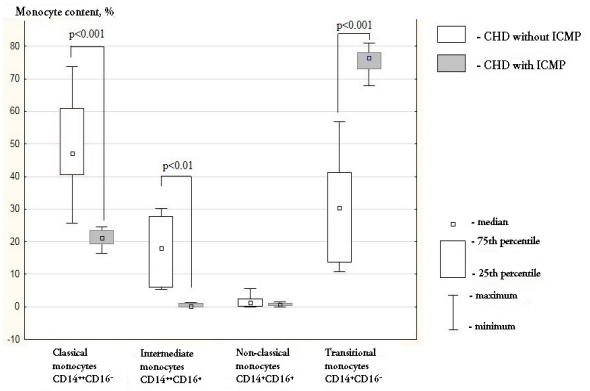


Fig. 1. *Subpopulations of bone marrow monocytes in CHD patients with and without ICMP. P – between groups of patients.*

In patients without ICMP, classical and intermediate monocytes were the most abundant in BM among studied subpopulations, while in patients with ICMP, transient monocytes were predominant (Fig.1). In both groups, a negative correlation between percentages of transitional and classical cells was observed (r=-0.69; $P<0.01$ and r=-0.72; $P<0.01$ in ICMP+ and ICMP- groups of patients, respectively). Additionally, in patients with ICMP, the number of transitional monocytes inversely correlated with the percentage of intermediate cells (r=-0.54; $P<0.05$). These results allow us to speculate that transitional monocytes represent the precursors for maturation of classical and intermediate cells, rather than their derivatives. Evidently, this process is impaired in ICMP, and mature forms of monocytes in BM are not sufficiently generated. Differentiation of non-classical monocytes apparently occurs outside BM, since the level of these cells in both groups (ICMP+ and ICMP-) of patients with CHD did not correlate with the percentage of other monocyte subpopulations. Generally, considering that atherosclerotic lesions of coronary arteries are observed both in CHD without ICMP and with ICMP,[4] the differences in the monocyte subpopulation ratio in BM of patients with ICMP relative to patients without ICMP can be viewed as a dysfunction of monocytopoiesis in the setting of atherosclerosis.

Concentrations of IL-1β, IL-13, TNF-α, and M-CSF in blood plasma and BM supernatants did not differ significantly between the studied groups. At the same time, we observed a multifold increase in the BM level of IFN-γ in patients with ICMP compared to patients without ICMP (Table 1). Generally, potential sources of IFN-γ include T helper lymphocytes type 1 (Th1), cytotoxic T lymphocytes, natural killer cells and sometimes regulatory T cells (Treg), which are present in various tissues, evidently including myeloid tissue.[17] Bansal and colleagues recently demonstrated that development of ICMP is associated with expansion of Treg lymphocytes expressing IFN-γ in an experimental murine model.[18] This process could be linked to tissue hypoxia characteristic for ICMP, specifically to the associated production of hypoxia-inducible factors (HIF). During chronic hypoxia, HIF synthesis switches from HIF-1 to HIF-2. As shown previously, a predominance of HIF-2 not only initiates a long-term adaptation to hypoxic condition (by activating angiogenesis, tissue remodeling, etc.), but also contributes to immunosuppression,[19,20] as a deficit of HIF-1 in lymphocytes disinhibits differentiation of $Foxp3^+$ regulatory T cells.[21] Collectively, it can be speculated that the dysregulation of monocytopoiesis observed in patients with ICMP could be induced by an excessive medullary production of IFN-γ, potentially derived from Treg lymphocytes. IFN-γ was shown to exert antimitotic and pro-apoptotic effects toward hematopoietic stem cells.[22] It is plausible that IFN-γ may also inhibit the differentiation of mature classical and intermediate monocytes, which would explain the observed association of a high medullary concentration of IFN-γ with a low content of these cells in BM of patients with ICMP (Fig. 1, Table 1). As for the remaining BM cytokines, in patients with ICMP there was a tendency toward an increase in the content of IL-1β, IL-13, TNF-α and a decrease in the concentration of M-CSF relative to their BM levels in patients without ICMP (Table 1).

Table 1.

Concentrations of cytokines in the bone marrow and in the blood of CHD patients with and without ICMP, Me [Q_1 – Q_3]

Concentrations of cytokines	CHD patients without ICMP		CHD patients with ICMP	
	Bone marrow	Blood	Bone marrow	Blood
IL-1β, pg/ml	5.00 [2.80; 24.56]	2.06 [0.35; 2.74] $P_1<0.05$	8.73 [4.70; 18.45]	2.93 [0.04; 3.65] $P_1<0.01$
IL-13, pg/ml	1.00 [0.80; 1.23]	0.60 [0.41; 0.82]	1.22 [0.80; 2.41]	0.82 [0.40; 0.95]
TNF-α, pg/ml	10.80 [9.90; 21.84]	1.16 [0.90; 1.82] $P_1<0.001$	18.06 [14.15; 19.40]	2.08 [1.04; 3.60] $P_1<0.001$
IFN-γ, pg/ml	0.02 [0; 0.15]	0	10.00 [0.65; 18.23] $P_2<0.01$	0
M-CSF, pg/ml	7.16 [3.45; 16.33]	0.40 [0.12; 2.37] $P_1<0.01$	3.22 [1.20; 8.04]	2.00 [1.21; 3.24]
M-CSF/IL-13 ratio	9.00 [2.13; 22.09]	-	1.02 [0.41; 2.00] $P_2<0.05$	-

P_1 – between cytokine concentration in blood and in bone marrow supernatants; P_2 – compared to CHD patients without ICMP.

Interestingly, despite a significant increase in medullary concentration of IFN-γ in patients with ICMP, its level was not directly associated with the percentage of individual monocyte subpopulations in BM. Apparently, IFN-γ influences monocytopoiesis indirectly, which is further substantiated by the observed correlations between the concentration of M-CSF, a specific inducer of monocytopoiesis,[23] and levels of IFN-γ and other medullary cytokines. Concentrations of M-CSF in BM of patients without ICMP correlated positively to the levels of IL-1β (r=0.62; $P<0.01$), TNF-α (r=0.60; $P<0.01$), and IFN-γ (r=0.72; $P<0.05$). In patients with ICMP, however, these associations were not observed, although the M-CSF level correlated to the BM content of IL-13 (r = 0.72; $P<0.01$). Taking into account the data on medullary concentration of studied cytokines and the results of correlation analysis, we can conclude that in CHD patients without ICMP, relatively low medullary levels of IL-1β, TNF-α, and IFN-γ (as compared to ICMP+ group of patients) are associated with hypersecretion of M-CSF (almost a two-fold increase compared to patients with ICMP) (Table 1). Such reciprocal changes could be explained by the mutually potentiating effects of these cytokines on the synthesis of M-CSF. Apparently, a high medullary level of IFN-γ in patients with ICMP promotes dissociation of the effects of M-CSF secretion inducers. Thus, an excess of IFN-γ appears to indirectly downregulate the differentiation of classical and intermediate monocytes in BM by affecting cytokine-dependent regulation of M-CSF production.

There were no associations between medullary concentrations of cytokines and the percentage of different monocyte subpopulations in BM within ICMP+ and ICMP- groups of patients. Consequently, correlation analysis of these parameters was performed in a pooled sample consisting of all patients with myocardial ischemia, irrespective of the presence or absence of ICMP. In this sample, the medullary concentration of IL-13 correlated positively with the number of transitional monocytes (r=0.60; $P<0.05$) and negatively with the percentage of classical cells (r=-0.53; $P<0.05$). In contrast, M-CSF level was negatively associated with the content of transitional monocytes (r=-0.52; $P<0.05$) and positively associated with the number of classical monocytes (r=0.52; $P<0.05$). These data, together with the observed correlations among various subpopulations of BM monocytes, allow us to speculate that M-CSF promotes BM differentiation of transitional monocytes into classical cells, while IL-13 inhibits this process.

Despite a putative role of M-CSF and IL-13 in generation of different monocyte subsets, levels of these cytokines in BM did not differ significantly between CHD patients with and without ICMP. However, the medullary content in both ICMP+ and ICMP- groups of patients showed contrasting tendencies. Considering this fact, as well as opposite effects of M-CSF and IL-13 on generation of monocyte subpopulations, as observed in the study, we used M-CSF/IL-13 coefficient as a parameter reflecting the ratio of medullary concentration of cytokines with antagonistic effects on monocytopoiesis. It was found that in CHD patients without ICMP, the M-CSF/IL-13 ratio was nine times higher compared to patients with ICMP (Table 1). This allows us to consider the M-CSF/IL-13 coefficient as an indicator parameter for the regulation of monocytopoiesis, as well as an additional (together with IFN-γ) indicator of BM cytokine profile, which differs reliably between CHD patients with and without ICMP.

In order to delineate local and distant cytokine-dependent mechanisms of monocytopoiesis regulation, a comparative analysis of the concentrations of studied cytokines in BM and in the blood was performed. It was shown that in both groups of patients with CHD, concentrations of IL-1β and TNF-α in BM were higher than in the blood, while medullary and plasma levels of IL-13 were comparable (Table 1). In patients without ICMP, the medullary concentration of M-CSF exceeded its plasma level; IFN-γ was present in a very low concentration in BM, and was not detected in the blood. In contrast, in patients with ICMP, medullary and plasma levels of M-CSF did not differ, while BM concentration of IFN-γ was higher compared to patients without ICMP (Table 1). No statistically significant difference was observed in the plasma concentrations of IL-1β, IL-13, TNF-α, IFN-γ, and M-CSF between CHD patients with and without ICMP (Table 1), which indicates the inexpediency of differentiating ICMP+ and ICMP- CHD based on the plasma levels of these cytokines.

To further investigate cytokine-dependent regulation of monocytopoiesis, we searched for correlations between medullary concentration and plasma levels of IL-1β, IL-13, TNF-α, IFN-γ, and M-CSF in the pooled sample of patients, which proved to be more informative. It was found that the level of IL-13 in BM positively correlated with its concentration in the blood (r=0.78, $P<0.001$), while no similar associations were identified for IL-1β, TNF-α, or M-CSF. Taken together, the results of comparative and correlation analysis of medullary and plasma cytokine levels indicate that IL-13 represent a distant factor of monocytopoiesis regardless of the clinical form of chronic myocardial ischemia, as its concentrations in BM and blood were comparable and interconnected (Table 1). IL-1β and TNF-α, on the other hand, seem to exert a local regulatory effect on monocytopoiesis, since their medullary levels exceeded and were not associated with their plasma concentrations in both groups of patients (Table 1). At the same time, some differences were observed between the studied groups regarding the role of M-CSF and IFN-γ. Thus, in CHD patients without ICMP, M-CSF apparently functions as a local factor of hemopoiesis (as its medullar concentration was higher than its plasma level), while IFN-γ presumably does not have a considerable effect on monocytopoiesis due to an extremely low concentration of the cytokine in BM. In contrast, in patients with ICMP, M-CSF can be considered a distant hemopoietin (as its plasma and bone marrow levels were comparable, Table 1), while IFN-γ can be viewed as a local factor of hematopoiesis. Supposedly, in CHD patients with ICMP, bone marrow-derived IFN-γ could restrain an activating effect of M-CSF on the differentiation of transitional monocytes into classical and intermediate cells. The local influence of IFN-γ on monocytopoiesis in ICMP indicates that it is not feasible to control its medullary level through modulation of plasma concentration of the cytokine. At the same time, M-CSF and IL-13, which realize a distant mechanism of monocytopoiesis regulation in patients with

ICMP, can be considered as potential molecular targets for correction of the monocyte differentiation process via modulating levels of these cytokines in the blood. Demonstrated patterns could open new perspectives on cytokine (or anti-cytokine) therapy for ICMP, thus improving the effectiveness of otherwise largely ineffective treatments for this condition.

Taken together, the current study shows that development of ICMP in patients with CHD is associated with impairment of BM differentiation of classical and intermediate monocytes, accompanied by local hypersecretion of IFN-γ and a decreased ratio of distant hemopoietins M-CSF/IL-13 in BM (as compared with patients without ICMP). Dysregulation of monocytopoiesis in ICMP underlies the suppression of differentiation of mature monocyte subsets, which could affect the subpopulation constitution of blood monocytes and contribute to diffuse atherosclerosis with the development of ICMP. Targeted regulation of these processes through modulation of M-CSF and IL-13 plasma levels could represent a potential treatment modality of therapy for ICMP.

Conclusions:

In CHD patients with ICMP, percentages of classical and intermediate monocyte subpopulations in BM were lower, while the number of transitional cells and concentration of IFN-γ (as a local factor of monocytopoiesis regulation) were higher compared to CHD patients without ICMP. Plasma and medullary levels of IL-1β, IL-13, TNF-α, and M-CSF in chronic myocardial ischemia did not depend on its clinical form.

M-CSF activates, while IL-13 inhibits, BM differentiation of classical monocytes from transitional cells. A high M-CSF/IL-13 ratio in CHD patients without ICMP was associated with efficient generation of mature forms of monocytes. A decreased M-CSF/IL-13 ratio in CHD patients with ICMP was associated with dysregulation of this process.

IL-13 realizes distant, while IL-1β and TNF-α realize local, mechanisms of monocytopoiesis, irrespective of the clinical form of myocardial ischemia. M-CSF in CHD without ICMP exerts a local regulatory effect on monocyte differentiation. In CHD with ICMP, in contrast, M-CSF can be considered a distant hemopoietin.

Competing Interests

The authors declare the absence of obvious and potential conflicts of interest related to the publication of the article.

Sources of Funding

The research was supported by the Russian Foundation for Basic Research (№18-015-00160\18) and Grant Council of the President of the Russian Federation for state support of the leading scientific schools (SS-2690.2018.7).

Acknowledgments

The authors are grateful to Alexandra G. Pershina (PhD, Senior Researcher of Central Research Laboratory at Siberian State Medical University) for assistance with flow cytometry, and Prof. Alexander N. Baikov (Honored Worker of Higher Education of the Russian Federation, Head of the Central Research Laboratory at Siberian State Medical University) for the possibility of conducting research on the basis of the Central Research Laboratory.

References

1. Gutor SS, Kazakov VA, Suhodolo IV, Shipulin VM, Babokin VYe, Ogurkova ON, et al. [Natriuretic peptide and his progenitors as predictors of progressive postoperative left ventricle remodeling in patients with ischemic cardiomyopathy]. Bulletin of Siberian Medicine. 2013; 12(6):25-30. [Article in Russian].
2. Alzahrani T, Tiu J, Panjrath G, Solomon A. The effect of angiotensin-converting enzyme inhibitors on clinical outcomes in patients with ischemic cardiomyopathy and midrange ejection fraction: a *post hoc* subgroup analysis from the PEACE trial. Ther Adv Cardiovasc Dis. 2018;12(12):351–359. doi: 10.1177/1753944718809266.
3. Gavrish AS, Paukov VS. Ischemic cardiomyopathy. Moscow: "GEOTAR-Media"; 2015. [in Russian].
4. Kaski JC, Crea F, Gersh BJ, Camici PG. Reappraisal of ischemic heart disease. Circulation. 2018;138(14):1463-1480. doi: 10.1161/CIRCULATIONAHA.118.031373.
5. Kozhevnikov ML, Shipulin VM, Sukhodolo IV. [The virus-immune hypothesis for cardiac dilatation]. Ter Arkh. 2017;89(11):79-83. doi: 10.17116/terarkh2017891179-83. [Article in Russian].
6. Xi R, Fan Q, Yan X, Zhang H, Xie H, Gu G, et al. Increased serum interleukin-34 levels are related to the presence and severity of cardiac dysfunction in patients with ischemic cardiomyopathy. Front Physiol. 2018;9:904. doi: 10.3389/fphys.2018.00904.
7. Rojas J, Salazar J, Martínez MS, Palmar J, Bautista J, Chávez-Castillo M, et al. Macrophage heterogeneity and plasticity: impact of macrophage biomarkers on atherosclerosis. Scientifica (Cairo). 2015;2015:851252. doi: 10.1155/2015/851252.
8. Wang LX, Zhang SX, Wu HJ, Rong XL, Guo J. M2b macrophage polarization and its roles in diseases. J Leukoc Biol. 2018 Dec 21. doi: 10.1002/JLB.3RU1018-378RR.
9. Ziegler-Heitbrock L. Monocyte subsets in man and other species. Cell Immunol. 2014; 289(1-2):135-9. doi: 10.1016/j.cellimm.2014.03.019.
10. Ziegler-Heitbrock L, Hofer TPJ. Toward a refined definition of monocyte subsets. Front Immunol. 2013;(4):23. doi:10.3389/fimmu.2013.00023.
11. Fadini GP, Simoni F, Cappellari R, Previato L, Avogaro A. Pro-inflammatory monocyte-macrophage polarization imbalance in human hypercholesterolemia and atherosclerosis. Atherosclerosis. 2014;237(2):805-808. doi: 10.1016/j.atherosclerosis.2014.10.106.
12. Hulsmans M, Sam F, Nahrendorf M. Monocyte and macrophage contributions to cardiac remodeling. J Mol Cell Cardiol. 2016;93:149–155. doi: 10.1016/j.yjmcc.2015.11.015.
13. Shahid F, Lip GYH, Shantsila E. Role of monocytes in heart failure and atrial fibrillation. J Am Heart Assoc. 2018;7(3). pii: e007849. doi: 10.1161/JAHA.117.007849.
14. Azarova DA, Chumakova SP, Urazova OI, Vins MV, Shipulin VM, Pryakhin AS, et al. [Interleukins 4 and 6 as

factors of modulation of subpopulation composition of blood monocytes in patients with ischemic cardiomyopathy]. Kazan Medical Journal. 2018;99(6):900-905. [Article in Russian].
15. Felker GM, Shaw LK, O'Connor CM. A standardized definition of ischemic cardiomyopathy for use in clinical research. J Am Coll Cardiol. 2002;39(2):210-8.
16. Dutta P, Nahrendorf M. Monocytes in myocardial infarction. Arterioscler Thromb Vasc Biol. 2015;35(5):1066-70. doi: 10.1161/ATVBAHA.114.304652.
17. Urazova OI, Esimova IE, Kononova TE, Zakharova PV, Kolobovnikova YuV, Churina EG. [Molecular mechanisms of the immune response suppression in pulmonary tuberculosis]. Med Immunol. 2017;19(Suppl):206. [Article in Russian].
18. Bansal SS, Ismahil MA, Goel M, Zhou G, Rokosh G, Hamid T, et al. Dysfunctional and proinflammatory regulatory T-lymphocytes are essential for adverse cardiac remodeling in ischemic cardiomyopathy. Circulation. 2019;139(2):206-221. doi: 10.1161/CIRCULATIONAHA.118.036065.
19. Koh MY, Powis G. Passing the baton: the HIF switch. Trends Biochem Sci. 2012;37(9):364-72. doi: 10.1016/j.tibs.2012.06.004.
20. Lin N, Simon MC. Hypoxia-inducible factors: key regulators of myeloid cells during inflammation. J Clin Invest. 2016;126(10):3661-3671. doi: 10.1172/JCI84426.
21. Hsiao HW, Hsu TS, Liu WH, Hsieh WC, Chou TF, Wu YJ, et al. Deltex1 antagonizes HIF-1α and sustains the stability of regulatory T cells in vivo. Nat Commun. 2015;6:6353. doi: 10.1038/ncomms7353.
22. Morales-Mantilla DE, King KY. The role of interferon-gamma in hematopoietic stem cell development, homeostasis, and disease. Curr Stem Cell Rep. 2018;4(3):264-271. doi: 10.1007/s40778-018-0139-3.
23. Jeannin P, Paolini L, Adam C, Delneste Y. The roles of CSFs on the functional polarization of tumor-associated macrophages. FEBS J. 2018;285(4):680-699. doi: 10.1111/febs.14343.

International Journal of Biomedicine 9(2) (2019) 97-101
http://dx.doi.org/10.21103/Article9(2)_OA2

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Predictors of Coronary Atherosclerosis: HSP70, Markers of Oxidative Stress and Endothelial Dysfunction

Julia A. Kotova, PhD*; Anna A. Zuikova, PhD, ScD; Aleksander N Pashkov, PhD, ScD; Natalia V. Strahova, PhD; Olga N. Krasnorutskaya, PhD

Voronezh State Medical University named after N.N. Burdenko
Voronezh, Russian Federation

Abstract

The aim of this study was to evaluate the role of HSP70, and markers of oxidative stress and endothelial dysfunction, as determinants of the severity of coronary atherosclerosis. The study revealed significant differences between patient groups with and without coronary atherosclerosis in terms of HSP70, superoxide dismutase, total homocysteine (tHcy) and markers of oxidative modification of proteins. Significant correlations between Gensini score, lipid profile parameters and studied markers were determined. The results of multiple linear regression analysis allow us to consider the levels of HSP70, tHcy, LDL-C and ketone derivative of 2.4-dinitrophenylhydrazine as factors associated with the risk of coronary atherosclerosis.(**International Journal of Biomedicine. 2019;9(2):97-101.**)

Key Words: coronary atherosclerosis • heat shock protein 70 • superoxide dismutase • homocysteine

Abbreviations

ADPH, aldehyde derivative of DNPH; **CHD**, coronary heart disease; **CAG**, coronary angiography; **CA**, coronary atherosclerosis; **DNPH**, 2.4-dinitrophenylhydrazine; **ED**, endothelial dysfunction; **GS**, Gensini score; **HSP**s, Heat shock proteins; **HSP70**, heat shock protein 70; **HDL-C**, high-density lipoprotein cholesterol; **KDPH**, ketone derivative of DNPH; **LDL-C**, low-density lipoprotein cholesterol; **MLRA**, multiple linear regression analysis; **OS**, oxidative stress; **OMP**, oxidative modification of proteins; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TC**, total cholesterol; **TG**, triglycerides; **tHcy**, total homocysteine.

Introduction

Coronary artery disease (CAD) is the leading cause of death and disability worldwide.[1,2] Atherosclerosis, which is the primary pathophysiologic mechanism for the development of plaque leading to CAD, is a multifactorial and multifaceted process. In this process, fundamental roles for inflammation and OS have been established.[3-6] In CHD, a decrease in intracellular protection against reactive oxygen species, primarily due to a decrease in the level of SOD—the key enzyme of the antioxidant system—has been demonstrated by a number of researchers.[7] The imbalance between pro-oxidants and antioxidants leads to oxidative damage of proteins—an early indicator of the cell damage,[8,9] including the endothelium.In contrast to their inherent harms, ROS also function as signaling molecules, inducing stress tolerance mechanisms. OS can be responsible for the increased expression of HSPs. HSPs have been reported to work together with the antioxidant system to inhibit or neutralize the cellular effects of ROS.[10-12] OS is one of the most important factors that produce ED. ED contributes to atherogenesis at every phase of atherosclerosis.[13] In addition, Hcy is an established biomarker for ED and vascular disease, and is linked to increased OS.[14] Elevated Hcy promotes atherosclerosis through increased OS, impaired endothelial function, and induction of thrombosis.[15]

In this regard, the aim of this study was to evaluate the role of HSP70, and markers of OS and ED, as determinants of the severity of CA.

***Corresponding author:** Julia A. Kotova, PhD. Voronezh State Medical University n. a. N.N. Burdenko. Voronezh, Russia. E-mail: kotova_u@inbox.ru*

Materials and Methods

We examined 354 CHD patients (175 women and 179 men aged between 47 and 75 years, mean age of 61.8±8.1 years) who had CA of varying degrees, according to CAG.

All patients underwent the following examinations: assessment of traditional risk factors, physical examination, clinical and biochemical laboratory methods, 12-lead ECG, echocardiography, Holter ECG monitoring, treadmill test, and coronary angiography. Blood samples were obtained in the morning after a 12 h overnight fast. The levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) were determined in the blood plasma using "Daytona» analyzer (RANDOX, Ireland).

CAG was performed by the Judkins technique using General Electric Innova 3100 (GE Healthcare, USA).

The severity of CAD was evaluated by the Gensini score (GS).[16] The severity of the disease is expressed as the sum of the scores for individual lesions and the functional importance index of the area of each lesion in the coronary tree. According to the calculated GS, patients were divided into two groups: GS0 – 152 patients with normal coronary artery and GS>1 – 202 patients with mild to severe coronary stenosis. In Group GS0, 53(35%) patients received statins for more than 6 months before the study, 99(65%) patients did not receive statins for 6 months before the study. In Group GS>1, 79(39%) patients received statins for more than 6 months before the study, 123(61%) patients did not receive statins for 6 months before the study.

The determination of OMP in the blood serum was carried out using the methods by Dubinina et al.[17] The assay is based on the spectrophotometric detection of the reaction between 2.4-dinitrophenylhydrazine (DNPH) with protein carbonyl to form protein hydrazone. The optical density of 2,4-dinitrophenylhydrazones derivates was recorded on an SF-36 spectrophotometer. The optical density of aldehyde- and ketone derivatives of a neutral character was recorded at 356 nm and 370 nm, respectively (ADPHn and KDPHn). The optical density of aldehyde- and ketone derivatives of a basic character was recorded at 430 nm and 530 nm, respectively (ADPHb and KDPHb).

The SOD activity was determined by the spectrophotometric method. The serum level of tHcy was determined by EIA using «Axis-Shield» test kit. Extracellular Hsp70 was measured by ELISA (Elisa Kit for Hsp70, Cloud-Clone Corp.) in blood samples.

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Chicago, IL). Median values are presented with interquartile (IQ) ranges (IQR; 25th to 75th percentiles). The Mann-Whitney test was used to compare median values. The frequencies of categorical variables were compared using Pearson $\chi 2$. The Spearman correlation coefficient (r_s) was used to assess the relationship between variables. Stepwise multiple linear regression analysis (MLRA) was done to determine the variables with independent significant association with the severity of coronary atherosclerosis, and included all variables (biochemical markers and lipid metabolism indicators) with significant relationship with coronary atherosclerosis in univariate analyses ($P<0.05$ after correction for multiple comparisons). Probability values less than 0.05 were considered statistically significant. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by the Voronezh State Medical University Ethics Committee. Written informed consent was obtained from all patients.

Results

The main characteristics of the patients are presented in Table 1. The patients of the two groups were comparable in age. In Group GS>1, with a predominance of men, an average statistical power was found between the presence of CA, determined by the GS index, and the gender of patients ($\chi^2=14.174$, $P=0.0001$; $\varphi=0.459$, $P=0.0001$). In addition, the statistical relationship between gender and the number of affected vessels was determined: An insignificant lesion was more common in women (83%), two-vessel lesions were common in men (58.7%) and women (41.3%), and the three-vessel or multivessel lesions were predominant in men (61.6%) ($\chi^2=8.116$, $P=0.017$). The blood levels of TC, LDL-C and HDL-C also differed significantly between the two groups. GS>1 patients had higher levels of tHcy and OMP and lower levels of HSP70 and SOD activity (Table 2).

Table 1.

The main characteristics of the patients (Me, IQR [P_{25}; P_{75}])

Variable	GS0	GS1	*P*-value
Men	51	128	
Women	101	74	
Age, years	59.1 [51;67.5]	60.59 [57;65]	0.742
TC, mmol/l	4.54 [4.2;3.8]	5.82 [5.15;6.8]	0.001
TG, mmol/l	1.2 [1.1;1.3]	1.43 [1.1;1.57]	0.259
LDL-C, mmol/l	2.44 [2.05; 2.76]	2.94 [2.37; 2.94]	0.05
HDL-C, mmol/l	1.19 [1.1;1.3]	1.01 [0.9;1.1]	0.003

Table 2.

The blood levels of studied markers (Me, IQR [P_{25}; P_{75}])

Variable	GS0	GS1	*P*-value
HSP70, ng/ml	4.47 [4.12;4.98]	3.11 [2.63;3.71]	0.000
SOD, %	39.10 [39.10;43.89]	34.99 [32.26;36.14]	0.000
tHcy, μmol/l	8.47 [8.47;9.97]	11.46 [10.36;12.0]	0.000
ADPHn, IU/mg	21.88 [21.54;22.29]	25.9 [24.30;27.31]	0.000
KDPHn, IU/mg	20.32 [19.88;20.32]	21.43 [20.51;22.74]	0.003
ADPHb, IU/mg	10.73 [10.54; 10.88]	11.07 [10.67; 11.71]	0.075
KDPHb, IU/mg	2.54 [2.34;8.72]	6.87 [6.34;8.50]	0.006

A correlation analysis revealed direct correlations between GS and tHcy, indicators of OMP, as well as inverse correlations between GS and SOD activity. In addition, inverse

correlations were found between HSP70 and TC and LDL-C; SOD activity and TC; ADPHn, KDPHn and HDL-C. Direct correlations were found between HSP70 and SOD activity and HDL-C; ADPHn, KDPHn, ADPHb, KDPHb and TC; ADPHn and TG, LDL-C. We also found significant correlations between the GS and lipid profile parameters (TC [r_s=0.61, *P*=0.000], TG [r_s=0.27, *P*=0.04], LDL-C [r_s=0.45, *P*=0.001], and HDL-C [r_s=-0.46, *P*=0.000]).

On the first step of MLRA, the inclusion of HSP70 into the regression model (negative regression coefficient) explained 67% of the variance of the dependent variable (Table 3). On the second step, with the tHcy inclusion into the model, the relationship between HSP70 level and the presence of coronary atherosclerosis was maintained, and this model explained 73.1% of the variance of the dependent variable. The third predictor in the model was LDL-C. With the continued contribution of HSP70 and tHcy to the development of coronary atherosclerosis, this model explained 75.3% of the variance of the dependent variable. On the next step, the input variable was found to be KDPHb, and the model explained 76.7% of the variance of the dependent variable. At this step, the regression analysis was completed, which confirms the greatest importance of 4 markers in predicting coronary atherosclerosis: HSP70, tHcy, LDL-C, and KDPHb. The forced inclusion of other biochemical markers did not improve the model: The regression coefficients for them, as independent predictors, were insignificant, the standardized regression coefficients were low, the incremental R^2 was insignificant, and the partial F-test did not reveal significant differences among the models.

Table 3.

Results of the multiple linear regression analysis

Variable	B±Std. Error	β	*P*	adR²	F	*P*
Model 1				0.670	106.632	0.000
HSP70	-0.851 ±0.082	-0.822	0.000			
Model 2				0.731	71.785	0.000
HSP70	-0.524 ±0.118	-0.506	0.000			
tHcy	3.290 ±0.926	0.406	0.001			
Model 3				0.753	53.702	0.000
HSP70	-0.537 ± 0.114	-0.519	0.000			
tHcy	2.660 ±0.930	0.328	0.006			
LDL-C	2.210 ±0.962	0.173	0.026			
Model 4				0.767	39.025	0.000
HSP70	-0.511 ±0.019	-0.490	0.000			
tHcy	2.576 ±0.956	0.312	0.032			
LDL-C	1.982 ±0.908	0.150	0.047			
KDPHb	0.903 ±0.254	0.164	0.049			

Analyzing the ratios of standardized regression coefficients included in the regression analysis, it can be noted that the relative unique prognostic importance of HSP70, as an independent predictor of coronary atherosclerosis, is about -0.5. That is, when other independent predictors remain unchanged, and the HSP70 level increases by 1 standard deviation, then the severity of coronary atherosclerosis decreases by 0.5 standard deviation. This once again confirms the high importance of HSP70 as a protective factor for coronary atherosclerosis within these regression models.

Discussion

Identifying patients at early stages of coronary atherosclerosis is still a major problem. In the past two decades, numerous studies have demonstrated the importance of oxidative stress in the development of atherosclerosis. Elevated concentrations of a variety of oxidative stress markers were linked to a more frequent occurrence of cardiac events.[18] The study performed by Y Huo et al.[19] revealed metabolic disturbances in the model of long-term hyperhomocysteinemia together with vascular remodeling. Authors suggested that OS, ED, and decreased PPARγ expression in the vessel wall could be underlying mechanisms. Activation of ROS transduce matrix metalloproteinase, renders eNOS ineffective and promotes endothelial-smooth muscle disconnection/uncoupling by antagonizing PPARγ.[20] Elevated levels of plasma Hcy cause endothelial dysfunction and vascular diseases. [21,22] The importance of homocysteine in vascular function and arteriosclerosis was discovered by demonstration of arteriosclerotic plaques in children with homocystinuria caused by inherited enzymatic deficiencies of cystathionine synthase, methionine synthase, or methylene-tetrahydrofolate reductase.[23,24] Nonfasting plasma tHcy levels were independently associated with increased rates of all-cause and CVD mortality in the elderly Framingham men and women.[25] The elevated levels of homocysteine and indicators of oxidative stress were also found in our study. At the same time, a significant difference between GS0 and GS>1 precisely in ketone derivatives indicates the duration of oxidative stress and the degree of destruction of the protein molecule.

The activation of HSP70 may play a role in protecting the cells against oxidative stress and inflammatory damage.[26] When assessing the level of HSP70, we found a decrease in its level in the presence of coronary atherosclerosis, as well as a high association with GS. The first evidence that high levels of human HSP70 are associated with low CAD risk, probably through its multiple protective effects on a cell's response to stress, was provided by Zhu et al.[27] However, there are conflicting reports that preclude assigning HSP70 a definite role in atherosclerosis at present. Plasma levels of HSP70 have been found to have an inverse [27,28] as well as a direct association [29,30] with the severity of atherosclerosis. HSP70 is presently a matter of debate.[31,32] High levels of circulating HSP70 (HSPA1A) are associated with low risk of CAD;[27] they appear in hypertensive subjects with a lesser intima media, thickening after 4 years of follow-up.[33] In the study by E. Dulin, extracellular HSP70 and anti-HSP70 antibody concentrations have been proposed as biomarkers for the progression of atherosclerotic disease.[34] At least 4 studies have demonstrated that the transgenic overexpression of HSP70 in the heart of mice significantly protects against ischemia/reperfusion injury.[35-38]

Thus, our findings emphasize the significance of the studied markers in the pathogenesis of coronary atherosclerosis and make it possible to use the indicators of HSP70, tHcy, LDL-C and KDPHb in screening the risk for the development of coronary atherosclerosis.

Conclusion

The study revealed significant differences between groups with and without coronary atherosclerosis in terms of HSP70, superoxide dismutase, tHcy and markers of oxidative modification of proteins (except ADPHb). Significant correlations between Gensini score, lipid profile parameters and studied markers were determined. The results of multiple linear regression analysis allow us to consider the levels of HSP70, total homocysteine, low-density lipoprotein cholesterol and KDPHb as factors associated with the risk of coronary atherosclerosis.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

This work was partially supported by the Council on Grants of the President of the Russian Federation for State Support of Young Scientists and Leading Scientific Schools (Grant MK-552.2018.7).

References

1. Mendis S, Puska P, Norrving B, Organization WH, Federation WH, Organization WS. Global atlas on cardiovascular disease prevention and control. Geneva: World Health Organization; 2011. Available from: http://www.who.int/iris/handle/10665/44701
2. WHO | Cardiovascular diseases (CVDs]. Geneva: World Health Organization; 2017. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/
3. Ragino YuI, Chernyavskij AM, Eremenko NV, Shakhtshnejder EV, Polonskaya YaV, Tsimbal SYu, et al. [Key laboratory diagnostic biomarkers of coronary atherosclerosis]. Kardiologiia. 2011;51(3):42-6. [Article in Russian].
4. Vertkin AL, Topolyanskij AV. [The problem of hyperhomocysteinemia in cardiac patients]. Farmateka. 2007;15:10-14. [Article in Russian].
5. L'vovskaya EI, Sahankova EN. [The ratio of levels of lipid peroxidation and oxidative modification of proteins in students 17-23 years (Kungur)]. Vestnik YUrGU. 2012; 21: 112-116. [Article in Russian].
6. Musthafa QA, Abdul Shukor MF, Ismail NAS, Mohd Ghazi A, Mohd Ali R, M Nor IF, et al. Oxidative status and reduced glutathione levels in premature coronary artery disease and coronary artery disease. Free Radic Res. 2017 Oct;51(9-10):787-798. doi: 10.1080/10715762.2017.1379602.
7. Zanozina OV, Brovkova NN, Shcherbatyuk TE. [Oxidized modified proteins in the atherosclerosis genesis at a diabetes mellitus of the 2nd type]. Sovremennye Tehnologii v Medicine. 2009;2:72-75. [Article in Russian].
8. Bykova AA, Azizova OA, Dumikyan ASh, Shvachko AG, Sergienko VI, Syrkin AL. [Oxidative modification of fibrinogen in patients with coronary heart disease]. Rossijskij Kardiologicheskij Zhurnal. 2015;1:24. [Article in Russian].
9. Fomina MA, Abalenihina YuV. Oxidative modification of tissue proteins with a change in the synthesis of nitric oxide. M: Gehotar-Media; 2018. [In Russian].
10. Wick G, Knoflach M, Xu Q. Autoimmune and inflammatory mechanisms in atherosclerosis. Annu Rev Immunol. 2017;22:361-403.
11. Trott A, West JD, Klaić L, Westerheide SD, Silverman RB, Morimoto RI, Morano KA. Activation of heat shock and antioxidant responses by the natural product celastrol: transcriptional signatures of a thiol-targeted molecule. Mol Biol Cell. 2008;19(3):1104-12. doi: 10.1091/mbc.E07-10-1004.
12. Wu CW, Biggar KK, Zhang J, Tessier SN, Pifferi F, Perret M, Storey KB. Induction of Antioxidant and Heat Shock Protein Responses During Torpor in the Gray Mouse Lemur, Microcebus murinus. Genomics Proteomics Bioinformatics. 2015;13(2):119-26. doi: 10.1016/j.gpb.2015.03.004.
13. Juonala M, Viikari JS, Laitinen T, Marniemi J, Helenius H, Rönnemaa T, Raitakari OT. Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. Circulation. 2004;110(18):2918-23.
14. Davydchyk EV, Snezhitskiy VA, Nikonova LV. [Relationship of hyperhomocysteinemia with coronary heart disease and diabetes mellitus]. Journal of the Grodno State Medical University. 2015; (1):9-13.[Article in Russian].
15. Guthikonda S, Haynes WG. Homocysteine: role and implications in atherosclerosis. Curr Atheroscler Rep. 2006;8(2):100-6.
16. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. Am J Cardiol. 1983;51(3):606.
17. Dubinina EE, Burmistrov SO, Khodov DA, Porotov IG. [Oxidative modification of human serum proteins. A method of determining it].Vopr Med Khim. 1995;41(1):24-6. [Article in Russian].
18. Vichova T1, Motovska Z. Oxidative stress: Predictive marker for coronary artery disease. Exp Clin Cardiol. 2013 Spring;18(2):e88-91.
19. Huo Y, Wu X, Ding J, Geng Y, Qiao W, Ge A, et al. Vascular Remodeling, Oxidative Stress, and Disrupted PPARγ Expression in Rats of Long-Term Hyperhomocysteinemia with Metabolic Disturbance. PPAR Res. 2018;2018:6738703. doi: 10.1155/2018/6738703.
20. Tyagi N, Qipshidze N, Sen U, Rodriguez W, Ovechkin A, Tyagi SC. Cystathionine beta synthase gene dose dependent vascular remodeling in murine model of hyperhomocysteinemia. Int J Physiol Pathophysiol Pharmacol. 2011;3(3):210-22.
21. McCully KS. Homocysteine Metabolism, Atherosclerosis, and Diseases of Aging. Compr Physiol. 2015;6(1):471-505. doi: 10.1002/cphy.c150021.
22. Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ. Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: results from the Third National Health and Nutrition Examination Survey, 1988-1994. Stroke. 1998;29(12):2473-7.
23. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol. 1969;56(1):111-28.
24. Kanwar YS, Manaligod JR, Wong PW. Morphologic

studies in a patient with homocystinuria due to 5, 10-methylenetetrahydrofolate reductase deficiency. Pediatr Res. 1976;10(6):598-609.
25. Bostom AG, Silbershatz H, Rosenberg IH, Selhub J, D'Agostino RB, Wolf PA, et al. Nonfasting plasma total homocysteine levels and all-cause and cardiovascular disease mortality in elderly Framingham men and women. Arch Intern Med. 1999;159(10):1077-80.
26. Ren J, Liu C, Zhao D, Fu J. The role of heat shock protein 70 in oxidant stress and inflammatory injury in quail spleen induced by cold stress. Environ Sci Pollut Res Int. 2018 Jul;25(21):21011-21023. doi: 10.1007/s11356-018-2142-8.
27. Zhu J, Quyyumi AA, Wu H, Csako G, Rott D, Zalles-Ganley A, et al. Increased serum levels of heat shock protein 70 are associated with low risk of coronary artery disease. Arterioscler Thromb Vasc Biol. 2003;23(6):1055-9.
28. Martin-Ventura JL, Leclercq A, Blanco-Colio LM, Egido J, Rossignol P, Meilhac O, Michel JB. Low plasma levels of HSP70 in patients with carotid atherosclerosis are associated with increased levels of proteolytic markers of neutrophil activation. Atherosclerosis. 2007;194(2):334-41.
29. Wright BH, Corton JM, El-Nahas AM, Wood RF, Pockley AG. Elevated levels of circulating heat shock protein 70 (Hsp70) in peripheral and renal vascular disease. Heart Vessels. 2000;15(1):18-22.
30. Xie F, Zhan R, Yan LC, Gong JB, Zhao Y, Ma J, Qian LJ. Diet-induced elevation of circulating HSP70 may trigger cell adhesion and promote the development of atherosclerosis in rats. Cell Stress Chaperones. 2016;21(5):907-14. doi: 10.1007/s12192-016-0716-2.
31. Rodríguez-Iturbe B, Johnson RJ. Heat shock proteins and cardiovascular disease. Physiol Int. 2018;105(1):19-37. doi: 10.1556/2060.105.2018.1.4.
32. Bielecka-Dabrowa A, Barylski M, Mikhailidis DP, Rysz J, Banach M. HSP 70 and atherosclerosis--protector or activator? Expert Opin Ther Targets. 2009;13(3):307-17. doi: 10.1517/14728220902725149.
33. Pockley AG, Georgiades A, Thulin T, de Faire U, Frostegård J. Serum heat shock protein 70 levels predict the development of atherosclerosis in subjects with established hypertension. Hypertension. 2003;42(3):235-8.
34. Dulin E, García-Barreno P, Guisasola MC. Extracellular heat shock protein 70 (HSPA1A) and classical vascular risk factors in a general population. Cell Stress Chaperones. 2010;15(6):929-37. doi: 10.1007/s12192-010-0201-2.
35. Marber MS, Mestril R, Chi SH, Sayen MR, Yellon DM, Dillmann WH. Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. J Clin Invest. 1995;95(4):1446-56.
36. Plumier JC, Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G, Pagoulatos GN. Transgenic mice expressing the human heat shock protein 70 have improved post-ischemic myocardial recovery. J Clin Invest. 1995;95(4):1854-60.
37. Radford NB, Fina M, Benjamin IJ, Moreadith RW, Graves KH, Zhao P, et al. Cardioprotective effects of 70-kDa heat shock protein in transgenic mice. Proc Natl Acad Sci U S A. 1996;93(6):2339-42.
38. Trost SU, Omens JH, Karlon WJ, Meyer M, Mestril R, Covell JW, Dillmann WH. Protection against myocardial dysfunction after a brief ischemic period in transgenic mice expressing inducible heat shock protein 70. J Clin Invest. 1998;101(4):855-62.

International Journal of Biomedicine 9(2) (2019) 102-105
http://dx.doi.org/10.21103/Article9(2)_OA3

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Association of *ACE* Gene Polymorphism with Hypertension and Metabolic Risk Factors among Indigenous People of the Northern Territory of Yakutia

Sargylana I. Sofronova, PhD*; Maria P. Kirillina, PhD; Vyacheslav M. Nikolaev, PhD; Sardaana K. Kononova; Oksana G. Sidorova; Anna N. Romanova

Yakut Science Center of Complex Medical Problems
Yakutsk, the Republic of Sakha (Yakutia), Russia

Abstract

The research objective was to study the association of the *ACE* gene I/D polymorphism with essential hypertension (EH) and metabolic risk factors among indigenous people of the northern territory of Yakutia. The obtained data show that representatives of indigenous people of the North of Yakutia with the *ACE* ID genotype are characterized with high levels of systolic blood pressure. The carriage of DD genotype in EH patients was associated with a high frequency of hypercholesterolemia and hyper-LDL cholesterolemia. The carriage of ID genotype in EH patients, compared to subjects without EH, was characterized by higher blood levels of TC, LDL-C, TG, and FPG and associated with a high frequency of obesity. Thus, the *ACE* I/D polymorphism was found to be associated with metabolic risk factors in indigenous EH patients of the North of Yakutia. (**International Journal of Biomedicine. 2019;9(2):102-105.**)

Key Words: essential hypertension • *ACE* gene • indigenous people • risk factors

Abbreviations

ACE, angiotensin-converting enzyme; **AO**, abdominal obesity; **EH**, essential hypertension; **FPG**, fasting plasma glucose; **HDL-C**, high-density lipoprotein cholesterol; **LDL-C**, low-density lipoprotein cholesterol; **OGTT**, oral glucose tolerance test; **RAS**, renin–angiotensin system; **SBP**, systolic blood pressure; **WS**, waist circumference.

Introduction

Hypertension is one of the main risk factors worldwide for cardiovascular disease and the main reason for a high mortality rate among the adult population.[1,2] Essential hypertension (EH), the most common form of hypertension,[3] is defined as an elevation in blood pressure of unknown cause, which increases the risks for cerebral, cardiac, and renal complications.[4] EH is considered a multifactorial disease.[5] From a genetic perspective, many single nucleotide polymorphisms (SNPs), genes and epigenetic factors are associated with EH.[6] Currently, increasing attention is being paid to RAS genes in the development of EH, and the value of *ACE* gene I/D polymorphism has been investigated in many studies.[7-9] A strong association between the DD genotype and the D allele with EH, abdominal obesity and coronary artery disease was revealed in a number of studies.[10-12] Grigor'eva found a significant association between *ACE* gene polymorphism and the risk of myocardial infarction in Yakut men.[13]

The research objective was to study the association of *ACE* gene polymorphism with hypertension and metabolic risk factors among indigenous people of the northern territory of Yakutia.

Materials and Methods

This study was done to determine the clinico-epidemiological aspects of EH in the remote districts in the North of Yakutia (Kolymskoye and Andryushkino rural

***Corresponding author:** Sargylana I. Sofronova, PhD. Yakut Science Center of Complex Medical Problems. Yakutsk, the Republic of Sakha (Yakutia), Russia. E-mail: sara2208@mail.ru*

localities of the Nizhnekolymsky District, Topolinoe rural locality of the Tomponsky District, Nelemnoye rural locality of the Verkhnekolymsky District). A total of 348 indigenous people of Yakutia (Evens, Chukchi, Yukaghirs, Yakuts) (225 women and 123 men) were examined. The patient sample consisted of adults aged between 20 and 70 years, with an average age of 45.71±0.67 years. All patients were divided into 2 groups. Group 1 consisted of 175 patients (mean age of 53.11±0.51 years) with EH; Group 2 (control group) included 173 people (mean age of 38.88±0.60 years) without elevated blood pressure.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from each patient.

A comprehensive clinical examination and laboratory tests included the following procedures:

- Anthopometrical reference data: BMI was calculated using Quetelet's formula (in kg/cm^2). Measurement of WC was made at the uppermost lateral border of the ilium using a tape measure (in cm)
- Assessment of blood pressure by Korotkov's method.
- Assessment of FPG, OGTT, and blood levels of TG, HDL-C, LDL-C.

Glucose and lipid metabolism disorders were diagnosed according to the Russian national recommendations (the All-Russian Scientific Society of Cardiologists, 2009)[14] based on the IDF consensus criteria (2006)[15]: TG ≥1.7 mmol/l; HDL-C <1.0 mmol/l in males and <1.2 mmol/l in females; LDL-C >3.0 mmol/l; FPG >6.1 mmol/l; IGT 2Hr PG ≥7.8 mmol/l and ≤ 11 mmol/l . Abdominal obesity (AO) was confirmed at WC ≥ 94 cm in males and ≥ 80 cm in females.

The diagnosis of hypertension was based on 2017 ACC/AHA Guideline for or the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults.[16]

The insertion/deletion (I/D) polymorphism of the *ACE* gene was examined by PCR in the laboratory of molecular genetics at Yakut Science Center of Complex Medical Problems. From each patient, 2ml of peripheral blood were drawn into an EDTA tube. Genomic DNA was isolated from the peripheral blood leukocytes using standard phenol–chloroform extraction technique (Maniatis et al., 1982) Genotyping was carried out with the allele specific primers method.

Reactions were performed with 10 pmol of each primer:

F: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'
R: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'.

PCR products were analyzed on 2% agarose gels after staining with ethidium bromide and were visualized using a UV transilluminator. Two alleles were identified: a 490-bp fragment I (with the insertion) and a 190-bp fragment D (without the insertion). In heterozygous samples, two bands (490 and 190 bp) were detected. To avoid mistyping of heterozygotes (ID) DNA samples identified as a DD genotype were subsequently amplified with second set of primers designed for the insertion specific allele.

Statistical analysis was performed using SPSS (version 17.0). 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. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by χ2- test with 1 degree of freedom (df). A probability value of P<0.05 was considered statistically significant.

Results and Discussion

In the general population, the *ACE* II, ID, and DD genotype frequencies were 19.5% (n=68), 65.2% (n=227), and 15.2% (n=227). We did not find statistically significant differences in the frequency distribution of *ACE* I/D alleles and genotypes between the group of patients and control group (Table 1). The distribution of the genotype frequency was not in HWE for patients (χ^2=19.17, p<0.05) and for controls (χ^2=13.95, p<0.05). The occurrence of the departure from HWE in controls is probably due to population substructure. We further used the three types of genetic models to test the association between the *ACE* I/D polymorphism and EH; the results are shown in Tables 2a-2c. We found no association between the *ACE* I/D polymorphism and EH in our case–control study.

Table 2a.
General model of inheritance (df = 2)

Genotype	Genotype frequencies		χ^2	*P*	OR	95% CI
	Patients	Control				
II	0.200	0.191	0.63	0.73	1.06	0.62-1.80
ID	0.663	0.642			1.10	0.71-1.71
DD	0.137	0.168			0.79	0.44-1.42

Table 1.
Frequencies of the genotypes and alleles of the ACE I/D polymorphism and deviations from HWE (df = 1)

Genotype	Patients	HWE	χ^2	*P*	Control	HWE	χ^2	*P*	Allele	Allele frequencies	
										Patients	Control
II	0.200	0.282	19.17	1.0E-5	0.191	0.262	13.95	0.0002	I	0.531	0.512
ID	0.663	0.498			0.642	0.500			D	0.469	0.488
DD	0.137	0.220			0.169	0.239					

Table 2b.

Dominant model of inheritance (df = 1)

Genotype	Genotype frequencies		χ^2	*P*	OR	95% CI
	Patients	Control				
II+ID	0.863	0.832	0.63	0.43	1.27	0.70-2.28
DD	0.137	0.168			0.79	0.44-1.42

Table 2c.

Recessive model of inheritance (df = 1)

Genotype	Genotype frequencies		χ^2	*P*	OR	95% CI
	Patients	Control				
II	0.200	0.191	0.05	0.83	1.06	0.62-1.80
ID+DD	0.800	0.809			0.94	0.55-1.60

In the general population (n=348), we did not find significant differences in average values of TG, LDL-C, and HDL-C depending on the carriage of genotypes of the *ACE* I/D polymorphism. The frequency of hypercholesterolemia was 51.5% in carriers of II homozygous genotype, 42.7% in carriers of ID heterozygous genotype, and 49.1% in carriers of DD heterozygous genotype (*P*>0.05). Hyper-LDL cholesterolemia was found in 64.2% of DD homozygotes, 54.4% of II homozygotes, and 54.6% of ID heterozygotes (*P*>0.05). Hypo-HDL cholesterolemia was found in 33.8% of II homozygotes, 34.8% of ID heterozygotes, and 35.8% of DD homozygotes (*P*>0.05).The frequency of hyperglycemia was as follows: ID carriers - 6.6%, II carriers - 2.9%, and DD carriers - 3.8%.

In Croup 1 (patients with EH), the average level of SBP in ID carriers, II carriers and DD carriers was 144.2±1.2mmHg, 136.6±2.8mmHg and 138.8±2.1mmHg, respectively, *P*=0.0072. Table 3 presents the relationship between *ACE* genotype carriage and parameters of lipid and glucose metabolism in two groups. In ID carriers, the blood levels of TC, LDL-C, TG, and FPG were significantly higher in Group 1 than in Group 2. In II carriers, the blood level of TG was significantly higher in Group 1 than in Group 2.

We found a high frequency of hyper-LDL cholesterolemia in DD carriers of Group 1 (70.8%) compared to Group 2 (58.4%) (*P*=0.015). In DD carriers, the frequency of hypercholesterolemia was also significantly higher in Group 1 than in Group 2 (66.8% versus 34.7%, *P*=0.000)

In the general population, the frequency of AO in ID carriers, II carriers and DD carriers was as follows: 59.9%, 55.9%, and 50.9%, respectively, *P*>0.05. The average level of WC in ID carriers, II carriers and DD carriers was 89.61±0.65 cm, 86.46±1.49 cm, and 86.75±1.52 cm, respectively, *P*=0.0391.

In Group 1, the average level of WC in ID carriers, II carriers and DD carriers was 96.23±0.89 cm, 91.43±1.99 cm, and 92.63±1.27cm (*P*=0.0205), respectively. The frequency of AO was as follows: ID carriers – 84.5%, II carriers – 71.4%, and DD carriers – 66.7% (*P*>0.05). AO frequency in ID carriers vs. DD carriers was significantly higher (*P*<0.05).

In Group 2 the average level of WC in ID carriers, II carriers and DD carriers was 82.68±0.68 cm, 81.18±1.85 cm, and 81.90±1.21 cm (*P*>0.05). The frequency of AO was as follows: ID carriers – 34.2%, II carriers – 39.4%, and DD carriers – 37.9% (*P*>0.05)

Conclusion

The obtained data show that representatives of indigenous people of the North of Yakutia with the *ACE* ID genotype are characterized with high levels of SBD. The carriage of DD genotype in EH patients is associated with a high frequency of hypercholesterolemia and hyper-LDL cholesterolemia. The carriage of ID genotype in EH patients, compared to subjects without EH, is characterized by higher blood levels of TC, LDL-C, TG, and FPG and associated with a high frequency of AO. A number of studies have also found a high frequency of metabolic syndrome in ID carriers.[17-20] Thus, the *ACE* I/D polymorphism was found to be associated with metabolic risk factors in indigenous EH patients of the North of Yakutia.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

The study was carried out within the framework of the project "Contribution of metabolic syndrome to the development of coronary artery atherosclerosis in residents of Yakutia" and R&D "Development of new technologies for the treatment and risk prediction of hypertension and stroke in the Republic of Sakha (Yakutia)" (State Contract No. 1133).

Table 3.

Mean concentrations of lipid spectrum and glucose among hypertensive patients and persons without hypertension

Blood parameters	Genotype II			Genotype ID			Genotype DD		
	Group 1	*P*	Group 2	Group 1	*P*	Group 2	Group 1	*P*	Group 2
TC	5.05±0.14	>0.05	4.68 ±0.16	5.15±0.09	<0.01	4.79±0.07	5.13±0.16	>0.05	4.71±0.16
LDL-C	3.24±0.13	>0.05	2.88±0.13	3.32±0.08	<0.01	3.05±0.06	3.32±0.14	>0.05	3.07±0.12
HDL-C	1.26±0.05	>0.05	1.37±0.06	1.26±0.03	>0.05	1.33±0.03	1.28±0.06	>0.05	1.14±0.05
TG	1.21±0.09	<0.02	0.92±0.07	1.21±0.05	0.000	0.91±0.03	1.16±0.10	>0.05	1.07±0.08
FPG	4.48±0.18	>0.05	4.41±0.14	5.02±0.17	0.000	4.19±0.08	4.48±0.27	>0.05	4.25±0.14

References

1. Appel LJ; American Society of Hypertension Writing Group, Giles TD, Black HR, Izzo JL Jr, Materson BJ, Oparil S, Weber MA. ASH Position Paper: Dietary approaches to lower blood pressure. J Clin Hypertens (Greenwich). 2009;11(7):358-68. doi: 10.1111/j.1751-7176.2009.00136.x.
2. Oganov RG, Timofeeva TN, Koltunov IE, Konstantinov VV, Balanova YA, Kapustina AV, et al. [Arterial hypertension epidemiology in Russia; the results of 2003-2010 federal monitoring]. Cardiovascular Therapy and Prevention. 2011;10(1):9-13. [Article in Russian].
3. Oparil S1, Zaman MA, Calhoun DA. Pathogenesis of hypertension. Ann Intern Med. 2003;139(9):761-76.
4. Messerli FH1, Williams B, Ritz E. Essential hypertension. Lancet. 2007 ;370(9587):591-603.
5. Nakayama T1. [Genetic factors of hypertension]. Rinsho Byori. 2013;61(2):144-9. [Article in Japanese]
6. Natekar A, Olds RL, Lau MW, Min K, Imoto K, Slavin TP. Elevated blood pressure: Our family's fault? The genetics of essential hypertension. World J Cardiol. 2014;6(5):327-37. doi: 10.4330/wjc.v6.i5.327.
7. Fox CS1, Heard-Costa NL, Vasan RS, Murabito JM, D'Agostino RB Sr, Atwood LD; Framingham Heart Study. Genomewide linkage analysis of weight change in the Framingham Heart Study.J Clin Endocrinol Metab. 2005;90(6):3197-201.
8. Penesova A, Cizmarova E, Kvetnansky R, Koska J, Sedlakova B, Krizanova O. Insertion/deletion polymorphism on ACE gene is associated with endothelial dysfunction in young patients with hypertension. Horm Metab Res. 2006;38(9):592-7.
9. Zivko M, Kusec R, Galesić K. Impact of angiotensin-converting enzyme gene polymorphism on proteinuria and arterial hypertension. Coll Antropol. 2013;37(3):765-70.
10. Irijanto F, Rahajeng H, Kamajaya S, Sja'bani M, Sadewa A, Tomino Y. High D Allele Frequency of ACE I/D Gene Polymorphism in Familial Hypertension in Javanese Indonesian. Juntendo Medical Journal, 2012. 58(3):224-230.
11. Kotlovsky MYu, Kotlovskaya OS, Osedko OYa, Kirichenko DS, Pokrovsky AA, Osedko AV, et al. Gene polymorphism of the angiotensin-converting enzyme in people with hypertension and chronic IHD. Fundamentalnye issledovania. 2011;11-1:49-52.
12. Strazzullo P, Iacone R, Iacoviello L, Russo O, Barba G, Russo P, et al.; Olivetti Prospective Heart Study. Genetic variation in the renin-angiotensin system and abdominal adiposity in men: the Olivetti Prospective Heart Study. Ann Intern Med. 2003;138(1):17-23.
13. Grigor'eva, L.V. Molecular-genetic study of myocardial infarction in the Yakut population. PhD Thesis. Ufa; 2006.[In Russian].
14. Metabolic syndrome diagnostics and treatment. Compilation of national recommendations. Moscow: Silicea-Poligraf Publishers; 2009:106–143. [In Russian].
15. IDF Consensus Worldwide Definition of the Metabolic Syndrome, 2006. Available from: https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome
16. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2018;71(19):e127-e248. doi: 10.1016/j.jacc.2017.11.006.
17. Bhavani BA, Padma T, Sastry BK, Krishna Reddy N, Nausheenm K. The Insertion I/ Deletion D polymorphism of Angiotensin- Converting Enzyme (ACE) Gene Increase the Susceptibility to Hypertension and / or Diabetes. Int J Hum Genet. 2017;5(4):247-252.
18. Randhawa NK, Kumar A, Matharoo K, Bhanwer AS. Association Study of Angiotensin-Converting Enzyme Ins/Del Polymorphism with Hypertension in Punjabi Population. Int J Hum Genet. 2006;6:317-321. D
19. Sameera AS, Syeeda N, Takc S, Bashird S, Nissara S, Siddiqi M. ACE I/D Polymorphism in Hypertensive Patients of Kashmiri Population. Cardiology Research. 2010;1(1):1-7.
20. Sun F, He N, Zhang K, Wu N, Zhao J, Qiu C. Association of ACE gene A2350G and I/D polymorphisms with essential hypertension in the northernmost province of China. Clin Exp Hypertens. 2018;40(1):32-38. doi: 10.1080/10641963.2017.1291659.

International Journal of Biomedicine 9(2) (2019) 106-110
http://dx.doi.org/10.21103/Article9(2)_OA4

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Synchronization of Wave Flows of Arterial and Venous Blood and Phases of the Cardiac Cycle: Part 4

Alexander G. Kruglov, PhD, ScD*; Valery N. Utkin; Alexander Yu. Vasilyev, PhD, ScD; Andrey A. Kruglov, PhD

Central Research Institute of Radiation Diagnosis
Moscow, the Russian Federation

Abstract

Hemodynamic indices of healthy people obtained by catheterization in such vascular areas as the chambers of the heart (both ventricles, both atria, coronary sinus), IVC, SVC, RHV, right renal vein, sigmoid sinus, and aorta were analyzed. Using the mean values of hemodynamic parameters, we constructed graphs of the curves of the central, arterial, and venous pressure, synchronized with each other, with an ECG, and with the peripheral pulse wave.

In the present work, to generalize the results obtained, we constructed integral curves of the dynamics of the blood substrate ("bolus," BB) vector from the entrance (the fusion of venous flows: SVC, IVC, RHV, and coronary sinus) to the exit from the heart-lung-heart system in the form of LVEF. It was revealed that the complete CC consists of two BBs, simultaneously passing the hemodynamic pathway: heart-lung-heart. One BB completes the transformation, leaving PC after contact with the external gaseous medium in the lung and the formation of the pulse wave spheroid in LV, going into SC in the form of LVEF; another BB, after the formation of the pulse wave spheroid in RV, goes into PC, followed by hemodynamic, metabolic and gas transformation. CMIP is a universal indicator of the quality and stability of the relationship of all hemodynamic structures (flows in vessels and heart chambers) included in integral hemodynamic flows (IC-1, IC-2) throughout the complete CC. Changes in the configuration or level of CMIP is an indicator of imbalance and restructuring of the integral indicators of blood flow (therefore, components of hemodynamic flows), indicating a deviation in the parameters of stability of homeostasis as a whole.(**International Journal of Biomedicine. 2019;9(2):106-110.**)

Key Words: cardiac cycle • hemodynamic parameters • synchronization • wave flows

Abbreviations

Ao, aorta; **AV**, aortic valve; **BB**, "bolus" of the blood; **CC**, cardiac cycle; **CS**, coronary sinus; **CMIP**, cardiac mean integral pressure; **IC**, integral curve; **IVC**, inferior vena cava; **LVEF**, left ventricular ejection fraction; **LV**, left ventricle; **MV**, mitral valve; **PC**, pulmonary circulation; **PV**, pulmonary valve; **PPW**, peripheral pulse wave; **PT**, pulmonary trunk; **RVEF**, right ventricular ejection fraction; **RV**, right ventricle; **RA**, right atrium; **RHV**, right hepatic vein; **SC**, systemic circulation; **SN**, the sinus node; **SS**, sigmoid sinus; **SVC**, superior vena cava; **TV**, tricuspid valve; **ZTEP**, zone of temporal equalization of pressure.

Basic Part

The purpose of this part of the work was to determine the dynamics of the vector of the BB passage from merging venous blood flows in RA (entry into the heart-lung-heart system) to the exit from the system in the structure of LVEF.

Completing the analysis and discussion of data from previous publications,[1-3] we present Table 1, which includes integral indicators of hemodynamics of "complete CC."

Based on the previously cited digital and graphic data on the hemodynamic and metabolic indices of the heart activity as a whole, we put forward the task of plotting the dynamics of the BB passage vector from the merging of venous blood flows (IVC, SVC, CS, VH), which are feedback channels (wave and metabolic) between the body and the heart.

***Corresponding author**: *Alexander G. Kruglov, PhD, ScD. Central Research Institute of Radiation Diagnosis. Moscow, the Russian Federation. E-mail: krag48@mail.ru*

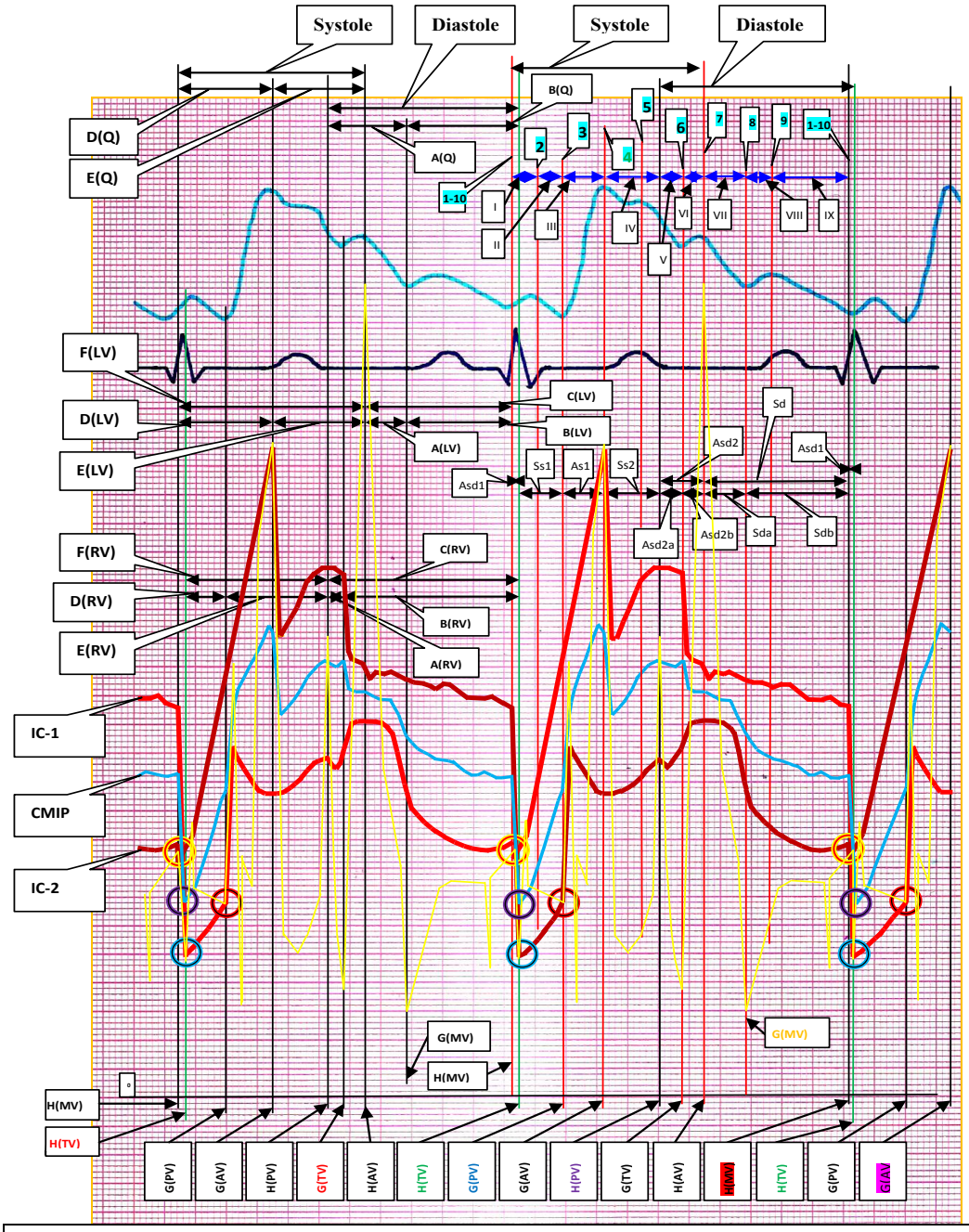


Table 1. Norm

1-10 – characteristic points of PPW (V.V. Boronoev)	**H(MV)** – closing of MV
A(Q) – isometric ventricular relaxation	**G(PV)** – opening of PV
B(Q) – actual ventricular diastole	**H(PV)** – closing of PV
C(LV) – LV diastole	**Asd1**– asynchronous period of ventricular systole-diastole -1
A(LV) – isometric LV relaxation	**Ss1**– synchronization period of isometric ventricular contraction-1
B(LV) – actual LV diastole	**As1** – asynchronous period of ventricular systole -1
C(RV) – RV diastole	**Ss2** – synchronization of the actual ventricular systole -2
A(RV) – isometric RV relaxation	**Asd2** – asynchronous period of ventricular systole-diastole -2
B(RV) – actual RV diastole	**Asd2a** – from closing of PV to opening of TV
D(Q) – isometric ventricular contraction	**Asd2b** – from opening of TV to closing of AV
E(Q) – actual ventricular systole	**Sd** – period of synchronization of ventricular relaxation
F(LV) – LV systole	**Sda** – isometric relaxation of LV
D(LV) – isometric LV contraction	**Sdb** – actual LV diastole
E(LV) – actual LV systole	**Ad1** – asynchronous period of ventricular diastole -1
F(RV) – RV systole	**Sd1** – period of synchronization of ventricular diastole-1
D(RV) – isometric RV contraction	←→ – PW phases; ○ – ZTEP: SS-VH-SVC-CS-RV-LA
E(RV) – actual RV systole	– dynamic curve ZTEP
G(AV) – opening of AV	– coincidence between ZTEP and IPPW
H(PV) – closing of PV	– integral curves (IC-1 and IC2)
G(TV) – opening of TV	– intersection of the integral curve with ZTEP of TV
H(AV) – closing of AV	– point of "stabilization" of ICs of the right and left parts of the heart
G(MV)– opening of MV	– CMIP
H(TV) – closing of TV	– intersection of CMIP with ZTEP (SS-RV и PT-SS)

We emphasize the presence in the metabolic component of the blood of parameters having, along with chemical properties, physical characteristics (volume, density, total pressure of gases, etc.), whose effects on hemodynamic parameters have been repeatedly cited.[4-6]

IC-1 and IC-2, given in Table 1, show the dynamics of the average pressure between the hemodynamic parameters of two extreme (consecutive) points (RA, RV, PT, etc. on the Norm Graphs[1-3]) relevant for each period of CC (Asd 1, Ss1, Ss2, etc.). CMIP is a graph of the calculated pressure between IC-1 and IC-2 passing synchronously each period and phase of CC in the right and left regions of the heart during the complete CC.

The formation of the vector of BB passing the complete CC (from entering RV to LVEF) is determined by the contraction of the myocardium and the creation of pressure gradients in the system of intracardiac valves (closing-opening) between the heart chambers.

We would like to note that the initial period of the BB formation inside RA occurs as a result of the inflow of hemodynamic flows to the heart from the whole body, which have metabolic characteristics that are expressed, among other indicators, in the variable physical characteristics (density and viscosity) of blood. We consider the possible direct pressure of BB located inside RV on baroreceptors of the SN, which is separated from the blood flow by only one endocardial layer (without muscular inclusions and elastic structures). The mechanism of autogeneration of the heart contractions, in our opinion, is realized by the TV closure wave (outrunning zeroing of the pressure gradient between RA and RV), including impulses of CS and the RA appendage, that (when TV is closing) forms a retrograde wave impulse propagating in the direction of SVC, topographically falling into the SN area.

Graphically (the Norm Graphs[1-3]), this is an ascending part of wave "a" of RA, corresponding to the P wave on the ECG (i.e. the onset of the RA systole passing into the contraction of the spongy part of RA [the RA appendage]), which in the final phase of the systole is isolated from the sinus part by border bundles,[7] and which corresponds to the PQ interval on the ECG. We believe that this mechanism (including the physical properties of the RA BB) can be a physical initiator of the electrical impulse in SN, which launches the work of the cardiac conduction system (autorun).

Thus, taking into account the autonomy of the myogenic nature of heart contractions[8] (the ability for long-term uninterrupted work of a denervated heart[9,10]), a pressor action of BB (inside RA) on SN can be considered as a factor affecting the autogeneration of electrical impulses in SN. In other words, BB inside RA—which is the informational result of the body's activity as a whole, whose thesaurus includes wave, hemodynamic, metabolic, and gas-dynamic information resources—can be the initiator of an impulse trigger (SN), which launches a synchronized sequence of heart activity phases.

In Table 2, the corresponding designations identify the sequence of two parallel complete CCs of BB from the entrance (RA) to the exit (LVEF) from the heart system. Column 1 (extreme calculation points) describes the synchronized sequence of the valve apparatus of the heart (opening-closing). Columns 2 and 3 describe the periods, phases, and vectors of the complete CC of the IC-1 curve (red) and the IC-2 curve (burgundy) with a step-by-step interval for BB movement, determined by the average pressure values of successive hemodynamic areas of the heart system and the vascular system (bypassing the exchange zones: lung, brain, etc.).

Thus, RV-PT-LA (during the actual RV systole, with closed TV and MV) determines the BB vector on the hemodynamic, metabolic and gas exchange pathways: RV-LA (represented by the average pressure curve at each point of RV and LA); Ao-SS-RV (during the period of actual RV diastole, with opened TV and closed MV) determines the BB vector after ejection from Ao at the nearest pressure recording point (SS) after passing through the pial chamber of the brain in the direction of RA-RV(transmission pressure).

In Table 2, all given calculated points and parameters of phase indicators are synchronized with each other, with the ECG and a graph of PPW. The initial data, which became the basis of logical and constructive calculations, were cited earlier by us in the Norm tables.[1-3,5,6]

IC-1 and IC-2, which were presented in Table 1, as well as a description of the synchronized sequences and vectors of these curves in Table 2, demonstrate the content of the complete CC, consisting of the dynamics of two BBs simultaneously passing through the hemodynamic heart-lung-heart systems. One BB completes the transformation, leaving the vascular bed in the structure of LVEF; the second BB is transferred to the system (lung) of further hemodynamic, gas and metabolic transformations, with subsequent metamorphoses in the left heart up to the ejection into Ao.

In other words, the complete CC of each BB of RA, which has the maximum information thesaurus of the body, including the hemodynamic feedback (information) from a previous cardiac output, consists of two completed CCs in the traditional sense (where each myocardial contraction equals one CC). We gave baseline data earlier in the form of tables and graphs.[1-3]

We believe that the stabilization point (Table 1) of the integral curves in ZTEP (TV and MV) determines: 1) the pressure dynamics of parallel processes in the right and left parts of the heart—a sequential launch of isometric contraction phases (the right side of the ventricular block after the left side); 2) the PT opening pressure level, synchronizing the work of the valve apparatus of the heart (opening-closing), the ventricular block as a whole and the formation of chambers (arterial and venous) of variable capacity. At the stabilization point (ZTEP of the heart valves) of both integral curves, the regulatory mechanism (synchronization) of the ventricular block function is initiated during the formation of spheroids of RVEF (current cycle) and LVEF (the end of the previous cycle). At the same time, the emerging LVEF participates in the formation of RVEF.

CMIP, which affects the formation of the RV spheroid, determines the pressure level at the trigger point (Table 1); after ZTEP (SS-VH-SVC-CS-RV-LA) is formed, PV opens and the spheroid is released (BB of RV) in the lung system. When pCMIPmax is reached, AV opens and the second spheroid (BB of LV) is released into the bloodstream, rushing towards the exchange

zones. We believe that CMIP is an integral hemodynamic indicator of the stability of homeostasis as a whole.

CMIP is a calculated parameter for the mean pressure values of the extreme values of IC-1 and IC-2, which generalizes the dynamics of two parallel current processes during the complete CC. CMIP is an indicator of the mutual hemodynamic influence of the arterial and venous BBs by changing the character of influence at the point of stabilization (Table 1). CMIP contains a total amount of information from all the hemodynamic flows that we studied and is a universal indicator of the quality and stability of the relationships of all the components of the hemodynamics of the vascular bed throughout the complete CC.

Changes in the configuration or level of CMIP is an indicator of imbalance and restructuring of the integral indicators of blood flow (therefore, components of hemodynamic flows), indicating a deviation in the parameters of stability of homeostasis as a whole. We consider the cardiovascular system as a single integrated system of inflow and outflow from the heart (quantum generator), which operates according to the trigger principle, where the regulation of the wave and metabolic processes occurs at the input (IVC, SVC, CS, RA) and output (LV, Ao) from the system. The regulation of processes (wave and metabolic) in SC begins at the exit from the ventricular block (Ao), with a phase change during CC in the arterial chamber of variable capacity (Ao, Ao-LV), and then by wave feedbacks through the zones of exchange of the venous part of the ventricular block, with phase changes of variable capacity (IVC, SVC-RA; IVC, SVC, CS, RA-RV) connected through a lung exchange zone with entrance into the arterial block of the heart (LA), forming a closed system of the information control and regulation of the heart and body as a whole.

In other words, the wave impulses generated by the heart are formed while taking into account the integral hemodynamic pressure at the inlet, intracardiac route, and exit from the working parts of the heart. We believe that the trigger point[2,3] and ZTEP[1-3] of the valvular apparatus of the heart play a leading role in the synchronization of these processes. We believe that the role of fibrous structures and ligamentous apparatus of the heart is very significant. For example, the ligamentum arteriosum, connecting the left pulmonary artery (54.2%) or the bifurcation of PT (41.1%) with the beginning of the downward section Ao (75.7%) outside the epicardium, has also the role of a two-way feedback channel between Ao and PT with periodic vector changes. The impact of the ligamentum arteriosum on BB hemodynamics, in our opinion, is realized by means of a transmission pressor-depressor (a change in the tension vector) interaction between Ao and PT with pressure changes: 1) in the trigger point at the opening of PV (venous BB); 2) in case of LV Ejection into Ao (arterial BB). In other words, it is one of the mechanisms of mutual regulation (two-way feedback) at both "exits" from the heart to both circulatory systems: SC and PC. For exchange zones, in addition to the main ZTEP (heart valves), there is a specific ZTEP for each organ, which allows for dual regulation (random filter): a systemic regulation at the input-output level of a trigger zone and more subtle regulation at the level of each exchange zone.

Thus, we believe that the cardiovascular system autonomously generates and controls the values of CMIP, a universal parameter, which regulates and reflects the synchronization of all wave impulses and hemodynamic flows, adequate to the metabolic needs of the body as a whole.

Table 2. Norm

Step-by-step calculation of the average on the gradient between the extreme points for each period (Asd1, Ss1, As1, Ss2, etc.) of CC

	Extreme points of CC	IC-1 (—)	IC-2 (—)
Cycle 1	H(MV) (closing of MV) – H(TV) (closing of TV)	Asd1(actual RV diastole) – ΔAO-RV	Asd1 (isometric LV contraction) – LV
	H(TV) (closing of TV) – G(PV) (opening of PV)	Ss1 (isometric RV contraction) – RV	Ss1 (isometric LV contraction) – LV
	G(PV) (opening of PV) – G(AV) (opening of AV)	Ac1 (actual RV systole) – ΔRV-LA	Ss1 (isometric LV contraction) – LV
	G(AV) (opening of AV) – H(PV) (closing of PV)	Ss2 (actual RV systole) – ΔRV-LA	Ss2 (actual LV systole) – ΔLV-RA
	H(PV) (closing of PV) – G(TV) (opening of TV)	Asd2a (isometric RV relaxation) – ΔPT-LA	Asd2a (actual LV systole) – ΔLV-RA
	G(TV) (opening of TV) – H(AV) (closing of AV)	Asd2b (actual RV diastole – ΔPT-LA	Asd2b (actual LV systole) – ΔLV-RV
	H(AV) (closing of AV) – G(MV) (opening of MV)	Sda (isometric LV relaxation) – ΔPT-LA	Sda (isometric LV relaxation) – ΔAo- RV
	G(MV) (opening of MV) – H(MV) (closing of MV)	Sdb (actual LV diastole) – ΔPT-LV	Sdb (actual LV diastole) – ΔAo- RV
Cycle 2	H(MV) (closing of MV) – H(TV) (closing of TV)	Asd1 (isometric LV contraction) – LV	Asd1 (actual RV diastole) – ΔAO-RV
	H(TV) (closing of TV) – G(PV) (opening of PV)	Ss1 (isometric LV contraction) – LV	Ss1 (isometric RV contraction) – RV
	G(PV) (opening of PV) – G(AV) (opening of AV)	Ss1 (isometric LV contraction) – LV	As1 (actual RV diastole) – ΔRV-LA
	G(AV) (opening of AV) – H(PV) (closing of PV)	Ss2 (actual LV systole) – ΔLV-RA	Ss2 (actual RV diastole) – ΔRV-LA
	H(PV) (closing of PV) – G(TV) (opening of TV)	Asd2a (actual LV systole) – ΔLV-RA	Asd2a (isometric RV relaxation) – ΔPT-LA
	G(TV) (opening of TV) – H(AV) (closing of AV)	Asd2b (actual LV systole) – ΔLV-RV	Asd2b (actual RV diastole) – ΔPT-LA
	H(AV) (closing of AV) – G(MV) (opening of MV)	Sda (isometric LV relaxation) – ΔAo-RV	Sda (isometric RV relaxation) – ΔPT-LA
	G(MV) (opening of MV) – H(MV) (closing of MV)	Sdb (actual LV diastole) – ΔAo- RV	Sdb (actual LV diastole) – ΔPT-LV

We believe that a high degree of similarity of graphic profiles and the coincidence of key points (max-min) PPW and CMIP throughout the complete cardiac cycle are significant for promising diagnostic technologies.

Conclusion

Complete cardiac cycle, during which BB from the venous substrate of RA is transformed into arterial LVEF, consists of two completed heart contraction cycles. Two BBs pass the hemodynamic route (heart - lung - heart) at the same time. One BB completes the transformation, leaving SC in the form of arterial LVEF; the second BB goes into PC in the form of a venous RVEF for further hemodynamic, metabolic and gas transformations until the new arterial ejection fraction of the next cycle is formed.

CMIP contains a total amount of information from all the hemodynamic flows that we studied and is a universal indicator of the quality and stability of the relationships of integral hemodynamic flows (IC-1; IC-2) throughout the complete cardiac cycle. Changes in the configuration or level of CMIP indicates a deviation in the parameters of stability of homeostasis as a whole.

References

1. Kruglov AG, Utkin VN, Vasilyev AY. Synchronization of Wave Flows of Arterial and Venous Blood with Phases of the Cardiac Cycle in Norm (Part 1). International Journal of Biomedicine. 2018;8(2):123-128.
2. Kruglov AG, Utkin VN, Vasilyev AY, Kruglov AA. Synchronization of Wave Flows of Arterial and Venous Blood and Phases of the Cardiac Cycle (Part 2). International Journal of Biomedicine. 2018;8(3):177-181.
3. Kruglov AG, Utkin VN, Vasilyev AY, Kruglov AA. Synchronization of Wave Flows of Arterial and Venous Blood and Phases of the Cardiac Cycle (Part 3). International Journal of Biomedicine. 2018;8(4):288-291.
4. Kruglov AG, Utkin VN, Vasilyev AY, Sherman VA. Human Homeostatic Control Matrix in Norm. International Journal of Biomedicine. 2016;6(3):184-9.
5. Kruglov AG, Gebel GY, Utkin VN, Vasilyev AY, Sherman V. Dynamic Networks of Human Homeostatic control in Norm (Part 1). International Journal of Biomedicine. 2016;6(2):101-105.
6. Kruglov AG, Gebel GY, Vasilyev AY. Impact of Intra-Extracranial Hemodynamics on Cerebral Ischemia by Arterial Hypertension (Part 2). International Journal of Biomedicine. 2012;2(2):96-101.
7. Yuden GG. Bundle structure of the **atrial myocardium**. Proceedings of the Smolensk Medical Institute. 1947;1:10-14.
8. Zavarzin AA, Rumyantsev AA. Course of Histology. 6th edition. M. Medgiz; 1946.
9. Donald DE, Shepherd JT. Initial Cardiovascular adjustment to exercise in dogs with chronic cardiac denervation. Am J Physiol. 1964;207:1325-9.
10. Donald DE, Shepherd JT. Sustained capacity for exercise in dogs after complete cardiac denervation. Am J Cardiol. 1964;14:853-9.

International Journal of Biomedicine 9(2) (2019) 111-116
http://dx.doi.org/10.21103/Article9(2)_OA5

ORIGINAL ARTICLE

Polar Medicine

INTERNATIONAL JOURNAL OF BIOMEDICINE

Relationships between Parameters of the Cardiovascular System, Salivary Lactoferrin Level and Body Temperature during a Short-Term Human Whole-Body Exposure to Cold Air

Liliya V. Poskotinova, PhD, ScD*; Elena V. Krivonogova, PhD; Olga V. Krivonogova; Denis B. Demin, PhD, ScD; Irina N. Gorenko; Elena V. Tipisova, PhD, ScD; Victoria A. Popkova, PhD; Alexandra E. Elfimova, PhD

N. Laverov Federal Center for Integrated Arctic Research FCIARctic of the RAS
Arkhangelsk, Russia

Abstract

Background: The problem of maintaining body temperature in people working outdoors in the cold air of the Arctic remains relevant. The purpose of this study was to determine the autonomic nervous mechanisms of regulation of heart rate (HR), blood pressure (BP) and local immunity on the example of the dynamics of salivary lactoferrin during a decrease in body temperature in humans (the core and the skin of hands) during and after a short-term, whole-body exposure to cold air.

Materials and Methods: A total of 15 healthy Russian male volunteers aged between18 and 20 years, born and living in Arkhangelsk, were examined in the winter. Research stages: the registration of indicators before exposure to the cold air (Stage 1), during the 10-minute exposure to the cold air at -20 °C (Stage 2) and after the 10-minute exposure to the cold air (Stage 3). The registration of indicators in Stages 1 and 3 was carried out indoors at an air temperature of +20 °C. HR (bpm), the heart rate variability (HRV) parameters, systolic and diastolic blood pressure (BPsyst, BPdiast, mm Hg), salivary lactoferrin level (ng/ml) by ELISA and the body temperature in the ear canal (Tear, °C) and on the skin of the dorsum of the right hand (Tskin, °C) were determined at each stage of the study.

Results: In Stage 2 with significantly decreased Tear and Tskin, compared to the initial indicators in Stage 1, there was a significant increase in HRV indices reflecting the overall HRV and vagal effects on the heart rhythm. At the same time, HR was significantly decreased, as well as stress index. An increase in the total power (TP) of the HRV spectrum was revealed due to a predominant increase in HF and VLF, and to a lesser degree in LF. Both BPsyst and BPdiast significantly increased. After cooling in Stage 3, HR increased, but remained significantly lower than the initial values. The overall HRV according to SDNN and TP decreased, reaching baseline values. Concentrations of salivary lactoferrin during cooling in Stage 2 tended to increase, which was also maintained in Step 3 after cooling. Correlation analysis in the entire sample (n=15) revealed a positive correlation between SDNN and Tear in Stage 2 (r_s=0.56, *P*=0.035). In Stage 2, the change in Tskin was significantly correlated with the salivary lactoferrin level (r_s=-0.73, *P*=0.003); this relationship was also found in Stage 3 (r_s=-0.65, *P*=0.015).

***Conclusion*:** The successful return of body temperature after general cooling occurs under the condition of increasing the overall HRV, enhancing vagal influences on the heart rhythm, HF and VLF components of HRV during cooling. An increase in the level of salivary lactoferrin, while maintaining vagal reserves of the vegetative regulation of heart rhythm against a decrease in skin temperature during general cooling, as well as a decrease in the level of lactoferrin against the background of recovery of body temperature after cooling, at least 10 minutes, can be regarded as an adaptive response of the body to exposure to cold temperature with minimal risk of cold inflammation. (**International Journal of Biomedicine. 2019;9(2):111-116.**)

Key Words: heart rate variability • heart rate • blood pressure • salivary lactoferrin • exposure to cold air

Abbreviations

BP, blood pressure; **CIG**, cardiointervalogram; **HR**, heart rate; **HRV**, heart rate variability; **SI**, stress index; **TP**, total power

Introduction

General cooling of the human body can cause different ratios between the activity of the neurophysiological mechanisms, sympathetic and parasympathetic regulation of heart rhythm and vascular tone,[1-4] and mobilization of the immune system in the form of enhanced pro- and anti-inflammatory reactions.[5] Against the background of general cooling, the core body temperature is maintained through increased vasoconstriction in peripheral tissues (skin, subcutaneous fat) and an increase in BP. It is believed that stable, increased BP in Arctic residents subjected to moderate exposure to cold (-10°C) may not lead to aggravation of symptoms of arterial hypertension, as this action reduces the body's sympathetic response to exposure to cold.[6] However, an excessive redistribution of blood supply, to the detriment of blood supply to peripheral tissues, can lead to impaired microcirculation, pronounced activation of inflammatory reactions up to necrosis of supercooled parts of the body. Thus, there is the problem of determining the physiological criteria for the balance of autonomic-immune relationships that maintain body temperature and the immune response under conditions of general cooling.

Lactoferrin in the biosubstrates of a living organism reflects the process of the acute phase of inflammation and activation of local nonspecific immunity—in particular, the activity of phagocytosis in response to the stress factor—and also regulates the effect of redistribution of immune competent cells during cooling.[7,8] The level of lactoferrin in biosubstrates is a marker of stress-induced modulation of congenital secretory immune response, the dynamics of which determines a person's susceptibility to infectious diseases under stress.[9] The consumption of food enriched with lactoferrin contributes to the preservation of the daily rhythm of the bioelectric structures of the brain under stress,[10] and its prior introduction into the tissues of the body reduces the stress-induced effects of cortisol.[8] When exercising on the background of high levels of cortisol, the level of lactoferrin may increase,[11] decrease[12] or remain stable[13]—which level occurs is associated with different stress-reactivity of the immune and autonomous nervous systems of the human body. Currently, the conditions of the optimal ratio of the parameters of the cardiovascular system and immune factors to maintain temperature of the corpus and peripheral tissues of the human body during experimental exposure to cold air have not been determined.

The purpose of this study was to determine the autonomic nervous mechanisms of regulation of HR, BP and local immunity on the example of the dynamics of salivary lactoferrin during a decrease in body temperature in humans (the core and the skin of hands) during and after a short-term, whole-body exposure to cold air.

Materials and Methods

A total of 15 healthy Russian male volunteers aged between18 and 20 years, born and living in Arkhangelsk, were examined in the winter. Volunteers had no signs of deficiency or excessive body weight (BMI of 18.5-25 kg/m^2), or fever (axillary temperature ≤37°C) and inflammatory processes of the oral cavity.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the FCIARctic Ethics Committee. Written informed consent was obtained from all participants.

The study stages were conducted in the morning between 9.30AM and 12.00AM and included the registration of indicators before (1), during (2) and after the 10-minute exposure to the cold air (3). Volunteers dressed in underwear, cotton trousers, winter boots and a cotton robe were examined in a sitting position at resting-state. The registration of indicators in Stages 1 and 3 was carried out indoors at an air temperature of +20 °C. The registration of indicators in Stage 2 was carried out in a cold chamber at -20 °C. Determining the body temperature in the ear canal (Tear, °C) and on the skin of the dorsum of the right hand (Tskin, °C) was performed using a B.Well WF-1000 medical electronic infrared thermometer (Switzerland) before entering the chamber (Stage 1), on the 10th minute of being in the chamber (Stage 2) and the 10th minute after leaving the chamber (Stage 3). The thermometer was installed in the ear canal and on the skin of the hand perpendicular to the surface of the body.

We calculated additional indicators that demonstrate the degree of preservation of body temperature during cooling in Stage 2 - Tear% and Tskin%.

Tear%=Tear(2)/Tear(1)×100%, where Tear (2) is the temperature value in the ear canal in Stage 2, Tear (1) is the temperature value in the right ear canal in Stage 1.

Tskin%=Tskin(2)/Tskin(1)×100%, where Tskin (2) is the temperature value on the skin of the dorsum of the right hand in Stage 2, Tskin (1) is the temperature value on the skin of the dorsum of the right hand in Stage 1.The high rates of Tear% and Tskin% indicated good preservation of body temperature when exposed to cold.

The registration of the cardiointervalograms (CIG) was performed during 5 minutes before entering the chamber (Stage 1), 10 minutes in the chamber (Stage 2) and 6-10 minutes (during 5 minutes) after leaving the chamber (Stage 3). For the possibility of CIG recording under cold room conditions, a one-lead electrocardiogram channel of the Neuron-Spectrum-SM device (Neurosoft, Russia), placed in a tank with thermal insulation, was used. Subsequently, CIGs of the last 5 minutes in the cold chamber and Stages 1 and 3 were processed using the Varicard instrument software (Ramena, Russia), and the HRV parameters were calculated.[14]

The following HRV parameters were evaluated: HR – heart rate, bpm; RMSSD (ms) – the root mean square differences of successive R-R intervals; SDNN (msec) – the standard deviation of the normal-to-normal RR intervals; SI (unit) – Stress Index, calculated by the formula [SI=Amo50/2×VAR×Mo, where Mo (ms) is the cardiointerval value dividing the CIG series in half, VAR – variation range between the minimum and maximum values in the CIG series, and Amo50,% –amplitude of mode – number of R-R intervals]; TP (ms^2) – Total Power, HF (ms^2) – high frequency power of HRV (0.15 to 0.40 Hz); LF (ms^2) – low-frequency power of HRV (0.04-0.155 Hz); VLF (ms^2) –

very low frequency power of HRV (0.0033–0.04 Hz).

With short CIG recordings (up to 5 min), the SDNN, RMSSD, TP indices reflect both the general reactivity of the vegetative regulation of the heart rhythm and the parasympathetic activity. The HF HRV power spectrum or respiratory band coincides with respiration as the parasympathetic nervous system operates using signaling mechanisms that can change HR in phase with respiration. The LF index shows baroreflex activity with the predominant participation of the sympathetic centers of vegetative regulation. Stress Index is associated with increased sympathetic activity. VLF index indicates the activity of humoral regulation of heart rhythm, the activity of thermogenesis, endothelial function and renin-angiotensin mechanism.[15,16]

BP (systolic – BPsyst and diastolic – BPdiast) was determined using an A&D medical device (Japan) before entering the chamber, immediately after leaving the chamber and in the 10th minute after leaving the chamber.

The saliva was collected on an empty stomach before entering the cold chamber, immediately after leaving the chamber and in the 10th minute after leaving the chamber. The collected samples of saliva were stored in eppendorfs at –20 °C, then immediately before analysis were thawed and centrifuged at 3,000 rpm for 15 minutes. Biological material was taken from the middle of the eppendorf for further analysis, without touching the walls and sediment at the bottom of the tube. The level of lactoferrin in saliva (ng/ml) was determined by ELISA using One-plate Fully Automated ELISA Analyzer (Elisys Uno; Human, Germany) and a commercial test kit (Hycult biotechnology b.v.; Netherlands) with a 20-fold dilution of samples using solution for dilution (Sample Dilution buffer), which is part of the test kit.

Statistical analysis was performed using the statistical software «STATISTICA 10». The normality of distribution of continuous variables was tested by Shapiro-Wilk's W test. Median (Me), interquartile range (IQR; 25th to 75th percentiles), and the 95% confidence interval (95% CI) were calculated. Spearman's rank correlation coefficient (r_s) was calculated to measure the strength and direction of the relationship between two variables. The Friedman Test was used to test for differences between 3 dependent samples. A probability value of $P<0.017$ was considered statistically significant.

Results

Table 1 presents the parameters of HRV, blood pressure, body temperature and salivary lactoferrin levels on the study stages. When exposed to cold in Stage 2, compared to the initial indicators in Stage 1, there was a significant increase in HRV indices reflecting the overall HRV and vagal effects on the heart rhythm: SDNN ($P_{1-2}<0.001$), RMSSD ($P_{1-2}<0.001$), and TP ($P_{1-2}=0.004$). At the same time, HR was significantly decreased ($P_{1-2}<0.001$), as well as SI ($P_{1-2}=0.004$). An increase in the TP of the HRV spectrum was revealed due to a predominant increase in HF ($P_{1-2}<0.001$) and VLF ($P_{1-2}=0.012$), and to a lesser degree in LF ($P_{1-2}=0.017$). Both BPsyst and BPdiast significantly increased ($P_{1-2}=0.005$). The temperature of Tear and Tskin decreased significantly with total cooling ($P_{1-2}<0.001$).

After cooling in Stage 3, HR increased ($P_{2-3}=0.001$), but remained significantly lower than the initial values ($P_{1-3}<0.001$). The overall HRV according to SDNN and TP decreased, reaching baseline values ($P_{2-3}<0.001$ and $P_{2-3}=0.003$, respectively). Indicators of RMSSD and HF, which characterize vagal activity, despite a significant decrease, remained elevated relative to baseline values ($P_{1-3}=0.010$ and $P_{1-3}=0.008$, respectively). Indicators of SI and LF, reflecting sympathetic activity, increased ($P_{2-3}=0.002$ and $P_{2-3}=0.009$, respectively) to the level of baseline values. VLF and BPsyst, despite the downward trend, had large intragroup differences; therefore, in general, they did not achieve a significant decrease by the 10th minute after exposure to cold ($P_{2-3}=0.069$ and $P_{2-3}=0.033$, respectively). BPdiast after exposure to cold significantly decreased to baseline values ($P_{2-3}<0.001$). The temperatures Tear and Tskin increased significantly after cooling; however, these values remained lower compared to background values ($P_{1-3}<0.001$).

Concentrations of salivary lactoferrin during cooling in Stage 2 tended to increase ($P>0.05$), which was also maintained in Step 3 after cooling. An individual analysis of baseline levels of lactoferrin revealed that 3 people had a significant excess of the value of 75th percentile (1203.6 ng/ml, 1482.6 ng/ml and 1805.6 ng/ml). In these 3 people during cooling, the dynamics of lactoferrin was found to be opposite to that of the whole group: a decrease in concentration instead of increasing. With the exclusion of these individuals from the sample (n=12), the increase in the level of lactoferrin was more pronounced—from 202.2 (41.8; 693.4) ng/ml to 1038.0 (257.6; 1934.0) ng/ml, $P=0.026$. In Stage 3, the lactoferrin level was almost unchanged - 1066.0 (91.2; 1787.2) ng/ml.

Correlation analysis in the entire sample (n=15) revealed a positive correlation between SDNN and Tear in Stage 2 ($r_s=0.56$, $P=0.035$). RMSSD and SDNN in Stage 2 correlated with Tear in Stage 3 ($r_s=0.57$, $P=0.035$; $r_s=0.61$, $P=0.020$, respectively). The HF, VLF and SI indicators in Stage 2 were also in correlation with Tear in Stage 3: $r_s=0.64$ ($P=0.014$), $r_s=0.62$ ($P=0.019$), and $r_s=-0, 60$ ($P=0.023$)), respectively.

Significant correlations of HRV indices and Tskin during cooling and after it were not revealed. However, Tskin dynamics was associated with changes in the level of salivary lactoferrin. In Stage 2, the change in Tskin was significantly correlated with the salivary lactoferrin level ($r_s=-0.73$, $P=0.003$); this relationship was also found in Stage 3 ($r_s=-0.65$, $P=0.015$). An inverse correlation was found between Tskin% and the salivary lactoferrin level in Stage 3 ($r_s=-0.72$, $P=0.008$).

The relationship between salivary lactoferrin levels and cardiovascular parameters during cooling was multidirectional. Exclusion from the group of 3 people with baseline high levels of lactoferrin (more than 75th percentile) allowed us to obtain significant correlations between these indicators in a sample of 12 people.

Thus, an increase in BPdiast was associated with an increase in lactoferrin level in Stage 2 ($r_s=0.61$, $P=0.047$). The remaining correlations reflected the relationship between

the lactoferrin dynamics upon cooling with baseline levels of HRV parameters. Thus, the level of lactoferrin in Stage 2 negatively correlated with the indicators of RMSSD, SDNN, and HF in Stage 1: r_s=-0.75 (P=0.007), r_s=- 0.65 (P=0.031), r_s=- 0, 64 (P=0.028), respectively.

Table 1.

Parameters of HRV, blood pressure, body temperature and salivary lactoferrin levels on the study stages (Me, IQR [P_{25}; P_{75}])

Variable	Stage 1	Stage 2	Stage 3	*P*-value
HR, bpm	74.5 (69.1;79.0)	59.0 (57.0;62.0)	65.4 (62.4;70.2)	P_{1-2}<0.001 P_{2-3}=0.001 P_{1-3}<0.001
SDNN, ms	53.8 (40.9;68.1)	82.8 (71.8;131.4)	51.78 (36.5;76.1)	P_{1-2}<0.001 P_{2-3}<0.001 P_{1-3}=0.070
RMSSD, ms	34.39 (22.79;48.48)	57.95 (47.99;82.01)	47.23 (28.24;56.43)	P_{1-2}<0.001 P_{2-3}=0.009 P_{1-3}=0.010
Stress Index, unit	84.2 (54.0;177.9)	39.2 (17.6;56.2)	76.33 (41.76;142.77)	P_{1-2}=0.002 P_{2-3}=0.002 P_{1-3}=0.191
TP, ×1000, ms^2	2.75 (1.46;3.85)	4.88 (2.97;13.9)	2.85 (1.80;4.61)	P_{1-2}=0.004 P_{2-3}=0.003 P_{1-3}=0.232
HF, ×1000, ms^2	0.42 (0.22;0.84)	1.49 (0.85;2.02)	0.76 (0.38;1.23)	P_{1-2}<0.001 P_{2-3}=0.001 P_{1-3}=0.008
LF, ×1000, ms^2	1.11 (0.66;1.79)	1.52 (0.84;4.89)	1.17 (0.67;1.62)	P_{1-2}=0.017 P_{2-3}=0.009 P_{1-3}=0.776
VLF, ×1000, ms^2	0.39 (0.26;0.85)	0.71 (0.43;2.77)	0.64 (0.35;0.80)	P_{1-2}=0.012 P_{2-3}=0.069 P_{1-3}=0.256
BPsyst, mm Hg	122.0 (117.0;130.0)	132.0 (123.0;138.0)	125.0 (120;130)	P_{1-2}=0.005 P_{2-3}=0.033 P_{1-3}=0.781
BPdiast, mm Hg	83.0 (81.0;86.0)	92.0 (85.0;102.0)	89.0 (78.0;90)	P_{1-2}=0.005 P_{2-3}<0.001 P_{1-3}=0.285
Tear, ^{0}C	36.3 (36.1;36.4)	34.7 (33.8;35.4)	35.6 (35.5;36.1)	P_{1-2}<0.001 P_{2-3}=0.001 P_{1-3}<0.001
Tskin,^{0}C	33.0 (27.0;35.7)	19.1 (17.0;20.8)	23.9 (22.8;26.7)	P_{1-2}<0.001 P_{2-3}<0.001 P_{1-3}<0.001
Lactoferrin, ng/ml	496.0 (90.0;917.4)	910.7 (257.6;1867.8)	1081.3 (148.0;1765.2)	P_{1-2}=0.157 P_{2-3}=0.753 P_{1-3}=0.116

Discussion

Increased blood pressure, especially BPdiast, is a natural reaction of the body in response to vasoconstriction in peripheral tissues, wherein the processes of thermogenesis, the renin-angiotensin mechanism, and endothelial function are activated. This was reflected in our study in an increase in the VLF component of HRV.[15,16] The enhancement of the HRV VLF component during cooling reflected the adaptive response of the body, confirmed by the correlation analysis: the higher VLF was during cooling, the stronger trend towards the original body temperature values was found 10 minutes after leaving the cold chamber. In response to an increase in blood pressure, the arterial baroreflex was activated, which led to a decrease in HR. An increase in the LF component of HRV has traditionally been associated with the activation of the baroreflex,[6,15] but in our study there was no significant correlation between this part of HRV and body temperature indices either during cooling or after it. At the same time, an increase in HF components of HRV during cooling is associated with an increase in body temperature at the warming stage after exposure to cold. This may indicate that the frequency range of the arterial baroreflex is consistent with the HF component of HRV (0.15-0.40 Hz) during cold exposure, which reflects the activation of respiration during hypoxia.[17] The results obtained for changes in the cardiovascular system with general air cooling are in tune with the results of studying a different type of cold exposure in the form of an isolated face cooling, when BP rises and HR decreases.[1] However, Hodjes et al., with a general human cooling at a temperature of 0°C for 30 minutes, found an increase in the LF and HF components, as well as RMSSD against the background of an increased HR.[18] These results show clearly that neurovegetative changes in humans depend on the level of temperature and the duration of cold temperature exposure. The vegetative manifestations we observed during general cooling differed from changes in HRV during local cooling of the head when an increase in sympathetic activity was observed against the background of a decrease in total HRV.[19] We observed no increase in vagal activity in 3 people with a high baseline level of lactoferrin (more than 75th percentile), in which SI was either elevated or did not significantly change against the background of stable values during the whole observation. A decrease in the sympathetic pressor response and an activation of the parasympathetic response to the cold is regarded as an adaptive response to cold in both the Aborigines and the newcomers of the Arctic.[1] Thus, the successful return of body temperature after general cooling occurs under the condition of increasing the overall HRV, enhancing vagal influences on the heart rhythm, HF and VLF components of HRV during cooling.

A moderate increase in the level of lactoferrin as a marker of the secretory response in stress-induced activation of local immunity is recognized as logical. Thus, Bosch et al.[9] revealed an increase in the lactoferrin secretion against the background of increased vagal activity, according to HRV during stress with low emotionality and against the background of increased sympathetic activity during stress with high emotionality among volunteers. However, with a pronounced sympathoadrenal stress reaction, the level of lactoferrin may decrease.[13] In our study, the presence of a significant correlation between skin temperature and lactoferrin level reflects the importance of transferrin system activation with

a moderate baseline as a response to the pro-inflammatory effect of cold hypoxia of peripheral tissues. The correlation of lactoferrin and BPdiast suggests that the greater BPdiast due to cold vasoconstriction, the more pronounced the oxidative processes due to cold hypoxia in the tissues. Moreover, if the skin temperature fell lower and BPdiast increased, the increase in salivary lactoferrin was more pronounced, which reflected the intensity of oxidative processes in the tissues, not only during cooling, but also in the 10th minute of warming. Data of correlation analysis showed that pronounced vagal activity in humans before exposure to cold contributes to repression of the level of growth of lactoferrin, reflecting the activity of local immune responses during exposure to cold. Stress-protective effects of lactoferrin administration before stress are known, in particular, the severity of inflammation decreases with subsequent exposure to stress.[20] However, the presence of marginal high basic values of salivary lactoferrin may reflect a pronounced activity of oxidative processes during the development of inflammation in the tissues, which does not allow an adequate vascular and immune response to develop with subsequent exposure to cold and to ensure the preservation of body temperature. Therefore, the focus of vegetative and immune reactions in individuals with a high baseline lactoferrin level (more than 75th percentile)in terms of adaptation and/or maladaptation at low temperatures needs further special study.

Thus, the maintenance of body temperature during short-term (10 min) general air cooling at -20 °C is ensured by the activation of vagal influences in combination with the enhancement of suprasegmental regulatory influences on the heart rhythm. An increase in the level of salivary lactoferrin, while maintaining vagal reserves of the vegetative regulation of heart rhythm against a decrease in skin temperature during general cooling, as well as a decrease in the level of lactoferrin against the background of recovery of body temperature after cooling, at least 10 minutes, can be regarded as an adaptive response of the body to exposure to cold temperature with minimal risk of cold inflammation. The data obtained reflect the need to use health-saving technologies to enhance vagal influences on the heart rhythm to restrain the pressor response of the sympathetic nervous system, maintain body temperature in cold conditions and minimize the cold-dependent inflammatory reactions in peripheral tissues.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

The reported study was funded by the FCIARctic according to the research project No 115050610141.

Acknowledgments

We would like to thank M.A. Ovsyankina, L.K. Dobrodeeva, and A.V. Samodova for their assistance in this study.

References

1. Le Blanc J, Dulac S, Cote J, Girard B. Autonomic nervous system and adaptation to cold in man. J ApplPhysiol. 1975;39(2):181-6. doi:10.1152/jappl.1975.39.2.181.
2. Demin DB, Poskotinova LV, Krivonogova EV. [Variants of neurophysiological reactions during short-term experimental hypothermia in humans]. J Biomedical Radioelectronics. 2018;12:48-50. doi: 10.18127/j15604136-201812-06. [Article in Russian].
3. Mäkinen TM, Mäntysaari M, Pääkkönen T, Jokelainen J, Palinkas LA, Hassi J, Leppäluoto J, Tahvanainen K, Rintamäki H. Autonomic nervous function during whole-body cold exposure before and after cold acclimation. Aviat Space Environ Med. 2008;79(9):875-82.
4. Peng RC, Yan WR, Zhou XL, Zhang NL, Lin WH, Zhang YT. Time-frequency analysis of heart rate variability during the cold pressor test using a time-varying autoregressive model. Physiol Meas. 2015 Mar;36(3):441-52. doi: 10.1088/0967-3334/36/3/441.
5. Patrakeeva VP, Basova EE. Effects of Low Temperatures on the Formation of Adaptive Reactions: A Review. International Journal of Biomedicine. 2018;8(2):95-101. doi: 10.21103/Article8(2)_RA1.
6. Hintsala HE, Kiviniemi AM, Tulppo MP, Helakari H, Rintamämaki H, Mäntysaari M, et al. Hypertension Does Not Alter the Increase in Cardiac Baroreflex Sensitivity Caused by Moderate Cold Exposure. Front Physiol. 2016;7:204. doi: 10.3389/fphys.2016.00204.
7. Kuznetsov IA, Potiyevskaya VI, Kachanov IV, Kuraleva OO. [Laktoferrin's role in biological circles of the person. Modern problems of sciences and education]. 2017:3 [Electronic source] http://www.science-education.ru/ru/article/view?id=26522. [Article in Russian].
8. Aleshina GM, Yankelevich IA, Zhakharova ET, Kokryakov VN. [Stress-protective effect of human lactoferrin]. Ross Fiziol Zh Im I M Sechenova. 2016;102(7):846-51. [Article in Russian].
9. Bosch JA, de Geus EJ, Veerman EC, Hoogstratan J, Nieuw Amerongen AV. Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. Psychosom Med. 2003;65(2):245-58.
10. Thompson RS, Roller R, Mika A, Greenwood BN, Knight R, Chichlowski M, et al. Dietary Prebiotics and Bioactive Milk Fractions Improve NREM Sleep, Enhance REM Sleep Rebound and Attenuate the Stress-Induced Decrease in Diurnal Temperature and Gut Microbial Alpha Diversity. Front Behav Neurosci. 2017;10:240. doi: 10.3389/fnbeh.2016.00240.
11. Li CY, Hsu GS, Suzuki K, Ko MH, Fang SH. Salivary Immuno Factors, Cortisol and Testosterone Responses in Athletes of a Competitive 5,000 m Race. Chin J Physiol. 2015; 58(4):263-9. doi: 10.4077/CJP.2015.BAE367.
12. He CS, Tsai ML, Ko MH, Chang CK, Fang SH. Relationships among salivary immunoglobulin A, lactoferrin and cortisol in basketball players during a basketball season. Eur J Appl Physiol. 2010;110(5):989-95. doi: 10.1007/s00421-010-1574-8.

***Corresponding author:** *Liliya V. Poskotinova, PhD, ScD. Department of Biorhythmology of N. Laverov Federal Center for Integrated Arctic Research FCIARctic of the RAS, Arkhangelsk, Russia. E-mail: liliya200572@mail.ru*

13. Svendsen IS, Hem E, Gleeson M. Effect of acute exercise and hypoxia on markers of systemic and mucosal immunity. Eur J Appl Physiol. 2016;116(6):1219-29. doi: 10.1007/s00421-016-3380-4.
14. Malik M, Bigger J, Camm A, Kleiger R, Malliani A, Moss AJ, Schwartz PJ. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology: Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Eur Heart J. 1996;17(3):354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868.
15. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. Front Public Health. 2017;5:258. doi: 10.3389/fpubh.2017.00258.
16. Millis RM, Austin RE, Hatcher MD, Bond V, Goring KL. Metabolic energy correlates of heart rate variability spectral power associated with a 900-calorie challenge. J Nutr Metab. 2011;2011:715361. doi: 10.1155/2011/715361.
17. Kazimierska A, Placek MM, Uryga A, Wachel P, Burzyrnska M, Kasprowicz M. Assessment of Baroreflex Sensitivity Using Time-Frequency Analysis during Postural Change and Hypercapnia. Comput Math Methods Med. 2019:4875231 doi.org/10.1155/2019/4875231.
18. Hodges GJ, Ferguson SAH, Cheung SS. Cardiac autonomic function during hypothermia and its measurement repeatability. Appl Physiol Nutr Metab. 2019;44(1):31-36. doi: 10.1139/apnm-2018-0248
19. Wang JJ, Chen CC. Study of the effect of short-time cold stress on heart rate variability. In: Lim CT, Goh JC (eds) 13th International Conference on Biomedical Engineering. IFMBE Proceedings. 2009: 23: 490-492. doi.org/10.1007/978-3-540-92841-6_120.
20. Shinjo T, Sakuraba K, Nakaniida A, Ishibashi T, Kobayashi M, Aono Y, Suzuki Y. Oral lactoferrin influences psychological stress in humans: A single-dose administration crossover study. Biomed Rep. 2018;8(5):426-432. doi: 10.3892/br.2018.1076.

International Journal of Biomedicine 9(2) (2019) 117-120
http://dx.doi.org/10.21103/Article9(2)_OA6

ORIGINAL ARTICLE

Pulmonology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Clinical Phenotype "Obesity-Asthma" as One of the Main Problems of Personalized Pulmonology

Ludmila V. Tribuntceva, PhD[1]; Yanina S. Shkatova[1]; Sergey N. Avdeev, PhD, ScD[2]; Andrey V. Budnevsky, PhD, ScD[1]; Evgeniy S. Ovsyannikov, PhD[1*]

[1]*Voronezh State Medical University named after N.N. Burdenko, Voronezh, Russia*
[2]*I. M. Sechenov First Moscow State Medical Univesity, Moscow, Russia*

Abstract

Background: Asthma is a heterogeneous disease comprising different phenotypes. One of the most common asthma phenotypes is the obesity-asthma phenotype, since obesity affects over a third of the world's population today. It is important to continue investigating possible underlying mechanisms of the interaction between asthma and obesity. ***The purpose*** of this study was to evaluate levels of leptin, adiponectin, neuropeptide Y (NPY), total oxidative damage (TOD), and total antioxidant status (TAS) in patients with asthma and different body weight (BW), and to analyze their association with spirometry parameters.

***Materials and Methods*:** The study included 27 men and 86 women diagnosed with moderate asthma (mean age of 57.81±13.05 years). During the study, all asthma patients were divided into 3 groups. Group 1 included 37 patients with normal BW, Group 2 included 38 overweight patients, and Group 3 included 38 patients with obesity. We analyzed complaints, anamnesis data, objective status data, and laboratory (blood levels of NPY, adiponectin, leptin, total antioxidant status, and total oxidative damage) and instrumental data (spirometry). Two questionnaires were used: Asthma Quality of Life Questionnaire (AQLQ) and Asthma Control Test (ACT).

***Results*:** The leptin level was significantly higher in Group 3 compared to Groups 1 and 2 (P=0.000). The NPY level was significantly lower in Group 1 compared to Groups 2 and 3 (P=0.000). The TAS value was significantly higher in Group 1 compared to Groups 2 and 3 (P=0.000) and the TOD value in Group 1 was significantly lower than in Group 3 (P=0.038).The leptin level positively correlated with BMI and waist circumference, and had an inverse correlation with FEV_1 and vital capacity (VC). The adiponectin level had a positive correlation with the Tiffno index, FEF_{50}, and peak expiratory flow (PEF). The NPY level had an inverse correlation with VC, FEV_1, FEF_{25}, FVC, Tiffno index, FEF_{50}, and PEF.

***Conclusion*:** The severity of the clinical course of moderate asthma in obese patients is associated with different factors, including oxidative stress and levels of leptin, adiponectin and NPY. (**International Journal of Biomedicine. 2019;9(2):117-120.**)

Key Words: asthma • body weight• oxidative stress • leptin • adiponectin • neuropeptide Y

Abbreviations

BMI, body mass index; **BW**, body weight; **FEV_1**, forced expiratory volume in 1 sec; **FEF**, forced expiratory flow; **FVC**, forced vital capacity; **NPY**, neuropeptide Y; **OS**, oxidative stress; **PEF**, peak expiratory flow; **TAS**, total antioxidant status; **TOD**, total oxidative damage; **VC**, vital capacity; **WC**, waist circumference.

Introduction

According to the Global Initiative for Asthma (GINA), asthma is a serious disease, which affects around 300 million people worldwide. One of the most important factors for clinicians is that asthma is a heterogeneous disease. It is the heterogeneity of the disease that dictates the need for the most individualized treatment.[1,2] The heterogeneity of both clinical manifestations of asthma and response to therapy is considered nowadays.[3,4] We distinguish many asthma phenotypes, and even subphenotypes, based on those factors.[5-7] Currently, the search for special pathological and molecular features of asthma phenotypes continues, which can become the basis for the development of individual therapy. One of

***Corresponding author**: Evgeniy S. Ovsyannikov, PhD. Voronezh State Medical University named after N.N. Burdenko. Voronezh, Russia. E-mail: ovses@yandex.ru.*

the most common asthma phenotypes is the obesity-asthma phenotype, since obesity affects over a third of the world's population today. It is also estimated that the prevalence of obesity will continue to grow and by 2030, about 38% of the world's adult population will be overweight and 20% will be obese.[8] Considering all of the above, it is important to continue investigating possible underlying mechanisms of the interaction between asthma and obesity. Adiponectin, leptin, OS, and NPY remain relevant in this field of study.

The purpose of this study was to evaluate levels of leptin, adiponectin, NPY, TOD, and TAS in patients with asthma and different BW, and to analyze their association with spirometry parameters.

Materials and Methods

The study included 27 men and 86 women diagnosed with moderate asthma aged from 18 to 75 years (mean age of 57.81±13.05 years).

The study was approved by the Ethics Committee of Voronezh State Medical University named after N.N. Burdenko. (Protocol № 1 from February 21, 2018). Written informed consent was obtained from each patient.

Exclusion criteria were patients' refusal to participate in this study; asthma exacerbation; acute and chronic neurological, psychiatric and endocrinological disorders at the time of examination; chronic diseases in the acute stage; severe and decompensated diseases of liver and kidneys; severe and decompensated cardiovascular diseases (acute period of myocardial infarction, unstable angina, transient ischemic attack, intracerebral hemorrhage, acute heart failure, etc.); cancer; multiple organ failure of different genesis; cancer; pregnancy and lactation; severe infectious diseases.

The asthma diagnosis was based on the integral assessment of symptoms, medical history, health status, and spirometry values according to the Global Strategy for Asthma Management and Prevention.[9]

We analyzed complaints, anamnesis data, objective status data, and laboratory and instrumental data (spirometry with a 400-mg salbutamol test), and we measured levels of NPY, adiponectin, leptin, total antioxidant status, and total oxidative damage.

The levels of leptin and adiponectin were measured using the appropriate reagent kits for quantitative determination of leptin and adiponectin in serum. The determination of TOD was carried out with use of a reagent kit to determine the degree of TOD to biological molecules (PerOx (TOS) (Oxidative Capacity)). We determined the overall antioxidant status by using reagents for determining TAS (ImAnOx (TAS) (Antioxidative Capacity)). NPY levels were measured with a reagent kit for NPY determination in serum.

Two questionnaires were used in this study: Asthma Quality of Life Questionnaire (AQLQ) and Asthma Control Test (ACT). All patients received standard asthma therapy.

All data was evaluated with STATGRAPHICS Plus 5.1. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Student's unpaired t-test was used to compare two groups for data with normal distribution. Group comparisons with respect to categorical variables are performed using the Chi-square test. Multiple comparisons were performed with one-way ANOVA and *Tukey's* HSD Post-hoc Test. Pearson's correlation coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A probability value of $P<0.05$ was considered statistically significant.

Results

During the study, all asthma patients were divided into 3 groups. Group 1 included 37 patients with normal BW, Group 2 included 38 overweight patients, and Group 3 included 38 patients with obesity. The three groups did not differ with regard to age and sex (Table 1).

Table 1.

General characteristics of patients

Parameters	Group 1 (n=37)	Group 2 (n=38)	Group 3 (n=38)
Women, n (%) Men, n (%)	12 (32.4) 25 (67.6)	8 (21.1) 30 (78.9)	7 (18.4) 31 (81.6)
	$P>0.05$		
Age. years	54.43±16.93	58.68±9.94	60.21±10.96
	F=0.0535; $P=0.9480$		
Higher education, n (%) Specialized secondary education, n (%) Secondary education, n (%)	25 (67.6) 8 (21.6) 4 (10.8)	16 (42.1) 14 (36.8) 8 (21.1)	9 (23.7) 22 (57.9) 7 (18.4)
	$\chi^2=15.875$; $P=0.003$		
Married, n (%) Single, n (%)	13 (35.1) 24 (64.9)	6 (15.8) 32 (84.2)	22 (57.9) 16 (42.1)
	$\chi^2=14.602$; $P=0.0007$		

Laboratory test results are presented in Table 2. The leptin level was significantly higher in Group 3 compared to Groups 1 and 2 ($P=0.0000$). No statistically significant differences in adiponectin levels were found in the studied groups. The NPY level was significantly lower in Group 1 compared to Groups 2 and 3 ($P=0.0000$). The TAS value was significantly higher in Group 1 compared to Groups 2 and 3 ($P=0.0000$). The value of total oxidative damage in Group 1 was significantly lower than in Group 3 ($P=0.0382$).

Correlation coefficients between laboratory and spirometry data are presented in Table 3. The leptin level positively correlated with BMI r=0.56; $P<0.05$) and WC (r=0.42; $P<0.05$), and had an inverse correlation with FEV_1 (r=-0.28; $P<0.05$) and VC (r=-0.29; $P<0.05$). The adiponectin level had a positive correlation with the Tiffno index (r=0.51; $P<0.05$), FEF_{50} (r=0.37; $P<0.05$), and PEF r=0.33; $P<0.05$). The NPY level had an inverse correlation with VC (r=-0.75; $P<0.05$), FEV_1(r=-0.57; $P<0.05$), FEF_{25} (r=-0.53; $P<0.05$), FVC (r=-0.45; $P<0.05$), Tiffno index (r=-0.32; $P<0.05$), FEF_{50} (r=-0.41; $P<0.05$), and PEF(r=-0.38; $P<0.05$).

Table 2.

Laboratory test results in the studied groups

Parameters	Group 1	Group 2	Group 3	Statistics
Leptin, ng/ml	13.01±1.97	11.32±1.99	22.36±1.97	F=343.0272 *P*=0.0000 P_{1-2}=0.0010 P_{1-3}=0.0000 P_{2-3}=0.0000
NPY, ng/ml	0.31±0.02	0.48±0.02	1.19±0.25	F=386.0549 *P*=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{2-3}=0.0000
TAS, μmol/l	535.78±64.35	277.59±63.49	287.96±63.49	F=196.0883 *P*=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{2-3}=0.7588
TOD, μmol/l	877.70±623.33	1177.75±1022.51	1454.69±1257.72	F=3.0876 *P*=0.0496 P_{1-2}=0.4026 P_{1-3}=0.0382 P_{2-3}=0.4553
Adiponectin, μg/ml	23.66±11.03	23.40±11.29	23.70±10.25	F=0.0085 *P*=0.9915

Table 3.

Correlation coefficients between laboratory and spirometry data

Parameters	FEV1	FVC	VC	Tiffno	FEF 25%	FEF 50%	FEF 75%	PEF	BMI	WC
Adiponectin	0.04	-0.08	-0.07	0.51*	0.05	0.37*	0.24*	0.33*	-0.06	0.12
Leptin	-0.28*	-0.25	-0.29*	-0.18	-0.11	-0.23	-0.15*	-0.15*	0.56*	0.42*
NPY	-0.57*	-0.45*	-0.75*	-0.32*	-0.53*	-0.41*	-0.18	-0.38*	0.20	0.05
TAS	-0.03	0.43*	0.36*	-0.40*	0.00	-0.33	-0.42*	-0.36*	-0.32*	-0.15
TOD	0.23	0.22	0.07	-0.43*	0.18	-0.08	0.04	-0.01	0.50*	0.48*

** -P-value <0.05.*

Discussion

Our study showed that levels of leptin, NPY and TOD were significantly higher and the TAS level was significantly lower in asthmatic patients with obesity compared with asthmatic patients who were overweight or had normal body weight, indicating inflammatory activity. According to GINA (2019), obesity is a state of chronic low-grade inflammation with increased adipocyte-driven proinflammatory activity. Adipose tissue cells secrete adipokines, and the role of such adipokines as leptin and adiponectin in asthma continues to be investigated. Studies show that there is a correlation between BW and levels of leptin, even though there is also a gender difference: women with the same BMI as men have higher concentrations of leptin.[10] Leptin is a proinflammatory cytokine and might be one of the contributing factors to the higher prevalence of asthma in obese patients. It stimulates release of NO, IL-6 and IL-8 and TNF-α.[6] At the same time, an experimental study on mice found that administration of TNF-α led to an increase of leptin levels.[11] Higher levels of leptin are associated with asthma; this association is stronger in women than men, and more pronounced in premenopausal women than in postmenopausal women.[11]

On the other hand, adiponectin plays an opposite role, inhibiting inflammation. Our study also showed that adiponectin functions as an inhibitor of inflammation in asthma, since it had a positive correlation with spirometry parameters. The data about the association between asthma and adiponectin are contradictory. Some studies suggested that low levels of adiponectin are associated with greater odds for asthma in women,[12,13] while others deny any correlation at all.[14,15] Other studies report that higher levels of adiponectin correlate with milder asthma, but only in women, while in men this association is the opposite: asthma that required more frequent use of medication was associated with higher adiponectin levels.[16,17] There was also a study that analyzed serum adiponectin levels and expression of adiponectin mRNA in abdominal adipose tissue in obese patients. Patients with obesity and asthma had lower adiponectin mRNA expression than obese patients without asthma, but levels of serum adiponectin did not differ significantly in these groups.[18] There is also information about decreased levels of serum adiponectin, but only during exacerbations in asthma patients.[19] These data invoke a thought that a decreased level of adiponectin might be not the predictor, but the consequence of asthma's clinical course.

OS plays an important role in asthma. According to the latest research, biomarkers of OS are higher in patients with obesity and correlate with BMI, [20] and at the same time antioxidant defense biomarkers have an inverse correlation

with BW.[21] In our study, we observed the same association in asthmatic patients with different BW.

There are limited studies on the association between NPY and asthma. A few studies report that certain genotypes of NPY are associated with asthma.[22,23] Y1 receptors of NPY play an important role in allergic inflammation of respiratory airways.[24] One study also reports that during asthma exacerbations, levels of NPY increase.[25] In our study, levels of NPY were significantly higher in obese and overweight asthmatics and had an inverse correlation with spirometry parameters, indicating a negative effect on the clinical course of asthma.

In conclusion, it can be said, that the severity of the clinical course of asthma in obese patients is associated with different factors, including OS and levels of leptin, adiponectin and NPY. Exact mechanisms remain unclear. Also in this study, we included only patients with moderate asthma, did not analyze gender differences, and did not differentiate between women of premenopausal and postmenopausal age. Further research is required.

Competing Interests

The authors declare that they have no competing interests.

References

1. Budnevsky AV, Tribuntceva LV, Kozhevnikova SA, Ovsyannikov ES. Impact of metabolic syndrome components on asthma control and life quality of patients. International Journal of Biomedicine. 2018;8(1):33-36. doi: 10.21103/article8(1)_OA4.
2. Budnevsky AV, Malysh EY, Ovsyannikov ES, Drobysheva ES. [Asthma and metabolic syndrome: Clinical and pathogenetic relationships]. Ter Arkh. 2015;87(10):110-114. doi: 10.17116/terarkh20158710110-114. [Article in Russian].
3. Tsvetikova LN, Budnevsky AV, Ovsyannikov ES, Kudashova EA. [Melatonin: Possibilities for use in the treatment of asthma]. Ter Arkh. 2017;89(3):112-115. doi: 10.17116/terarkh2017893112-115. [Article in Russian].
4. Provotorov VM, Budnevsky AV, Filatova YI. [Clinical manifestations of asthma during combination therapy using ceruloplasmin]. Ter Arkh. 2016;88(3):36-39. doi: 10.17116/terarkh201688336-39. [Article in Russian].
5. Hirose M, Horiguchi T. Asthma phenotypes. J Gen Fam Med. 2017;18(5):189-194. doi: 10.1002/jgf2.7.
6. Matsusaka M, Fukunaga K, Kabata H, Izuhara K, Asano K, Betsuyaku T. Subphenotypes of type 2 severe asthma in adults. J Allergy Clin Immunol Pract. 2018;6(1):274-276.e2. doi: 10.1016/j.jaip.2017.06.015.
7. Zacharias J, Ishmael FT, Henao M, Rael EL. Identification of Sub-Phenotypes of Eosinophilic Asthma: Eosinophilic Asthma Is Not Always Allergic Asthma. The Journal of Allergy and Clinical Immunology. 2017;139(2):AB170. doi: 10.1016/j.jaci.2016.12.557.
8. Hruby A, Hu FB.The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics. 2015;33(7):673-89. doi: 10.1007/s40273-014-0243-x.
9. Global strategy for asthma management and prevention (GINA, 2017 REPORT). Available from: file:///C:/Users/Admin/Downloads/wmsGINA-2017-main-report-final_V2%20(1).pdf
10. Ruhl CE, Everhart JE. Leptin concentrations in the United States: relations with demographic and anthropometric measures. Am J Clin Nutr. 2001;74(3):295-301.doi: 10.1093/ajcn/74.3.295.
11. Vuolteenaho K, Koskinen A, Kukkonen M, Nieminen R, Päivärinta U, Moilanen T, et al. Leptin enhances synthesis of proinflammatory mediators in human osteoarthritic cartilage--mediator role of NO in leptin-induced PGE2, IL-6, and IL-8 production. Mediators Inflamm. 2009;2009:345838. doi: 10.1155/2009/345838.
12. Sood A, Ford ES, Camargo CA Jr. Association between leptin and asthma in adults. Thorax. 2006;61(4):300-5. doi: 10.1136/thx.2004.031468.
13. Sood A, Cui X, Qualls C, Beckett WS, Gross MD, Steffes MW, et al. Association between asthma and serum adiponectin concentration in women. Thorax. 2008;63(10):877-82. doi: 10.1136/thx.2007.090803.
14. Nagel G, Koenig W, Rapp K, Wabitsch M, Zoellner I, Weiland SK. Associations of adipokines with asthma, rhinoconjunctivitis, and eczema in German schoolchildren. Pediatr Allergy Immunol. 2009;20(1):81-8. doi: 10.1111/j.1399-3038.2008.00740.x.
15. Jartti T, Saarikoski L, Jartti L, Lisinen I, Jula A, Huupponen R, et al. Obesity, adipokines and asthma. Allergy. 2009;64(5):770-7. doi: 10.1111/j.1398-9995.2008.01872.x.
16. Sutherland TJ, Sears MR, McLachlan CR, Poulton R, Hancox RJ. Leptin, adiponectin, and asthma: findings from a population-based cohort study. Ann Allergy Asthma Immunol. 2009;103(2):101-7. doi: 10.1016/S1081-1206(10)60161-5.
17. Sood A, Dominic E, Qualls C, Steffes MW, Thyagarajan B, Smith LJ, et al. Serum Adiponectin is Associated with Adverse Outcomes of Asthma in Men but Not in Women. Front Pharmacol. 2011;2:55. doi:10.3389/fphar.2011.00055.
18. Tsaroucha A, Daniil Z, Malli F, Georgoulias P, Minas M, Kostikas K, et al. Leptin, adiponectin, and ghrelin levels in female patients with asthma during stable and exacerbation periods. J Asthma.2013;50(2):188-97.doi:10.3109/02770903.2012.747101.
19. Sideleva O, Suratt BT, Black KE, Tharp WG, Pratley RE, Forgione P, et al. Obesity and asthma: an inflammatory disease of adipose tissue not the airway. Am J Respir Crit Care Med. 2012;186(7):598-605. doi: 10.1164/rccm.201203-0573OC.
20. Pihl E, Zilmer K, Kullisaar T, Kairane C, Mägi A, Zilmer M. Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes. Int J Obes (Lond). 2006;30(1):141-6. doi: 10.1038/sj.ijo.0803068.
21. Chrysohoou C, Panagiotakos DB, Pitsavos C, Skoumas I, Papademetriou L, Economou M, et al.The implication of obesity on total antioxidant capacity in apparently healthy men and women: the ATTICA study. Nutr Metab Cardiovasc Dis. 2007;17(8):590-7. doi: 10.1016/j.numecd.2006.05.007.
22. Lu Y, Andiappan AK, Lee B, Ho R, Lim TK, Kuan WS, et al. Neuropeptide Y associated with asthma in young adults. Neuropeptides.2016;59:117-121.doi:10.1016/j.npep.2016.07.003.
23. Jaakkola U, Kakko T, Juonala M, Lehtimäki T, Viikari J, Jääskeläinen AE, et al. Neuropeptide Y polymorphism increases the risk for asthma in overweight subjects; protection from atherosclerosis in asthmatic subjects--the cardiovascular risk in young Finns study. Neuropeptides. 2012;46(6):321-8. doi: 10.1016/j.npep.2012.09.005.
24. Macia L, Rao PT, Wheway J, Sierro F, Mackay F, Herzog H. Y1 signalling has a critical role in allergic airway inflammation. Immunol Cell Biol. 2011;89(8):882-8. doi: 10.1038/icb.2011.6.
25. Cardell LO, Uddman R, Edvinsson L. Low plasma concentrations of VIP and elevated levels of other neuropeptides during exacerbations of asthma. Eur Respir J. 1994;7(12):2169-73.

International Journal of Biomedicine 9(2) (2019) 121-124
http://dx.doi.org/10.21103/Article9(2)_OA7

ORIGINAL ARTICLE

Reumatology

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Molecular Genetic Features of Patients with Juvenile Arthritis in Yakutia

Khariton A. Kurtanov, PhD[1*]; Fekla V. Vinokurova[1]; Nadezhda I. Pavlova, PhD[1]; Aitalina S. Golderova, PhD, ScD[2]; Aleksandra T. Diakonova[1]; Galina A. Apsolikhova[1]; Vlad A. Alekseev[1]

[1]Yakut Science Center of Complex Medical Problems
[2]M. K. Ammosov North-Eastern Federal University Yakutsk, Russia
Yakutsk, the Republic of Sakha (Yakutia), Russia

Abstract

The research objective was to conduct a retrospective study of patients with Juvenile arthritis (JA) in association with the carriage of the *HLA-B27* allele.

Materials and Methods: A total of 73 patients (39 boys and 34 girls aged from 1 to 16 years, mean age of 10.28±4.24 years) living in Yakutia with Juvenile Chronic Arthritis (JCA), Juvenile Ankylosing Spondylitis (JAS), Juvenile Psoriatic Arthritis (JPsA), and reactive arthritis (RA) were examined. Among them, 62(84.9%) children were of Yakut nationality, and 11(15.1%) – the Russian nationality. The control group included 85 Yakuts without clinical diagnosis of arthritis. Testing for the *HLA-B27* allele was performed according to Dominquez et al. (1992) as modified by Steffens-Nakken et al. (1995).

Results: According to the genotyping results, in 30 of 73 examined samples an association was found between the *HLA-B27* allele carriage and JA. The *HLA-B27* allele was diagnosed in 24 (38.7%) Yakuts and 6 (54.5%) ethnic Russians. For further analysis, all patients (Yakuts, Russians) were divided into diagnosis-related groups. Diagnoses of JAS (n=10) and JCA (n=9) prevailed in Yakuts. In the Russian children, RA was more common (n=4). In population sampling of Yakuts, the frequency of the *HLA-B27* allele was 32.9%. A comparison of the frequencies of the *HLA-B27* allele among the Yakut patient groups and the control group found a statistically significant association with JAS. The carriage of the *HLA-B27* allele in Yakut females did not increase the risk of JAS development, whereas in male Yakuts this risk increased by 5.6 times.(**International Journal of Biomedicine. 2019;9(2):121-124.**)

Key Words: Juvenile ankylosing spondylitis • HLA-B27 • Yakuts

Introduction

Juvenile arthritis (JA) is a group designation of the numbers of rheumatic children's diseases presented in ICD-10, heading M.08 and M.09, including Juvenile Rheumatoid Arthritis (JRA), Juvenile Ankylosing Spondylitis (JAS), Juvenile Chronic Arthritis (JCA) not otherwise specified, Juvenile Psoriatic Arthritis (JPsA), and arthritis with inflammatory bowel diseases (Crohn's disease, Whipple's disease, non-specific enterocolitis).

JA is an umbrella term used to describe the many autoimmune and inflammatory conditions that can develop in children under the age of 16.[1-3] Characteristics associated with these conditions are familial susceptibility, existence of the pathogenetic or associated markers of the disease predisposition, the variability of clinical implications depending on gender and age, lower level of coincidence on a disease at monochorial twins, and others.[4]

The human leukocyte antigen (HLA) class I molecule *HLAB27* was the first genetic risk factor identified as associating with JAS and remains the most important risk locus for this archetypal spondyloarthropathy.[5] The important role of *HLA-B27* in JA pathogenesis has been known for a long time. The number of the first immunogenic works revealed the

***Corresponding author:** Khariton A. Kurtanov, PhD. Yakut Science Center of Complex Medical Problems**.** Yakutsk, the Republic of Sakha (Yakutia), Russia. E-mail: hariton_kurtanov@mail.ru*

upregulation of *HLA-B27* among children with JA.[6.7]

According to contemporary views, the basis of pathogenesis of immune inflammatory rheumatic diseases is the complex combination of genetically determined and acquired defects ("imbalance") of immune regulatory mechanisms, limiting pathological activation of the immune system in response to potentially pathogenic factors of the external environment.[8-11] With rheumatic diseases, under the influence of the IL-23 excess production, the *folding* of a *HLA-B27* heavy chain appears to be *slower* than other HLA-alleles, leading to misfolding.[12] In the presence of endoplasmic reticulum stress there is an abnormal accumulation of misfolded heavy chains leading to activation not only of the unfolded protein response, but also of a nuclear factor of NF-kB—a key transcriptional regulator of synthesis of pro-inflammatory cytokines, including IL-17 and TNFα, which also play an important role in the development of inflammation. There are data showing that an adjournment of β2m caused by a high rate of dissociation between a *HLA-B27* heavy chain and β2m occurs also in a synovial tissue and can lead to chronic inflammation.[13-15]

Numerous studies have found a close connection between *HLA-B27* and diseases of this group. Detection of the carrier state of *HLA-B27* is one of modern approaches in preliminary diagnosis and the choice of treatment. Well-timed definition of *HLA* antigens before the emergence of symptoms allows identifying a risk group for the development of a particular disease. Thus, studying the prevalence of the *HLA-B27* gene and features of a clinical aspect of inflammatory joint diseases among the children's population of Yakutia will help to develop effective preventive programs, which, carried out, will reduce the disease burden among children and teenagers. The research objective was to conduct a retrospective study of patients with JA in association with the carriage of the *HLA-B27* allele.

Materials and Methods

Genotyping of HLA-B27 was performed in the laboratory of molecular genetics at Yakut Science Center of Complex Medical Problems. A total of 73 patients (39 boys and 34 girls aged from 1 to 16 years, mean age of 10.28±4.24 years) living in Yakutia with JCA (30 Yakuts and 3 Russians), JAS (15 Yakuts and 1 Russians), JPsA (2 Yakuts), and reactive arthritis (RA) (15 Yakuts and 7 Russians) were examined. Among them, 62(84.9%) children were of Yakut *nationality,* and 11(15.1%) – the Russian nationality. The control group included 85 Yakuts without clinical diagnosis of arthritis. The ethnic origin was considered to the third generation.

For specification of the clinical diagnosis and for the purpose of *HLA-B27* identification, we conducted a molecular and genetic analysis of 73 children from 73 families. From each patient, 2 mL of peripheral blood were drawn into an EDTA tube. Genomic DNA was isolated from the peripheral blood leukocytes using standard phenol–chloroform extraction technique (Maniatis et al., 1982)

Testing for the *HLA-B27* allele was performed according to Dominquez et al. (1992) as modified by Steffens-Nakken et al. (1995). Primers are amplifying codons 91-136, E91S (5'-GGG TCT CAC ACC CTC CAG AAT-3') and 136AS (5'-CGG CGG TCC AGG AGC T-3') (amplificate length 135 bp). For internal control, the β-globin gene was genotyped by primers PCO4 (5' - CAA CTT CAT CCA CGT TCA CC-3') and GH20 (5' - GAA GAG CCA AGG ACA GGT AC-3') (amplificate length 268 bp). All primers described above were synthesized in MNPK Biotekhindustriya. (Moscow, Russia).[16-18]

The reaction mixture (20 μL) contained : 50 ng of DNA, 0.15-0.9 mmol/l. of primers (MNPK Biotekhindustriya), 200 mmol/L of each nucleotide triphosphate (NTP) (Sileks, Russia), 2 units of DNA polymerase (Sileks, Russia), 10×PCR buffer (500 mmol/l KCl, 100 mmol/l. Tris-HCl (pH=8.3), gelatin 1 g/l, 11 mmol/l $MgCl_2$) (Sileks, Russia).

PCR was conducted in the MJ Mini Gradient Thermal Cycler (BioRad). The DNA was amplified using the following thermocycling steps: 94°C for 100 sec, 94°C for 1 min, 57 °C for 1 min, 30 cycles of 72 °C for 2 min, 72 °C for 10 min.

PCR products were analyzed on 2% agarose gels after staining with ethidium bromide and were visualized using a UV transilluminator (Vilber Lourmat, France) (Fig.1).

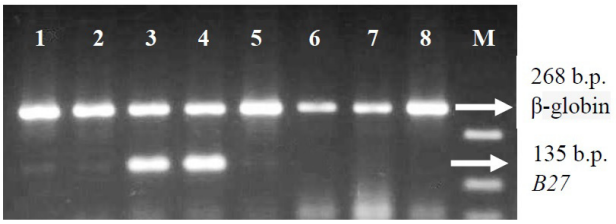


Fig.1. *Electrophoretogram of the PCR products on a 2% agarose gel.*

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). Differences in the HLA-B27 allele distribution between the two groups were assessed by χ^2- test with 1 degree of freedom (df) or Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

According to the genotyping results, in 30 of 73 examined samples an association was found between the *HLA-B27* allele carriage and JA. We did not find the HLA-B27 allele in 43 patients with JA. The *HLA-B27* allele was diagnosed in 24 (38.7%) Yakuts and 6(54.5%) ethnic Russians. For further analysis, all patients (Yakuts, Russians) were divided into diagnosis-related groups. Diagnoses of JAS (n=10) and JCA (n=9) prevailed in Yakuts. In the Russian children, RA was more common (n=4).

For comparison, population sample of healthy Yakuts (without JA and family burden for JA) (n=85) was tested. All DNA samples of population sampling were genotyped for the carriage of the *HLA-B*27 allele. The frequency of the HLA-B27 allele varies widely across populations, from 0.4% to 39.6%—the lowest frequency in Japanese from the southern regions of

Japan, the highest frequency in Koryaks from settlements of the Koryak Autonomous Area (Tymlat and Voyampolka).[19] In populations of Europe, the frequency of the *HLA-B27* allele is from 4% to 8%, in ethnic Russians - 10.4%.[20,21] Studies conducted on different populations of the world have shown that the highest and lowest frequency of *HLA-B27* is detected in populations belonging to the Mongoloid race (Fig.2). In our research, in population sampling of Yakuts, the frequency of the *HLA-B27* allele was 33%. It can possibly be defined by similarity of profiles of the HLA system and ethnogenetic bases of populations of the Arctic mongoloids and Yakuts. The distribution of Yakut patients by gender showed an insignificant prevalence of male patients (54.8%). In sampling of Russians, this distribution appeared approximately identical, probably because of a small number of patients (n=11).

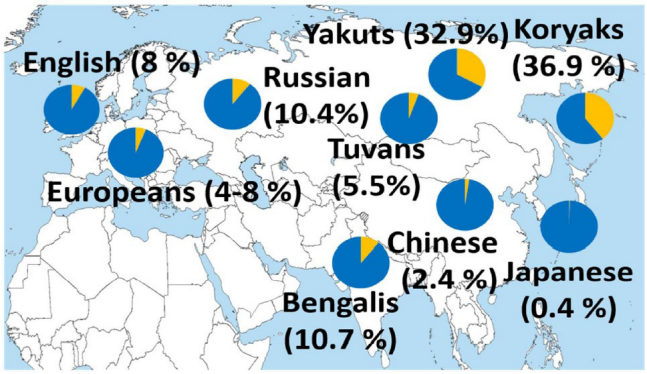


Fig. 2. *The frequency of the HLA-B27 allele in the different populations.*

While comparing the frequency of the *HLA-B27* allele in the group of patients (JA and RA) of the Yakut nationality (38.7%) with population sampling of Yakuts (32.9%), an association with the *HLA-B27* allele carriage was not found (Table 1). A similar analysis for the Russian patients was impossible due to the lack of comparative control. The Yakut patients were divided into diagnosis-related groups: JAS (n=15), JCA (n=30), JPsA (n=2) and RA (n=15). A comparison of the frequencies of the HLA-B27 allele among the Yakut patient groups and the control group found a statistically significant association with JAS (Table 1).

Table 1.

The frequency of the HLA-B27 allele in the studied groups of Yakut patients

№	Group	*HLA-B27* n (%)	OR (95% CI)	*P*-value
1	JAS (n=15)	10 (66.7)	4.071 (1.270-13.052)	0.0182
2	JCA (n=30)	9 (30)	0.872 (0.354-2.151)	0.7670
3	JPsA (n=2)	0 (0)	0.000	1.0000
4	RA (n=15)	5 (33.3)	1.018 (3.217-3.263)	0.9762
Total (n=62)		24 (38.7)	1.286 (0.650-2.544)	0.4704
Control group (Yakuts) (n=85)		28 (32.9)	-	-

We found also a statistically significant association with the carriage of the *HLA-B27* allele in the male subgroup (OR=5.6471, 95% CI: 1.3474-23.6676; *P*=0.018) (Table 2). Thus, the carriage of the *HLA-B27* allele in Yakut males increases the risk of JAS by 5.6 times.

Table 2.

The frequency of the HLA-B27 allele in the studied groups of Yakut patients depending on gender

№	Group	Male				Female			
		n	HLA-B27 n (%)	OR (95% CI)	*P*	n	HLA-B27 n (%)	OR (95% CI)	*P*
1	JAS	12	9 (75)	5.647 (1.347- 23.668)	0.018	3	1 (33.3)	1.136 (0.093-13.886)	0.920
2	JCA	12	4 (33.3)	0.941 (0.247-3.582)	0.930	18	5 (27.8)	0.874 (0.250-3.056)	0.833
3	RA	9	3 (33.3)	0.941 (0.209-4.242)	0.938	6	2 (33.3)	1.1364 (0.180-7.152)	0.892
4	JPsA	1	0	0	1	1	0	0	1
Total		34	16 (47.1)	1.6732 (0.684-4.092)	0.260	28	8 (28.6)	0.909 (0.308-2.688)	0.863
Control group (n=85)		49	17 (34.7)	-	-	36	11 (30.6)	-	-

In conclusion, the population frequency of the *HLA-B27* allele in Yakuts was 32.9%. In the Yakut population, the association between the carriage of the *HLA-B27* allele and JAS was not found, which might be due to a highly heterogeneous sample of patients. The carriage of the *HLA-B27* allele in Yakut females did not increase the risk of JAS development, whereas in male Yakuts this risk increased by 5.6 times.

Competing Interests

The authors declare that they have no competing interests.

References

1. Bochkov N.P. Human genetics (heredity and pathology). M.: Meditsina; 1978.[in Russian].
2. Murphy EA, Chase GA. Principles of Genetic Counseling. Yearbook Medical Publishers, Chicago; 1975
3. Benevolenskaya L, Myakotkin V, Ondrashik M, Gemer B. Clinical and genetic aspects of rheumatic diseases. M.: Meditsina; 1989. [in Russian].
4. Zolobova ES, Yasdovsky VV, Voronin AV, Boldyreva MP. [Immunogenetic features of juvenile rheumatoid arthritis]. Rheumatology Science and Practice. 2007;45(5):66-73. [Article in Russian].
5. Thomas GP, Brown MA. Genetics and genomics of ankylosing spondylitis. Immunol Rev. 2010;233(1):162-80. doi: 10.1111/j.0105-2896.2009.00852.x.

6. Morling N, Friis J, Fugger L, Georgsen J, Heilmann C, Pedersen FK, et al. DNA polymorphism of HLA class II genes in pauciarticular juvenile rheumatoid arthritis. Tissue Antigens. 1991;38(1):16-23.
7. Wordsworth P. Progress in the immunogenetics of rheumatoid arthritis. Hosp Pract (Off Ed). 1995;30(4):77-81.
8. Nasonov EL. Genetically engineered biological medications in the treatment of rheumatoid arthritis. M.: IMA- PRESS; 2013. [in Russian].
9. Nasonov EL, Denisov LN, Stanislav ML. [Interleukin-17 is a new target for anti-cytokine therapy of immune inflammatory rheumatic diseases]. Rheumatology Science and Practice. 2013;51(5):545-552. [Article in Russian].
10. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep. 2006;7(9):880-5.
11. Kitamura M. Endoplasmic reticulum stress and unfolded protein response in renal pathophysiology: Janus faces. Am J Physiol Renal Physiol. 2008;295(2):F323-34. doi: 10.1152/ajprenal.00050.2008.
12. DeLay ML, Turner MJ, Klenk EI, Smith JA, Sowders DP, Colbert RA. HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with Th17 activation in transgenic rats. Arthritis Rheum. 2009;60(9):2633-43. doi: 10.1002/art.24763.
13. Schaeverbeke T, Truchetet ME, Richez C. Gut metagenome and spondyloarthritis. Joint Bone Spine. 2013;80(4):349-52. doi: 10.1016/j.jbspin.2013.02.005.
14. Sherlock JP, Cua DJ. Interleukin-23: a promising therapeutic target in seronegative spondyloarthropathy. Curr Opin Pharmacol. 2013;13(3):445-8. doi: 10.1016/j.coph.2013.03.002.
15. Colbert RA, Tran TM, Layh-Schmitt G. HLA-B27 misfolding and ankylosing spondylitis. Mol Immunol. 2014;57(1):44-51. doi: 10.1016/j.molimm.2013.07.013.
16. Steffens-Nakken HM, Zwart G, van den Bergh FA. Validation of allele-specific polymerase chain reaction for DNA typing of HLA-B27. Clin Chem. 1995;41(5):687-92.
17. Dominguez O, Coto E, Martinez-Naves E, Choo SY, López-Larrea C. Molecular typing of HLA-B27 alleles. Immunogenetics. 1992;36(5):277-82.
18. Bon MA, van Oeveren-Dybicz A, van den Bergh FA. Genotyping of HLA-B27 by real-time PCR without hybridization probes. Clin Chem. 2000;46(7):1000-2.
19. Fefelova VV, Khamnagadaev II., Polikarpov LS. [HLA-B27 antigen and spondyloarthropathies in Arctic Mongoloids]. Bulletin of the SB RAMS] 2010;30(6):136-139. [Article in Russian].
20. Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and HL-A 27. Lancet. 1973;1(7809):904-7.
21. Konenkov VI, Golovanova OV, Prokof'ev VF, Shevchenko AV, Zonova EV, Korolev MA, et al. [Distribution of allelic variants of promotor sites of cytokine genes and endothelial growth factor gene among healthy subjects and patients with rheumatoid arthritis in a Russian Europeoid population]. Vestn Ross Akad Med Nauk. 2010;(9):9-14. [Article in Russian].

International Journal of Biomedicine 9(2) (2019) 125-127
http://dx.doi.org/10.21103/Article9(2)_OA8

ORIGINAL ARTICLE

Dermatology and Venereology

INTERNATIONAL JOURNAL OF BIOMEDICINE

The *CYP17A1* rs743572 Gene Polymorphism and Risk of Development and Clinical Features of Acne Vulgaris in the Uzbek Population

Nilufar N. Malikova[1*]; Khamid Ya. Karimov, PhD, ScD[2];
Kodirjon T. Boboev, PhD, ScD[2]; Saidkasim S. Arifov, PhD, ScD[3]

[1]*Republican Dermatovenerologic Clinical Hospital*
[2]*Scientific Research Institute of Hematology and Blood Transfusion*
[3]*Tashkent Institute of Postgraduate Medical Education*
Tashkent, Uzbekistan

Abstract

The purpose of this study was to analyze the association between the *CYP17A1* rs743572 SNP and the development and clinical course of acne vulgaris (AV) in the Uzbek population.

The study included 165 AV patients aged from 18 to 30 years (Group AV). All patients were divided into 3 subgroups in accordance with the severity of the disease. SAV1 included 59(35.8%) patients with a mild degree of AV, SAV2 included 64(38.8%) patients with a moderate degree of AV, and SAV3 included 42(25.4%) patients with a severe degree of AV. The control group (CG) consisted of 97 healthy volunteers without a burdened dermatological history. The study revealed a significant association of the *CYP17A1* rs743572 SNP with the risk of AV development in the Uzbek population. The study results showed that the presence of the A2 allele and A2/A2 genotype of the *CYP17A1* rs743572 SNP might be a risk factor for AV in Uzbek ethnicity. It is obvious that the A1/A1 genotype of the *CYP17A1* rs743572 SNP can have a protective effect not only in the formation of AV, but also in the severity of the disease. The functionally unfavorable A1/A2 genotype of the *CYP17A1* rs743572 SNP is more characteristic of patients with a moderate degree of AV, and the homozygote minor allele genotype A2/A2 is more characteristic of patients with a severe degree of AV.(**International Journal of Biomedicine. 2019;9(2):125-127.**)

Key Words: acne vulgaris • single nucleotide polymorphism • *CYP17A1* gene • rs743572

Abbreviations

SNP, single nucleotide polymorphism; **RMI**, recessive model of inheritance; **DMI**, dominant model of inheritance; **GMI**, general model of inheritance

Introduction

Acne vulgaris (AV) is a polymorphic, multifactorial, chronic relapsing inflammatory disease of the sebaceous glands and hair follicles. Epidemiological studies have shown that the prevalence of acne among adolescents and young people reaches of 80%-90%. However, the disease can occur in infants and older adults.[1,2] AV has a multifactorial pathogenesis, of which the key factors are genetic predisposition and hormonal abnormalities (androgens play the key role).[3]

Cytochrome P450 family 17 (CYP17) is one of the key enzymes for the steroidogenic pathway.[4-6] CYP17 is encoded by the *CYP17A1* gene, which is located on the long arm of chromosome 10q24.32.[7] The enzyme mediates steroid 17α-hydrolase and 17, 20-lyase activity in the endoplasmic reticulum.[8] SNP (rs743572) (a common single base pair substitution [−34 T→C]) in the 5'-untranslated region of the *CYP17A1* gene is widely studied, but the functional impact of the T/C change is presently, to our knowledge, not known. The common T allele is referred as A1

[*]***Corresponding author:*** *Dr. Nilufar B. Malikova, Republican Dermatovenerologic Clinical Hospital of the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan. E-mail: dr.malikova@gmail.com*

and the variant C allele as A2. The minor allele A2, compared to A1, is postulated to correlate with higher serum levels of various sex steroids in some studies.[9,10]

The purpose of this study was to analyze the association between the *CYP17A1* rs743572 SNP and the development and clinical course of AV in the Uzbek population.

Materials and Methods

The study included 165 AV patients aged from 18 to 30 years (Group AV). All patients were divided into 3 subgroups in accordance with the severity of the disease. SAV1 included 59(35.8%) patients with a mild degree of AV, SAV2 included 64(38.8%) patients with a moderate degree of AV, and SAV3 included 42(25.4%) patients with a severe degree of AV. The control group (CG) consisted of 97 healthy volunteers without a burdened dermatological history. The groups were comparable in sex and age. We used the standard method for analysis of case-control data.

Genomic DNA was isolated from the peripheral blood leukocytes using a modified phenol–chloroform extraction method.[11] PCR was performed in Rotor Gene 6000 (Corbett Research, Australia) using Sintol reagent kits (Russia) according to the manufacturer's instructions (Table 1, Fig. 1).

Table 1.

Polymerase chain reaction amplification conditions

Step	Temperature	Time	Detection	Repeats
Hold	94	3 min	No acquiring	1
Cycling 1	94	20 sec	No acquiring	10
	58	20 sec	No acquiring	
	61	30 sec	No acquiring	
Cycling 2	94	20 sec	No acquiring	30
	58	20 sec	No acquiring	
	61	30 sec	Acquiring on Green, Yellow	

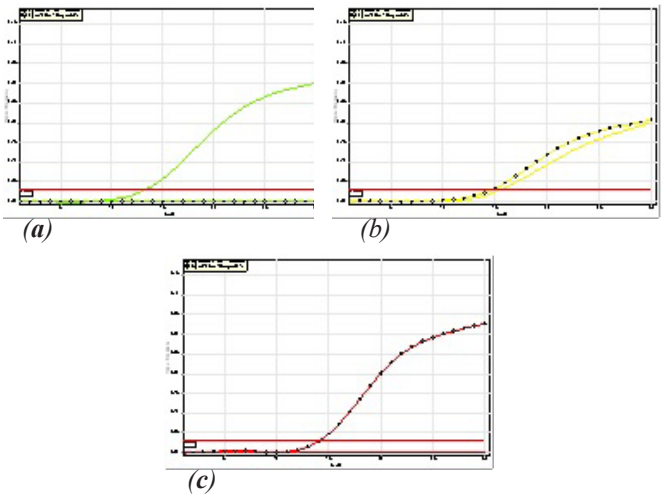


Fig. 1. *The results of real-time PCR for the CYP17A1 rs743572 SNP (a)-homozygous wild-type; (b)-heterozygous mutant; (c)-homozygous mutant.*

Statistical data processing was performed using OpenEpi 2009, Version 2.3. Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by χ2- test with 1 degree of freedom (df). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Chi-square test was used to assess the categorical variables. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by our Institutional Ethics Committee. Written informed consent was obtained from each patient.

Results and Discussion

The frequency of genotypes and alleles of the *CYP17A1* rs743572 SNP in the studied groups is presented in Table 2. The distribution of the genotype frequency was in HWE for both groups ($P>0.05$). In Groups AV and CG, the frequencies of the A1 and A2 alleles were as follows: 56.7% and 43.3% versus 80.4% and 19.6%, respectively. We further used the 3 types of genetic models to test the association between the *CYP17A1* rs743572 SNP and AV. We found a significant prevalence of the carriage of the minor A2 allele in Group AV, compared to CG ($\chi^2=30.5$; $P<0.05$; OR=3.14; 95% CI: 2.07–4.76). The frequency distribution of the A1/A1, A1/A2 and A2/A2 genotypes in Groups AV and CG were as follows: 33.9%, 45.5% and 20.6% versus 67.0%, 26.8% and 6.2%, respectively. We found a significant prevalence of the carriage of the heterozygous genotype A1/A2 and homozygote minor allele genotype A2A2 in Group AV, compared to CG ($\chi^2=26.55$; $P<0.05$; OR=2.28; 95% CI: 1.35–3.92; and OR=3.94; 95% CI: 1.59–9.76). The frequency of the wild A1/A1 genotype in Group AV was significantly lower than in CG, which may indicate a protective effect of this genotype for the development of AV (OR=0.25; 95% CI: 0.15–0.43).

Table 2.

The frequency of genotypes and alleles of the CYP17A1 rs743572 SNP in the studied groups

Group	Allele				Genotype					
	A1		A2		A1/A1		A1/A2		A2/A2	
	n	%	n	%	n	%	n	%	n	%
Group AV	187	56.7	143	43.3	56	33.9	75	45.5	34	20.6
SAV3	18	21.4	66	78.6	2	4.8	14	33.3	26	61.9
SAV2	70	54.7	58	45.3	13	20.3	44	68.7	7	10.9
SAV1	99	83.9	19	16.1	41	69.5	17	28.8	1	1.7
CG	156	80.4	38	19.6	65	67.0	26	26.8	6	6.2

In SAV3, the minor A2 allele was found approximately 5 times more often than in CG ($\chi^2=87.1$; $P<0.05$; OR=15.05; 95% CI: 8.014–28.27). In contrast, the frequency of the A1/A1 genotype was significantly lower than in CG ($\chi^2=45.5$; $P<0.05$; OR=0.02; 95% CI: 0.01–0.11). The carriage of the

A2/A2 genotype was associated with an increased risk of a severe degree of AV in RMI (χ^2=51.3; P<0.05; OR=24.6; 95% CI: 8.76–69.35). The frequency of the heterozygous A1/A2 genotype was not significantly different than in CG.

In SAV2, the minor A2 allele was also significantly more frequent than in CG (χ^2=24.4; P<0.05; OR=3.4; 95% C: I2.07–5.39). The carriage of the A1/A2 genotype was also associated with an increased risk of a moderate degree of AV in GMI (χ^2=34.04; P<0.05; OR=6.01; 95% CI: 3.00–12.02). There were no differences in the frequency distribution of the homozygous A2/A2 genotype between the patient sample and CG, and significant risk was not found in RMI (χ^2=1.17; P=0.28; OR=1.86; 95 % CI: 0.60–5.82).

In SAV1, no significant differences were found in the frequency of the alleles and genotypes of the *CYP17A*1 rs743572 SNP, compared to CG.

The distribution of alleles and genotypes of the *CYP17A1* rs743572 SNP differed significantly depending on the severity of the clinical course of AV. It was found that the minor A2 allele was significantly more frequent in SAV3 than in SAV2 and SAV1 (χ^2=75.721; P=0). It was also noted that the minor A2 allele was significantly more frequent in SAV2 than in SAV1 (45.3% versus 16.1%, respectively; χ^2=24.363; P=0.000). A similar pattern with a more pronounced statistical significance was found in a comparative analysis of the distribution of the homozygous A2/A2 genotype. The A2/A2 genotype was significantly more frequent in SAV3 than in SAV2 and SAV1 (χ^2=60.341, P=0.000). The A2/A2 genotype was also significantly more frequent in SAV2 than in SAV1 (χ^2=4.313; P=0.038). The heterozygous A1/A2 genotype was significantly more frequent in SAV2 than in SAV3 and SAV1 (χ^2=23.087; P=0.0000). Differences in the frequency of the A1/A1 genotype were also significant between SAV1 and SAV2 (χ^2=30.148; P=0.0000), as well as between SAV1 and SAV3 (χ^2=42.047; P=0.0000). It is obvious that the A1/A1 genotype of the *CYP17A1* rs743572 SNP can have a protective effect not only in the formation of AV, but also in the severity of the disease.

Thus, the functionally unfavorable A1/A2 genotype of the *CYP17A1* rs743572 SNP is more characteristic of patients with a moderate degree of AV, and the homozygote minor allele genotype A2/A2 is more characteristic of patients with a severe degree of AV.

The role of the *CYP17A1* rs743572 SNP in the formation and development of acne is not completely clear. Thus, in the Chinese and Iranian populations, there was higher acne risk related to CYP17 (T-34C),[12,13] but in the Polish and Indonesian populations this risk was not found.[14,15] Our study revealed a significant association of the *CYP17A1* rs743572 SNP with the risk of AV development in the Uzbek population. The results of our study showed that the presence of the A2 allele and A2/A2 genotype of the *CYP17A1* rs743572 SNP might be a risk factor for AV in Uzbek ethnicity. Obviously, populations and ethnic groups differ in the frequency of distribution of polymorphic variants of the *CYP17A1* rs743572 SNP and their contribution to the development of various nosologies associated with an imbalance of sex hormones. Further studies need to obtain a more accurate result.

Competing Interests

The authors declare that they have no competing interests.

References

1. Goldberg DJ, Berlin AL. Acne and Rosacea: Epidemiology, Diagnosis, and Treatment. Manson Publishing LTD; 2012.
2. Spencer EH, Ferdowsian HR, Barnard ND. Diet and acne: a review of the evidence. Int J Dermatol. 2009;48(4):339-47. doi: 10.1111/j.1365-4632.2009.04002.x.
3. James WD. Clinical practice. Acne. N Engl J Med. 2005;352(14):1463-72.
4. Miller WL. Early steps in androgen biosynthesis: from cholesterol to DHEA. Baillieres Clin Endocrinol Metab. 1998;12(1):67-81.
5. Auchus RJ, Miller WL. Molecular modeling of human P450c17 (17alpha-hydroxylase/17,20-lyase): insights into reaction mechanisms and effects of mutations. Mol Endocrinol. 1999;13(7):1169-82.
6. Missaghian E, Kempná P, Dick B, Hirsch A, Alikhani-Koupaei R, Jégou B, Mullis PE, Frey BM, Flück CE. Role of DNA methylation in the tissue-specific expression of the CYP17A1 gene for steroidogenesis in rodents. J Endocrinol. 2009;202(1):99-109. doi: 10.1677/JOE-08-0353.
7. Fan YS, Sasi R, Lee C, Winter JS, Waterman MR, Lin CC. Localization of the human CYP17 gene (cytochrome P450(17 alpha)) to 10q24.3 by fluorescence in situ hybridization and simultaneous chromosome banding. Genomics. 1992;14(4):1110-1
8. Nguyen PT, Conley AJ, Sneyd J, Lee RS, Soboleva TK, Shorten PR.The role of enzyme compartmentalization on the regulation of steroid synthesis.J Theor Biol. 2013;332:52-64. doi: 10.1016/j.jtbi.2013.04.021.
9. Carey AH, Waterworth D, Patel K, White D, Little J, Novelli P, Franks S, Williamson R. Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. Hum Mol Genet. 1994;3(10):1873-6.
10. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome P450c17alpha gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. Cancer Res. 1998;58(4):585-7.
11. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor NY; Cold Spring Harbor Lab Press; 1987.
12. He L, Yang Z, Yu H, Cheng B, Tang W, Dong Y, Xiao C. The relationship between CYP17 -34T/C polymorphism and acne in Chinese subjects revealed by sequencing. Dermatology. 2006;212(4):338-42.
13. Chamaie-Nejad F, Saeidi S, Najafi F, Ebrahimi A, Rahimi Z, Shakiba E, Rahimi Z. Association of the CYP17 MSP AI (T-34C) and CYP19 codon 39 (Trp/Arg) polymorphisms with susceptibility to acne vulgaris. Clin Exp Dermatol. 2018;43(2):183-186. doi: 10.1111/ced.13321.
14. Sobjanek M, Zabłotna M, Dobosz-Kawałko M, Michajłowski I, Mędrzycka-Dąbrowska W, Nowicki R, Sokołowska-Wojdyło M. Polymorphisms in the cytochrome P-450 (CYP) 1A1 and 17 genes are not associated with acne vulgaris in the Polish population. Postepy Dermatol Alergol. 2015;32(5):323-6. doi: 10.5114/pdia.2014.44004.
15. Anwar AI, Agusni I, Massi MN, Yusuf I. The Immunogenetic Analysis of Acne Vulgaris. Science Journal of Clinical Medicine. 2013;2(2):58-63.

International Journal of Biomedicine 9(2) (2019) 128-130
http://dx.doi.org/10.21103/Article9(2)_OA9

ORIGINAL ARTICLE

General Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Results of Applying the Original Colostomy in Patients with Acute Large Bowel Obstruction

Alexei L. Charyshkin, PhD, ScD*; Erik A. Keshyan; Oleg V. Midlenko, PhD, ScD; Antonina V. Smolkina, PhD, ScD; Nikolai I. Belonogov, PhD, ScD

Institute of Medicine, Ecology and Physical Education of Ulyanovsk State University
Ulyanovsk, the Russian Federation

Abstract

The results of the original method of colostomy formation in 67 patients with acute large bowel obstruction (ALBO) were studied. All patients underwent sigmoid colon resection with the colostomy formation. In total, postoperative complications of a purulent-inflammatory nature (skin maceration, suppuration of postoperative and paracolostomic wounds, necrosis of colostomy, abscess of the abdominal cavity, and paracolostomal fistula) in both groups were observed in 30 (44.8%) patients. In Group 1 (n=40), with the classical method of colostomy formation, purulent-inflammatory complications were observed in 21 (52.5%) patients, in Group 2 (n=27) with the original method of colostomy formation in 4 (14.8%) patients. Bleeding from colostomy and colostomy prolapse occurred only in Group 1 in 8 (20%) patients. The proposed method of applying a colostomy helps reduce purulent-inflammatory complications by more than 3 times and provides prevention of bleeding and colostomy prolapse. (**International Journal of Biomedicine. 2019;9(2):128-130.**)

Key Words: colon cancer • acute large bowel obstruction • colostomy • postoperative complications

Introduction

In patients with acute large bowel obstruction (ALBO), the main cause of the disease is neoplasm of the left colon and rectum.[1-4] The leading method of treatment for ALBO is emergency surgery [5-7] with the formation of a temporary or permanent colostomy.[1-4,8]

The presence of colostomy in patients reduces the quality of life; often there are specific complications: maceration of the skin, suppuration of the postoperative and paracolostomal wounds, necrosis of the colostomy, abscess of the abdominal cavity, paracolostomal fistula, colostomy prolapse, and parastomal hernia.[3,4,7] Improving the colostomy formation and methods for drainage of the paracolostomal space is an urgent task in surgery of the large intestine.

The aim of our study was to improve the method of colostomy formation in patients with ALBO.

***Corresponding author**: Prof. Alexei L. Charyshkin, PhD, ScD, Head of the Faculty Surgery Department, Institute of Medicine, Ecology and Physical Education, Ulyanovsk State University. Ulyanovsk, the Russian Federation. E-mail: charyshkin@yandex.ru*

Materials and Methods

We analyzed surgical outcomes in 67 patients (aged 50 to 70 years, mean age of 55.2) with ALBO, who underwent surgical treatment in Ulyanovsk City Clinical Emergency Hospital and O.M. Filatov City Clinical Hospital №15 (Moscow) in the period from 2010 to 2019.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the by Ethics Committees at our institutions. All patients underwent sigmoid colon resection with the colostomy formation. Patients were divided into two groups depending on the method of colostomy formation. In Group 1 (n=40), we performed the classical colostomy; in Group 2 (n=27), a colostomy was performed according to the developed technique (Fig.1): "A method for the prevention and treatment of inflammatory complications of colostomy" (Application for invention No. 2014122739; Priority of 06/21/2018) (Authors: A.L. Charyshkin and E.A. Keshyan).

Technique of the original method of colonostomy formation

In the anterior abdominal wall in the direction of the intended projection of the stoma, we form a hole; then we

place interrupted sutures between skin, aponeurosis and parietal peritoneum, and the colostomy is fixed with these uncut ligatures. The peculiarity is that before the interrupted sutures are applied around the hole for the stoma and away from the hole edge by 3-4 cm, the first catheter is installed through holes throughout the way into the preperitoneal space of the paracolostomal zone. This is done by fixing catheter to the parietal peritoneum with two interrupted sutures from absorbable material. The distal end of the catheter is displayed on the anterior abdominal wall through a separate hole (contour section) in the skin, departing 2.0 cm from the lower edge of the skin wound. The second catheter is installed through holes in the subaponeurotic space around the hole for the stoma, away from the hole edge by 3-4 cm, by fixing two interrupted sutures from absorbable material to the aponeurosis. The distal end of the catheter is displayed on the anterior abdominal wall through a separate hole (contra-aperture) on the skin, departing 2.0 cm from the upper edge of the skin wound. In the postoperative period, local anesthetic for anesthesia and an antibacterial drug for the prevention and treatment of purulent-inflammatory complications are alternately administered through catheters.

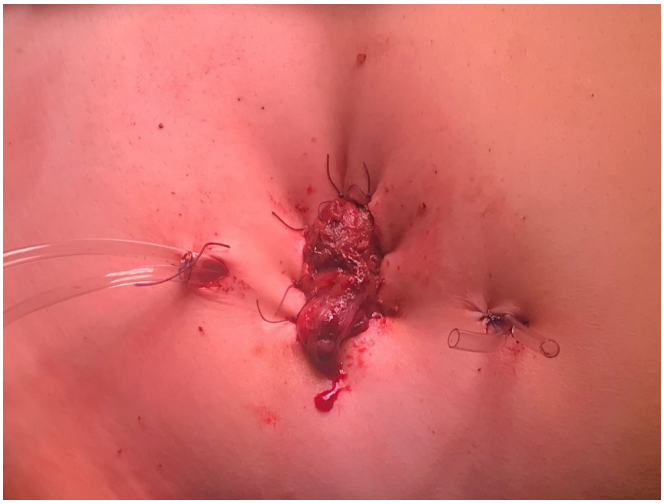


Fig. 1. *The final appearance of the original colostomy.*

All patients underwent general clinical and laboratory, radiographic, endoscopic, ultrasound, and histological methods of investigation; whenever required, echocardiographic study was carried out. Leukocyte index of intoxication (LII) was calculated by the formula of V.K. Ostrovsky.[9]

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). All values are presented as mean±SEM). The inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Analysis of the data showed that in the early postoperative period pain disappeared within 5.1±0.2 days and 8.3±0.4 days after surgery in Group 2 and Group 1, respectively, Group 2 being significantly shorter by 3 days.

Comparison of data on the timing of intestinal motility recovery in the early postoperative period (Table 1) showed that in Group 1 paresis was resolved within 6.2±0.3 days, the active functioning of the colostomy occurred within 5.7±0.3 days, and in Group 2 - 2.8±0.2 days and 2.7±0.2 days, respectively ($P<0.05$).

Table 1.

The timing of intestinal motility recovery

Duration of clinical symptoms (day)	Group 1	*P*-value	Group 2
Paresis	6.2±0.3	0.000	2.8±0.2
Active functioning of thecolostomy	5.7±0.3	0.000	2.7±0.2

On the eighth day after the operation, the LII decreased to the standard value in Group 2 and exceeded the standard values by 2 times in Group 1 (Table 2).

Table 2.

Dynamics of LII in the postoperative period

Group	LII index after the operation			
	Day 4	Day 6	Day 8	Day 10
Group 1	8.7±0.3	4.8±0.3	4.3±0.3	2.5±0.2
P-value	>0.05	0.000	0.000	0.007
Group 2	8.4±0.4	3.1±0.2	1.4±0.2	1.5±0.3

In total, postoperative complications of a purulent-inflammatory nature (skin maceration, suppuration of postoperative and paracolostomic wounds, necrosis of colostomy, abscess of the abdominal cavity, and paracolostomal fistula) in both groups were observed in 30 (44.8%) patients. In Group 1, with the classical method of colostomy formation, purulent-inflammatory complications were observed in 21 (52.5%) patients, in Group 2 with the original method of colostomy formation in 4 (14.8%) patients. Bleeding from colostomy and colostomy prolapse occurred only in Group 1 in 8 (20%) patients.

Reducing the incidence of postoperative inflammatory complications by more than 3 times, eliminating complications such as bleeding from the colostomy and colostomy prolapse in Group 2 is associated with the original method of draining the preperitoneal and subaponeurotic spaces of paracolostomal zone and introducing local anesthetics and antibacterial drugs through catheters in the postoperative period.

Findings

1. Active functioning of the original colostomy occurs 72 hours earlier than with the traditional colostomy.

2. The proposed method of applying a colostomy helps reduce purulent-inflammatory complications by more than 3 times and provides prevention of bleeding and colostomy prolapse.

Competing Interests

The authors declare that they have no competing interests.

References

1. Gataullin IG, Savinkov VG, Frolov SA, Kozlov AM. [Predictor of septic complications in colorectal cancer surgery]. Journal of Experimental and Clinical Surgery. 2018;11(1):33-37. doi: 10.18499/2070-478X-2018-11-1-33-37. [Article in Russian].
2. The state of cancer care for the population of Russia in 2016. In: Kaprina AD, Starinsky VV, Petrova GV, editors. M.: MNIOI named after PA. Herzen; 2017. [In Russian].
3. Tsarkov PV, Tulina IA, Tsugulya PB, Kochetkov VS, Khmelik SV. [Post-rectectomy choice of preventive intestinal stoma formation method: prospective multicenter randomized clinical trial protocol]. Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2017;27(2):102-110. doi:10.22416/1382-4376-2017-27-2-102-110. [Article in Russian].
4. Charyshkin AL, Aberyasev SYu, Mokhovikov VA. [Formation of reservoir colostomy]. Kazan Medical Journal. 2010;91(2):215-8. [Article in Russian].
5. Geng HZ, Nasier D, Liu B, Gao H, Xu YK. Meta-analysis of elective surgical complications related to defunctioning loop ileostomy compared with loop colostomy after low anterior resection for rectal carcinoma. Ann R Coll Surg Engl. 2015 Oct;97(7):494-501. doi: 10.1308/003588415X14181254789240.
6. Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A, Arnold D; ESMO Guidelines Committee. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(suppl_4):iv22-iv40. doi: 10.1093/annonc/mdx224.
7. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2016 for the treatment of colorectal cancer. Watanabe T, Muro K, Ajioka Y, Hashiguchi Y, Ito Y, Saito Y, et al.; Japanese Society for Cancer of the Colon and Rectum. Int J Clin Oncol. 2018;23(1):1-34. doi: 10.1007/s10147-017-1101-6.
8. Yanishev AA, Bazaev AV, Kokobelyan AR, Abelevich AI. [Current technologies for the prevention of parastomal herniation (review)]. Sovremennye Tehnologii v Medicine 2018;10(3):175–183. doi: 10.17691/stm2018.10.3.22. [Article in Russian].
9. Ostrovskiĭ VK, Alimov RR, Mashchenko AV, Semenova OP, Kurapova MI. [Normal parameters of leukocytic index of intoxication]. Klin Lab Diagn. 2003;(1):45-6. [Article in Russian].

International Journal of Biomedicine 9(2) (2019) 131-133
http://dx.doi.org/10.21103/Article9(2)_OA10

ORIGINAL ARTICLE

General Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

Minimally Invasive Treatment of Patients with Acute Appendicitis

Alexei L. Charyshkin, PhD, ScD*; Maksim M. Yartsev; Oleg V. Midlenko, PhD, ScD; Antonina V. Smolkina, PhD, ScD; Nikolai I. Belonogov, PhD, ScD

Institute of Medicine, Ecology and Physical Education of Ulyanovsk State University
Ulyanovsk, the Russian Federation

Abstract

The aim of our study was to improve the surgical treatment of patients with acute appendicitis (AA) by improving the mini-incision approach.

Materials and Methods: The study included 220 patients (mean age of 38.9±14.3 years) with AA, who underwent surgical treatment in the period from 2008 to 2017. A mini-incision appendectomy was performed on all patients. Patients were divided into 2 groups. The groups were comparable with respect to age and sex. Group 1 included 140 patients who underwent appendectomy by the traditional method of minilaparotomy with the classical sanitation and drainage of the abdominal cavity. Group 2 included 80 patients who underwent appendectomy by the developed method of a minimally invasive approach and sanitation and drainage of the abdominal cavity.

Results: The developed method of minilaparotomy expands and improves the area of accessibility by an average of 4.8 cm^2 for surgical manipulations during an appendectomy. The proposed method of minilaparotomy reduces the duration of an appendectomy by an average of 11.5 minutes, and the rate of complications during surgery and purulent-inflammatory complications by 3.9%. (**International Journal of Biomedicine. 2019;9(2):131-133.**)

Key Words: acute appendicitis • appendectomy • minilaparotomy • complications

Introduction

Minimally invasive surgical interventions are designed to reduce the trauma of surgery and the duration of hospitalization and rehabilitation of patients, and to improve the cosmetic effect.[1-5] In patients with acute appendicitis (AA) and the presence of such complications as typhlitis, abscesses or atypical location of the appendix, the performance of a minilaparotomy is preferable to video laparoscopy. Appendectomy using minilaparotomy in patients with subhepatic location of the appendix is difficult to perform; these cases are often converted to laparotomy.[6-9]

Mini-incision surgery in certain situations has significant advantages over laparotomy and, in some cases, over video laparoscopy.[1-6] We believe that the optimization of abdominal mini-incision surgery is an urgent task at the present stage of development of minimally invasive technologies.

***Corresponding author**: Prof. Alexei L. Charyshkin, PhD, ScD, Head of the Faculty Surgery Department, Institute of Medicine, Ecology and Physical Education, Ulyanovsk State University. Ulyanovsk, the Russian Federation. E-mail: charyshkin@yandex.ru*

The aim of our study was to improve the surgical treatment of patients with AA by improving the mini-incision approach.

Materials and Methods

The study included 220 patients (mean age of 38.9±14.3 years) with AA, who underwent surgical treatment in Ulyanovsk Regional Clinical Center for Specialized Types of Medical Aid and the surgical department at the Nikolaev district hospital of the Ulyanovsk region in the period from 2008 to 2017.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the by Ethics Committees at our institutions. All patients underwent general clinical and laboratory, radiographic, endoscopic, ultrasound, histological methods of investigation. A mini-incision appendectomy was performed on all patients. Patients were divided into 2 groups. The groups were comparable with respect to age and sex. Group 1 included 140 patients who underwent appendectomy by the traditional method of minilaparotomy with the classical sanitation and

drainage of the abdominal cavity. Group 2 included 80 patients who underwent appendectomy by the developed method of a minimally invasive approach and sanitation and drainage of the abdominal cavity.[6,10]

In the postoperative period, adequate infusion therapy was performed. To prevent purulent complications, cephalosporins of the third generation were intravenously prescribed.

To assess the proposed method of minilaparotomy, we measured the area of accessibility during the operation in both groups.[1,2] Leukocyte index of intoxication (LII) was calculated by the formula of V.K. Ostrovsky. [11]

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). All values are presented as mean±SEM). The inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The age and sex distribution of patients is shown in Table 1. There were 92(41.8%) men and 128(58.2%) women. The working-age patients were predominant (96.6%). Twelve (5.4%) patients were over 60 years old. In Group 1, the rate of conversion to laparotomy was 17.1%. Table 2 lists the reasons for the conversion. In Group 2, there were no conversions to laparotomy.

Table 1.

The age and sex distribution of AA patients

Age (year)	Sex		n/%
	Men	Women	
18 –29	52	54	106/48.2
30 - 39	18	29	47/21.4
40 - 49	12	28	40/18.2
50 -59	3	12	15/6.8
60 - 69	3	3	6/2.7
≥70	4	2	6/2.7
Total:	92/41.8	128/58.2	220/100

Table 2.

The reasons for the conversion to laparotomy in Group 1.

Reasons	n/%
Loose appendicular infiltrate, difficulty in identifying the appendix	6/4.3
Limited availability (subhepatic or pelvic location of the appendix)	18/12.8
Total	24/17.1

The area of accessibility was 13.1±1.1cm^2 in Group 1 and 17.9±1.2 cm^2 in Group 2 ($P<0.05$). On the fourth day after the appendectomy, the LII was 5.7±0.1 in Group 1 and 3.2±0.2 in Group 2. During the other days, the LII decreased equally. The wounds were fully healed within 8.1±0.2 days and 6.0±0.1 days after surgery in Group 1 and Group 2, respectively, which was significantly shorter by 2 days in Group 2 ($P<0.05$).

The duration of the appendectomy was 29.7±10.1 minutes in Group 1 and 18.2±11.1 minutes in Group 2, which reduced the time of surgery by 11.5 minutes in Group 2 ($P<0.05$). The intraoperative bleeding from a. appendicularis was detected in 8 (5.7%) patients of Group 1 and 1(1.25%) patient of Group 2. An injury to the dome of the cecum (damage of serous membrane, wall hematoma) was observed only in Group 1 in 6(4.3%) patients.

Thus, the proposed method of minilaparotomy contributes to reducing the number of complications during surgery. We believe that the reduction of complications in Group 2 is associated with the original mini-access, which contributed to the increase and improvement of the area of accessibility for surgical manipulations during appendectomy.

Purulent-inflammatory complications were detected in 9(6.4%) patients of Group 1 and 2(2.5%) of Group 2.

Performing an appendectomy with the developed mini-incision contributes to reducing the traumatic impact of retractors on the skin, subcutaneous tissue, and muscle layer, as well as anoneurosis of the anterior abdominal wall in the surgical area, which eliminates tissue ischemia, thereby significantly reducing the number of inflammatory complications of the wound.

Findings

1. Conversion to laparotomy during the traditional method of minilaparotomy is 17.1% of cases; the main indication for conversion (up to 12.8%) is the limited availability of the appendix with a subhepatic and pelvic location.

2. The developed method of minilaparotomy expands and improves the area of accessibility by an average of 4.8cm^2 for surgical manipulations during an appendectomy.

3. The proposed method of minilaparotomy reduces the duration of an appendectomy by an average of 11.5 minutes, and the rate of complications during surgery and purulent-inflammatory complications by 3.9%.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

This study was funded by the Ministry of Education and Science of the Russian Federation (the science project No. 18.7236.2017/BCh).

References

1. Bebурshvili AG, Mikhin SV. Operations from the open small access combined with other minimally invasive

technologies. Mini-access surgery. Yekaterinburg, 2005:111-112.[in Russian].
2. Prudkov MI. Basics of minimally invasive surgery. Yekaterinburg: Publishing House of the Ural State University; 2007. [in Russian].
3. Remizov OV, Maskin SS, Karsanov AM, Vakhotskiy VV, Gudushauri MK. Possibility of radiation imaging in acute appendicitis. Medical News of the North Caucasus. 2017;12(1):105-110. doi: 10.14300/mnnc.2017.12031
4. Charyshkin AL, Yakovlev SA. [Preperitoneal blockade in prevention of postoperative complications at patients with appendicular peritonitis]. Modern problems of science and education. 2014;(1). Available from: https://www.science-education.ru/en/article/view?id=12261. [Article in Russian].
5. Charyshkin AL, Bikbaeva KI. [Comparative results of application of the minilaparotomy at patients with sharp cholecystitis]. Modern problems of science and education. 2014;(2). Available from: https://science-education.ru/en/article/view?id=12262. [Article in Russian].
6. Charyshkin AL, Yartsev MM. The assessment of quality of life in patients with acute appendicitis after a minimally invasive approach. International Journal of Biomedicine. 2018;8(2):139-141. doi: 10.21103/Article8(2)_OA6
7. Rickert A, Bönninghoff R, Post S, Walz M, Runkel N, Kienle P. Appendix stump closure with titanium clips in laparoscopic appendectomy. Langenbecks Arch Surg. 2012;397(2):327-31. doi: 10.1007/s00423-011-0869-5.
8. Suh YJ, Jeong SY, Park KJ, Park JG, Kang SB, Kim DW, et al. Comparison of surgical-site infection between open and laparoscopic appendectomy. J Korean Surg Soc. 2012;82(1):35-9. doi: 10.4174/jkss.2012.82.1.35.
9. Lim SG, Ahn EJ, Kim SY, Chung IY, Park JM, Park SH, Choi KW. A Clinical Comparison of Laparoscopic versus Open Appendectomy for Complicated Appendicitis. J Korean Soc Coloproctol. 2011;27(6):293-7. doi: 10.3393/jksc.2011.27.6.293.
10. Charyshkin AL, Yartsev MM. Patent of the Russian Federation, "A Method of a minimally invasive approach for appendectomy". (RU #2612983; priority of 05.11.2016, register. of 03.14.2017; Bull #8). [in Russian]
11. Ostrovskiĭ VK, Alimov RR, Mashchenko AV, Semenova OP, Kurapova MI. [Normal parameters of leukocytic index of intoxication]. Klin Lab Diagn. 2003;(1):45-6. [Article in Russian].

International Journal of Biomedicine 9(2) (2019) 134-138
http://dx.doi.org/10.21103/Article9(2)_OA11

ORIGINAL ARTICLE

General Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Choice of Surgical Tactics for Appendicular Peritonitis in Children

Alexander A. Sleptsov[1,2*]; Valentina A. Savina, PhD, ScD[1,2]; Ahmed R. Varfolomeev, PhD[1]; Valentin N. Nikolaev[1]; Eduard I. Petukhov[2]; Alexey L. Zuev[2]; Tumen E. Erdyneev[2]; Sergey O. Kupryakov[1,2]; Vasily A. Grigoriev[1,2]; Olga S. Stepanova[1,2]; Evgeny A. Petrov[1]

[1]North-Eastern Federal University named after MK Ammosov, Yakutsk, Russia
[2]Republican Hospital №1, National Centre of Medicine, Yakutsk, Russia

Abstract

This article presents the experience of using the modified Mannheim Peritonitis Index (MPI) score with an intraoperative assessment of the severity of peritonitis in children, taking into account the duration of the disease before surgery.

Materials and Methods: We analyzed the results of treatment of 186 children under age 16 who were operated on for appendicular peritonitis in the period between 2011 and 2018. The method of scoring for appendicular peritonitis index (API) consists in summing up the points reflecting the risk factors for the disease. API provides 2 criteria for the severity of peritonitis: the first degree - API<10 points, the second degree - API≥10 points. Ten or more points are an indication for laparotomy.

Results: With the definition of criteria for intraoperative assessment of the severity of peritonitis (API), the number of indications for laparotomy decreased. So, for the period from 2011 to 2014, the number of laparotomies performed with the subsequent programmed sanitation of the abdominal cavity was 17/14% cases; from 2015 to 2018, the inflammatory process in the abdominal cavity were assessed with the determination of the intraoperative index of peritonitis severity—the number of conversions was 15/11% cases. (**International Journal of Biomedicine. 2019;9(2):134-138.**)

Key Words: appendicular peritonitis • appendicular peritonitis index • Mannheim peritonitis index

Abbreviations

AP, appendicular peritonitis; **API**, appendicular peritonitis index; **MPI**, Mannheim peritonitis index

Introduction

Operations with acute appendicitis are the most frequently performed emergency operations on the abdominal organs and reach up to 70% of all abdominal interventions.[1,2] Diffuse peritonitis in children is often a complication of destructive appendicitis.[3] In the treatment of diffuse peritonitis, the reduction of postoperative complications,[4-6] including the frequency of repeated interventions and adhesive obstruction, is important. The choice of the optimal volume of surgery is influenced by intraoperative findings—the nature of the effusion, fibrin, and the prevalence and presence of adhesions and abscesses. Also important is the duration of peritonitis before surgery, that is, the stage of peritonitis.[6]

In pediatric surgery, there are no clear criteria for determining the extent of the operation; typically, surgeons assess the severity of peritonitis.[7,8] Laparotomy is the method of choice in most cases, and underestimating the severity of peritoneal inflammatory changes leads to repeated operations.[9]

To assess the severity of peritonitis and select the extent of surgical intervention, we proposed using an index. As the basis, we used the MPI. In 1987, a group of German surgeons in Mannheim developed the MPI to predict the outcome of purulent peritonitis in cancer patients.[10] Subsequently, the MPI was used to assess the severity and prognosis of peritonitis outcome in patients with a general surgical profile.

***Corresponding author**: *Alexander A. Sleptsov, North-Eastern Federal University named after MK Ammosov, Yakutsk, Russia. E-mail: sashaogh@mail.ru*

The calculation of the MPI includes 8 factors, each of which is scored from 0 to 12. Quite quickly, the MPI received international recognition as an accurate and reliable assessment method with high sensitivity, from 83% to 98%.[11-13]

Materials and Methods

The study was performed in the Department of Pediatrics and Pediatric Surgery at North-Eastern Federal University named after MK Ammosov and in the Department of Purulent Surgery of the Pediatric Center of the Republican Hospital №1, National Centre of Medicine.

We analyzed the results of treatment of 186 children under age 16 who were operated on for AP in the period between 2011 and 2018. The diagnosis was confirmed by objective examination, radiographic examination of the abdominal organs, abdominal ultrasound examination, and laboratory data.

All patients (115/61.8% boys and 76/38.2% girls) were admitted to hospital for emergency reasons. The age of patients ranged from 4 months to 14 years (mean age of 8.2±3.7 years). The terms of admission of patients into the hospital with signs and symptoms of AP are presented in Table 1. According to the classification of K. Simonyan,[14] the distribution of patients was as follows: a reactive stage (the first 24 hours) – 88/47.3% patients, a toxic stage (24-72 hours) – 88/47.3% patients, and a terminal stage (over 72 hours) – 10/5.3% patients

Table 1.

The terms of admission of patients into the hospital with AP

Disease duration	Absolute	%
Up to 1 day inclusive	88	47.3%
From 1 to 2 days	63	33.8%
From 2 to 3 days	25	13.4%
More than 3 days	10	5.3%
Total	186	100%

Between 2011 and 2018, 1,868 children under age 16 were operated on for acute appendicitis and its complications. Until 2015, diagnostic laparoscopy was used in 95% of cases; appendectomy was performed endoscopically in 87% of cases. Since 2015, diagnostic laparoscopy has been used for all forms of appendicitis and its complications; conversions to *open* appendectomy were performed in 15 cases.

In 159/85.5% cases of peritonitis, the operation was started with diagnostic laparoscopy. During diagnostic laparoscopy, API was performed, taking into account the following factors:

1. Disease duration was considered, up to or over 72 hours.

2. The nature of effusion in the abdominal cavity during peritonitis is purulent or with the presence of fecal contents.

3. The nature of fibrin was assessed by its density and friability, the possibility of separation from the walls of the intestine and peritoneum.

4. The prevalence of peritonitis was assessed according to the anatomical regions of the abdominal cavity, with 5 or more areas affected; the prevalence of peritonitis was assessed by 2 points.

5. The presence of adhesions, their friability and density during separation.

6. The presence of inter-intestinal abscesses is a prognostic for a severe course of peritonitis, which indicates a violation of the primary immune response, and it suggests a risk of postoperative complications.

API provides 2 criteria for the severity of peritonitis: the first degree - API<10 points, the second degree - API≥10 points. The method of scoring for API consists in summing up the points reflecting the risk factors for the disease (Table 2). Ten or more points are an indication for laparotomy.

Table 2.

Appendicular peritonitis index

Factor	Severity	Points
Disease duration	up to 72 hours	1
	over 72 hours	3
Nature of effusion	Purulent	1
	Fecal	2
Nature of fibrin	Loose	1
	Dense	2
Prevalence of peritonitis	Up to 4 areas	1
	5 or more areas	2
The presence of adhesions	Loose adhesions	1
	Dense adhesions	2
The presence of inter-intestinal abscesses	None	1
	Plural	3

When evaluating less than 10 points, it is recommended to continue the operation endoscopically. In the presence of inflammatory effusion, the primary sanitation of the abdominal cavity is carried out, which begins with the evacuation of purulent exudate from the focus area using an electric suction device. Then the coagulation of the appendix mesentery by a monopolar coagulator is performed; the monopolar coagulator of the "hook" type is used for the stump. On the basis of the stump of the appendix, Raeder's loop with a conductor is superimposed, intracorporeally tightened, and suture material vicryl 2/0 or 3/0 is used. The appendix is evacuated through a 10 mm or 12 mm trocar. If it is impossible to remove the appendix through the trocar, the evacuation of the latter is carried out in a container, with an extension of the wound. The final sanitation of the abdominal cavity is performed with an aqueous solution of 0.02% chlorhexidine with a revision of all areas and subphrenic spaces. In our cases, the abdominal cavity was drained according to A. Generalov,[15] through the trocar installation site in 14/8.8% cases with marked inflammatory changes; a "cigar" tampon was installed locally in 58/36.5% cases.

Complications of acute appendicitis were observed in 186 children: local peritonitis was detected in 98 cases, including appendicular infiltration in 1 case, periappendicular abscess – in 25 cases, and diffuse peritonitis – in 57 cases (Fig.1). In complicated appendicitis, drainage was performed; with severe "late" peritonitis, conversion was performed and programmed rehabilitation of the abdominal cavity was the treatment of choice.

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA).

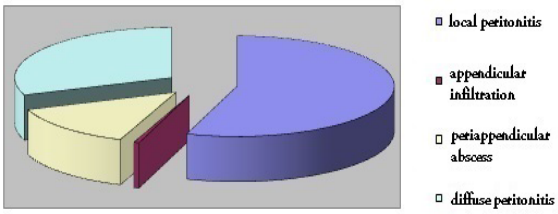


Fig. 1. *Complications of acute appendicitis.*

Results

AP at the beginning of the disease in the acute period is a controlled pathology in terms of surgical treatment. The volume of surgical treatment for peritonitis is limited by appendectomy and washing the abdominal cavity, and a drainage of pelvic cavity is performed according to indications. In our study, local peritonitis was detected in 4.89% of cases from performed appendectomies, and in the structure of peritonitis, it was 52.6%.

In acute peritonitis with duration up to 48-72 hours, an effusion into the abdominal cavity is more often exudative-purulent, as the response of the peritoneum to inflammation. Usually it is delimited by the small pelvis and the right iliac region. With a retrocecal, retroperitoneal location, periappendicular abscesses may form; abscesses also have a loose character without pronounced adhesions.

As a rule, when assessing the severity of peritonitis, the surgeon focuses on the presence of a purulent effusion in the abdominal cavity. Purulent effusion in the abdominal cavity up to 48-72 hours is of a liquid, exudative character, and its volume depends on the secretory features of each organism. In the study of exudate from the abdominal cavity in the first 48 hours from the onset of the disease, in most cases the microflora did not grow. Thus, in our opinion, the presence of a purulent effusion in the abdominal cavity, even in large quantities, is not an indication for conversion to open laparotomy.

In "late" peritonitis, with duration more than 48-72 hours, the nature of the effusion in the abdominal cavity is purulent and fecal, infiltrative-inflammatory changes in the peritoneum are expressed, and there is a spread of pus in almost all anatomical areas of the abdominal cavity. The resulting abscesses are dense, with thick fibrinous walls, with a pronounced adhesive process.

In the description of abscesses, we follow the classification of Doletsky et al.[8] In Stages 1-2, disease duration up to 72 hours, local changes are characteristic of abscesses with loose walls; Stage 3 of abscess formation corresponds to the disease duration more than 72 hours - "late peritonitis." In our study, periappendicular abscesses (n=25) made up 13.4% of the total number of complicated appendicitis, abscesses of Stages 1-2 were detected in 20/80% patients, abscesses of Stage 3 were in 5/20% children. In patients with periappendicular abscesses, the duration of the disease ranged from 2 to 12 days. Combined peritonitis is a combination of periappendicular abscess and free purulent effusion. This form is considered the outcome of an abscess; it is one of the most severe inflammatory processes, when infection can spread beyond the primary delimitation of purulent exudate. When determining the relationship between periappendicular abscesses and duration of disease, the correlation coefficient was 0.2 (P=0.01).

We analyzed the effectiveness of methods for draining the abdominal cavity in children admitted with complications from different central district hospitals and treated in the Department of Purulent Surgery from 2006 to 2010. In most cases, the pelvic cavity was drained according to A. Generalov;[15] since 2010, the method of choice for drainage in delimited peritonitis has been a "cigar" type tampon. Thus, over the past 8 years, the pelvic cavity drainage according to A. Generalov in AP was used in 10 cases, cigar-shaped drainage - in 59 cases. After pelvic cavity drainage according to A. Generalov,[15] complications (n=4) in the form of pericultitis, pelvic abscess, stump failure, inter-intestinal abscess, and noncropped peritonitis were observed. After cigar-shaped drainage, complications were observed in 3 cases: perforation of the dome of the cecum and failure of the stump. Since some of the children arrived after an operation performed in the central district hospital with drains, it cannot be excluded that the method of installing the drains was not disturbed.

The volume of surgical treatment for peritonitis depends on the disease duration and the intraoperative picture, on the basis of which we identified the evaluation criteria.

Surgical tactics based on the results of diagnostic laparoscopy*:*

-In the presence of widespread pus, which has a liquid character, endoscopic tactics consisted of primary sanitation of the abdominal cavity, aspiration of a free purulent effusion, appendectomy, and drainage of the abdominal cavity.

-If there is a pronounced inflammatory infiltration of the tissues around the appendix, which create technical difficulties in excretion, it is necessary to switch to Volkovich-Dyakonov access,[16] and finish the operation by draining the periappendicular abscess bed with cigar-shaped drainage.

-With pronounced inflammatory changes with a criterion for assessing the severity of peritonitis above 10 points, a laparotomy with a laparostomy is the method of choice for surgical treatment.

-In appendicular abscesses of Stages 2-3, the method of choice is mini-laparotomic access and cigar-shaped drainage of the cavity.

With the definition of criteria for intraoperative assessment of the severity of peritonitis, the number of indications for laparotomy decreased. So, for the period from

2011 to 2014, the number of laparotomies performed with the subsequent programmed sanitation of the abdominal cavity was 17/14% cases; from 2015 to 2018, the inflammatory process in the abdominal cavity were assessed with the determination of the intraoperative index of peritonitis severity—the number of conversions was 15/11% cases.

With the creation of criteria for intraoperative assessment of the severity of peritonitis, the number of indications for laparostomy has decreased. So, until 2014, the number of laparostomies was 15 cases, since 2015 - 13 cases; the frequency of programmed rehabilitation in patients has decreased. In total "late" peritonitis, endoscopic surgery is technically impossible. In patients with API<10 points, the frequency of programmed sanitations decreased to 2 cases, which reduced the frequency of unreasonable laparotomy.

There is no marked change in the average number or frequency of laparostomies before the introduction and after the introduction of API, which indicates the validity of laparostomy. Median laparotomy with subsequent programmed sanitation of the abdominal cavity allows adequate intraoperative rehabilitation of the abdominal cavity and control of the course of the infectious process in the postoperative period. We adhere to the classical methodology of laparostomy proposed by N. Makoha.[9]

When comparing the average duration of the disease for patients with local and diffuse peritonitis in 2 independent samples, the differences in the average duration of the disease for patients with different types of peritonitis were statistically significant.

When determining the relationship between diffuse peritonitis and the duration of disease, the correlation coefficient was 0.32 (P=0.001), with statistical significance. When analyzing the duration of inpatient treatment of children operated on for appendicular peritonitis, there was a decrease in the average number of bed-days (Fig.2).

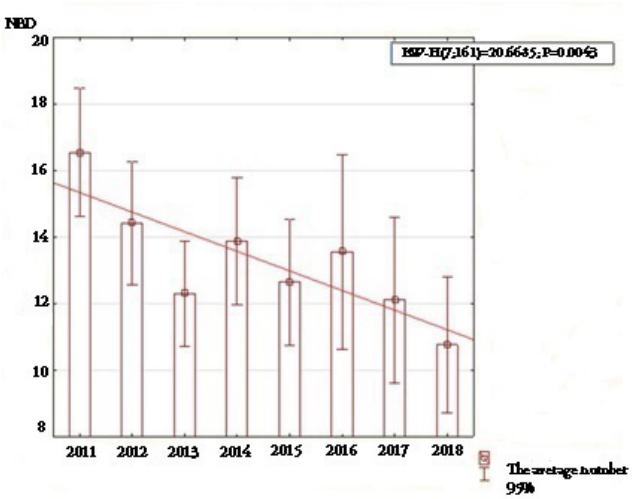


Fig. 2. *The average number of bed-days (NBD) in the year.*

Conclusion

With the introduction of the criteria for intraoperative assessment of the severity of peritonitis, the number of indications for laparotomy decreased (P<0.05). So, for the period from 2011 to 2014, the number of laparotomies performed with the subsequent programmed sanitation of the abdominal cavity was 17/14% cases; from 2015 to 2018, the inflammatory process in the abdominal cavity was assessed to determine the intraoperative index of peritonitis severity—the number of conversions was 15/11% cases. Thus, not only the number of laparoscopy performed, but also the frequency of programmed sanitations in patients decreased. In total "late" peritonitis, endoscopic surgery is technically impossible. The frequency of programmed sanitations decreased to 2 cases in patients with API<10 points, which reduced the frequency of unreasonable laparotomy.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

We thank Sardana A. Ivanova for design assistance and translation of this manuscript.

References

1. Yudin YAB, Prokopenko YD, Fedorov KK, Gabinsky TA. Acute appendicitis in children. M.: Meditsina;1998. [In Russian].
2. Muehlstedt SG, Pham TQ, Schmeling DJ. The management of pediatric appendicitis: a survey of North American Pediatric Surgeons. J Pediatr Surg. 2004;39(6):875-9; discussion 875-9.
3. Barskaja MA. Complex program of diagnosis and treatment of purulent surgical infection in children: Abstract of ScD Thesis. Ufa; 2000. [In Russian].
4. Dronov AF, Kotlobovsky VI, Poddubny IV. Laparoscopic surgery for acute appendicitis in children: real ways to improve the results of treatment. In: Proceedings of the symposium «Endoscopic surgery in children». Ufa; 2002:41-45. [In Russian].
5. Roshal' LM, Kapustin VA, Granikov OD, Karaseva OV. [Laparoscopic cleansing of the abdominal cavity in appendicular peritonitis in children]. Khirurgiia (Mosk). 1996;(4):35-7.[Article in Russian]
6. Tursunov KT, Ormantaev AK, Ruziddinov DB, Rakhmanov MA, Sagymbaeva AA. [Acute appendicitis and appendicular peritonitis]. Russian Journal of Pediatric Surgery, Anesthesia and Intensive Care. 2014;4(2):37-40. [Article in Russian].
7. Karaseva OV, Kapustin VA, Bryantsev AV. [Laparoscopic surgery for abscess forms of appendicular peritonitis in children]. Pediatric Surgery. 2005;3:25-29. [Article in Russian].
8. Doletsky SYa, Shchitinin VE, Arapova AV. Complicated appendicitis in children. M.: Meditsina; 1982. [In Russian].
9. Makokha NS. Treatment of severe forms of diffuse purulent peritonitis by the open method. In: Materials of the X Plenum Scientific Society of Surgeons of the Ukrainian SSR. Kiev; 1967:116-117. [In Russian].
10. Linder MM, Wacha H, Feldmann U, Wesch G, Streifensand RA, Gundlach E. [The Mannheim peritonitis index. An instrument for the intraoperative prognosis of

peritonitis]. Chirurg. 1987;58(2):84-92. [Article in German].
11. Krylov NN, Babkin OV, Babkin DO. [Mannheim peritonitis index, criteria for the selection of surgical intervention for perforated duodenal ulcer]. Surgery. 2016;7:18-22. [Article in Russian].
12. Sivaprahasam N, Vijayalakshmi S. Predictive value of mannheim peritonitis index (MPI) for mortality in perforated peptic ulcer. International Journal of Emerging Trends in Science and Technology. 2014;12:3:566-568.
13. Tushar D, Ramachandra L, Nair R, Digvijoy Sh. Evaluation of prognosis in patients, with perforation peritonitis using Mannheim's peritonitis index. International Journal of Scientific and Research Publication. 2015;5:2250-3153.
14. Simonyan KS. Peritonitis. M.: Meditsina; 1971. [In Russian].
15. Generalov AI, Kovalev VI, Baurova LV. [Abdominal cavity drainage in children with appendicular peritonitis (a review of the literature)]. Vopr Okhr Materin Det. 1977;22(12):16-22. [Article in Russian].
16. Anisimov AIu, Podshivalov AG. [Method for creating approach by Volkovich-D'iakonov in technically complicated appendectomies]. Khirurgiia. (Mosk). 1999;(12):19-20.[Article in Russian].

International Journal of Biomedicine 9(2) (2019) 139-143
http://dx.doi.org/10.21103/Article9(2)_OA12

ORIGINAL ARTICLE

Wound Repair and Regeneration

INTERNATIONAL JOURNAL OF BIOMEDICINE

Dynamics of Regenerative Processes in Patients with Trophic Ulcers against the Background of Chronic Venous Hemodynamic Disturbances Using Therapy with Polydesoxyrinobonucleotides

Yelena Yu. Shapovalova, PhD, ScD*; Yuriy G. Baranovskiy, PhD; Tatyana A. Boyko, PhD; Fedor N. Ilchenko, PhD, ScD; Nicolay P. Barsukov, PhD, ScD; Pavel V. Vaschenko, PGS

Medical Academy named after S.I. Georgievsky of Vernadsky CFU, Simferopol, Russia

Abstract

The aim of this study was to analyze the healing of trophic ulcers (TUs) of the skin against the background of chronic venous insufficiency (CVI) after applying PDRN-therapy in a complex of therapeutic measures.

Materials and Methods: The study included 19 patients with TUs of various sizes and shapes (from 4 to 12 cm^2) in the lower extremities with average duration from 4 weeks to 12 months, against the background of CVI. Patients were divided into two groups. Group 1 included 9 patients who received complex treatment along with using desoxyribonucleotide polymers (PDRN-therapy) according to the proposed methodology. Group 2 included 10 patients treated according to standard treatment of TUs. The area of wound defects was determined using the LesionMeter program. Skin thermometry was carried out using a B.Well WF-5000 medical infrared non-contact thermometer.

Results: We found that in patients of Group 1, who received PDRN-therapy in addition to standard treatment, by Day 20 there was a reduction in the TU area to 51.06±0.1% of the initial size; in patients of Group 2, who received only standard treatment, the TU area was 85.56±0.1% of the initial size. The temperature in the center of the ulcer and in its periphery increased in patients of Group 1 compared to patients of Group 2 by 2.79% and 2.74%, respectively, indicating a better blood supply to the skin defect, possibly due to more active angiogenesis

Conclusion: The use of PDRN-therapy in the complex treatment of trophic ulcers on the background of CVI is clinically effective. The applied method contributed to accelerating the repair processes of the skin defect and improving vascularization, which reduced the healing time of trophic ulcers. (**International Journal of Biomedicine. 2019;9(2):139-143.**)

Key Words: trophic ulcer • polydesoxyribonucleotides • planimetry • thermometry

Abbreviations

CVI, chronic venous insufficiency; **PDRN**, polydesoxyribonucleotide; **TU**, trophic ulcer.

Introduction

Treatment of nonhealing defects of the skin, which are called ulcers, is one of the most ancient surgical problems, which has not lost its relevance up to the present day. An ulcer is a defect in tissues with a small tendency to heal, which arises against the background of impaired tissue reactivity due to external or internal causes that are, in intensity, beyond the limits of the body's adaptive capabilities.[1] The formation of trophic ulcers (TUs) is a consequence of chronic venous insufficiency (CVI), accompanied by a violation of hemodynamics at the microcirculatory level and aggravated by the influence of external factors.[2] CVI affects 25-30% of women and 10-20% of men In 40%–90% of patients, venous insufficiency is complicated by TUs.[3] TUs are characterized by a low tendency to heal, frequent relapses, complexity and high cost of treatment.[4] Chronic TUs that exist for more

***Corresponding author**: Prof. Yelena Yu. Shapovalova, PhD, ScD. Head of the Department of Histology & Embryology of the Medical Academy named after S.I. Georgievsky of Vernadsky CFU. Simferopol, Russia. E-mail: Shapovalova_L@mail.ru*

than 3 months do not heal spontaneously.[5] They affect 2% of the US population and require treatment costing more than $3 billion annually.[6] At the same time, the problem of treating TUs of venous etiology is not currently resolved: The treatment is ineffective in 70% of patients. In this group, periods of temporary disability are high, resulting in 12.5% permanent disability.[7] According to statistics, in industrialized countries, TUs lead to disability more often than tuberculosis, rheumatism and traffic injuries, taken together.[8]

Despite the development of various dressings and methods of treatment using a wide range of drugs, TUs against the background of CVI remain a significant problem.[3,8,9] All this necessitates the search for new methods of treating this pathology.

Polydesoxyribonucleotides (PDRNs) are extracted from the semen of trout (*Oncorhynchus mykiss*) and salmon (*Oncorhynchus keta*). They are a combination of purine and phosphodiester bonds that make up the pyrimidine nucleotide monomer.[10] Although PDRNs can be extracted from human and cattle placenta, PDRN preparations from salmon sperm are preferable in terms of possible contamination. A recent study evaluating the wound-healing effect of *O. keta*-derived PDRN for injection (Rejuvenex) and PDRN cream(Rejuvenex Cream), compared to the effects of *O. mykiss*-derived PDRN injection(Placentex), showed a slightly higher efficiency of *O. keta*-derived PDRN for injection (Rejuvenex).[11] The use of polynucleotides is the newest specialized local therapy for the treatment of TUs. PDRNs are "prodrugs" that enrich cells with the right amount of mitogenic desoxyribonucleotides, desoxyribonucleosides, and also nitrogenous bases. PDRN, a DNA fragment of 50–2,000 bases, does not cause transformation at the genetic level and is completely harmless to the human body. PDRNs have the ability to replace the "broken" nitrogen bases of DNA with significant PDRN effectiveness. As a result, PDRN-based preparations remove incapable cell fragments, promote their recovery and activate the growth of young functional intracellular structures.[12]

Information about the positive effect of PDRNs on the healing of skin TUs of various etiologies is found in the scientific literature, but it is rare.[11,12]

The aim of this study was to analyze the healing of TUs of the skin against the background of CVI after applying PDRN-therapy in a complex of therapeutic measures.

Materials and Methods

The study included 19 patients with TUs of various sizes and shapes (from 4 to 12 cm^2) in the lower extremities with average duration from 4 weeks to 12 months (Table 1), against the background of CVI.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee of Medical Academy n.a. S.I. Georgievsky of Vernadsky CFU. Written informed consent was obtained from all participants.

Patients were divided into two groups. Group 1 included 9 patients who received complex treatment along with using PDRN according to the proposed methodology. Group 2 included 10 patients treated according to standard treatment of TUs. The groups were comparable in age, sex, severity of general condition, and nature of concomitant pathology.

Table 1.

Clinical characteristics of the patients according to the Clinical-Etiology-Anatomy-Pathophysiology (CEAP) classification

CEAP Clinical class	Gender (n/%)		Average duration of the skin defect, months	Average age, years
	M	F		
C5	4/21.1	5/26.3	8.9±0.2	56.3±2.5
C6	5/26.3	5/26.3	9.2±0.3	56.4±1.6

At the pre-hospital stage, all patients of the studied groups consulted with an angiosurgeon, received outpatient treatment at their place of residence, and in the case of a long wound history - in a general surgical hospital.

Standard treatment

All patients with TUs and CVI were subjected to compression with elastic knitwear; phlebotonics and systemic phleboprotectors were prescribed in accordance with the existing recommendations.

All patients of both groups were treated locally for TUs in a standard way, which included surgical treatment of a purulent focus, a removal of dead tissues, existing scab and fibrin layers, which often covered granulation tissue. Later, various antiseptics, including a 0.05% chlorhexidine digluconate solution, a 3% hydrogen peroxide solution, and a 1% dioxidine solution were topically used to clean the wound surface; we irrigated the wound surface during surgery or daily dressings. Then a gauze aseptic dressing was applied with a 0.1% betadine solution or ointment on a water-soluble basis (levocin, levomekol). All patients were prescribed antibiotic therapy from the first day of hospitalization in accordance with generally accepted regimens.

PDRN –therapy

Patients of Group 1 received PDRN extracted from the salmon semen with the commercial name "Plenhyage Medium" (I.R.A. Istituto Ricerche Applicate Sri; Italy) containing desoxyribonucleotide polymers with 50–2,000 pairs of nitrogenous bases.[13] PDRN solution (1.6 ml) was in a syringe with a needle in factory packaging. The drug was administered via subcutaneous and *intracutaneous* injections one time in 6 days along the periphery of the wound with a supplied 30G 0.3×13 mm needle (about 20 injections of 0.05 ml each).[14] The injections were made at a distance of 1 cm from the edge of the TU every 5 mm around the perimeter, distributing evenly the dose of the drug at the rate of 1 ml per 10 cm^2 of skin defect.

Determination of TU area

To study and objectivize the healing process of TUs in patients of both groups, the area of wound defects was determined using the LesionMeter program for the Android operating

system, which is installed on a smartphone. To measure the surface area of the ulcer, a limb segment with a wound defect was photographed using this program, with a standard plastic bank card placed in view as a scaling standard. After that, in the photo the perimeter of the wound defect was encircled by the cursor and its area was automatically received in cm^2. The result was recorded in the program archive. It should also be noted that this program is able to build line diagrams based on the accumulated data over time, which allows calculating the rate of epithelialization of a TU with high accuracy.

The area of the initial wound defect was determined upon admission of the patient to the hospital before the start of treatment and during the treatment period to determine the dynamics of wound healing on Days 4, 8, 12, 16 and 20, as well as at the end of the treatment period.

Thermometry of TU

Skin thermometry was performed to assess the effect of PDRN-therapy on the status of a TU. Skin thermometry was carried out using a B.Well WF-5000 medical infrared non-contact thermometer (UK, registration certificate for a medical product No. RZN 2013/1078 dated June 8, 2017) at 6 points of a TU: 3 points in the center of the ulcer and 3 points along its perimeter. Then the arithmetic mean temperature was determined according to the measurement localization.

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA) and Microsoft Excel 2007. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P<0.05$ was considered statistically significant.

Results

Before starting treatment in all patients of Groups 1 and 2, the mean values of the area of the wound defect were comparable and did not show a statistically significant difference (Table 2). By Day 4 of treatment, the area of a TU on the background of CVI slightly decreased in both groups, but a statistically significant difference between the groups was still not detected.

Table 2.

Healing dynamics of TUs in the patients of study groups

Day of observation	Group 1 (area of TU in cm^2)	Group 2 (area of TU in cm^2)	*P*-value
1	9.4±0.3	9.7±0.3	>0.05
4	9.1±0.2	9.4±0.4	>0.05
8	8.5±0.7	9.2±0.2	<0.05
12	7.3±0.4	8.8±0.4	<0.05
16	6.5±0.7	8.6±0.2	<0.05
20	4.8±0.4*	8.3±0.4	<0.05

*- *P<0.05 between Day 20 and Day 1 in Group 1*

After 8 days of complex treatment in patients of Group 1, to whom PDRN was administered twice (on Days 1 and 6), the ulcer area decreased by 6.59% compared to Day 4, which was statistically significant compared with Group 2.

On Days 12, 16 and 20, with continuing PDRN-therapy, the area of TUs decreased by 14.11%, 10.96% and 26.15%, respectively, compared with the previous stage of observation. In Group 2, a decrease in the area of TUs was 4.35%, 2.27% and 3.49%, respectively. Statistically significant differences between the two groups were observed on Days 8, 12, 16 and 20 (Table 2).

Data of Table 2 clearly show the rate of reduction in the skin defect area due to epithelialization of TUs in patients of both groups. Accelerated wound healing was observed in patients of Group 1 after Day 8 of treatment; in Group 2 without PDRN-therapy, similar acceleration was not observed.

In Group 1, after discharge from the hospital, TUs were completely healed by 32±1.2 days (Figures 1 and 2). In Group 2, we did not find complete epithelialization of TUs by this period of time.

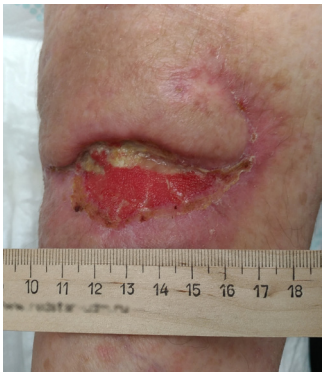


Fig. 1. *Trophic ulcer before treatment.*

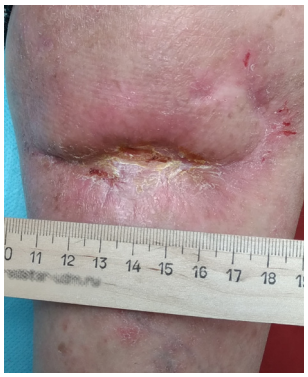


Fig. 2. *Trophic ulcer on Day 20 of treatment*

Since the temperature of the TU surface directly depends on the degree of microcirculation recovery, we used skin thermometry to indirectly characterize the changes in the blood supply to the plane of the ulcerative defect of the skin after PDRN injections. On Day 1 of treatment, the analysis of the temperature in the center of a TU and along its perimeter around the defect showed that this indicator had no statistically significant differences between the two groups ($P<0.05$) (Table 3). In Group 1, by Day 20 a significant increase in the temperature of TU in the examined areas was found.

Table 3.

Dynamics of the TU surface temperature in the patients of study groups

Temperature °C	Group 1		Group 2	
	Day 1	Day 20	Day 1	Day 20
In the center of TU	29.7±0.3°	32.3°±0.3°*	29.6±0.1°	31.4±0.3°
Along the perimeter around TU	30.8±0.2°	32.9±0.3°*	30.7±0.1°	32.0±0.1°

*- *P<0.05 between Day 20 and Day 1 in Group 1*

Discussion

The skin wound-healing process includes 3 stages: an inflammation, a proliferation with the formation of granulation tissue, and remodeling or fibrosis.[15] In long-term, non-healing ischemic wounds, elements of all three phases are simultaneously present, but inflammation predominates.[16] Despite the fact that the exact mechanism of the influence of polynucleotides on the regeneration processes is not completely clear, it is known that they contribute to the enhancement of angiogenesis and collagen formation during the healing of nonhealing defects of the skin of various etiologies.[17] PDRNs are expected to realize a significant regenerative effect, enhancing the metabolic processes in the dermis cells, increasing collagen and elastin production by fibroblasts, reducing the level of pro-inflammatory cytokine TNF-alpha and accelerating fibroblast differentiation into the proliferative phase of the wound process.[18] Granulation tissue is formed earlier, more actively and moderately. Experimentally proven reduction of the inflammatory response and a decrease in the presence of inflammatory cells after PDRN-therapy accelerates scarring and re-epithelialization of the skin.[19]

As a result of our comparative study, we found that in patients of Group 1, who received PDRN-therapy in addition to standard treatment, by Day 20 there was a reduction in the TU area to 51.06±0.1% of the initial size; in patients of Group 2, who received only standard treatment, the TU area was 85.56±0.1% of the initial size. In Group 1, there also was a tendency toward accelerating epithelization, which was not observed in Group 2. We did not find in the available literature any information on the effect of PDRNs on the dynamics of epidermis regeneration in humans. According to our experimental data, the thickness of the epidermis was significantly greater in mice in all studied periods of skin wound healing after the introduction of polynucleotides than in the control group.[20]

Since wound regeneration is accompanied by fluctuations in the temperature of damaged tissues caused by exo- and endothermic biochemical mechanisms of the healing process, the study of the dynamics of the perifocal and local temperature makes it possible to assess the nature of the course of the wound process. Many authors use thermometry in the complex of a common objective marker of wound healing.[21,22]) Thermometry of wounds in the clinic successfully uses various designs of non-contact infrared thermometers, which, compared to conventional mercury thermometers and electrothermometers, make it possible to measure the temperature with greater accuracy and discreteness, as well as being faster, more ergonomic and more comfortable for patients.[23] Due to the absence of direct contact of the infrared thermometer with the tissues and body fluids of the patient, there is no need for special treatment and preparation for work. Also, this method of remote temperature measurement allows one to measure the temperature of an object without disturbing the heat balance of the process being studied. This study does not harm the patients, does not violate the physiological processes in their bodies and does not cause those inconveniences that arise during the direct contact of any thermometers with the object being examined.

According to our data, the temperature in the center of the ulcer and in its periphery increased in patients of Group 1 compared to patients of Group 2 by 2.79% and 2.74%, respectively, indicating a better blood supply to the skin defect, possibly due to more active angiogenesis. Inadequate perfusion is an important factor in impaired wound healing.[10] Previously conducted experimental studies,[17] including those in our laboratory,[19] showed that PDRNs accelerate wound healing and improve angiogenesis, possibly due to an increase in vascular endothelial growth factor (VEGF) synthesis and binding to adenosine A2 receptors in endotheliocytes, thereby improving blood supply and ulcer perfusion.[11,12]

Conclusion

Thus, the study of planimetric indices of TU healing on the background of CVI in patients of Group 1, for whom conventional therapeutic measures were supplemented by local application of PDRN-therapy, showed that the area of ulcers was statistically significantly reduced by Day 20 of treatment to 51.06±0.1% of the original size. At the same time, in Group 2, this indicator remained at the level of 85.56±0.1% of the initial size. The surface temperature in the center of the ulcer and in its periphery on Day 20 of a combination of standard therapy and PDRN- therapy increased by 2.79% and 2.74%, respectively, compared to standard treatment. Temperature differences in the center and around the skin defect remained in both groups. The use of PDRN-therapy in the complex treatment of TUs on the background of CVI is clinically effective. The applied method contributed to accelerating the repair processes of the skin defect and improving vascularization, which reduced the healing time of trophic ulcers.

Competing Interests

The authors declare that they have no competing interests.

References

1. Tolstoy PI, Tamrazova OB, Pavlenko VV, Kuleshov IYu, Tolstoy MP. Prolonged non-healing wounds and ulcers (pathogenesis, clinic, treatment). M.: Deepak; 2009. [In Russian].
2. Obolensky VN, Rodoman GV, Nikitin VG, Karev MA. Trophic ulcers of the lower extremities - a review of the problem. Russian Medical Journal. 2009;(25):1647–63. [Article in Russian].
3. Brem H, Tomic-Canic M, Tarnovskaya A, Ehrlich HP, Baskin-Bey E, Gill K, et al. Healing of elderly patients with diabetic foot ulcers, venous stasis ulcers, and pressure ulcers. Surg Technol Int. 2003;11:161-7.
4. Budgen V. Evaluating the impact on patients of living with a leg ulcer. Nurs Times. 2004;100(7):30-1.
5. Saltmarche AE. Low level laser therapy for healing acute and chronic wounds- the extendicare experience. Int Wound J. 2008;5(2):351-60. doi: 10.1111/j.1742-481X.2008.00491.x.
6. Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. Impaired wound healing. Clin Dermatol. 2007;25(1):19-25.

7. Mikhailov AYu, Pronichev VV, Solov'ev AA, Styazhkina SN, Chernenkova LM, Ledneva AL. The effectiveness of stimulating auto-factors for the regeneration of local inflammatory and ulcerative processes. Perm Medical Journal. 2014;33(5):58-64. [Article in Russian].
8. Fraccalvieri M, Salomone M, Di Santo C, Ruka E, Morozzo U, Bruschi S. Quantum molecular resonance technology in hard-to-heal extremity wounds: histological and clinical results. Int Wound J. 2017;14(6):1313-1322. doi: 10.1111/iwj.12805.
9. Pang C, Ibrahim A, Bulstrode NW, Ferretti P. An overview of the therapeutic potential of regenerative medicine in cutaneous wound healing. Int Wound J. 2017;14(3):450-459. doi: 10.1111/iwj.12735
10. Shin J, Park G, Lee J, Bae H. The effect of polydeoxyribonucleotide on chronic non-healing wound of an amputee: a case report. Ann Rehabil Med. 2018;42(4):630-633. doi.org/10.5535/arm.2018.42.4.630.
11. Lee JH, Han JW, Byun JH, Lee WM, Kim MH, Wu WH. Comparison of wound healing effects between *Oncorhynchus keta*-derived polydeoxyribonucleotide (PDRN) and *Oncorhynchus mykiss*-derived PDRN. Arch Craniofac Surg. 2018;19(1):20-34. doi: 10.7181/acfs.2018.19.1.20.
12. Kim JY, Pak CS, Park JH, Jeong JH, Heo CY. Effects of polydeoxyribonucleotide in the treatment of pressure ulcers. J Korean Med Sci. 2014;29 Suppl 3:S222-7. doi: 10.3346/jkms.2014.29.S3.S222.
13. Noh TK, Chung BY, Kim SY, Lee MH, Kim MJ, Youn CS, Lee MW, et al. Novel anti-melanogenesis properties of polydeoxyribonucleotide, a popular wound healing booster. Int J Mol Sci. 2016;17(9):E1448. doi: 10.3390/ijms17091448.
14. Hwang KH, Kim JH, Park EY, Cha SK. An effective range of polydeoxyribonucleotides is critical for wound healing quality. Mol Med Rep. 2018;18(6):5166-72. doi: 10.3892/mmr.2018.9539.
15. Danilov RK. Wound process: Histogenetic basis. SPb.:VMedA; 2008. [In Russian].
16. Fedorov DN, Ivashkin AN, Shinin VV, Vasil'ev AV, Ivanov AA. [Morphological and immunohistochemical characteristics of repar processes in nonhealing wounds]. Arh Patol. 2002;64(1): 8-11. [Article in Russian].
17. Bitto A, Polito F, Altavilla D, Minutoli L, Migliorato A, Squadrito F. Polydeoxyribonucleotide (PDRN) restores blood flow in an experimental model of peripheral artery occlusive disease. J Vasc Surg. 2008;48(5):1292-300. doi: 10.1016/j.jvs.2008.06.041.
18. Bitto A, Oteri G, Pisano M, Polito F, Irrera N, Minutoli L, Squadrito F, Altavilla D. Adenosine receptor stimulation by polynucleotides (PDRN) reduces inflammation in experimental periodontitis. J Clin Periodontol. 2013;40(1):26–32. doi: 10.1111/jcpe.12010.
19. Shapovalova YeYu, Boyko TA, Baranovskiy YuG, Harchenko SV, Yunsi GA. [Influence of polynucleotides on the content of macrophages in regenerating ischemic wound]. Modern Problems of Science and Education. 2017;6. URL: http://www.science-education.ru/ru/article/view?id=27352 [Article in Russian].
20. Shapovalova YeYu, Boyko TA, Baranovskiy YuG, Kolomoyetz TA, Vasilenko SA. [The morphological peculiarities of the skin tissue are under the skin of the polydeoxyribonucleotide]. Crimea Journal of Experimental and Clinical Medicine. 2017;7(4):85-90. [Article in Russian].
21. Mufti A, Somayaji R, Coutts P, Sibbald RG. Infrared Skin Thermometry: Validating and Comparing Techniques to Detect Periwound Skin Infection. Adv Skin Wound Care. 2018;31(1):607-611. doi: 10.1097/01.ASW.0000527352.75716.70.
22. Sibbald RG, Mufti A, Armstrong DG. Infrared skin thermometry: an underutilized cost-effective tool for routine wound care practice and patient high-risk diabetic foot self-monitoring. Adv Skin Wound Care. 2015;28(1):37-44; quiz 45-6. doi: 10.1097/01.ASW.0000458991.58947.6b.
23. Langemo DK, Spahn JG. A Reliability Study Using a Long-Wave Infrared Thermography Device to Identify Relative Tissue Temperature Variations of the Body Surface and Underlying Tissue. Adv Skin Wound Care. 2017;30(3):109-119. doi: 10.1097/01.ASW.0000511535.31486.bb.

International Journal of Biomedicine 9(2) (2019) 144-149
http://dx.doi.org/10.21103/Article9(2)_OA13

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Results of Microbiological Investigations into Preterm Labor

Agamurad A. Orazmuradov, PhD, ScD[1]; Igor N. Kostin, PhD, ScD[1]; Sergey V. Apresyan, PhD, ScD[1]; Anna N. Parygina, PGS[1]; Alexandra A. Gavrilova, PGS[1]; Gayane A. Arakelyan, PGS[1]; Irina V. Savenkova, PGS[1]; Aleksey A. Lukaev, PhD[2]

[1]*RUDN University, Moscow Russia*
[2]*Mytishchi City Clinical Hospital Mytishchi, Moscow Region, Russia*

Abstract

The purpose of this study was to identify the relationship between the microbiological status of women and newborns, and the development of premature labor.

Materials and Methods: The study included 227 pregnant women at gestational age between 28 and 36 weeks and 6 days. All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge; the newborns underwent bacteriological examination of the auricle, pharynx and anus.

Results: Disturbances in the vaginal biotope were diagnosed in every second woman. We found that the shorter the gestation period, the higher the frequency of disturbances in the vaginal biotope, and the risk of premature birth at an earlier time correlates with the presence of infection in the mother. The risk of giving birth to an infected child is 4.2 times higher at birth from a mother who has disturbances in the biotope, compared to a woman with a normal biotope.(**International Journal of Biomedicine. 2019;9(2):144-149.**)

Key Words: premature labor • vaginal biotope • vaginal infections • newborns

Abbreviations

PL, premature labor; **BG**, bacterial growth; **GA**, gestational age; **PB**, premature birth.

Introduction

The causes of spontaneous PL are heterogeneous. An important risk factor for PL is a high infectious index of the genital tract [1,2] during pregnancy and chronic placental insufficiency.[3] The achievements of clinical microbiology in recent decades have largely changed our understanding of the possible causative agents of vaginal infections.[4-9] In women with bacterial vaginosis, PB occurs 3–4 times more often [10] and the likelihood of postpartum endometritis is 5–7 times higher than in women without the infection.

Long-term clinical observations confirm the connection between vaginal infections and adverse pregnancy outcomes for the mother and fetus.[11-13] To date, convincing evidence of the linkage between vaginal infections and PL, fetal intrauterine infection, and inflammatory complications after childbirth has been published.[14-20] That study, of the reproductive health of the examined women, showed that inflammatory diseases of the body and uterine appendages occurred in 18.2% of cases. There is evidence of repeated, and often recurrent, inflammatory diseases of the external genital organs and the vagina in 36.9% of women.[17] Currently, among the infections of the vagina, bacterial vaginosis, aerobic vaginitis and candidal vulvovaginitis play a leading role.[21] Unfavorable predictors of PB are a combination of bacterial vaginosis and the persistence of Ureaplasma urealyticum, Mycoplasma hominis and Mycoplasma genitalium in the cervical canal of pregnant women.[22] Inflammation of the mucous membrane in these cases is due to the activation of various representatives of opportunistic flora. Usually, the pathogens are different

[*]***Corresponding author:*** *Aleksey A. Lukaev, PhD. Mytishchi municipal clinical hospital, Mytishchi, Moscow Region, Russia E-mail: aleksei_lukaev@mail.ru*

types of staphylococci and streptococci, enterococci, E. coli, Klebsiella, Proteus and some other bacteria and their associations.[23]

However, the development of an imbalance in the vaginal microbiota is still poorly understood.[16,24] Therefore, etiotropic therapy is a difficult task, especially in the early stages of pregnancy. The use of antibacterial drugs during pregnancy is debatable, and treatment approaches do not have solid standards.[25] Numerous clinical studies and systematic reviews based on meta-analysis present conflicting views on antimicrobial therapy for opportunistic infections of the vagina during pregnancy.[26-29] The purpose of this study was to identify the relationship between the microbiological status of women and newborns, and the development of PL.

Materials and Methods

Our study included 227 pregnant women at gestational age between 28 and 36 weeks and 6 days. Depending on the gestational age, they were divided into 3 groups. Group 1 included 73 women at gestational age between 28 and 30 weeks and 6 days; Group 2 included 81 women at gestational age between 31 and 33 weeks and 6 days, Group 3 included 73 women at gestational age between 34 and 36 weeks and 6 days. All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge; the newborns underwent bacteriological examination of the auricle, pharynx and anus.

In order to study the state of vaginal microcenosis, as well as the diagnosis of trichomonas and gonococcal infections, we performed a microscopic examination of the vaginal discharges, which were taken with a sterile gynecological universal probe (Centrmed LLC, Russia) from the posterior and lateral vaginal vaults. The resulting material was applied to separate areas of a defatted slide, dried in air, and stained with methylene blue or Gram. We then evaluated the leukocyte reaction, the morphology of the microorganisms and their total number, the presence of "key" cells in the vaginal biotope.

To analyze the nature of the quantitative and qualitative composition of the biotope of the cervical discharge, a tampon-probe and test tubes with a transport medium ("Medical Wire & Equipment", England) were used. Seeding was performed on a series of nutrient media to isolate and cultivate various groups of microorganisms: 5% blood agar based on Brucella agar with the addition of vitamin growth factors to isolate and cultivate anaerobes, mannitol salt agar to isolate and cultivate gram-negative bacteria, and Saburo medium to isolate and cultivate fungi. Blood agar media were cultivated in a thermostat with a high content of carbon dioxide (5-10%). To cultivate anaerobes we used anaerostats (Becton Dickinson, USA). The isolated microorganisms were identified and their sensitivity to antibacterial drugs was determined using the Witek bacteriological analyzer.

The results obtained were recorded in accordance with the NCCLS standards (1999–2000). The number of isolated microorganisms was determined by the density of their growth on the sectors of the agar plate.

Bacteriological study of auricular secret and pharynx

The material obtained and transported to the laboratory was seeded on Petri dishes with 5% blood agar, chocolate agar, and yolk-salt agar, on Endo medium, Saburo medium, and in a tube with glucose broth. Seeding on dense nutrient media was carried out metered (according to Gould), which made it possible to quantify the number of grown colonies. Seedings were incubated at 37°C for 24–48 hours, and were examined daily. Plates with 5% blood agar were incubated under conditions with a high CO_2 content. With the appearance of growth on nutrient media, we counted colonies of various morphologies, taking into account their ratio and species identification of microorganisms, as well as determining their sensitivity to antibacterial drugs. A negative result of the study was issued in the absence of growth on all nutrient media for 72–96 hours.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the RUDN University Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. The Pearson's correlation coefficient (r) and Spearman's rank correlation coefficient (r_s) were was used to determine the strength and direction of the relationship between two variables. The two-proportions z-test was used to compare two observed proportions. Group comparisons with respect to categorical variables are performed using chi-square tests. A value of $P<0.05$ was considered statistically significant.

Results

Bacterioscopy of the contents of the *vaginal and cervical canal contents*

According to the results of bacterioscopic examination (Table 1), normocenosis of the genital tract was diagnosed only in 84(37.0%) women. Disturbances in the vaginal biotope were diagnosed in every second woman. We found that the shorter the gestation period, the higher the frequency of disturbances in the vaginal biotope (82.2%). We found a moderate inverse correlation between the term of labor and the presence of bacteria (r=-0.3657, $P<0.001$). Significant differences were found between Group 1 and Groups 2 and 3 ($\chi^2=23.036$, $P=0.0000$), and between Groups 2 and 3 ($\chi^2 5.653$, $P= 0.017$).

Table 1.

The results of bacterioscopic examination

Group	normocenosis		vaginal infections		Statistics
	n	%	n	%	
Group 1 (n=73)	13	17.8	60	82.2	$\chi^2=23.036$ $P=0.0000$
Group 2 (n-81)	30	37.0	51	63.0	
Group 3 (n=73)	41	56.2	32	43.8	$P_{1-2}=0.0079$ $P^{2-3}= 0.0174$
Total (n=227)	84	37.0	143	63.0	$P^{2-3}_{1-3}=0.0000$

Table 2.

Bacterial cultures of the cervical canal of pregnant women

Bacterial cultures	Total	Group 1	Group 2	Group 3	P_{1-2}	P_{1-3}	P_{2-3}
E. coli	27 (11.9 %)	11 (15.1 %)	10 (12.3 %)	6 (8.2 %)	0.6249	0.1965	0.3979
Candida spp.	38 (16.7 %)	15 (20.5 %)	13 (16 %)	10 (13.7 %)	0.8389	0.5472	0.6773
Enterococcus faecalis	22 (9.7 %)	8 (11 %)	5 (6.2 %)	9 (12.3 %)	0.2924	0.7967	0.1909
Strep. agalactiae	8 (3.5 %)	7 (9.6 %)	0 (0 %)	1 (1.4 %)	0.1002	0.0281	0.7782
Strep. oralis	7 (3.1 %)	5 (6.8 %)	2 (2.5 %)	0 (0 %)	0.2025	0.2515	0.6507
Staphyl. epirmidis	17 (7.5 %)	7 (9.6 %)	9 (11.1 %)	1 (1.4 %)	0.7568	0.0281	0.0103
Staph.aureus	15 (6.6 %)	6 (8.2 %)	3 (3.7 %)	6 (8.2 %)	0.2413	1.0000	0.2413
Staph. haemolyticus	6 (2.6 %)	2 (2.7 %)	3 (3.7 %)	1 (1.4 %)	0.7346	0.5601	0.3522
Staph.saprophyticus	6 (2.6 %)	2 (2.7 %)	4 (4.9 %)	0 (0 %)	0.4755	0.6195	0.3874
Gemella morbillorum	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Enterobacter aerogenes	3 (1.3 %)	2 (2.7 %)	1 (1.2 %)	0 (0 %)	0.5084	0.6195	0.8163
Strep.viridans	3 (1.3 %)	1 (1.4 %)	1 (1.2 %)	1 (1.4 %)	0.9412	1.0000	0.9412
Staph. warneri	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Serratia odorifera	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Enterobacter cloacae	1 (0.4 %)	1 (1.4 %)	0 (0 %)	0 (0 %)	0.7782	0.7979	1.0000
Streptococcus mitis	3 (1.3 %)	0 (0 %)	2 (2.5 %)	1 (1.4 %)	0.6507	0.7979	0.6174
Corynebacterium spp.	4 (1.8 %)	1 (1.4 %)	2 (2.5 %)	1 (1.4 %)	0.6174	1.0000	0.6174
Acinetobacter	6 (2.6 %)	1 (1.4 %)	2 (2.5 %)	3 (4.1 %)	0.6174	0.3106	0.5716
Klebsiella pneumoniae	1 (0.4 %)	0 (0 %)	0 (0 %)	1 (1.4 %)	1.0000	0.7979	0.7782

Table 3.

Data of bacteriological results from the studied loci (the anus. ears and pharynx) in newborns

Bacterial cultures	Total	Group 1	Group 2	Group 3	P_{1-2}	P_{1-3}	P_{2-3}
E.coli	20 (8.8 %)	9 (12.3 %)	8 (9.9 %)	3 (4.1 %)	0.6299	0.0695	0.1563
Candida spp	9 (4 %)	5 (6.8 %)	3 (3.7 %)	1 (1.4 %)	0.3869	0.0944	0.3522
Enteroccocus faecalis	20 (8.8 %)	8 (11 %)	5 (6.2 %)	7 (9.6 %)	0.2924	0.7855	0.4347
Enterobacter cloacae	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Strep.agalactiae	9 (4 %)	4 (5.5 %)	4 (4.9 %)	1 (1.4 %)	0.8804	0.1716	0.1988
Strep.oralis	5 (2.2 %)	2 (2.7 %)	1 (1.2 %)	2 (2.7 %)	0.5084	1.0000	0.5084
Streptococcus mitis	2 (0.9 %)	0 (0 %)	1 (1.2 %)	1 (1.4 %)	0.8163	0.7979	0.9412
Staph. aureus	18 (7.9 %)	8 (11 %)	7 (8.6 %)	3 (4.1 %)	0.6306	0.1160	0.2460
Staph. saprophyticus	3 (1.3 %)	2 (2.7 %)	1 (1.2 %)	0 (0 %)	0.5084	0.6195	0.8163
Staph. epirmidis	15 (6.6 %)	8 (11 %)	5 (6.2 %)	2 (2.7 %)	0.2924	0.0482	0.2979
Staph. haemolyticus	4 (1.8 %)	3 (4.1 %)	0 (0 %)	1 (1.4 %)	0.4313	0.3106	0.7782
Staph. warneri	3 (1.3 %)	2 (2.7 %)	1 (1.2 %)	0 (0 %)	0.5084	0.6195	0.8163
Strep. viridans	1 (0.4 %)	0 (0 %)	0 (0 %)	1 (1.4 %)	1.0000	0.7979	0.7782
Neisseria spp.	10 (4.4 %)	3 (4.1 %)	5 (6.2 %)	2 (2.7 %)	0.5611	0.6495	0.2979
Genella morbillorum	1 (0.4 %)	1 (1.4 %)	0 (0 %)	0 (0 %)	0.7782	0.7979	1.0000
Serratia marcescens	4 (1.8 %)	3 (4.1 %)	1 (1.2 %)	0 (0 %)	0.2756	0.4691	0.8163
Serratia odorifera	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Corynebacterium spp.	3 (1.3 %)	0 (0 %)	1 (1.2 %)	2 (2.7 %)	0.8163	0.6195	0.5084
Acinebacter	1 (0.4 %)	1 (1.4 %)	0 (0 %)	0 (0 %)	0.7782	0.7979	1.0000
Klebsiella pneumoniae	2 (0.9 %)	2 (2.7 %)	0 (0 %)	0 (0 %)	0.5873	0.6195	1.0000

The study of cervical canal cultures

In all pregnant women (n=227), immediately upon admission to the hospital, a discharge content from the cervical canal was taken for bacteriological examination with the determination of sensitivity to antibacterial drugs. The data of the first seeding are the most important, since after the course of antibiotic therapy, the subsequent results of bacteriological seeding are uninformative for the diagnosis of vaginal microflora. The growth of microorganisms was observed in 143(63.0%) pregnant women; in 29(12.8%) women who gave birth, the growth of the bacterial flora in the newborns was not observed.

The leading positions among the sowed microorganisms were occupied by *Candida spp.* (38/16.7%), *E. coli* (27/11.9%), and *Enterococcus faecalis* (22/9.7%), and the growth of 50% of the detected bacteria was observed in Group 1 (Table 2). In bacterial vaginosis, microorganisms of intestinal origin were predominant against the background of a significant decrease in the frequency of lactobacilli. In vaginitis, associations of microorganisms represented by 2–5 species with an anaerobic or aerobic component were found in all cases. At the same time, 84 women with normal flora gave birth to 16(28.6%) children, who showed bacterial growth in diagnostically significant titers. Thus, respectively, only 68(30%) of the women gave birth to children who did not show an increase in bacterial opportunistic flora.

All newborns were bacteriologically examined (the anus, ears and pharynx). The growth of microorganisms was observed in 131(57.7%) newborns (Table 3). The leading positions among the sowed microorganisms in children were occupied by *E. coli* (20/8.8%), *Enteroccocus faecalis* (20/8.8%), *Candida spp.* (9/4%), and *Streptococcus agalactiae* (9/4%). At the same time, the growth of microflora was found significantly more often in newborns from mothers of Group 1. According to the study, in 127(55.9%) of 227 children, the growth of bacteria from the studied loci was detected in diagnostically significant titers (>10^5cfu/ml). At the same time, in 114(89.8%) of these children, the growth of microflora was associated with the identified infection in mothers (Table 4, Fig.1). The birth of a child with microorganisms correlated with the presence of infection in the mother (r_s=0.706, P<0.0001).

Table 4.

Comparative analysis of bacterial growth in women and newborns

	Total (n/%)	Group 1 (n/%)	Group 2 (n/%)	Group 3 (n/%)	P_{1-2}	P_{1-3}	P_{2-3}
BG in women	143/63	60/82.2	51/63	32/43.8	0.007	0.000	0.017
BG in newborns	127/55.9	54/74	43/53.1	30/41.1	0.006	0.000	0.136
BG in women and newborns	114/50.2	50/68.5	38/46.9	26/35.6	0.006	0.000	0.154
BG in women, 100%	143	60	51	32			
The proportion of newborns with BG in % of mothers with BG	114/79.7	50/83.3	38/74.5	26/81.3	0.259	0.805	0.466

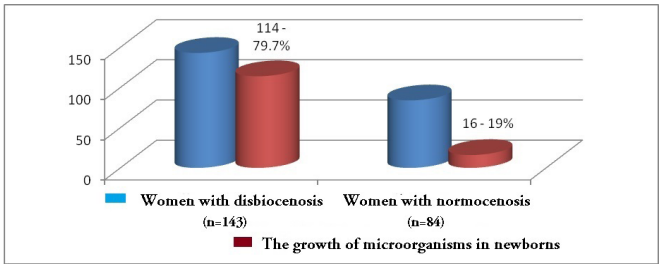


Fig. 1. *Bacterial growth in women and newborns.*

Discussion

High contamination of the genital tract of pregnant women with opportunistic and pathogenic bacterial and viral microflora is a high risk factor for PB. It is noted that the shorter the gestation period, the higher the frequency of disturbances in the vaginal biotope (82.2%), and the risk of PB at an earlier time correlates with the presence of infection in the mother (r=-0,327, P<0.001; r_s=-0.331, P=0.004) (Fig.2). The study by D. Nelson[16] showed that the prevalence of bacterial vaginosis among pregnant women was 3.88%, and the presence of G. vaginalis was 67.48%; but the presence of G. vaginalis was not enough to cause bacterial vaginosis. However, the presence of G. vaginalis may be considered a significant risk factor for PB.

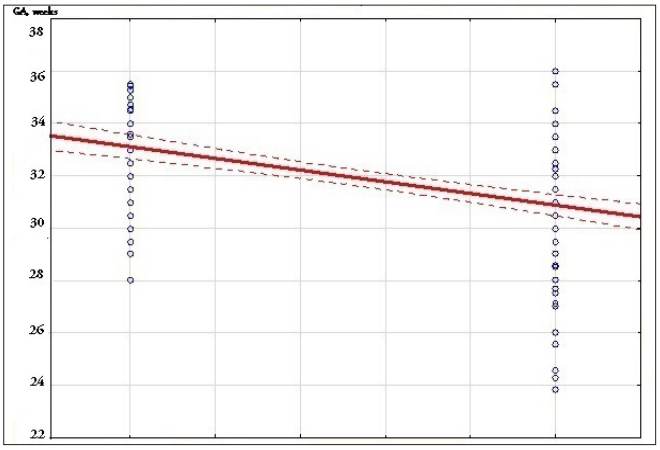


Fig.2. *The growth of microorganisms in the cervical canal of pregnant women (r=-0.327, P<0.001; OR=4.19, 95% CI: 2.67 – 6.55)*

In the previous study,[17] we showed the main risk factors leading to PL: early sexual debut, inflammatory diseases of the urinary organs, sexually transmitted infections, reproductive losses in history, anemia, etc. The present study shows the association of PL with an infectious factor. In our opinion, and according to a number of authors, a high bacterial contamination of the genital tract, already at the stage prior to the onset of pregnancy, contributes to the infection of the fetal membranes, with impaired amniotic fluid production, starting from the early stages of gestation.

Comparing the data obtained with the results of neonatal outcomes, according to a previous study,[30] we can note the

high risk of having children with congenital pneumonia in women of Group 1. With an increase in the period of gestation, the frequency of this complication is significantly reduced. In the future, we plan to perform a placental histology study and identify the presence of possible relationships among the data of microbiological studies.

Conclusion

The risk of giving birth to an infected child is 4.2 times higher at birth from a mother who has disturbances in the biotope, compared to a woman with a normal biotope. The result is a long-term presence of the newborn in the intensive care unit, antibiotic therapy, and a long rehabilitation period. Despite the ongoing preventive antibiotic therapy in pregnant women and mothers, the frequency of detectable pathogenic microorganisms in newborns has not decreased. The data obtained are explainable from several positions:

- First, microorganisms are resistant to antibacterial drugs used.
- Second, and probably the most important: A contamination of the newborn with pathogenic microorganisms occurs in-utero (prenatally) at earlier gestational periods.

In our study, 7(3.4%) women showed resistance to antibacterial drugs during bacteriological examination of discharge content from the cervical canal: E. coli was found in 5 women and Staphylococcus epidermidis in 2 women.

Thus, the results of the study allow us to:

- identify the group of pregnant women at risk for the infection process
- carry out therapeutic measures for the prevention and treatment of microflora biocenosis disorders in the early stages of gestation, and
- carry out measures to improve the reproductive potential of the population.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

The study was supported by the RUDN University Program 5-100.

References

1. Khadartseva KA, Kusibaeva RK. [The role of pregravid preparation in improving perinatal outcomes in women with inflammatory diseases of the genital tract]. Russian Journal of Woman and Child Health. 2017;(25):1934-1937. [Article in Russian].
2. Dolgushina VF, Kurnosenko IV, Astashkina MV, Nadvigova TV. [Infectious Pathology of the vagina and the cervix in women with spontaneous preterm birth]. Ural medical journal. 2017;(1):62-64. [Article in Russian].
3. Katkova NYu, Bodrikova OI, Bezrukova IM, Guseva OI, Lebedeva NV, Pokusayeva KB, Mikhaylenko OA. [Clinical and Anamnestic Features of Different Types of Premature Birth (Retrospective Review)]. Effektivnaya Farmakoterapiya. 2017; (26):12-17. [Article in Russian].
4. Beghini J, Giraldo PC, Linhares IM, Ledger WJ, Witkin SS. Neutrophil Gelatinase-Associated Lipocalin Concentration in Vaginal Fluid: Relation to Bacterial Vaginosis and Vulvovaginal Candidiasis. Reprod Sci. 2015;22(8):964-8. doi: 10.1177/1933719115570914.
5. Hilbert DW, Smith WL, Chadwick SG, Toner G, Mordechai E, Adelson ME, et al. Development and Validation of a Highly Accurate Quantitative Real-Time PCR Assay for Diagnosis of Bacterial Vaginosis. J Clin Microbiol. 2016;54(4):1017-24. doi: 10.1128/JCM.03104-15.
6. Vieira-Baptista P, Lima-Silva J, Pinto C, Saldanha C, Beires J, Martinez-de-Oliveira J, Donders G. Bacterial vaginosis, aerobic vaginitis, vaginal inflammation and major Pap smear abnormalities. Eur J Clin Microbiol Infect Dis. 2016;35(4):657-64. doi: 10.1007/s10096-016-2584-1.
7. Vitali B, Cruciani F, Picone G, Parolin C, Donders G, Laghi L. Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. Eur J Clin Microbiol Infect Dis. 2015;34(12):2367-76. doi: 10.1007/s10096-015-2490-y.
8. Wang ZL, Fu LY, Xiong ZA, Qin Q, Yu TH, Wu YT, et al. Diagnosis and microecological characteristics of aerobic vaginitis in outpatients based on preformed enzymes. Taiwan J Obstet Gynecol. 2016;55(1):40-4. doi: 10.1016/j.tjog.2015.06.012.
9. Xiao B, Niu X, Han N, Wang B, Du P, Na R, et al. Predictive value of the composition of the vaginal microbiota in bacterial vaginosis, a dynamic study to identify recurrence-related flora. Sci Rep. 2016;6:26674. doi: 10.1038/srep26674.
10. Petrov YuA, Galushchenko EM, Arndt IG. [Premature birth taking into account the influence of the vaginal microbiocenosis]. Mezhdunarodnii Zhurnal Prikladnih i Fundamental'nih Sssledovanii. 2018;(11-2):384-388. [Article in Russian].
11. Radzinsky VE, Orazmuradov AA. Early gestational age. From pregravid preparation to healthy gestation. 3rd., rev. version. M.: StatusPraesens; 2018. [In Russian].
12. Subramaniam A, Kumar R, Cliver SP, Zhi D, Szychowski JM, Abramovici A, et al. Vaginal Microbiota in Pregnancy: Evaluation Based on Vaginal Flora, Birth Outcome, and Race. Am J Perinatol. 2016;33(4):401-8. doi: 10.1055/s-0035-1565919.
13. Yang S, Reid G, Challis JR, Kim SO, Gloor GB, Bocking AD. Is there a role for probiotics in the prevention of preterm birth? Front Immunol. 2015;6:62. doi: 10.3389/fimmu.2015.00062.
14. Haahr T, Ersbøll AS, Karlsen MA, Svare J, Sneider K, Hee L, et al. Treatment of bacterial vaginosis in pregnancy in order to reduce the risk of spontaneous preterm delivery - a clinical recommendation. Acta Obstet Gynecol Scand. 2016;95(8):850-60. doi: 10.1111/aogs.12933.
15. Heczko PB, Tomusiak A, Adamski P, Jakimiuk AJ, Stefański G, Mikołajczyk-Cichońska A, et al. Supplementation of standard antibiotic therapy with oral probiotics for bacterial vaginosis and aerobic vaginitis: a randomised, double-blind, placebo-controlled trial. BMC Womens Health. 2015;15:115. doi: 10.1186/s12905-015-0246-6.
16. Nelson DB, Hanlon AL, Wu G, Liu C, Fredricks DN. First Trimester Levels of BV-Associated Bacteria and Risk of Miscarriage Among Women Early in Pregnancy. Matern Child

Health J. 2015;19(12):2682-7. doi: 10.1007/s10995-015-1790-2.
17. Orazmuradov AA, Soyunov MA, Efendieva ZM, Lukaev AA, Gradova IE, Mukhtarova AV, Kaplina IN. Influence of Perinatal Risk Factors on Premature Labor Outcome. International Journal of Biomedicine. 2016;6(3):199-201
18. Kuzmina TE, Pashkov VM, Klindukhov I.A. [Pregravid preparation. Modern conceptions]. Gynecology, Obstetrics and Perinatology. 2015;14(5):47–54. [Article in Russian].
19. Pustotina OA, Akhmedova AE. [Pregravid preparation for women with a history of miscarriage]. Meditsinskiy Sovet. 2016;(4):130-136. [Article in Russian].
20. Khamadyanov UR, Gumerova IA, Khamadyanova AU, Galimov AI, Khamadyanova SU, Tikhonova TF. [Role of genital nontransmissible infections in preterm pregnancy termination]. Russian Bulletin of Obstetrician-Gynecologist. 2016;16(6):32-37. [Article in Russian].
21. Karapetyan TE, Muraveva VV, Ankirskaya AS, Lyubasovskaya LA, Priputnevich TV. [Treatment options for opportunistic vaginal infections during pregnancy]. Ginekologiia. 2017;19(6):11-15. [Article in Russian].
22. Radzinskiy VE, Orazmuradov AA, Arakelyan VF. [Biocenosis vagina at the very early preterm labor]. Medical Herald of the South of Russia. 2014;(4):90-94. [Article in Russian].
23. Tyutyunnik VL, Kan NE, Lomova NA. [Modern aspects of therapy of non-specific vulvovaginitis during pregnancy]. Meditsinskiy Sovet. 2018;(7):102-105. [Article in Russian].
24. Nasioudis D, Linhares IM, Ledger WJ, Witkin SS. Bacterial vaginosis: a critical analysis of current knowledge. BJOG. 2017;124(1):61-69. doi: 10.1111/1471-0528.14209.
25. Podgornaya AV, Makhmutkhodzhaev ASh, Mikheenko GA, Koch LI. [Management of pregnant women with recurrent bacterial vaginosis]. Modern problems of science and education. 2016(6):172. [Article in Russian].
26. Amaya-Guio J, Viveros-Carreño DA, Sierra-Barrios EM, Martinez-Velasquez MY, Grillo-Ardila CF. Antibiotic treatment for the sexual partners of women with bacterial vaginosis. Cochrane Database Syst Rev. 2016;10:CD011701.
27. Catallozzi M, Fraiz LD, Hargreaves KM, Zimet GD, Stanberry LR, Ratner AJ, et al. Pregnant women's attitudes about topical microbicides for the prevention and treatment of bacterial vaginosis during pregnancy. Int J STD AIDS. 2017;28(9):881-886. doi: 10.1177/0956462416679067.
28. Liang Q, Li N, Song S, Zhang A, Li N, Duan Y. High-dose nifuratel for simple and mixed aerobic vaginitis: A single-center prospective open-label cohort study. J Obstet Gynaecol Res. 2016;42(10):1354-1360. doi: 10.1111/jog.13052.
29. Sobel R, Sobel JD. Metronidazole for the treatment of vaginal infections. Expert Opin Pharmacother. 2015;16(7):1109-15. doi: 10.1517/14656566.2015.1035255.
30. Orazmuradov AA, Zakharova NI, Lukaev AA, Zholobova EV. Neonatal outcomes in the postpartum period depending on perinatal risk factors, terms and mode of delivery. International Journal of Biomedicine. 2016;6(3):202-206.

International Journal of Biomedicine 9(2) (2019) 150-154
http://dx.doi.org/10.21103/Article9(2)_OA14

ORIGINAL ARTICLE

Histology & Cytology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Blocking L-type Voltage-Gated Calcium Ion Channels Changes the Intensity of Protein Synthesis in Metanephric Cells

Yelena Yu. Shapovalova, PhD, ScD*; Liliana A. Kutuzova, PhD; Svetlana V. Kharchenko, PhD; Svetlana A. Vasilenko

Medical Academy named after S.I. Georgievsky of Vernadsky CFU, Simferopol, Russia

Abstract

The purpose of this research was to study the effect of L-type voltage-gated calcium channels (L-VGCC) blocking on mRNA content and intensity of protein synthesis in cells of the metanephros.

Materials and Methods: The study was performed on 27 outbred Wistar rats weighing 250-270 g from 5 to 7 months of age. Pregnant females were divided into 3 groups of 9 individuals each. Group1 (the control group) included intact pregnant females treated with distilled water; Group 2 (the first experimental group) included animals receiving a therapeutic dose of nifedipine; Group 3 (the second experimental group) included animals receiving a toxic dose of nifedipine. The dihydropyridine nifedipine (Sigma-Aldrich, Gillingham, UK) was used as a Ca2+ channel blocker. Nifedipine was dissolved in distilled water. The dose of nifedipine was calculated based on therapeutic and toxic doses for humans. The therapeutic dose for rats was 127 mg/kg, toxic dose – 762 mg/kg. In each group, when rats reached Day 14, 15, 16 and consistently up to 22 of gestation, they were removed from the experiment by decapitation under ether anesthesia. The embryos and fetuses were removed, subjected to external examination and quickly fixed with 10% neutral formalin entirely up to the age of 15 days. At an older age, both metanephros were removed from the fetuses by preparation and fixed with 10% neutral formalin. The intensity of protein synthesis was estimated by the content of mRNA in the cytoplasm.

Results: The development of the metanephros in rats of the control group was accompanied by a gradual decrease in the content of mRNA, indicating the activity of protein synthesis in the cell cytoplasm of epithelial and mesenchymal buds of the metanephros. In the fetuses of rats whose mothers received therapeutic and toxic doses of nifedipine, statistically significant changes in the content of mRNA in the cytoplasm of the metanephric cells were observed. These changes were most significant in the metanephric cells after administration of the toxic dose. On ED 17, an L-VGCC blockade with a therapeutic dose of nifedipine led to a paradoxical reaction of cells with an increase in protein synthesis, compared with the control group.

Conclusion: Both doses of nifedipine have a greater effect on the content of mRNA in the cytoplasm of epithelial cells, compared to mesenchymal buds. (**International Journal of Biomedicine. 2019;9(2):150-154.**)

Key Words: metanephros • voltage-gated calcium channels • nifedipine • protein synthesis

Introduction

Calcium ions play an important role in regulation of many *vital* physiological functions and metabolic processes. By penetrating the cell, they activate bioenergetic processes, as the transformation of ATP into cyclic AMP and protein phosphorylation.[1] Physiological activity is characteristic only of ionized calcium (Ca^{2+})—unbound calcium. Ca^{2+} ions are unique in that they not only carry a charge but they are also the most widely used of diffusible second messengers. Voltage-gated calcium channels (VGCCs) are the primary mediators of depolarization-induced calcium entry into the cell.[2]

Based on pharmacological and biophysical properties, VGCCs are classified into 3 families: the L-type, the P/Q/R/N-type and the T-type channels.[2] The long-lasting (L-type) VGCCs (L-VGCCs) have been shown to play crucial roles in Ca^{2+} homeostasis of excitable cells. L-VGCCs are present in the heart, smooth and skeletal muscle, and some neurons. The importance of L-VGCCs is demonstrated by the clinical efficacy of $Ca2^{+}$ channel blockers in certain disease conditions.

A number of postnatal kidney diseases are known to be associated with abnormalities during prenatal organogenesis. Fetal development occurs in a hypercalcemic environment

***Corresponding author**: *Prof. Elena Shapovalova, PhD, ScD. Head of the Department of Histology & Embryology* of the *Medical Academy named after S.I. Georgievsky of Vernadsky CFU. Simferopol, Russia. E-mail: Shapovalova_L@mail.ru*

compared with the postnatal period.[3] The few experimental studies found in the world's scientific bases suggest that calcium activity contributes to many aspects of kidney development and its decline leads to the formation of associated diseases.[4] Very few studies have been devoted to the effect of blocking the passage of calcium ions into the cell through L-VGCCs on the development of the metanephros and the associated protein synthesis in its cells.

The purpose of this research was to study the effect of L-VGCC blocking on mRNA content and intensity of protein synthesis in cells of the metanephros.

Materials and Methods

Design of the experiment

The study was performed on 27 outbred Wistar rats weighing 250-270g from 5 to 7 months of age. The animals were housed in keeping with the rules for good laboratory practice (GLP). The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by Directive 86/609/EEC on the protection of animals used for experimental and other scientific purpose .Dated (timed) gestational age was determined using a daily study of vaginal smears. Before the start of the experiment, the estrous cycle was investigated in all females. Animals with its disturbances were excluded from the experiment. The female rats included in the experiment, in the cycle stage corresponding to the late proestrus or early estrus, were placed together with male rats during the night, and in the morning of the next day, vaginal smears were taken from females. The fact of pregnancy was established by the presence of sperm in the vaginal secretion, and the day of its discovery was considered the first day of pregnancy. Pregnant females were divided into 3 groups of 9 individuals each.

Group1 (the control group) included intact pregnant females treated with distilled water; Group 2 (the first experimental group) included animals receiving a therapeutic dose of nifedipine; Group 3 (the second experimental group) included animals receiving a toxic dose of nifedipine. The dihydropyridine nifedipine (Sigma-Aldrich, Gillingham, UK) was used as a Ca2+ channel blocker. Nifedipine was dissolved in distilled water. The dose of nifedipine was calculated based on therapeutic and toxic doses for humans. The therapeutic dose for rats was 127 mg/kg, toxic dose – 762 mg/kg.

Nifedipine or distilled water was administered from the eighth day of pregnancy once, at the same time of day, intragastrically using a probe. During the experiment, the condition and behavior of the animals were monitored with mandatory weighing once a week to identify the toxic effects of the drug. In each group, when rats reached Day 14, 15, 16 and consistently up to 22 of gestation, they were removed from the experiment by decapitation under ether anesthesia. Given the presence of circadian rhythms, decapitation was performed at the same time of day. At the opening of the abdominal cavity and the horns of the uterus, the embryos and fetuses were removed, subjected to external examination and quickly fixed with 10% neutral formalin entirely up to the age of 15 days. At an older age, both metanephros were removed from the fetuses by preparation and fixed with 10% neutral formalin.

Morphological examination of metanephros

Serial paraffin sections 5 μm thick were made from the obtained material. Tissue sections were stained with H&E. The intensity of protein synthesis was estimated by the content of mRNA in the cytoplasm.[6] The researchers in our lab[7] found that cells of embryonic tissues synthesize protein intensively, both for plastic and metabolic purposes. mRNA in the cytoplasm of cells was determined by the method of Einarson using gallocyanin-chromic alum.[8] The intensity of cytoplasmic staining was assessed using the computer program Aperio Image Scope 2008.

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA) and Microsoft Excel 2007. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P<0.05$ was considered statistically significant.

Results

In Group 1, the metanephros in rat embryos was determined by embryonic day (ED) 14. On ED 15, a metanephrogenic zone was formed along the periphery of the bud, in which at this time and on the subsequent ED 16, primary renal tubules were formed, indicating the beginning differentiation of nephrons. Mesenchyme, which lies in the area of the future medulla, was represented by loose tissue. At the stage of prenatal embryo development, corresponding to 16 days, Einarson stains were intensively staining the cyan-blue cytoplasm of the cells of the primary renal tubules and the surrounding mesenchyme, indicating a high content of mRNA in them. On ED 17, the epithelial and mesenchymal buds of the metanephros rapidly underwent further changes. The metanephros contained four generations of the branches of the metanephritic diverticulum, and an active neoplasm of the nephron primordia occurred. Their number per unit area was much larger than in the previous stage. This process of differentiation was accompanied by a decrease in the amount of mRNA in the cytoplasm of mesenchymal cells by 41.2%, in the tubule epithelium - by 18.8% compared with the same structures of the metanephros on ED 16.

By ED 19, the metanephros continued to grow, surrounded by a pronounced capsule. The cortex and medulla were well defined. In the cortical substance, a small amount of the metanephric tissue was maintained, consisting of densely adjacent cells with round nuclei and basophilic cytoplasm. In the subcapsular zone, the rudiments of the fourth generation nephrons were located, which were transformed into S-shaped structures, the most immature tabs of the nephrons. As we approached the medulla, more and more differentiated nephrons were generated. The pelvis with a rather wide cavity was lined with transitional epithelium, and epithelial folds were found. The transitional epithelium also appeared in large

and small calyces. In the metanephros on ED 19, there was a weakening of the color, according to Einarson, concerning all the structures studied. In the cytoplasm of mesenchymal cells, the weakening was 61.1%, and in the tubular epithelium it was 34.7%, compared with the same structures of the metanephros on ED 17.

In the metanephros on ED 21-22, the renal structures tightly adhered to each other. The buds of the nephrons did not form, but the existing buds grew and differentiated. Four generations of nephrons were detected. Fourth generation immature nephrons occupied a subcapsular position. The nephrons of the first 3 generations were much more differentiated. Tubular differentiation continued. Thin tubules were lengthened and penetrated deeper into the medulla. Collecting tubules were dilated, covered with prismatic or cubic epithelial cells. In the metanephros, clear demarcation between cortex and medulla was observed. An allocation of renal pyramids and pillars continued. In the pelvis, large and small calyces were lined with transitional epithelium, and we detected folding of the epithelial lining. The kidney was surrounded on the outside by a well-formed connective tissue capsule. In the fetus of this age, the RNA content in the cells of the kidney buds continued to decline. The most pronounced decrease was found in the cytoplasm of mesenchymal cells, where it reached 74.3%, compared with ED 19. A decrease in the RNA content was not so active in the epithelium of the tubules and was 37.4%.

In Group 2, the changes in the mRNA content were already detected on ED 17. Its amount in epithelial cells increased significantly compared to Group 1 (Table 1) and more significantly than in mesenchymal cells (Table 2).

Table 1.

Dynamics of the mRNA content in the cytoplasm of epithelial cells of rat metanephros in study groups

Group	ED 17	ED 19	ED 21
Group 1	100%	100%	100%
Group 2	+10.79%	+13.86%	-26.60%
Group 3	-27.58%	-38.89%	-88.40%

Table 2.

Dynamics of the mRNA content in the cytoplasm of mesenchymal cells of rat metanephros in study groups

Group	ED 17	ED 19	ED 21
Group 1	100%	100%	100%
Group 2	+8.19%	-0.72%	-6.80%
Group 3	-22.12%	-31.7%	-72.10%

On ED 19, the positive dynamics of the RNA content in the cytoplasm of the bud cells of the metanephros was traced only for the epithelial cells of the tubules, while in the cells of mesenchymal buds, this content began to fall, although not statistically significantly. The most pronounced changes in the developing rat metanefrosis after the introduction of a therapeutic dose of nifedipine appeared on ED 21, when the RNA content in epithelial cells and mesenchyme decreased.

In Group 3, the changes in the mRNA content in the cytoplasm of metanephros cells were more pronounced. Significant shifts in the direction of the mRNA content inhibition began from ED 17 and by ED 21 reached peak values (Tables 1 and 2). Both doses of nifedipine had a greater effect on the mRNA content in the cytoplasm of epithelial cells than they did in cells of mesenchymal buds.

Discussion

In the literature, there is evidence that calcium signaling is necessary for regulating the processes of differentiation of the metanephric tubules.[9] It was also found that calcium activity contributes to multiple aspects of kidney development and associated diseases.[10] In recent years, several studies have pinpointed an unsuspected role of calcium in determining the pronephric territory and for converting metanephric mesenchyme into nephrons. Influx of calcium and calcium transients has been recorded in the pool of renal progenitors to allow tubule formation, highlighting the occurrence of calcium-dependent signaling events during early kidney development.[11] However, the presence of calcium channels directly in the metanephric tubules has not yet been described. They are found only in the smooth muscles of the kidney vessels.[12]

Studies over the last several decades have revealed that vascular smooth muscle cells (VSMs) express a variety of Ca^{2+}-permeable channels that coordinate a regulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). Changes in $[Ca^{2+}]i$ are produced by Ca^{2+} influx through voltage-dependent and -independent plasmalemmal Ca^{2+}-permeable channels, as well as Ca^{2+} release from intracellular stores. Ca^{2+} influx through L-VGCCs is the principal mediator of myogenic response. Ca^{2+} release from intracellular stores through ryanodine receptors (RyRs) and inositol-1,4,5,-trisphosphate receptors (IP3Rs) in the sarcoplasmic reticulum (SR) is an important contributor to $[Ca^{2+}]i$ and VSM excitability.[13]

Depending on the physiological stimuli, Ca^{2+} release from RyRs can cause global elevation of $[Ca^{2+}]i$ to activate actin–myosin interaction for vasoconstriction, and generate local Ca^{2+} signals in the sarcoplasmic reticulum - plasma membrane junctions to stimulate Ca^{2+}-activated channels to modulate membrane potential and Ca^{2+} influx via VGCCs.[14]

Specific blockade of L-type calcium channels using nifedipine decreases the myoplasmic calcium ion concentration, thus preventing the development of changes in the expression of myosin heavy chains (MHC) and sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) isoforms.[15]

In a series of theoretical studies, it was proposed that morphogenesis in kidney, lung, and mammary glands can be described as a self-organized process[16] with stochastic components that are modified and controlled by signaling molecules.[17,18] Calcium signals play a central role in these processes. Several studies of the embryonic kidney

demonstrated that genetically controlled and programmed events play a role in proper branching morphogenesis.[19]

JM Kim et al.[20] found spatially expressed L-VGCCs in the peripheral layers of developing epithelial buds and identified the VDCCs as a core signaling mediator for patterning branching architecture. JM. Fontana et al.[17] found that down-regulation of the calcium activity, both by blocking the sarco-endoplasmic reticulum Ca^{2+}-ATPase and by chelating cytosolic calcium, resulted in retardation of branching morphogenesis and a reduced formation of primitive nephrons but had no effect on cell proliferation. Authors proposed that the spontaneous calcium signals observed in metanephric mesenchyme cells are, along with stochastic components, among the factors that contribute to the process that drives branching morphogenesis.

Cells of embryonic tissues intensively synthesize protein, both for plastic and metabolic purposes.[7] The content of mRNA in the cytoplasm reflects the intensity of protein-synthetic processes in the cell. Protein synthesis is most active in the early stages of organ development, when the morphogenesis of organs is accompanied by the migration, proliferation and differentiation of cells that require a large number of protein molecules.[21] This fact is confirmed by the results of our research. The maximum high mRNA content, estimated by intensity of cytoplasmic staining, in the epithelial and mesenchymal buds of the metanephros was found in the earliest period (ED16) of embryogenesis. With an increase in the gestation period, the color intensity of the cytoplasm of epithelial cells and cells of mesenchymal buds gradually decreased, reaching the lowest values on ED 21. With the introduction of nifedipine in a therapeutic dose (a moderate channel blockade), the content of mRNA in the cytoplasm of the cells of the metanephric tubules, indicating the intensity of protein synthesis, depends on the duration of the L-VGCC blockade. On ED 17, a paradoxical reaction of the cells was recorded with an increase in protein synthesis, compared with the control group. Subsequently, on ED 19 and 21, the content of mRNA in the cytoplasm of epithelial cells and mesenchymal derivatives decreased steadily. With a complete L-VGCC blockade (toxic dose of nifedipine), an inhibition in protein synthesis in the epithelial and mesenchymal buds of the metanephros statistically reliably increased in all studied periods relative to the control group.

Conclusion

Thus, the development of the metanephros in rats of the control group was accompanied by a gradual decrease in the content of mRNA, indicating the activity of protein synthesis in the cell cytoplasm of epithelial and mesenchymal buds of the metanephros. In the fetuses of rats whose mothers received therapeutic and toxic doses of nifedipine, statistically significant changes in the content of mRNA in the cytoplasm of the metanephric cells were observed. These changes were most significant in the metanephric cells after administration of the toxic dose. On ED 17, an L-VGCC blockade with a therapeutic dose of nifedipine led to a paradoxical reaction of cells with an increase in protein synthesis, compared with the control group. Both doses of nifedipine have a greater effect on the content of mRNA in the cytoplasm of epithelial cells, compared to mesenchymal buds.

Competing Interests

The authors declare that they have no competing interests.

References

1. Kramer I. Intracellular Calcium. In: Signal Transduction. Third Edition. Elsevier; Amsterdam, The Netherlands; 2016:381-439.
2. Catterall WA. Voltage-gated calcium channels. Cold Spring Harb Perspect Biol. 2011;3(8):a003947. doi: 10.1101/cshperspect.a003947.
3. Finney BA, del Moral PM, Wilkinson WJ, Cayzac S, Cole M, et al. Regulation of mouse lung development by the extracellular calcium-sensing receptor, CaR. J Physiol. 2008;586:6007–6019.
4. Tena JJ, Neto A, de la Calle-Mustienes E, Bras-Pereira C, Casares F, Gómez-Skarmeta JL. Odd-Skipped Genes Encode Repressors That Control Kidney Development. Dev. Biol. 2007;301:518–531. doi: 10.1016/j.ydbio.2006.08.063.
5. Compendium 2006 - Medicinal Products. In Kovalenko VN, Viktorov AP, editors. K.: Morion; 2006. [In Russian].
6. Spirin AS. [Biosynthesis of proteins, the world of RNA and the origin of life]. Herald of the Russian Academy of Sciences. 2001;71(4):320-328. [Article in Russian].
7. Kharchenko SV. [Changes in the activity of protein synthesis in embryogenesis of the lungs and kidneys of rats after the administration of paracetamol and nimesulide to pregnant females]. World of Medicine and Morphology. 2009;5(3-2):185-188. [Article in Russian].
8. Semchenko VV, Barashkova SA, Nozdrin VI. Histological technique. Omsk, 2006. [In Russian].
9. Leclerc C, Webb SE, Miller A, Moreau M. An increase in intracellular Ca^{2+} is involved in the differentiation of prenodic tubules in amphibians of Xenopus laevis. Dev Biol. 2008;321(2):357-67. doi: 10.1016/j.ydbio.2008.06.029.
10. Paudel S, Sindelar R, Saha. Calcium Signaling in Vertebrate Development and Its Role in Disease. Int J Mol Sci. 2018;19(11):3390 doi: 10.3390/ijms19113390
11. Gilbert T, Leclerc C, Moreau M. Control of kidney development by calcium ions. Biochimie. 2011;93(12):2126-31. doi: 10.1016/j.biochi.2011.07.007.
12. Hughes AD. Calcium channels in vascular smooth muscle cells. J Vasc Res. 1995;32:353–370.
13. Ghosh D, Syed AU, Prada MP, Nystoriak MA, Santana LF, Nieves-Cintrón M, Navedo MF. Calcium Channels in Vascular Smooth Muscle. Adv Pharmacol. 2017;78:49-87. doi: 10.1016/bs.apha.2016.08.002.
14. Lin AH, Sun H, Paudel O, Lin MJ, Sham JS. Conformation of ryanodine receptor-2 gates store-operated calcium entry in rat pulmonary arterial myocytes. Cardiovasc Res. 2016;111(1):94-104. doi: 10.1093/cvr/cvw067.
15. Mukhina AM, Altaeva EG, Nemirovskaya TL, Shenkman BS. The role of L-type calcium channels in the accumulation of Ca2+ in soleus muscle fibers in the rat and changes in the ratio of myosin and serca isoforms in conditions of gravitational unloading. Neurosci Behav Physiol. 2008;38(2):181-8. doi: 10.1007/s11055-008-0027-x.

16. Xu H, Sun M, Zhao X. Turing mechanism underlying a branching model for lung morphogenesis. PLoS One. 2017;12(4):e0174946. doi: 10.1371/journal.pone.0174946.
17. Fontana JM, Khodus GR, Unnersjö-Jess D, Blom H, Aperia A, Brismar H. Spontaneous calcium activity in metanephric mesenchymal cells regulates branching morphogenesis in the embryonic kidney. FASEB J. 2019;33(3):4089-4096. doi: 10.1096/fj.201802054R.
18. Blake J, Rosenblum ND. Renal branching morphogenesis: morphogenetic and signaling mechanisms. Semin Cell Dev Biol. 2014;36:2-12. doi: 10.1016/j.semcdb.2014.07.011.
19. Hannezo E, Scheele CLGJ, Moad M, Drogo N, Heer R, Sampogna RV, van Rheenen J, Simons BD. A Unifying Theory of Branching Morphogenesis. Cell. 2017;171(1):242-255.e27. doi: 10.1016/j.cell.2017.08.026.
20. Kim JM, Choi S, Lee SW, Park K. Voltage-dependent Ca2+ channels promote branching morphogenesis of salivary glands by patterning differential growth. Sci Rep. 2018;8(1):7566. doi: 10.1038/s41598-018-25957-w.
21. Vetter MR, Gibley CW. Morphogenesis and histochemistry of the developing mouse kidney. Journal of Morphology. 2005;120(2):135-155.

International Journal of Biomedicine 9(2) (2019) 155-158
http://dx.doi.org/10.21103/Article9(2)_OA15

ORIGINAL ARTICLE

Experimental Nephrology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Anti-Lithogenic Activity of Tetrapeptide Leu-Ile-Lys-His in Experimental Urolithiasis

N. N. Yakushev, PhD[1]; A. Yu. Zharikov, PhD, ScD[1,2]; O. N. Mazko, PhD[1]; O. G. Makarova, PhD[1]; G. V. Zharikova[1*]; V. M. Brukhanov, PhD, ScD[1]; Yu. V. Korenovsky, PhD[1]; A. V. Lepilov, PhD, ScD[1]; I. P. Bobrov, PhD, ScD[1]; O. V. Azarova, PhD, ScD[1]

[1]*Altai State Medical University, Barnaul, Russia*
[2]*Scientific-Research Institute of Physiology and Basic Medicine, Novosibirsk, Russia*

Abstract

Background: The research objective was to study the effect of the tetrapeptide Leu-Ile-Lys-His on the course of experimental oxalate nephrolithiasis.

Methods and Results: Experiments were conducted on 30 male Wistar rats, which were divided equally into 3 groups: the control group (6 weeks of ON modeling), the test group (6 weeks of ON modeling+the tetrapeptide Leu-Ile-Lys-His introduction at a dose of 12 mg/kg), and the experimental group (determination of indicators of free radical oxidation process activity in intact rats). Every 7 days, indicators of the excretory renal function (the level of diuresis and creatinine excretion) and activity of marker enzymes of renal tissue damage (lactate dehydrogenase and γ-glutamyl transferase) were determined in the urine of rats. After six weeks, we determined parameters of activity of the free radical oxidation process in the kidneys of rats posthumously and conducted the morphological study for formation of calcium deposits.

Conclusion: Experiments showed that in the control group there were characteristic signs of nephrolithiasis development: increase of LDH activity by 5.4 times, increase of free radical oxidation process activity in the kidneys, and formation of large concrements in the kidneys, the mean size of which was 298.8±34.2 μm^2. In the test group, there was weakening of LDH and GGT activity and weakening of free radical oxidation process activity. There were no morphological signs of nephrolithiasis development in the kidneys. (**International Journal of Biomedicine. 2019;9(2):155-158.**)

Key Words: oxalate nephrolithiasis • Leu-Ile-Lys-His tetrapeptide • anti-lithogenic effect

Introduction

Previously, we found that the new pharmacological agent, the tetrapeptide Leu-Ile-Lys, showed pronounced anti-lithogenic activity in experimental oxalate nephrolithiasis.(1) The obtained results are in good agreement with the so-called "proteomic approach" in the development of new methods of treatment for urolithiasis and create prerequisites for further search for new peptide molecules with potential anti-lithogenic activity.(2)

Today, there is information about the important role of histidine amino acid in the renal reabsorption of calcium. It has been established that the activity of TRPV5 calcium channels in the kidneys, which are involved in the reverse absorption of calcium in distal tubules, is regulated by a histidine-kinase signal pathway, and that the presence of histidine in the carboxyl terminal of TRPV5 channels is required to activate these channels.(3) It is possible that external modulation of this signal pathway can change the character of intrarenal calcium transport, and thus change the intensity of calcium concrements formation.

Taking into account the above, we decided to experimentally assess the impact of the new Leu-Ile-Lys-His tetrapeptide on the processes of stone formation in experimental oxalate nephrolithiasis.

The research objective was to study the effect of the tetrapeptide Leu-Ile-Lys-His on the course of experimental oxalate nephrolithiasis

***Corresponding author**: Ganna V. Zharikova. Altai State Medical University, Barnaul, the Russian Federation. E-mail: anna1704@mail.ru*

Materials and Methods

The study was conducted on 30 male Wistar rats weighing 200–250g that were on a standard laboratory diet. Animals were grown in the Department of Animal and Human Genetics of the Federal Research Center "Institute of Cytology and Genetics" of the Siberian branch of the Russian Academy of Sciences (Novosibirsk). The work with animals was carried out in accordance with the principles of humanism laid down in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, in accordance with the "Animal experimentation legislations". Throughout the experiment, animals were in individual metabolic cells adapted for urine collection, in conditions of free access to fluids and food under the natural light mode. All procedures with the animals, namely weighing, introduction of study objects, and euthanasia, were carried out in the interval of time from 9 a.m. to 12 noon.

Rats were divided equally into 3 groups: Group 1 (n=10) – the control group; Group 2 (n=10) – the test group; Group 3 (n=10) – the experimental group. In Groups 1 and 2, oxalate nephrolithiasis was modeled according to the generally accepted "ethylene glycol model."[4] The bottom line is that rats receive a 1% ethylene glycol (EG) solution for free drinking. In hepatocytes, under the influence of microsomal enzymes, EG metabolizes to an oxalate-ion, which is then excreted with urine where it reacts with calcium ions, forming insoluble crystals of cipitating calcium oxalate. Under these conditions, nephrolithiasis is considered to develop up to the end of the third week of modeling, acquiring a stable form in a period of 3-6 weeks. Therefore, the duration of pathology modeling was 6 weeks. In Group 2, the tetrapeptide Leu-Ile-Lys-His was administrated from day 22 to day 42 orally through a probe in the form of a starch suspension at a dose of 12 mg/kg.

The tetrapeptide Leu-Ile-Lys-His was synthesized at the enterprise of SHANGHAI APEPTIDE CO., LTD (Shanghai, China), supported by "Evalar" ZAO (Biysk, Russia).

In Group 3, the indicators of free radical oxidation activity were measured in normal intact rats. In Groups 1 and 2, daily urine volume was collected every week during the experiment, in which creatinine concentration and activity of marker enzymes of nephrothelium damage—lactate dehydrogenase (LDH) and γ-glutamyl transferase (GGT)—were determined in order to assess the degree of renal tissue damage in ON modeling on the automatic biochemical analyzer DIRUI CS-T240. Creatinine concentration was determined by a kinetic method without deproteinization basing on the Jaffe reaction with formation of the red-orange colored complex. The principle of the method for determining LDH activity is based on the reaction of pyruvate recovery to lactic acid; the reaction rate is proportional to LDH activity in the sample. The determination of GGT activity is based on the reaction of transfer of L-γ-glutamyl-3-carboxy-p-nitroanilide to glycylglycine, which is catalyzed by this enzyme with the formation of the colored 5-amino-2-nitrobenzoate.

After 6 weeks of the experiment, the rats were euthanized under ethereal anesthesia and both kidneys were extracted, one of which was used to determine activity of the free radical oxidation process in the renal tissue, and the other one for morphological research.

The activity of the free radical oxidation process in the renal tissue was estimated by a combination of pro-oxidant and antioxidant indicators. The first ones included the content of thiobarbituric acid reactive products (TBARP) and the total pro-oxidant activity (TPA), the second ones – total antioxidant activity (TAA) and activity of antioxidant enzymes: glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT).

TBARP were determined by the colorimetric method for measuring the intensity of the coloring solution during the chemical reaction of TBRP with TBA. TPA was estimated by accumulation of Tween 80 peroxidation products reacting with TBA. TAA was assessed as an integrative activity indicator of all enzymatic and nonenzymatic factors of neutralization of free radicals by degree of oppression of Fe^{2+}/Tween-80 ascorbate-dependent oxidation by tissue homogenate. To determine the glutathione peroxidase (GPx) activity, the concentration of reduced glutathione was measured in a colored reaction with Ellman's reagent. The SOD activity was determined by suppressing the formation of nitro formazan, which is a colored product of nitrotetrazolium oxidation by superoxide radicals formed by interaction of phenazinmetasulfate and nicotinamidinucleotide (NADN). The CAT activity was determined by suppressing sodium molybdate with the oxidation enzyme of hydrogen peroxide.

For morphological studies, the kidneys were fixed in 10% formalin solution, treated according to the standard method, and a cross section 6 μm thick was produced through the renal papilla. The resulting sections were colored with H&E, methenamine-silver. Calcium deposits were identified by the von Kossa staining method. The number of calcium deposits pictured in the field of vision and their sizes were determined using a computer program. Morphometric studies were carried out using the ImageJ 1.43 and AxioVision 3.1 software packages.

Statistical analysis was performed using StatSoft Statistica v12.0. The results of biochemical studies are presented as the median (Me) and interquartile range (IQR; 25th to 75th percentiles). The results of morphometric studies are presented as the mean (M) and standard error of the mean (SEM). Statistical comparisons between groups were performed using the non-parametric Kruskal–Wallis test and Mann–Whitney U-test; comparisons within groups with respect to the reference level were made using the non-parametric Wilcoxon test. A probability value of $P<0.05$ was considered statistically significant.

Results

Conducted experiments showed that in rats of Group 1 there were characteristic signs of nephrolithiasis development. First, the activity of marker enzymes of renal epithelium damage increased (Table 1). The LDH activity increased 5.4 times during the experiment ($P=0.012$). The study of free radical oxidation process activity in the kidneys of rats

in Group 1 showed (Table 2) that the TBARP concentration increased 1.2 times compared to the level of intact rats of Group 3 (P=0.004). In addition, TAA decreased 3.5 times and SOD activity increased 1.4 times (P=0.0002).

Table 1.

Indicators of lactate dehydrogenase and γ-glutamyl transferase activity in the study of Leu-Ile-Lys-His peptide impact on the course of experimental oxalate nephrolithiasis

	LDH (U/mg of creatinine per day)		GGT (U/mg of creatinine per day)	
	Group 1	Group 2	Group 1	Group 2
Initial level	0.09 (0.06;0.15)	0.21 (0.18;0.39) P_c=0.061	0.74 (0.56;1.06)	0.93 (0.48;1.41) P_c=0.747
Week 1	0.11 (0.07;0.28)	0.13 (0.07;0.37) P_c=0.452	0.74 (0.68;1.03)	0.87 (0.54;1.00) P_c=0.875
Week 2	0.22 (0.15;0.33)	0.16 (0.12;0.19) P_c=0.333	-	-
Week 3	-	-	-	-
Week 4	0.22 (0.13;0.37)	0.23 (0.08;0.37) P_c=0.793	0.74 (0.59;1.29)	0.73 (0.35;1.23) P_c=0.875
Week 5	0.42 (0.34;0.55) P_{in}=0.012	0.12 (0.03;0.20) P_c=0.002	1.22 (0.55;1.88)	0.33 (0.25;0.36) P_{in}=0.028 P_c=0.0004
Week 6	0.49 (0.18;0.60) P_{in}=0.012	0.03 (0.02;0.05) P_{in}=0.028 P_c=0.0006	0.85 (0.49;2.37)	0.13 (0.08;0.18) P_n=0.028 P_c=0.001

P_n – level of statistical significance of differences in comparison with the initial level of the corresponding indicator; P_c – level of statistical significance of changes of the corresponding indicator in Group 2 (the test group) in comparison with Group 1 (the control group).

The morphological study of the kidneys of rats in Group 1 showed that when coloring with H&E on nephrolithiasis areas, tubular epithelium was in a state of hyaline-drop dystrophy. The apical edge of cells was destroyed in some places; the brush border was not visible in some places. The tubules looked cystic stretched at the same time. Nephrothelium cells covering the tubules were either destroyed, flattened or atrophic.

We determined that there was weak or moderate inflammatory lymphoplasmacytic infiltration around the tubules, and noted nephrosclerosis phenomena. The vessels were in a state of pronounced hyperemia.

During the histochemical coloring for calcium by von Kossa's staining method, we observed that the stone deposit crystals in singles or in groups were of brownish-black color and of various shapes and sizes in the tubules of the kidneys of rats in the control group. The number of deposits in channel lumens ranges from 6 to 19 and averaged 7.9±1.6 in the field of vision with an increase ×400 with a modal value of 6. When conducting computer morphometry, the area of stone deposits ranged from 48.8 μm^2 to 789.9 μm^2, with an average of 298.8±34.2 μm^2. A more detailed analysis of the distribution of stones depending on their area revealed that the number of stone deposits from 20 μm^2 to 100 μm^2 amounted to 16.7%, the number of deposits from 100 μm^2 to 300 μm^2 amounted to 47.2%, and the content of deposits over 300 μm^2 amounted to 36.1%.

Against this background, in the long-term use of the tetrapeptide Leu-Ile-Lys-His or the correction of modeled nephrolithiasis, significant differences in the pattern of pathology were observed, compared to the control group. It turned out that since Week 5 of the experiment, LDH activity decreased and statistically significantly differed from the same indicator of Group 1: in Week 5, it was 3.5 times less (P=0.002), in Week 6 – 16.3 times less (P=0.0006). By the end of Week 6, LDH activity was even 7 times less than its initial level (P=0.028).

On the whole, the same dynamics characterized the changes in GGT activity in Group 2. It turned out that in Week 5 and Week 6, it significantly decreased, compared to the initial level: 2.8 times and 7.2 times, respectively (P=0.028). However, it was statistically significantly less than in Group 1 in the same period of time: in Week 5 – 3.7 times (P=0.0004), and in Week 6 – 6.5 times (P=0.001).

The study of the activity of free radical oxidation process in the kidneys of rats in Group 2 revealed that after a 3-week application of the tetrapeptide Leu-Ile-Lys-His, TPA decreased 7 times, compared to Group 1 (Table 2). At the same time, TAA significantly increased: 4.7 times compared to the level of Group 1 (P=0.0002) and 1.3 times compared to intact rats of Group 3 (P=0.0002). In addition, GPx activity increased 1.4 times compared to Group 1 (P=0.0000) and 1.6 times compared to Group 3 (P=0.0000).

Table 2.

Indicators of free radical oxidation process activity in the kidneys of experimental rats

Group	TBRP (μmol/mg)	TPA (%)	TAA (%)	GPx (%)	CAT (%)	SOD (%)
Group 3 (Experimental)	6.1 (5.4;6.9)	65.1 (63.4;68.0)	41.7 (40.2;43.3)	38.5 (25.2;41.6)	14.4 (10.2;15.6)	18.2 (13.0;18.5)
Group 1 (Control)	7.6 (7.2;7.6) P_{in}=0.004	54.0 (49.9;56.8) P_n=0.0002	12.0 (10.4;13.2) P_{in}=0.0002	42.2 (32.2;47.9)	12.2 (9.0;15.8)	25.1 (23.6;32.1) P_{in}=0.0002
Group 2 (Test)	7.5 (6.1;10.7)	7.7 (5.0;13.3) P_n=0.0002 P_c=0.0002	56.1 (53.9;58.9) P_{in}=0.0002 P_c=0.0002	59.8 (58.3;66.4) P_{in}=0.0000 P_c=0.0000	13.8 (10.5;24.7)	6.5 (3.5;10.7) P_{in}=0.002 P_c=0.0002

P_{in} – level of statistical significance in comparison with Group 3; P_c – level of statistical significance in comparison with Group 1.

Finally, there was a decrease in SOD activity 3.9 times compared to Group 1 (P=0.0002), resulting in this indicator being even 2.8 times less than the level of intact rats (P=0.002).

The morphological study of the kidneys of rats in Group 2 showed that no nephrolithiasis phenomena were detected in this group. Renal tissues were in a normal state; neither inlay of tubules with calcium salts nor formation of large concrements was observed.

Discussion

Thus, the conducted experiments allowed us to determine high anti-lithogenic activity of the tetrapeptide Leu-Ile-Lys-His. It emerged that no morphological signs of nephrolithiasis development were revealed in the group where Leu-Ile-Lys-His was introduced. At the same time, oxidative stress in the renal tissue was weakening and LDH and GGT activity was decreasing against the background of treatment, which indicates normalization of the structure and function of urothelium.

The mechanisms of the identified anti-lithogenic effect have yet to be studied. However, there are data in the available literature that can clarify, to some extent, the ability of histidine-containing oligopeptides to weaken the formation of stones. For example, it is known that endogenous dipeptide carnosine (β-alanyl-L-histidine) plays a significant role in renal pathologies.[5,6] In particular, it reduces the synthesis of matrix products in the kidneys, such as fibronectin and type VI collagen. In this context, it is worth mentioning that a number of matrix large-molecular glycoproteins are powerful stimulants of crystallization in the kidneys.[7] In addition, carnosine is able to reduce the level of pro-inflammatory cytokines. It is well known that inflammatory processes in tubules largely determine the formation of the primary focus of lithogenesis.[8]

Is it possible that the Leu-Ile-Lys-His tetrapeptide we developed and studied has activity in the kidneys similar to that of carnosine, which contributes to the inhibition of stone formation. Of course, such assumptions require further in-depth study. However, the obtained results show for the first time the anti-lithogenic activity of histidine-containing tetrapeptide.

Conclusion

In experimental oxalate nephrolithiasis, the 3-week application of Leu-Ile-Lys-His was accompanied by a pronounced anti-lithogenic effect. There was a decrease in the activity of marker enzymes of renal epithelium damage and weakening of oxidative stress in the kidneys. No morphological signs of nephrolithiasis development were detected.

Competing Interests

The authors declare that they have no competing interests.

References

1. Zharikov AYu, Yakushev NN, Zharikova GV. Pharmacological agent for treatment of kidney stone disease. RU Patent (RU #2679120; priority of 29.10.2018).
2. Mittal A, Tandon S, Singla S.K, Tandon C. Mechanistic Insights into the Antilithiatic Proteins from Terminalia arjuna: Proteomic Approach in Urolithiasis. PLoS One. 2016;11(9):e0162600. doi: 10.1371/journal.pone.0162600.
3. Cai X, Srivastava S, Surindran S, Li Z, Skolnik EY. Regulation of the epithelial Ca^{2+} channel TRPV5 by reversible histidine phosphorylation mediated by NDPK-B and PHPT1. Mol Biol Cell. 2014;25(8):1244-50. doi: 10.1091/mbc.E13-04-0180.
4. Zharikov AYu, Brukhanov VM, Zverev YaF, Lampatov VV. [Current methods of modeling of oxalate nephrolythiasis]. Nephrology. 2008;12(4):28-35.[Article in Russian].
5. Kiliś-Pstrusińska K. [Carnosine, carnosinase and kidney diseases]. Postepy Hig Med Dosw (Online) 2012;66:215-21. [Article in Polish].
6. Peters V, Klessens CQ, Baelde HJ, Singler B, Veraar KA, Zutinic A, et al. Intrinsic carnosine metabolism in the human kidney. Amino Acids. 2015;47(12):2541-50. doi: 10.1007/s00726-015-2045-7.
7. Zharikov AYu, Zverev YaF, Brukhanov VM, Lampatov VV. [The current conceptions about the modulators of the oxalate nephrolythiasis. I. stimulators of crystallization]. Nephrology. 2009;13(1):56-72. [Article in Russian].
8. Zharikov AYu, Zverev YaF, Brukhanov VM, Lampatov V.V. [Mechanism of formation of crystals in oxalate nephrolithiasis]. Nephrology. 2009;13(4):37-50. [Article in Russian].

International Journal of Biomedicine 9(2) (2019) 159-162
http://dx.doi.org/10.21103/Article9(2)_OA16

ORIGINAL ARTICLE

Experimental Dentistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Lipid Peroxidation Activity and Immune Response in Modeling Inflammatory and Degenerative Damage to the Periodontium of Rats

Marina A. Darenskaya, PhD, ScD[1*]; Lyudmila A. Grebenkina, PhD, ScD[1]; Evgeniy V. Mokrenko, PhD[2]; Petr D. Shabanov, PhD, ScD[3]; Maria I. Suslikova, PhD[2]; Mark E. Mokrenko[2]; Yulia O. Sinyova[4]; Ivan S. Goncharov[2]; Marina I. Gubina, PhD[2]; Igor Yu. Kostritsky[2]; Alexandra F. Bulnaeva, PhD[2]; Elena V. Proskurnina, PhD, ScD[5]; Lyubov I. Kolesnikova, Academician of the RAS[1]; Sergey I. Kolesnikov, Academician of the RAS[1,5]

[1]*Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, the Russian Federation*
[2]*Irkutsk State Medical University, Irkutsk, the Russian Federation*
[3]*Russian military medical Academy, St. Petersburg, the Russian Federation*
[4]*Irkutsk National Research Technical University, Irkutsk, the Russian Federation*
[5]*M.V. Lomonosov Moscow State University, Moscow, the Russian Federation*

Abstract

The aim of this study was to assess changes in the lipid peroxidation activity and immune response in modeling inflammatory and degenerative damage to the soft tissues of the periodontium of Wistar rats.

Materials and Methods: The experiments were carried out on male Wistar rats weighing 220-250g. Modeling the inflammatory and degenerative damage to the soft tissues of the periodontium in animals (n=10) of the test group (TG) was carried out using a single dose of a 2% formaldehyde aqueous solution injected into each side of the outer part of the gums (0.15 ml) at the level of the lower molars of anesthetized animals. The control group (CG) of animals (n=10) received an injection of physiological saline solution in the same volume. In 3 days after damage modeling, the animals were killed by decapitation and blood samples were drawn for testing. The intensity of LPO processes in the blood was estimated by content of conjugated dienes and TBA-reactive substances (TBARS). The status of the AOD system was determined by the SOD activity and GSH level. The immune status of the animals was assessed by several indicators as leukocyte migration inhibition test (LMIT), the phagocytic reaction of neutrophils, and oxygen-dependent and oxygen-independent microbicidal activity of neutrophils.

Results: The results obtained showed that the modeling of inflammatory and degenerative damage to the periodontium in rats was characterized by significant changes, affecting both the LPO system and the immune response. An LMIT conducted with various mitogens demonstrated the development of immunodeficiency in TG characterized by high activity of the inflammatory process. Immune disorders in animals of TG were also supported by changes in the phagocytosis system. Low levels on the integral NBT test indicate disorders in the state of oxygen-dependent microbicidal systems of phagocytes. The assessment of the activity of oxygen-independent microbicidal systems of phagocytes in LCT also found disorders in the immune status of animals of TG.

Conclusion: Inflammatory and degenerative damage of the soft tissues of the periodontium in rats is accompanied by pronounced disorders in both the LPO system and the immune response.(**International Journal of Biomedicine. 2019;9(2):159-162.**)

Key Words: periodontitis • inflammation • rats • lipid peroxidation • phagocytes

Abbreviations

AOD, antioxidative defense; **CD**, conjugated dienes; **ConA**, concanavalin A; **GSH**, reduced glutathione; **LMIT**, leukocyte migration inhibition test; **LPO**, lipid peroxidation; **LCT**, lysosomal-cation test; **NBT test**, nitroblue tetrazolium reduction test; **OS**, oxidative stress; **PI**, phagocytic index; **PN**, phagocyte number; **PHA**, phytohemagglutinin; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TBARS**, TBA-reactive substances.

Introduction

The Global Burden of Disease Study (1990–2010) indicates that severe periodontitis is the sixth most prevalent disease worldwide.[1] Periodontal diseases are responsible for 3.5 million years lived with disability.[2] The overall prevalence of periodontitis increases with age, and the incidence rises steeply in adults aged 30–40 years.[3]

As shown in a number of experimental and clinical works, under the influence of various types of factors a number of pathological changes with a predominance of inflammatory and destructive manifestations appear in the periodontium tissues.[4-6] Periodontitis appears to have multiple etiologies with a microbial factor contributing to initiation of the disease and an immunological factor of the host propagating the disease.[7] Processes of free-radical oxidation connected with phagocytes, producing ROS, fall into unspecific defense mechanisms.[8-13] This evolved secretory function of phagocytes is necessary to eliminate bacteria. The intensive ROS generation that triggers LPO processes in cell biomembranes takes place under the conditions of oxygen overconsumption in phagocytosis.[14-16] As a result of damage, severe disturbances arise in the regulation of the biomembrane state. Thus, abnormal proteins and secondary destructive processes appear, which cause profound disorders in cell membrane architecture and cell death.[17,18] Despite numerous studies of redox-changes in the tissues of the periodontium, an interpretation of findings is still questionable.[17,19] Thus, modeling inflammatory and degenerative damage in the soft tissues of the periodontium seems to be very important for clarifying the pathogenesis of periodontitis.

The aim of this study was to assess changes in the lipid peroxidation activity and immune response in modeling inflammatory and degenerative damage to the soft tissues of the periodontium of Wistar rats.

Materials and Methods

The experiments were carried out on male Wistar rats weighing 220-250g. All animals were given access to food and water *ad libitum*.

Modeling the inflammatory and degenerative damage to the soft tissues of the periodontium in animals (n=10) of the test group (TG) was carried out using a single dose of a 2% formaldehyde aqueous solution injected into each side of the outer part of the gums (0.15 ml) at the level of the lower molars of anesthetized animals.[5] The control group (CG) of animals (n=10) received an injection of physiological saline solution in the same volume. In 24 hours, we found stable extensive inflammatory and degenerative changes in the soft tissues of the periodontium at the injection site. In 3 days after damage modeling, the animals were killed by decapitation and blood samples were drawn for testing.

The intensity of LPO processes in the blood was estimated by content of primary oxidation products (CD) and secondary oxidation products (TBARS).[20] The status of the AOD system was determined by the SOD activity and GSH level.[20]

The immune status of the animals was assessed by several indicators as LMIT, the phagocytic reaction of neutrophils, and oxygen-dependent and oxygen-independent microbicidal activity of neutrophils. LMIT assessment methodology is based on the ability of lymphocytes to secrete leukocyte migration inhibitory factor in response to the nonspecific mitogens PHA and ConA (LMIT was expressed in %).[21] The phagocytic activity of neutrophils was assessed by phagocytic index (PI), which demonstrates phagocyte count from among calculated neutrophils, as well as phagocyte number (PN): the average number of microbes absorbed by one active neutrophil (RU). To analyze the digesting function, IPC (the ratio of the number of digested microbes to the average number of absorbed microbes, both digested and undigested) was defined (in %).

The activity of oxygen-independent microbicidal systems of phagocytes was estimated by LCT (in %).[21,22] The principle of the test is based on cytochemical detection of nonenzyme lysosomal-cation proteins, whose relative count in studied cells indicates the presence of concerned antimicrobial systems. The activity of oxygen-dependent microbicidal systems of phagocytes was estimated by the NBT test (in %).[21] The NBT test checks if certain immune system cells can change a colorless chemical called nitroblue tetrazolium (NBT) into a deep blue color. In the NBT test, neutrophils change the colorless compound NBT into a compound with a deep blue color. If this test is negative (i.e., no blue color is produced), then this indicates a defect in superoxide-generating NADPH oxidase activity with inability to efficiently kill phagocytized bacteria.[22,23] The NBT test is a qualitative assay of ROS production.

Experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals (The institute of Laboratory Animal Resources,1996); Guidance on experimental (non-clinical) testing of new pharmaceutical substances, 2005);[24] Order of the Ministry of Health of the Russian Federation № 708n (23.08.2010) "On approval of the rules of Good Laboratory Practice» (GLP).

Statistical analysis was performed using the Statistica 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For descriptive analysis, results are presented as median (Me), interquartile range (IQR; 25th to 75th percentiles). F-test for testing equality of variance was used to test the hypothesis of the equality of two population variances. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The assessment of the LPO reactions in rats with inflammatory and degenerative damage to the soft tissues of the periodontium (TG) found a statistically significant increase in the content of CD (by 3.56 times) and TBARS (by 5.82

times) compared to intact animals (Fig.1). It was also noted that activity of SOD and GSH was reduced by 3.93 times and 3.41 times, respectively.

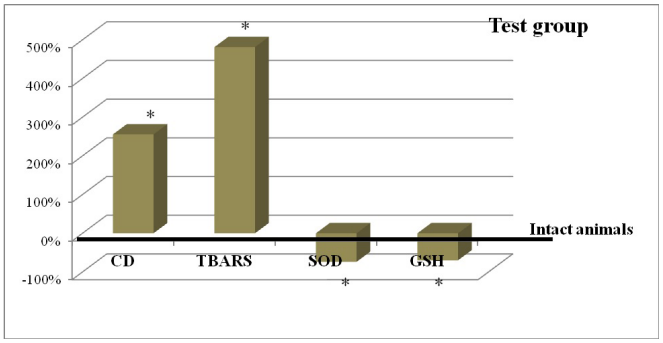


Fig. 1. *The status of LPO and AOD in rats with inflammatory and degenerative damage of the soft tissues of the periodontium. *- P<0.05 compared to intact animals.*

In TG, lymphokine production by T lymphocytes in LMIT with ConA and PHA was depressed by 1.64 and 1.58 times, respectively, compared to CG (Fig.2). In TG, PN and IPC it was increased a statistically significant amount (by 1.26 and 1.22 times), while PI decreased by 1.45 times compared to CG. The activity of oxygen-independent microbicidal systems of phagocytes estimated by LCT decreased by 1.16 times. In addition, indexes of the spontaneous NBT test and induced NBT test increased by 1.62 and 1.35 times, respectively, compared to CG (Fig.2).

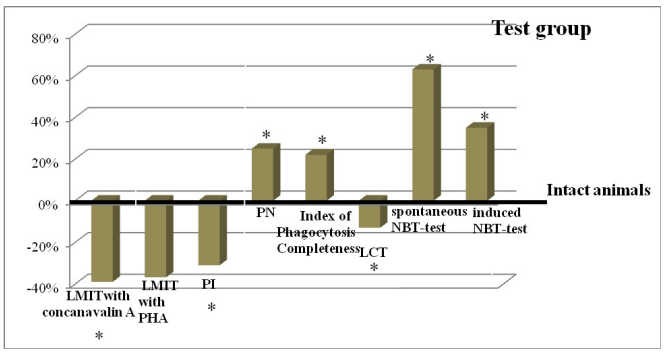


Fig. 2. *Changes in immunological indicators in rats with inflammatory and degenerative damage of the soft tissues of the periodontium. * - P<0.05 compared to intact animals.*

The results obtained showed that the modeling of inflammatory and degenerative damage to the periodontium in rats was characterized by significant changes, affecting both the LPO system and the immune response. Broken oxygen delivery under hypoxia in the tissues of the periodontium with the cascade of biochemical reactions, involving disturbances in the energy metabolism, formed the basis of this damage.[4] Dramatic changes in cellular redox systems triggered the suppression of antioxidative defense in biological tissues and internal environments.[17]

The prooxidative/antioxidative cellular imbalance between the production of ROS and the ability of the biological systems' defense mechanisms to eliminate the cellular stress disturbances leads to a vicious circle, since OS reciprocally aggravates ROS production. OS acts as a leading pathogenetic factor of microcirculatory disorders. The disorganization of homeostatic mechanisms of the periodontal microcirculation has been identified as a cause of chronic tissue hypoxia of the periodontal complex, in which the processes of LPO of biomolecules are activated, leading to a violation of the structure and function of the periodontal biomembranes.[5,25] Progression of periodontitis greatly depends on the state of the immune system, closely related to free radical oxidation, which determines the progression of inflammation as a typical pathological process.[4] An LMIT conducted with various mitogens demonstrated the development of immunodeficiency in TG characterized by high activity of the inflammatory process.

Immune disorders in animals of TG were also supported by changes in the phagocytosis system reflected in decreased PI values. Low levels on the integral NBT test indicate disorders in the state of oxygen-dependent microbicidal systems of phagocytes. The assessment of the activity of oxygen-independent microbicidal systems of phagocytes in LCT also found disorders in the immune status of animals of TG. Thus, inflammatory and degenerative damage of the soft tissues of the periodontium in rats is accompanied by pronounced disorders in both the LPO system and the immune response.

Competing Interests

The authors declare that they have no competing interests.

References

1. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression.J Dent Res. 2014;93(11):1045-53. doi: 10.1177/0022034514552491.
2. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1545-1602. doi: 10.1016/S0140-6736(16)31678-6.
3. Tonetti MS1,2, Jepsen S3, Jin L2, Otomo-Corgel J4. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. J Clin Periodontol. 2017;44(5):456-462. doi: 10.1111/jcpe.12732. Epub 2017 May 8.
4. Bykova NI, Odol'skii AV, Grigoryan VA. [Indicators of cellular and humoral immunity in experimental inflammation of periodontal tissues]. Kuban Scientific Medical Bulletin. 2016;6:20-26. [Article in Russian].
5. Mokrenko YeV, Shabanov PD. [Modeling of inflammatory and degenerative damages of paradont tissue for assessment of drugs and food supplements effect]. Obzory po klinicheskoi farmakologii i lekarstvennoi terapii. 2013;11(2):57-59. [Article in Russian].

Corresponding author: *Marina A. Darenskaya, PhD, ScD. Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia. E-mail: marina_darenskaya@inbox.ru*

6. Sycheva YuA, Gorbacheva IA, Orekhova LYu, Grigor'eva LG, Chudinova TN. [The role of regional micro-circulation and metabolic disorders in pathogenesis of hypertensive disease and inflammantary diseases of the periodontium]. Periodontology. 2014;2(71):32-35. [Article in Russian].
7. Nath SG, Raveendran R. Microbial dysbiosis in periodontitis. J Indian Soc Periodontol. 2013;17(4):543-5. doi: 10.4103/0972-124X.118334.
8. Kolesnikova LI, Semyonova NV, Grebenkina LA, Darenskaya MA, Suturina LV, Gnusina SV. Integral indicator of oxidative stress in human blood. Bull Exp Biol Med. 2014;157(6):715-7. doi: 10.1007/s10517-014-2649-z.
9. Darenskaya MA, Grebenkina LA, Sholokhov LF, Rashidova MA, Semenova NV, Kolesnikov SI, Kolesnikova LI. Lipid peroxidation activity in women with chronic viral hepatitis. Free Radical Biology & Medicine. 2016;100(S1):192.
10. Darenskaya MA, Rychkova LV, Kolesnikov SI, Gavrilova OA, Kravtsova OV, Grebenkina LA, Kolesnikova LI. Oxidative stress parameters in adolescent boys with exogenous-constitutional obesity. Free Radical Biology & Medicine. 2017;112:129-130.
11. Kolesnikova LI, Kolesnikov SI, Darenskaya MA, Grebenkina LA, Nikitina OA, Lazareva LM, et al. Activity of LPO processes in women with polycystic ovarian syndrome and infertility. Bull Exp Biol Med. 2017;162(3):320-322. doi: 10.1007/s10517-017-3605-5.
12. Ershova OA, Bairova TA, Kolesnikov SI, Kalyuzhnaya OV, Darenskaya MA, Kolesnikova LI. Oxidative stress and catalase gene. Bull Exp Biol Med. 2016;161(3):400-3. doi: 10.1007/s10517-016-3424-0.
13. Bairova TA, Kolesnikov SI, Kolesnikova LI, Pervushina OA, Darenskaya MA, Grebenkina LA. Lipid peroxidation and mitochondrial superoxide dismutase-2 gene in adolescents with essential hypertension. Bull Exp Biol Med. 2015;158(2):181-4. doi: 10.1007/s10517-014-2717-4.
14. Kolesnikova LI, Darenskaya MA, Semenova NV, Grebenkina LA, Suturina LV, Dolgikh MI et al. Lipid peroxidation and antioxidant protection in girls with type 1 diabetes mellitus during reproductive system development. Medicina (Lithuania). 2015;51(2):107-111.
15. Darenskaya MA, Gavrilova OA, Rychkova LV, Kravtsova OV, Grebenkina LA, Osipova EV, et al. The assessment of oxidative stress intensity in adolescents with obesity by the integral index. International Journal of Biomedicine. 2018;8(1):37-41.
16. Kolesnikova LI, Kolesnikov SI, Korytov LI, Suslikova MI, Darenskaya MA, Grebenkina L.A, Kolesnikova L.R. Oxidative stress as a mechanism of reduced glucose absorption under conditions of immobilization stress. Bull Exp Biol Med. 2017;164(2):132-135. doi: 10.1007/s10517-017-3941-5.
17. Omarov IA, Bolevich SB, Savateeva-Lyubimova TN, Silina EV, Sivak KV. [Oxidative stress and comprehensive antioxidative energy correction in treatment of periodontitis]. Stomatology. 2011;1:10-17. [Article in Russian].
18. Kolesnikova LI, Kolesnikov SI, Romanova ED, Chkhenkeli VA, Darenskaya MA, Grebenkina LA, et al. Effect of Preparation Based on Trametes Pubescens Xylotroph Fungi on Lipid Peroxidation in the Blood of Experimental Animals under Conditions of Dark Stress. Bull Exp Biol Med. 2017;162(6):762-764. doi: 10.1007/s10517-017-3707-0.
19. Mokrenko EV, Shabanov PD. Therapy of inflammatory and degenerative damage of the smooth parodont tissue with tooth pastes in rats. Pediatr. 2015;6(2):81–86. [Article in Russian].
20. Kamyshnikov VS. Handbook on clinical and biochemical studies and laboratory diagnostics. Moscow; 2009. [in Russian].
21. Potemkina EE, Pozdnyakova RZ, Manukyan LM. [Training Manuals for Laboratoty Clinical Immunology]. Moscow; 2003. [in Russian].
22. Nazarenko GI, Kishkun AA. [Clinical assessment of laboratory results]. Moscow; 2007. [Article in Russian].
23. Negative nitroblue tetrazolium reduction test. Available from: https://www.ncbi.nlm.nih.gov/medgen/336678
24. Khabriev RU. [Guidance on experimental (non-clinical) testing of new pharmaceutical substances]. Moscow; 2005. [Article in Russian].
25. Gumus P, Huseyinalemdaroglu B, Buduneli N. The role of oxidative stress in the interaction of periodontal disease with systemic diseases or conditions. Oxid Antioxid Med Sci. 2016;5(2):33-3817:18.

International Journal of Biomedicine 9(2) (2019) 163-167
http://dx.doi.org/10.21103/Article9(2)_OA17

ORIGINAL ARTICLE

Lasers in Medical Science

INTERNATIONAL JOURNAL OF BIOMEDICINE

Stress-Related Effects of Low-Intensity Laser Irradiation

Anna V. Deryugina, PhD, ScD[1]; Marina N. Ivashchenko, PhD[2*]; Pavel S. Ignatyev, PhD[3]; Tatyana I. Soloveva, PhD[4]; Evgenia V. Arkhipova[4]; Michael S. Lodyanoy, PhD[2]; Vladimir A. Petrov[2]; Aleksandr G. Samodelkin, PhD, ScD[2]

[1]*Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia*
[2]*Nizhny Novgorod State Agricultural Academy, Nizhny Novgorod, Russia*
[3]*Production Association «Urals Optical & Mechanical Plant» named after E.S. Yalamov, Ekaterinburg, Russia*
[4]*Privolzhsky Research Medical University, Nizhny Novgorod, Russia*

Abstract

The purpose of this study was to investigate the effect of low-level laser (light) therapy (LLLT) on the electrokinetic properties of red blood cells (RBCs), taking into account the activity of stress-realizing systems of the body. The RBC electrophoretic mobility (RBCEM) was used as an index of stress reaction. The experiment included two series: *in vivo* and *in vitro*. Analyzing the LLLT effect on RBCEM, we can assume that the body's response to LLLT is associated with a short-term activation of the sympathoadrenal system and the subsequent longer reaction of the hypothalamic-pituitary-adrenal axis. Through activation of the hypothalamic-pituitary-adrenal axis, LLLT can indirectly cause the development of adaptation processes in the body. (**International Journal of Biomedicine. 2019;9(2):163-167.**)

Key Words: low-level laser (light) therapy • stress • red blood cells • electrophoretic mobility of red blood cells

Introduction

A growing number of reports have shown a positive outcome for low-level laser (light) therapy (LLLT), sometimes known as photobiomodulation, in restorative and rehabilitative medicine. Although the exact mechanisms are yet to be fully understood, LLLT has been used to rescue neurons from neurotoxic injuries[1-3] and help tissue repair and wound healing in animal models.[4-8] LLLT is effective in pain relief and promotes the recovery of some pathologies, including tendinopathies, osteoarthritis, wound healing, and nerve injuries.[9-15]

The fundamental mechanism of photobiomodulation[16] is proposed to involve mitochondria as the primary cellular target for the photons leading to increased cytochrome C oxidase activity,[17] release of nitric oxide,[18,19] and an increase in ATP levels.[20] Changes in intracellular signaling molecules, such as calcium ions, reactive oxygen species and redox sensitive transcription factors, like NF-κB, are also thought to mediate the effects of light.[21,22] Low-level lasers have been reported to attenuate oxidative stress.[23,24]

LLLT at low doses has been shown to enhance cell proliferation of fibroblasts, keratinocytes, endothelial cells, and lymphocytes.[25-30] The mechanism of proliferation is thought to result from photo-stimulation of the mitochondria leading to activation of signaling pathways and up regulation of transcription factors, eventually giving rise to increases in growth factors.[31] It has been observed in many studies that LLLT exhibits a biphasic dose response curve,[32,33] whereby lower doses of light are more effective than much higher doses.

Although LLLT is mostly applied to localized diseases, and its effect is often considered to be restricted to the irradiated area, there are reports of systemic effects of LLLT acting at a site distant from the illumination.[34-36]

The purpose of this study was to investigate the effect of LLLT on the electrokinetic properties of red blood cells (RBCs), taking into account the activity of stress-realizing systems of the body. Of particular interest were RBCs, due to their participation in processes related to maintaining homeostasis at the level of the whole body.

***Corresponding author:** Marina N. Ivashchenko, PhD; Nizhny Novgorod State Agricultural Academy, Nizhny Novgorod, Russia. E-mail: kafedra2577@mail.ru*

Materials and Methods

The experiment included two series: *in vivo* and *in vitro*. The first series of the experiment was carried out on 75 non-pedigree female rats at 3.5-4 months of age weighing 200±20g. The animals were divided into 5 equal groups. Group 1 (control group) included intact animals (n=15). Animals of Group 2 (n=15) were treated with LLLT. For stress modeling, the animals of Group 3 received (n=15) a single intraperitoneal injection of adrenaline hydrochloride solution (0.1 mg/kg), and animals of Group 4 (n=15) received an intraperitoneal cortisol solution (0.4 mg/kg). The animals of Group 5 (n=15) received a single intraperitoneal injection of physiological saline solution.

Animals of Group 2 were given transcutaneous LLLT at the occipital region for 10 minutes. The laser irradiation parameters: a pulse regime, 800-900 nm wavelength, pulse repetition frequency of 415Hz. The minimum value of average power density in the plane of the output window was 193 μW/cm^2. Blood samples were taken from the sublingual vein in 15 min, 30 min, 60 min, and 120 min after starting the experiment.

The second series of the experiments *in vitro* was performed on the isolated RBCs. Blood samples were taken from the sublingual vein of the animals. The effect of stress factors was studied in experiments (20 experiments in each case) *in vitro* using washed erythrocytes. RBCs were incubated with adrenaline (1×10^{-9}g/ml and 1×10^{-10}g/ml) or cortisol (5×10^{-7}g/ml), or they were treated with LLLT (for 10 minutes at 2-5 mm from the applicators). The controls for experiments with LLLT were intact cells, for experiments with adrenaline and cortisol - cells incubated with physiological saline solution.

In order to modify the cell structure, RBCs were fixed with glutaraldehyde according to Walter and Krob.[37] The erythrocyte suspension was incubated with 0.1% glutaraldehyde solution at 22–24°C for 10 min. After a triple washing in salt solution, RBCs were incubated with adrenaline/cortisol or they were treated with LLLT.

The RBC electrophoretic mobility (RBCEM) was used as an index of stress reaction. The RBCEM level was measured by the microelectrophoresis method using a cytopherometer in our modification by registering the 100 μm RBC transmission time in Tris-HCL buffer with pH of 7.4 and amperage of 12мА. The RBC electrophoretic mobility value was defined using the formula: U=S/T×H, where S – a distance to which the cells moved, T – time, H – a gradient of electric potential. The value of potential gradient was determined using the formula: H=I/g×χ, where I – amperage, g – chamber cross section, χ – electrical conductivity of the media.[38,39]

Animals were housed in keeping with the rules for good laboratory practice. Experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals (the institute of Laboratory Animal Resources, 1996) and with approval of local Ethics Committee.

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA). The Shapiro-Wilk test was used in testing for normality. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student's unpaired t-test was used to compare two groups for data with normal distribution. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. A value of $P<0.05$ was considered statistically significant.

Results and Discussion

The results obtained by the first series of the experiment are represented in Table 1. LLLT provoked a decrease in the level of RBCEM 15 minutes after the beginning of the experiment, and it was increased by the 120th minute by 10% relative to the control group value. It is known that LLLT acts on the body at the cell and systemic levels. We may suppose that the development of a typical stress reaction is a response to LLLT at the systemic levels. Given that the sympathoadrenal system and hypothalamic-pituitary-adrenal (HPA) axis are central stress response systems, we analyzed the effects of adrenaline and cortisol. Thus, the intraperitoneal injections of adrenaline provoked a decrease in the RBCEM level during all the experiment, making 74% of the value of the control group at the last registration point (120the min). On the contrary, the cortisol injection provoked an increase in the RBCEM level at all the observation points.

Table 1.
The RBCEM level (μm cm B^{-1}s^{-1}) during the first series of the experiment in vivo

Group	Period after the beginning of the experiment (min)			
	15	30	60	120
Group 3	1.02±0.09*	1.05±0.06*	0.99±0.08*	0.92±0.08*
Group 4	1.28±0.08*	1.49±0.05*	1.73±0.05*	1.67±0.09*
Group 5	1.17±0.02	1.19±0.02	1.19±0.02	1.23±0.04
Group 2	0.96±0.02*	0.98±0.04	1.08±0.02	1.12±0.02*
Group 1	1.02±0.02	1.00±0.03	1.04±0.02	1.02±0.02

**P<0.05 - with animals receiving physiological saline solution (Group 5).*

A registered primary decrease in the RBCEM index under stressful effects may be mediated by an increase in the level of circulating catecholamines in the blood and/or an increase in the sensitivity of cell adrenoreceptors to them. It is known that in reactions to stress, the concentration of adrenaline in the blood plasma increases tenfold in a few minutes. In the stress reaction, catecholamines, by activating the release of ACTH, stimulate an increase in the blood of the adrenal hormone level. While catecholamines reflect the onset of an immediate trigger effect, corticosteroids have a long-lasting effect.[40] Under stress, the blood content of corticosterone in rats increases after 30 minutes and reaches a maximum after 2 hours. Probably, the excretion of corticosteroids, aimed at limiting the first phase of the stress reaction, determines the increase in the RBCEM index (the second phase). Analyzing the LLLT effect on RBCEM, we can assume that the body's

response to LLLT is associated with a short-term activation of the sympathoadrenal system and the subsequent longer reaction of the HPA axis. Through activation of the HPA axis, LLLT can indirectly cause the development of adaptation processes in the body.

Based on this point, the effect of LLLT at the cellular level can also be realized through the consequent starting of processes mediated by the influence of changing concentrations of catecholamines and corticosteroids in the peripheral blood.

In the experiments *in vitro*, we found that LLLT provoked a decrease in the RBCEM index by 20% ($P<0.05$) by the 15th minute after the beginning of the experiment, but after that, the RBCEM index increased considerably and reached 188% of intact cell value by the end of the experiment. The incubation of RBCs with adrenaline in a concentration corresponding to its concentration in the blood under the conditions of the physiological norm (1×10^{-10}g/ml) did not cause changes in the RBCEM level. Concentrations corresponding to the level during stress reactions (1×10^{-9}g/ml) provoked an irreversible decrease in RBCEM ($P<0.05$). In contrast to the experiments with epinephrine, the incubation with cortisol (5×10^{-7}g/ml) led to increasing RBCEM in the range from 30 minutes to 2 hours (Table 2).

Table 2.

The RBCEM level (μm cm $B^{-1}s^{-1}$) during the second series of the experiment in vitro

Group	Before the beginning of the experiment	Period after the beginning of the experiment (min)			
		15	30	60	120
Group 2	1.26±0.08	0.92±0.09*	1.55±0.18*	1.30±0.18	2.06±0.20*
Group 1	1.26±0.08	1.15±0.10	0.96±0.07	1.09±0.12	1.09±0.10
Group 3 (AHS: 1×10^{-9}g/ml)	1.32±0.02	1.24±0.05	1.19±0.08*	1.15±0.07*	1.14±0.05*
Group 3 (AHS: 1×10^{-10}g/ml)	1.25±0.07	1.10±0.11	0.94±0.08	1.1±0.12	1.05±0.07
Group 4 (CS: 5×10^{-7}g/ml)	1.32±0.02	1.52±0.06*	1.96±0.06*	1.72±0.08*	1.72±0.08*
Group 5	1.32±0.02	1.33±0.02	1.36±0.06	1.32±0.06	1.35±0.04

*AHS - adrenaline hydrochloride solution; CS - cortisol solution; *P<0.05 - with intact animals (Group 1).*

Thus, the change in RBCEM under the effect of LLLT on isolated RBCs has the same typical picture as when it affects the whole body. The first reaction of RBCEM, reflecting a decrease in the total charge of the erythrocyte membrane, is comparable to the change in RBCEM under the adrenaline action. The second phase (an increase in RBCEM) is similar to the changes in RBCEM detected for cortisol.

Thus, the detected changes in RBCEM under LLLT *in vitro* experiments are consistent with *in vivo* experiments and associated with changes in the electrokinetic properties of RBCs in stress response, which can be realized through a modification of the receptor apparatus of cells. Taking into account that the most important structural components of this apparatus are proteins, we may conclude that they are a target for stress factors. Thus, one of the likely effects of LLLT action is associated with a nonspecific effect on biopolymers, which leads to a change in the charge of proteins, in their conformational structure, and in their functional state.[41]

To confirm the role of proteins in modifying the activity of cellular receptors, we conducted experiments with pretreatment of RBCs with glutaraldehyde fixing protein molecules due to the formation of cross-links in –NH2-groups.[37] It was shown that pretreatment of RBCs with glutaraldehyde practically nullified the first phase of the reaction of intact erythrocytes to the stress effects of LLLT and epinephrine. At the same time, in the series with cortisol, it was found that the phase of increased RBCEM detected in previous experiments was preserved (Table 3).

Table 3.

The RBCEM level (μm cm $B^{-1}s^{-1}$) in the studied animals after 10 minutes of glutaraldehyde fixation

Kind of influence	Period after the influence (min)			
	15	30	60	120
LLLT	1.64±0.10*	1.88±0.08*	1.53±0.11*	1.55±0.09*
Cortisol	1.36±0.05	1.67±0.12*	1.68±0.10*	1.65±0.08*
Adrenaline	1.32±0.06	1.36±0.10	1.39±0.08	1.44±0.10

**P<0.05 with the RBCEM index of the glutaraldehyde fixed RBC value (1.38±0.06 μm cm $B^{-1}s^{-1}$).*

The results of the study indicate that, against the background of the fixation of protein molecules by glutaraldehyde, the reaction of dropping RBCEM in response to stress is nullified. It can be argued that the mechanism of cell response is realized in the same way and is associated with changes in cell receptor responsiveness. At the same time, if the first phase of the response reaction is associated with modification of membrane adrenoreceptors, the second phase may be due to the modification of intracellular steroid receptors located in the cytosol. Apparently, the effect of LLLI *in vitro* experiments is determined by the possibility of its influence on the hormonal stress-realizing components of the cell. At the same time, the first phase can be considered as an "anxiety stage" of the system, arising by the mechanism of excitation of the sympathoadrenal regulation with an increasing adrenaline effect on the cell membranes. The second stage of stress response is characterized by the return of a reduced function to the initial state, or an increased function of the system in new conditions, which may be one of the mechanisms of LLLT action.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

This study was supported by the Russian Foundation for Basic Research, research project No. 18-016-00195.

References

1. Wong-Riley MT, Liang HL, Eells JT, Chance B, Henry MM, Buchmann E, et al. Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome C oxidase. J Biol Chem. 2005;280(6):4761-71.
2. Liang HL, Whelan HT, Eells JT, Wong-Riley MT. Near-infrared light via light-emitting diode treatment is therapeutic against rotenone- and 1-methyl-4-phenylpyridinium ion-induced neurotoxicity. Neuroscience. 2008;153(4):963-74. doi: 10.1016/j.neuroscience.2008.03.042.
3. Wang CZ, Chen YJ, Wang YH, Yeh ML, Huang MH, Ho ML, et al. Low-level laser irradiation improves functional recovery and nerve regeneration in sciatic nerve crush rat injury model. PLoS One. 2014;9(8):e103348. doi: 10.1371/journal.pone.0103348. eCollection 2014.
4. Albertini R, Villaverde AB, Aimbire F, Salgado MA, Bjordal JM, Alves LP, et al. Anti-inflammatory effects of low-level laser therapy (LLLT) with two different red wavelengths (660 nm and 684 nm) in carrageenan-induced rat paw edema. J Photochem Photobiol B. 2007;89(1):50-5.
5. Draper WE, Schubert TA, Clemmons RM, Milse SA. Low-level laser therapy reduces time to ambulation in dogs after hemilaminectomy: a preliminary study. J Small Anim Pract. 2012;53(8):465–9.
6. Gigo-Benato D, Geuna S, de Castro Rodrigues A, Tos P, Fornaro M, Boux E, et al. Low-power laser biostimulation enhances nerve repair after end-to-side neurorrhaphy: a double-blind randomized study in the rat median nerve model. Lasers Med Sci. 2004;19(1):57–65.
7. Casalechi HL, Leal-Junior EC, Xavier M, Silva JA Jr, de Carvalho Pde T, Aimbire F, Albertini R. Low-level laser therapy in experimental model of collagenaseinduced tendinitis in rats: effects in acute and chronic inflammatory phases. Lasers Med. Sci. 2013;28(3):989-95.
8. Gigo-Benato D, Russo TL, Tanaka EH, Assis L, Salvini TF, Parizotto NA. Effects of 660 and 780 nm low-level laser therapy on neuromuscular recovery after crush injury in rat sciatic nerve. Lasers Surg Med. 2010;42:673–82.
9. Chow RT, Johnson M, Lopes-Martins RA, Bjordal JM. Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials. Lancet. 2009;374(9705):1897–908.
10. Brosseau L, Robinson V, Wells G, Debie R, Gam A, Harman K, et al. Low level laser therapy (Classes I, II and III) for treating rheumatoid arthritis. Cochrane Database Syst Rev. 2005;19:CD002049.
11. Tumilty S, Munn J, McDonough S, Hurley DA, Basford JR, Baxter GD. Low level laser treatment of tendinopathy: a systematic review with meta-analysis. Photomed Laser Surg. 2010;28(1):3–16.
12. Marcos RL, Arnold G, Magnenet V, Rahouadj R, Magdalou J, Lopes-Martins RÁ. Biomechanical and biochemical protective effect of low-level laser therapy for Achilles tendinitis. J Mech Behav Biomed Mater. 2014;29:272–85.
13. Kheshie AR, Alayat MS, Ali MM. High-intensity versus low-level laser therapy in the treatment of patient with knee osteoarthritis: a randomized controlled trial. Lasers Med Sci. 2014;29(4):1371–6.
14. Saber K, Chiniforush N, Shahabi S. The effect of low level laser therapy on pain reduction after third molar surgery. Minerva Stomatol. 2012;61(7–8): 319–22.
15. Alves AC, Vieira R, Leal-Junior E, dos Santos S, Ligeiro AP, Albertini R, et al. Effect of low-level laser therapy on the expression of inflammatory mediators and on neutrophils and macrophages in acute joint inflammation. Arthritis Res Ther. 2013;15(5):116.
16. Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. Ann Biomed Eng. 2012;40(2):516-33. doi: 10.1007/s10439-011-0454-7.
17. Wong-Riley MT, Liang HL, Eells JT, Chance B, Henry MM, Buchmann E, et al. Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase. J Biol Chem. 2005;280(6):4761–71.
18. Karu TI, Pyatibrat LV, Afanasyeva NI. Cellular effects of low power laser therapy can be mediated by nitric oxide. Lasers Surg Med. 2005;36(4):307-14.
19. Moriyama Y, Nguyen J, Akens M, Moriyama EH, Lilge L. In vivo effects of low level laser therapy on inducible nitric oxide synthase. Lasers Surg Med. 2009; 41(3):227-31.
20. Passarella S, Casamassima E, Molinari S, Pastore D, Quagliariello E, Catalano IM, Cingolani A. Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium-neon laser. FEBS Lett. 1984;175(1):95-9.
21. Chen AC, Arany PR, Huang YY, Tomkinson EM, Sharma SK, Kharkwal GB, et al. Low-level laser therapy activates NF-kB via generation of reactive oxygen species in mouse embryonic fibroblasts. PLoS One. 2011;6(7):e22453. doi: 10.1371/journal.pone.0022453.
22. Lavi R, Shainberg A, Friedmann H, Shneyvays V, Rickover O, Eichler M, et al. Low energy visible light induces reactive oxygen species generation and stimulates an increase of intracellular calcium concentration in cardiac cells. J Biol Chem. 2003;278(42):40917-22.
23. Giuliani A, Lorenzini L, Gallamini M, Massella A, Giardino L, Calzà L. Low infra red laser light irradiation on cultured neural cells: effects on mitochondria and cell viability after oxidative stress. BMC Complement Altern Med. 2009;9:8. doi: 10.1186/1472-6882-9-8.
24. Huang YY, Nagata K, Tedford CE, McCarthy T, Hamblin MR. Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons in vitro. J Biophotonics. 2013;6(10):829-38. doi: 10.1002/jbio.201200157.
25. AlGhamdi KM, Kumar A, Moussa NA. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers Med. Sci. 2012;27:237-49.
26. Lubart R, Wollman Y, Friedmann H, Rochkind S, Laulicht I. Effects of visible and near infrared lasers on cell cultures. J Photochem Photobiol B. 1992;12(3):305–10.
27. Frigo L, Fávero GM, Lima HJ, Maria DA, Bjordal JM, Joensen J, et al. Low-level laser irradiation (InGaAlP-660 nm) increases fibroblast cell proliferation and reduces cell death in a dose-dependent manner. Photomed Laser Surg. 2010;28(Suppl 1):S151–6.
28. Basso FG, Oliveira CF, Kurachi C, Hebling J, Costa CA. Biostimulatory effect of low-level laser therapy on keratinocytes in vitro. Lasers Med Sci. 2013;28(2):367–74.
29. Szymanska J, Goralczyk K, Klawe JJ, Lukowicz M, Michalska M, Goralczyk B, et al. Phototherapy with low-level laser influences the proliferation of endothelial cells and vascular endothelial growth factor and transforming growth factor-beta secretion. J Physiol Pharmacol. 2013;64(3):387–91.
30. Agaiby AD, Ghali LR, Wilson R, Dyson M.

Laser modulation of angiogenic factor production by T-lymphocytes. Lasers Surg Med. 2000;26(4):357–63.
31. Saygun I, Nizam N, Ural AU, Serdar MA, Avcu F, Tözüm TF. Low-level laser irradiation affects the release of basic fibroblast growth factor (bFGF), insulin-like growth factor-I (IGF-I), and receptor of IGF-I (IGFBP3) from osteoblasts. Photomed Laser Surg. 2012;30(3):149–54.
32. Huang YY, Chen AC, Carroll JD, Hamblin MR. Biphasic dose response in low level light therapy. Dose Response. 2009;7(4):358–83.
33. Huang YY, Sharma SK, Carroll J, Hamblin MR. Biphasic dose response in low level light therapy - an update. Dose Response. 2011;9(4):602–18.
34. Rochkind S1, Rousso M, Nissan M, Villarreal M, Barr-Nea L, Rees DG. Systemic effects of low-power laser irradiation on the peripheral and central nervous system, cutaneous wounds, and burns. Lasers Surg Med. 1989;9(2):174-82.
35. Coelho RC, Zerbinati LP, de Oliveira MG, Weber JB. Systemic effects of LLLT on bone repair around PLLA-PGA screws in the rabbit tibia. Lasers Med Sci. 2014;29(2):703-8.
36. Schindl A, Heinze G, Schindl M, Pernerstorfer-Schön H, Schindl L. Systemic effects of low-intensity laser irradiation on skin microcirculation in patients with diabetic microangiopathy. Microvasc Res. 2002;64(2):240-6.
37. Walter H, Krob EJ. Fixation with even small guantities of glutaraldehyde effects red bloods cell surface properties in a cell. Biosci Rep.1989;9(6):727-35.
38. Deryugina AV, Shumilova AV, Filippenko ES, Galkina YV, Simutis IS, Boyarinov GA. Functional and biochemical parameters of erythrocytes during mexicor treatment in posttraumatic period after experimental blood loss and combined traumatic brain injury. Bull Exp Biol Med. 2017;164(1):26-9. doi: 10.1007/s10517-017-3918-4.
39. Deryugina AV, Ivashchenko MN, Ignatyev PS, Zolotova MV, Samodelkin AG. Electrophoretic Mobility of Red Blood Cells and Micronucleus Test in Exfoliated Buccal Cells as Stress Intensity Markers. International Journal of Biomedicine. 2018;8(4):347-50.
40. Sunada, Rao BS, Raju TR. Restraint stress-induced alterations in the levels of biogenic amines, amino acids and AchE activity in the hippocampus. Neurochem Res. 2000;25(12):1547-52.
41. Deryugina AV, Samodelkin AG, Ivashchenko MN, Ignatyev PS, Zolotova MV. The use of low intensity laser therapy for the reduction of technology stress of cows. AER-Advances in Engineering Research. 2018:151;820-24.

International Journal of Biomedicine 9(2) (2019) 168-171
http://dx.doi.org/10.21103/Article9(2)_OA18

ORIGINAL ARTICLE

Reproductive Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Processes of Lipoperoxidation and Antioxidant Protection in Men with Different Variants of Spermograms

N. A. Kurashova, PhD, ScD*; B. G. Dashiev; M. I. Dolgikh, PhD; L.I. Kolesnikova, PhD, ScD

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

Abstract

Background: Currently, infertile couples represent a very complex and serious medical and social problem. In 40%-60% of cases, the reason for the absence of children in the family is the male factor. Oxidative stress (OS) has been identified as one of the many mediators of male infertility by causing sperm dysfunction. The purpose of this study was to identify the characteristics of the intensity indices of lipid peroxidation (LPO) and antioxidant system (AOS) in the ejaculate of men with different spermogram variants.

Materials and Methods: We performed a retrospective analysis of the results of examinations of 69 men of infertile couples with various disorders of the ejaculate and 155 healthy men with proven fertility. In the semen of the examined men, the level of CD (conjugated dienes), TBARS (thiobarbituric acid reactive substances), α-tocopherol, retinol and total antioxidant activity (AOA) was evaluated by spectrophotometric method.

Results: We found that the antioxidant protection system in patients with asthenozoospermia was characterized by a decrease in the level of total AOA in the semen by 50% and the α–tocopherol concentration by 52%, compared to healthy men in the control group. In patients with oligozoospermia, an increase in the CD concentration by 39% and a decrease in the TBARS concentration by 26% was found; the level of total AOA decreased by 37% and the concentration of α–tocopherol by 41%, compared to healthy men.

Conclusion: In general, the analysis of the obtained data of the survey of men with different variants of spermograms indicates a change in the parameters of the LPO–AOS and confirms the OS development. Thus, it can be noted that depending on the pathological state of the ejaculate in men of reproductive age, LPO processes have their own characteristics, and in men with oligozoospermia, LPO processes occur more intensively. Activation of LPO-AOS processes can be both a consequence and a cause of various metabolic changes in the human body. (**International Journal of Biomedicine. 2019;9(2):168-171.**)

Key Words: men • sperm • lipid peroxidation • antioxidants

Abbreviations

AOA, antioxidant activity; **AOS,** antioxidant system; **CD**, conjugated dienes; **ROS**, reactive oxygen species; **OS**, oxidative stress; **LPO**, lipid peroxidation; **TBARS**, thiobarbituric acid reactive substances.

Introduction

Currently, infertile couples represent a very complex and serious medical and social problem.[1-4] Despite the improvement of clinical-laboratory examinations and methods, and the introduction of auxiliary reproductive technologies into the wide clinical practice, the incidence of infertility in the marriage varies widely (7-28%) and does not tend to decrease.[5] In 40%-60% of cases, the reason for the absence of children in the family is the male factor.[4-7] According to the literature, many factors affect the quality of the ejaculate, including an unfavorable ecological situation, inadequate and unbalanced nutrition, smoking, alcohol, inflammatory diseases of the genitourinary system, varicocele, and food products.[1,4]

OS has been identified as one of the many mediators of male infertility by causing sperm dysfunction. Active forms

***Corresponding author:** Nadezhda A. Kurashova, PhD, ScD. Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia. E-mail: nakurashova@yandex.ru*

of oxygen (ROS) are necessary; under normal physiological conditions, they contribute to the reaction of capitation, regulation of maturation of spermatozoa and the development of cellular signaling pathways. Higher levels of ROS induce LPO, damage to sperm DNA and apoptosis.[8] To overcome these undesirable consequences, ROS are naturally stabilized by the components of the body's antioxidant defense. In a healthy body, pro-oxidants and antioxidants remain in balance. However, under pathological conditions, the uncontrolled production of ROS exceeds the antioxidant capacity of the seminal plasma, resulting in OS.[1,9-12] Spermatozoa are particularly vulnerable to OS because they do not have the necessary cytoplasmic antioxidant recovery systems. 20%-40% of infertile men have a higher level of ROS in the semen than in healthy men.[13]

Lipid composition of cell membranes of spermatozoa affects their functional characteristics.[14] Long-chain polyunsaturated fatty acids in a high concentration are present in the male germ cells. Their number in relation to saturated fatty acids and cholesterol is closely related to the fluidity of the membranes of spermatozoa.[15] Due to a significant number of double bonds, polyunsaturated fatty acids in the membranes of spermatozoa are particularly susceptible to LPO when there is an increase in the total amount of oxygen compounds formed and an imbalance in the components of the antioxidant system. In male infertility, the role of reactive oxygen species and decreased AOA in seminal plasma was established. [2,16]

The purpose of this study was to identify the characteristics of the intensity indices of LPO and AOS in the ejaculate of men with different spermogram variants.

Materials and Methods

We performed a retrospective analysis of the results of examinations of 69 men of infertile couples of Irkutsk city with various disorders of the ejaculate. All patients were divided into two groups: Group 1 included 45 men (mean age of 30.2±3.6 years) with astenozoospermia; Group 2 included 24 men (mean age of 31.9±7.5) with oligozoospermia. The control group consisted of 155 healthy men (mean age of 31.6±5.9 years) with normozoospermia and a realized reproductive function.

Exclusion criteria were obesity, type 1 and type 2 diabetes, arterial hypertension, endocrine infertility, inflammatory diseases of the urogenital tract, including sexually transmitted infections.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki (2000; revised October 2013, Fortaleza, Brazil). Written informed consent was obtained from all participants.

Methods of standard clinical examination of fertile and infertile men included: an ultrasonic scan of scrotum and prostate, macroscopic and microscopic examination of ejaculate, and biochemical analysis. The semen analysis was performed in accordance with the WHO recommendations.[17]

In the semen of the examined men, the content of CD (primary oxidation products) and TBARS, end products of LPO, was determined by the methods of V. Gavrilov et al.[18,19] The level of α-tocopherol and retinol was estimated by the method of R. Ch.Chernyauskene et al.[20] AOA according to GI Klebanov et al.[21] The measurements were performed using a Shimadzu RF-1501 spectrofluorophotometer (Japan).

The statistical analysis was performed using the statistical software STATISTICA 6.1 (StatSoft Inc., USA). The mean (M) and standard deviation (SD) were calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

OS has been identified as one of the many mediators of male infertility. It is shown that in 30%-80% of cases of male infertility, pathospermia are caused by high levels of ROS in seminal plasma.[2,13,14]

We found that the antioxidant protection system in patients with asthenozoospermia was characterized by a decrease in the level of total AOA in the semen by 50% and the α–tocopherol concentration by 52%, compared to healthy men in the control group (Table 1).

Table 1

Parameters of the LPO-AOS system in healthy men (Control group) and in men with asthenozoospermia (Group 1)

Parameter	Control group	Group 1	P-value
CD (μmol/l)	1.27±0.81	1.31±0.94	0.791
TBARS (μmol/l)	1.06±0.61	0.89±0.46	0.091
AOA (unit)	3.86±2.26	1.94±1.41	0.000
α–tocopherol (μmol/l)	5.27±2.99	2.53±1.90	0.000

As known, α–tocopherol helps to preserve both sperm motility and morphology, protecting the components of sperm membranes from damage by OS.[22] A number of studies have confirmed the positive effect of antioxidants on spermatogenesis disorders caused by OS.[23-25]

In patients with oligozoospermia (Table 2), we found an increase in the CD concentration by 39% and a decrease in the TBARS concentration by 26%; the level of total AOA decreased by 37% and the concentration of α–tocopherol by 41%, compared to healthy men.

Table 2

Parameters of the LPO-AOS system in healthy men (Control group) and in men with oligozoospermia (Group 2)

Parameter	Control group	Group 2	*P*-value
CD (μmol/l)	1.27±0.81	1.77±0.73	0.005
TBARS (μmol/l)	1.06±0.61	0.78±0.29	0.031
AOA (unit)	3.86±2.26	2.44±1.61	0.003
α–tocopherol (μmol/l)	5.27±2.99	3.14±2.10	0.001

The primary products of LPO, as a rule, are very unstable substances and are easily subjected to further transformations with the formation of more stable oxidation components—aldehydes, ketones, low-molecular acids—as a result of which they exhibit a wide range of changes. A decrease in the level of TBARS in men with oligozoospermia may indicate activation of the enzymatic component of antioxidant protection, in particular SOD, and oxidized and reduced glutathione. [1,24,26]

Thus, the low level of total AOA and α-tocopherol concentration in men with oligozoospermia and asthenozoospermia (Tabl. 1,2) indicates the activation of LPO process. Dramatic changes in cellular redox systems trigger the suppression of antioxidative defense in biological tissues and internal environments. Failure of antioxidant protection can lead to the following changes: damage to membranes, inactivation or transformation of enzymes, suppression of cell division, and accumulation of inert polymerization products in cells.[3]

Reduced sperm motility (asthenozoospermia) and low sperm count (oligozoospermia) are significant causes of male reproductive failure. Their origin is diverse and, in some cases, cannot be established. Reduced sperm motility can often be associated with ultrastructural flagellum disorders, which is a consequence of the genetic nature, as well as the result of external factors—adverse environment, smoking, alcohol, poor and micronutrient diet, sedentary lifestyle and much more.[27] OS causes an arrest of spermatogenesis at the early meiotic stage and induces apoptosis, leading to oligozoospermia.[28] Elevated ROS levels lead to the development of DNA mutations and damage to cell structures with the development of teratozoospermia.[7,28] In preventing oxidative stress and reducing its negative impact on spermatogenesis, the simultaneous use of fat- and water-soluble vitamins is promising.[8] In experiments to study the effects of α–tocopherol, an increase in the mobility and functioning of sperm, and in the frequency of fertilization, was established.[29-31]

Conclusion

In general, the analysis of the obtained data of the survey of men with different variants of spermograms indicates a change in the parameters of the LPO–AOS and confirms the OS development. Thus, it can be noted that depending on the pathological state of the ejaculate in men of reproductive age, LPO processes have their own characteristics, and in men with oligozoospermia, LPO processes occur more intensively. Activation of LPO-AOS processes can be both a consequence and a cause of various metabolic changes in the human body.[32]

Competing Interests

The authors declare that they have no competing interests.

References

1. Kolesnikova LI, Kurashova NA, Bairova TA, Dolgikh MI, Ershova OA, Dashiev BG, et al.. Role of Glutathione-S-Transferase Family Genes in Male Infertility. Bull Exp Biol Med. 2017;163(5):643-645. doi: 10.1007/s10517-017-3869-9.
2. Nikolaev AA, Loginov PV, Vetoshkin RV. [Participation of free radicals in the function of spermatozoa]. Astrakhan Medical Journal. 2014;(1):23-29. [Article in Russian].
3. Galimov SN, Akhmetov RM, Galimova EF, Bairamgulov FM, Bikkulova LR. [Molecular aspects of the impact of the Speroton complex on the male fertility in idiopathic infertility]. Urologiia. 2017;(2):88-92.[Article in Russian].
4. Shantanova LN, Osadchuk LV, Dashiev BG, Kleschev MA, Gutorova NV, Osadchuk AV, Bashelkhanov IS. [Assessment of reproductive health in young men of the Republic of Buryatia]. Bulletin of the East Siberian Scientific Center SB RAMS. 2012;88:44-46. [Article in Russian].
5. Artifeksova MS, Borodacheva IV, Sergeev MYu, Artiefeksov SB, Zhabin SG. [Indicators of life quality of the men from infertile couple]. Russian Journal of Human Reproduction. 2015;21(5): 84-88. [Article in Russian].
6. Moretti E, Collodel G, Fiaschi AI, Micheli L, Iacoponi F, Cerretani D. Nitric oxide, malondialdheyde and non-enzymatic antioxidants assessed in viable spermatozoa from selected infertile men. Reprod Biol. 2017;17(4):370-375. doi: 10.1016/j.repbio.2017.10.003.
7. Dorostghoal M, Kazeminejad SR, Shahbazian N, Pourmehdi M, Jabbari A. Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. Andrologia. 2017;49(10). doi: 10.1111/and.12762. Epub 2017 Jan 26.
8. Khodos MYa, Kazakov YaE, Vidrevich MB, Brainina HZ. [Oxidative stress and its role in pathogenesis]. Journal of Ural Medical Academic Science. 2017;4(4):381-398. [Article in Russian].
9. Kolesnikova LI, Darenskaya MA, Kolesnikov SI. [Free radical oxidation: a pathophysiologist's view]. Bulletin of Siberian Medicine. 2017;16(4):16-29. [Article in Russian]. doi: 10.20538/1682-0363-2017-4-16-29.
10. Darenskaya MA, Kolesnikov SI, Rychkova LV, Grebenkina LA, Kolesnikova LI. Oxidative stress and antioxidant defense parameters in different diseases: ethnic aspects. Free Radical Biology & Medicine. 2018;120(S1):S60. doi: 10.1016/j.freeradbiomed.2018.04.199.
11. Trussell JC. Optimal diagnosis and medical treatment of male infertility. Semin Reprod Med. 2013;31(4):235-6. doi: 10.1055/s-0033-1345269.
12. Henkel RR. Leukocytes and oxidative stress: dilemma for sperm function and male fertility. Asian J Androl. 2011;13(1):43-52. doi: 10.1038/aja.2010.76.
13. Lomteva SV, Savikina KG, Shestel AN, Sagamonova KYu, Shkurat TP. Oxidative stress and male reproductive system. Valeology. 2015;(1):59-67. [Article in Russian].
14. Ploskonos MV. [Biochemical changes in the membrane of spermatozoa of fertile men under the influence of an inducer of oxidative stress and correction of these changes]. Russian Journal of Human Reproduction. 2015;(5):102-108 [Article in Russian].
15. Rudneva SA, Bragina EE, Arifulin EA, Sorokina TM, Shileiko LV, Ermolaeva SA, et al. [DNA fragmentation in spermatozoa and its relationship with impaired spermatogenesis]. Andrology and Genital Surgery. 2014;15(4):26-33. [Article in Russian].
16. Kurashova NA, Dolgikh MI, Ershova OA, Gavrilova OA, Osipova EV, Dashiev BG, et al. Associations of Polymorphic Variants of the Biotransformation Genes with the Components of the Glutathione System in Men with Infertility. International

Journal of Biomedicine. 2017;7(3):226-230. doi: 10.21103/Article7(3)_OA13.
17. WHO laboratory manual for the examination and processing of human semen - 5th ed. Available from: https://apps.who.int/iris/bitstream/handle/10665/44261/9789241547789_eng.pdf;jsessionid=46F8915182775DD661AE4D5247CDA162?sequence=1.
18. Gavrilov VB, Mishkorudnaya MI. [Spectrophotometric determination of the content of lipid hydroperoxes in the blood plasma]. Lab Delo. 1983;(3)33-36. [Article in Russian].
19. Gavrilov VB, Gavrilova AR, Mazhul' LM. [Methods of determining lipid peroxidation products in the serum using a thiobarbituric acid test]. Vopr Med Khim. 1987;33(1):118-22. [Article in Russian].
20. Cherniauskene RCh, Varshkiavichene ZZ, Gribauskas PS. [Simultaneous fluorometric determination of the concentrations of vitamins E and A in blood serum]. Lab Delo. 1984;(6):362-5.[Article in Russian].
21. Klebanov GI, Babenkova IV, Teselkin IuO, Komarov OS, Vladimirov IuA. [Evaluation of the antioxidative activity of blood plasma using yolk lipoproteins]. Lab Delo. 1988;(5):59-62. [Article in Russian]
22. Kolesnikova LI., Darenskaya MA, Rychkova LV, Grebenkina LA, Dolgikh MI, Gavrilova OA. Ethnic Peculiarities of the Lipid Profile in Adolescent Representatives of Some Indigenous Ethnic Groups of Siberia. J Evol Biochem Phys. 2018;54(5):356-362. doi: 10.1134/S0022093018050034.
23. Kul'chenko NG. [Principal antioxidant therapy patospermia]. "REAVIZ" Bulletin: Rehabilitation, Doctor and Health. 2018;31(1):41-48. [Article in Russian].
24. Ahmadi S, Bashiri R, Ghadiri-Anari A, Nadjarzadeh A. Antioxidant supplements and semen parameters: An evidence based review. Int J Reprod Biomed. 2016;14(12):729-736.
25. Kolesnikova LI, Kurashova NA, Bairova TA, Osipova EV. Activity of components of lipid peroxidation system and antioxidant protection in men with infertility, carriers of non-functional genotypes GSTT1 and GSTM1. Free Radical Biology and Medicine. 2018;120(1):72-73. doi: 10.1016/j.freeradbiomed.2018.04.240
26. Kao SH, Chao HT, Chen HW, Huang Y-S, Liao TL, Wei YH. Increase of oxidative stress in human sperm with lower motility. Fertil Steril. 2008;89(5),1183-90.
27. Madaeva IM, Berdina ON, Semenova NV, Madaev VV, Kolesnikova LI. Erectile dysfunction and sleep apnea syndrome are risk factors of cardiovascular diseases. European Journal of Preventive Cardiology. 2017; 24(S1): S288.
28. Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J. The role of oxidative stress and antioxidants in male fertility. Cent European J Urol. 2013;66(1):60-67. doi: 10.5173/ceju.2013.01.art19
29. Belenkaia LV, Sholokhov LF, Kolesnikova LI. Levels of essential elements in pubic hair of men with type 1 diabetes. Endocrine Rev. 2017;38(S3):169-170.
30. Ammar O, Haouas Z, Hamouda B, Hamdi H, Hellara I, Jlali A, et al. Relationship between sperm DNA damage with sperm parameters, oxidative markers in teratozoospermic men. Eur J Obstet Gynecol Reprod Biol. 2019;233:70-75. doi: 10.1016/j.ejogrb.2018.12.003.
31. Intasqui P, Antoniassi MP, Camargo M, Nichi M, Carvalho VM, Cardozo KH, et al. Differences in the seminal plasma proteome are associated with oxidative stress levels in men with normal semen parameters. Fertil Steril. 2015;104(2):292-301. doi: 10.1016/j.fertnstert.2015.04.037.
32. Zagarskikh EIu, Labygina AV, Kurashova NA [Experience in the treatment of normogonadotropic infertility in men]. Urologiia. 2014;(5):87-9. [Article in Russian].

International Journal of Biomedicine 9(2) (2019) 172-175
http://dx.doi.org/10.21103/Article9(2)_OA19

ORIGINAL ARTICLE

Epidemiology & Population Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Serum Levels of Tumor Markers in the Elderly Population of Yakutia

Vyacheslav M. Nikolaev, PhD[1*]; Svetlana D. Efremova[1]; Sargylana I. Sofronova, PhD[1]; Nadezhda K. Chirikova, PhD[2]; Sardana A. Fedorova, PhD, ScD[2]; Anna N. Romanova, PhD, ScD[1]

[1]Yakut Science Center of Complex Medical Problems
[2]M. K. Ammosov North-Eastern Federal University
Yakutsk, the Republic of Sakha (Yakutia), Russia

Abstract

The purpose of our research was to assess the level of tumor markers in the blood serum of elderly residents of Yakutia.

***Material and Methods*:** We examined 204 elderly residents of Yakutia (52 men aged from 60 to 74 years and 152 women aged from 55 to 74 years). The study did not include the people who have oncological or preoncological diseases, or those with exacerbation of chronic diseases. Regarding ethnic origin, all examined people were Yakuts (indigenous people of Yakutia). The levels of tumor markers (AFP, CA 125, and PSA) in the blood serum were determined by ELISA.

Results: Among elderly people, the serum level of AFP was at higher levels within within the reference range in single working people and people having a low level of education and/or obesity. The serum level of CA125 in women increased depending on ML, discomfort of housing, and low level of material well-being. The serum level of PSA was at higher levels within the reference range in male smokers.(**International Journal of Biomedicine. 2019;9(2):172-175.**)

Key Words: cancer antigen 125 • prostate-specific antigen • alpha-fetoprotein • reference range

Abbreviations

AFP, alpha-fetoprotein; **BMI**, body mass index; **CA 125**, cancer antigen 125; **ML**, marriage length **PSA**, prostate-specific antigen; **QL**, the quality of life.

Introduction

Tumor markers (TMs) are specific substances, usually proteins, that are produced by the body in response to cancer growth or by the cancer tissue itself. TMs are assuming a growing role in all aspects of cancer care, from initial screening to follow-up after treatment.[1,2] TMs are also expressed by healthy fetal tissues and recognized as oncofetal. These antigens are associated with cell proliferation and differentiation. In pregnancy, they affect the maternal immune response, generating maternal tolerance toward the embryo, while in malignancy their biological role is to suppress the host's immune system.

TMs can also show up in certain non-cancerous conditions. So, CA125 level in the blood serum increases in patients with heart failure, endometriosis, and obesity.[3-6] Megaloblastic anemia leads to a substantial increase of average CA15-3 value.[7] The AFP level is significantly higher among people with metabolic syndrome, cirrhosis, hepatitis, ataxia-telangiectasia, nephritic syndrome, pregnancy and gastritis, in comparison with healthy people.[8-12] Furthermore, the following factors can influence the level of TMs: age, addictions (smoking, alcohol), ecology, and socio-economic conditions.[13-15] PSA is not a malignant, but a specific organ target marker; it means that the PSA level can be increased in the presence of nonmalignant conditions of a prostate, such as benign hyperthrophy of prostate or prostatitis.[16]

TMs quantitatively reflect any damage to tissues and bodies demanding restoration of a cellular homeostasis. Thus, TMs are

***Corresponding author**: Nikolaev V. Mikhaylovich, PhD. Yakut Scientific Center of Complex Medical Problems. Yakutsk, the Republic of Sakha (Yakutia), Russia. E-mail: Nikolaev1126@mail.ru*

integrated indicators of the general state of health of the body.

We examined the elderly population of the Sakha Republic (Yakutia) [(SR(Y)], which lives in extreme climatic conditions in the North. Screening a population of elderly residents of Yakutia for TMs has never been conducted. In this regard, the purpose of our research was to assess the level of TMs in the blood serum of this population.

Material and Methods

We examined 204 elderly residents of Yakutia (52 men aged from 60 to 74 years and 152 women aged from 55 to 74 years). The study did not include the people who have oncological or preoncological diseases, or those with exacerbation of chronic diseases. Regarding ethnic origin, all examined people were Yakuts (indigenous people of Yakutia). To assess quality of life, we used the standard questionnaire (SF-36), modified by the lab of medical-social research in our institution.

BMI was calculated using Quetelet's formula (kg/cm^2). BMI value between 25 kg/m^2 and 29.9 kg/m^2 was assessed as overweight (pre-obesity), BMI value ≥30 kg/m^2 was assessed as obesity.[17] The levels of TMs in the blood serum were determined by ELISA on a Multiskan FC microplate photometer (Thermo Fisher Scientific, USA) using the Vector-Best test systems (Russia).

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from each patient.

Statistical analysis was performed using SPSS (version 19.0). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean± SEM for continuous variables. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. The Pearson's correlation coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A probability value of P<0.05 was considered statistically significant.

Results and Discussion

The choice of these TMs was based on an analysis of data in the literature: AFP, PSA, and CA125 are mentioned most often in non-cancerous conditions. The mean levels of PSA, CA125 and AFP in the blood serum of elderly inhabitants were 1.85±0.78 ng/ml, 5.89±0.84 ng/ml, and 5.90±0.91 IU/ml, respectively, which were within the reference range.

According to the results of the questionnaire, respondents were divided into 3 groups, depending on marital status: married (n=89), widowers (n=75), and single (n=40). Results of the research showed that the serum TM content in elderly people depended on their marital status. We found a decrease in the AFP level in the group of widowers and married people by 1.22 times and 1.79 times, respectively, in comparison with single people (5.47±0.58 IU/ml versus 6.68±1.74 IU/ml (P=0.049) and 3.72±0.89 IU/ml versus 6.68±1.74 IU/ml (P=0.035), respectively) (Fig.1).

The PSA value was lower in widowers by 1.17 times, in comparison with single men (1.73±0.62 ng/ml vs. 2.04±0.85 ng/ml). The PSA level in married persons did not differ from single men. The serum level of CA125 in women did not depend on marital status. Possibly, significant reduction in the content of AFP in married people can be explained by protection of health by buffering stress reactivity and encouraging healthy behavior.

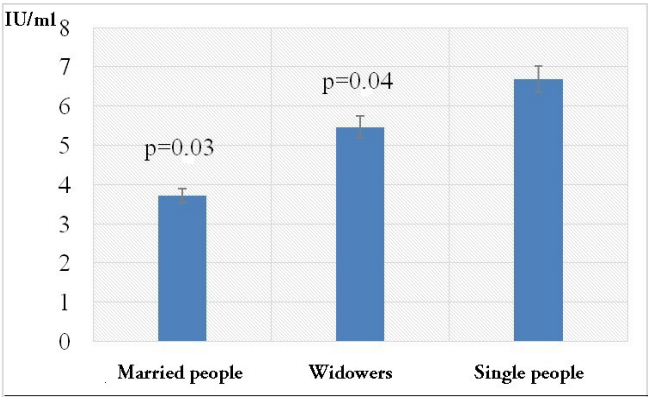


Fig.1. *The serum level of AFP depending on marital status*

The levels of TMs depended on the marriage length (ML). In groups of women with ML between 10 and 20 years (n=20), 20 and 30 years (n=35), and > 30 years (n=82), the serum levels of CA125 were as follows: 2.38±0.78 ng/ml 5.47±0.80 ng/ml and 6.46±1.32 ng/ml, respectively; P<0.05 between (n=20) and (n=35). However, we found a weak positive correlation between ML and CA125 (r=0.20, P=0.13). In addition, average values of PSA and AFP did not differ with respect to ML.

From numerous references, it is known that QL of elderly people is associated with such factors as employment and education.[18] The serum AFP level in persons with higher education (n=24) was 1.3 times lower (4.89±0.15 IU/ml) than in persons with secondary education (n=148) (6.36±0.23 IU/ml) (P<0.05). Low levels of AFP in people with higher education can be explained by the presence of established concepts aimed at maintaining a healthy lifestyle.

The serum level of AFP in working people (n=51) was 1.35 times higher (7.38±0.80 IU/ml) than in nonworking (n=153) people (5.45±0.31 IU/ml) (P<0.05)The levels of CA125 and PSA did not differ depending on education level (higher, secondary, and elementary) and employment status.

Type of housing had a significant impact on the level of tumor markers. In the women living in uncomfortable housing, the serum level of CA125 was 1.79 times higher (6.04±0.87 ng/ml; P=0.02) than in women with comfortable housing (3.44±0.79 ng/ml) (Fig.2). The levels of PSA and AFP did not depend on this factor.

According to the completed questionnaires, 83 respondents answered to a question of material well-being that they were well-off (Group A), 83 answered - means were enough for food and essentials (Group B), 32 - means were enough only for food (Group C), and 6 answered - means were not enough for food (Group D). According to our data, the serum levels of CA125 and AFP decreased depending on material well-being. Therefore, in Group B, the serum levels

CA125 and AFP were 1.51 and 1.42 times higher (6.61±0.49 ng/ml; *P*=0.026; 8.10±1.15 IU/ml) than in Group A. Thus, in Group C, the serum levels of CA125 and AFP were 2.13 and 1.35 times higher (9.33±1.33 ng/ml [*P*=0.03] and 17.68±1.76 IU/ml), respectively, than in Group A (CA125 – 4.38±0.67 ng/ml; AFP – 5.69±1.26 IU/ml). The PSA level did not depend on material well-being. Among the interviewed elderly people, 173 were non-smokers and 29 - long-term smokers. In smokers, we found a significant increase in the PSA level by 4.6 times in comparison with non-smokers 6.62±1.44 ng/ml vs. 1.44±0.01 ng/ml, *P*<0.05) (Figure 3). However, the literature data on this question are contradictory.

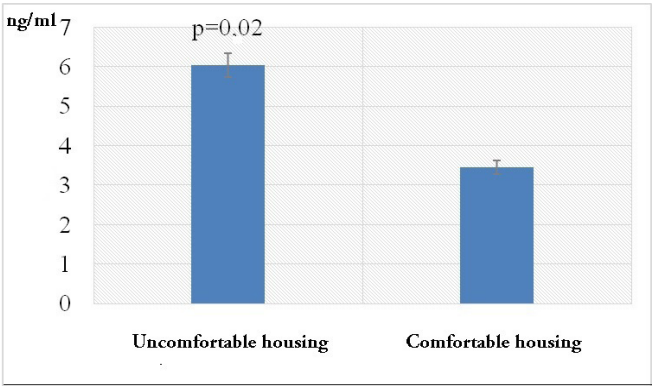


Fig. 2. *The serum level of CA125 and type of housing.*

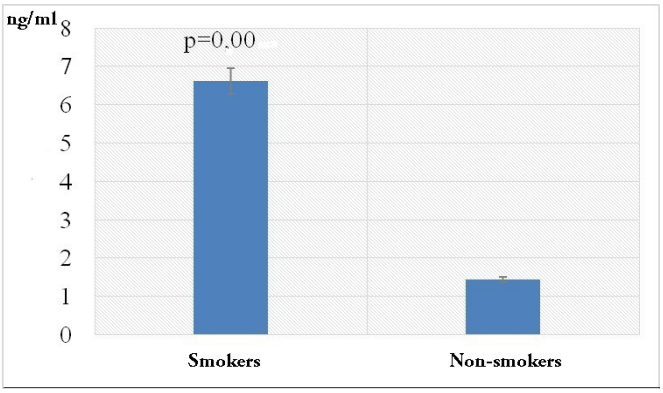


Fig.3. *The PSA level in the blood serum of smokers and non-smokers*

Multivariate linear regression analysis in the study by J.Li[19] showed that total PSA was 7.9% and 12.2% lower among current and former smokers, respectively, than among never smokers. In the study by G.Kog et al., [20] the PSA level was higher in smokers compared to nonsmokers, although it was not statistically significant.

In the circumstance of severe climatic conditions, 155 respondents had a sedentary lifestyle for a longer time during the year, and only 49 engaged in physical culture and sports. The levels of PSA, SA125 and AFP in elderly people not engaged in physical culture were 3.55 (1.98±0.21 ng/ml), 1.5 (5.98±0.71 ng/ml) and 4 (7.10±0.51 IU/ml) times higher, respectively, than in elderly people engaged in physical activity (PSA – 0.56±0.50 ng/ml, CA125 – 4.02±0.41 ng/ml, AFP – 1.77±0.39 IU/ml).. Thus, regular physical activity may be an important factor in stabilizing the level of the investigated TMs.

Consumption of unbalanced, high-calorific food by elderly people and insignificant physical activity in wintertime (6-7 months a year) promotes metabolic disorders with increasing BMI. We found a normal BMI in 77 cases, overweight in 65, and obesity in 62 cases. The AFP level depended on BMI. With BMI<25 kg/m^2, the AFP level was 3.16±0.50 IU/ml. In persons with overweight and obesity, the AFP level was 1.64 and 2.12 times higher than in persons with normal body weight (5.16±1.03 IU/ml and 6.68±0.62 IU/ml vs. 3.16±0.50 IU/ml, *P*<0.05). Therefore, obesity had a great influence on the AFP level. Y. Chen et al. [9] found a significant association between alpha-fetoprotein and metabolic syndrome in a Chinese asymptomatic population. Authors proposed that oxidative stress and oval cell proliferation were responsible for the elevation of serum AFP levels in patients with metabolic syndrome.

The serum levels of CA125 and PSA were not associated with BMI (r=0.017 and r=0.121, respectively). Despite the lack of reliable links between PSA and BMI in our study, there is evidence in the literature that obese males have a lower PSA level in blood serum.[19,21]

Thus, among elderly people of RS(Y), the serum level of AFP was at higher levels within the reference range in single working people and people having a low level of education and/or obesity. The serum level of CA125 in women increased depending on ML, discomfort of housing, and low level of material well-being. The serum level of PSA was at higher levels within the reference range in male smokers.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

The reported study was funded by RFBR, research project # 19-09-00361. The authors would like to acknowledge the financial support provided by the Ministry of Education and Science of Russia (0794-2017 0019 FSRG-2017-0019).

References

1. Liu F, Du F, Chen X. Multiple tumor marker protein chip detection system in diagnosis of pancreatic cancer. World J Surg Oncol. 2014;12:333. doi: 10.1186/1477-7819-12-333.
2. Gion M, Cardinali G, Trevisiol C, Zappa M, Rainato G, Fabricio ASC. Indicators of inappropriate tumour marker use through the mining of electronic health records. J Eval Clin Pract. 2017;23(4):895-902. doi: 10.1111/jep.12754.
3. Kouris NT, Zacharos ID, Kontogianni DD, Goranitou GS, Sifaki MD, Grassos HE, et al. The significance of CA 125 levels in patients with chronic congestive heart failure. Correlation with clinical and echocardiographic parameters.

Eur J Heart Fail. 2005;7(2):199–203.
4. Varol E, Ozaydin M, Dogan A, Kosar F. Tumour marker levels in patients with chronic heart failure. Eur J Heart Fail. 2005;7(5):840–3.
5. Zhong YC, Zhou XF, Hou CM, Li WP. Effect of danefukang on symptoms and biomarkers in women with endometriosis. Taiwan J Obstet Gynecol. 2019;58(2):218-222. doi: 10.1016/j.tjog.2019.01.009.
6. Kim JH, Park BR, Yang WJ. Dilution effect of serum CA125 and CA19-9 over a cutoff value, according to obesity. Int J Biol Markers. 2015;30(1):e122-6. doi: 10.5301/jbm.5000110
7. Karakoyun I, Duman C, Demet Arslan F, Baysoy A, Isbilen Basok B. Vitamin B12 and folic acid associated megaloblastic anemia: Could it mislead the diagnosis of breast cancer? Int J Vitam Nutr Res. 2019;11:1-6. doi: 10.1024/0300-9831/a000555.
8. Harada T, Shigeta K, Noda K, Fukumoto Y, Nishimura H, Mizuta M, Takemoto T. Clinical implications of alpha-fetoprotein in liver cirrhosis: five-year follow-up study. Hepatogastroenterology. 1980;27(3):169-75.
9. Chen Y, Zhao Y, Feng L, Zhang J, Zhang J, Feng G. Association between alpha-fetoprotein and metabolic syndrome in a Chinese asymptomatic population: a cross-sectional study. Lipids Health Dis. 2016;15:85. doi:10.1186/s12944-016-0256-x.
10. Nagpal M, Singh S, Singh P, Chauhan P, Zaidi MA. Tumor markers: A diagnostic tool. Natl J Maxillofac Surg. 2016;7(1):17–20. doi:10.4103/0975-5950.196135.
11. Yang N, Li Z, Yan M, Xiao W, Zhang W, Long Y, Cheng Y, Ming K, Xu B. Evaluation of Serum Alpha-Fetoprotein Level in Chronic Hepatitis C Patients. Clin Lab. 2019;65(1). doi: 10.7754/Clin.Lab.2018.180607.
12. He Y, Lu H, Zhang L Serum AFP levels in patients suffering from 47 different types of cancers and noncancer diseases. Prog Mol Biol Transl Sci. 2019;162:199-212. doi: 10.1016/bs.pmbts.2019.01.001.
13. Lopez LA, Villar VD, Ulla M, Fernandez F, Fernandez LA, Santos I, Rababdan L, Gutierrez M. Prevalence of abnormal levels of serum tumour markers in elderly people. Age Ageing. 1996;25(1):45-50.
14. Efremova SD, Golderova AS, Krivoshapkina ZN, Okhlopkova ED, Nikolaev VM, Tsypandina EV, et al. The water hardness and its relationships with the level of tumor markers among the inhabitants of Yakutia. Yakut Medical Journal. 2018;3(63):37-39.
15. Park SY, Haiman CA, Cheng I, Park SL, Wilkens LR, Kolonel LN, et al. Racial/ethnic differences in lifestyle-related factors and prostate cancer risk: the Multiethnic Cohort Study. Cancer Causes Control. 2015;26(10):1507–15. doi:10.1007/s10552-015-0644-y
16. Diamandis EP, Yu H. New biological functions of prostate-specific antigen? J Clin Endocrinol Metab. 1995;80(5):1515-7.
17. Yumuk V, Tsigos C, Fried M, Schindler K, Busetto L, Micic D, Toplak H; Obesity Management Task Force of the European Association for the Study of Obesity. European Guidelines for Obesity Management in Adults. Obes Facts. 2015;8(6):402-24. doi: 10.1159/000442721.
18. Adamowicz K, Zalewska M, Majkowicz M, Zaucha JM. Evaluation of the Impact of Different Types of Health Education on the Adoption and Preservation of Prohealth Attitudes in Preventing Cancer in Juveniles Younger than 18 Years. J Cancer Educ. 2014;30(3):432–8. doi:10.1007/s13187-014-0730-y.
19. Li J, Thompson T, Joseph DA, Master VA. Association between smoking status, and free, total and percent free prostate specific antigen. J Urol. 2012;187(4):1228–33. doi:10.1016/j.juro.2011.11.086.
20. Koc G, Akgul K, Yilmaz Y, Dirik A, Un S. The effects of cigarette smoking on prostate-specific antigen in two different age groups. Can Urol Assoc J. 2013;7(11-12):E704–E707. doi:10.5489/cuaj.358
21. Zhang J, Ma M, Nan X, Sheng B. Obesity inversely correlates with prostate-specific antigen levels in a population with normal screening results of prostate cancer in northwestern China. Braz J Med Biol Res. 2016;49(8). pii: S0100-879X2016000800704. doi: 10.1590/1414-431X20165272.

International Journal of Biomedicine 9(2) (2019) 176-178
http://dx.doi.org/10.21103/Article9(2)_CR1

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

A Case of Lung Cancer with a Patient with Situs Inversus Totalis

Alexei L. Charyshkin, PhD, ScD[1*]; Eugene A. Toneev[1,2]; Alexander A. Martynov[2]; Dmitrij V. Bazarov, PhD[3]; Yuliya A. Dergunova[1]; Oleg V. Midlenko, PhD, ScD[1]; Antonina V. Smolkina, PhD, ScD[1]; Nikolai I. Belonogov, PhD, ScD[1]

[1]Institute of Medicine, Ecology and Physical Education of Ulyanovsk State University, Russia
[2]Ulyanovsk Regional Oncology Center, Russia
[3]Petrovsky Russian Research Center for Surgery, Russia

Abstract

In the current study, we describe a clinical case of the right upper lobectomy at Situs inversus totalis (SIT). Our report is the first described case in the domestic literature of SIT in a patient with multiple primary metachronous cancer, when a patient with SIT had primary lung cancer and successful surgical treatment. SIT is a complex and extremely rare clinical situation for the surgeon. In all such patients, it is advisable to use all available methods of imaging and diagnosis in the preoperative period, to clarify the anatomical structure of the vascular bed and bronchial tree. (**International Journal of Biomedicine. 2019;9(2):176-178.**)

Key Words: situs inversus totalis • lung cancer • right upper lobectomy

Introduction

Situs inversus totalis (SIT)—a completely reversed location of the internal organs—is a rare congenital autosomal recessive disorder that is associated with a defect on the X chromosome. The frequency of this pathology ranges from 1:8000 to 1:20,000.[1] With this defect, an abnormal rotation of the heart tube occurs during embryogenesis. In 20% of cases, transposition is associated with the Kartagener syndrome, which includes a classical triad: situs inversus, sinusitis and bronchiectasis.[2] Most people do not know about their peculiarities until they start to undergo various types of surveys.[3] In the absence of functional disorders of the cardiovascular system, SIT can be attributed to normal variant anatomy, which does not have a significant impact on the quality of life. There are two main types of situs inversus. Dextrocardia with the normal position of the abdominal organs was first described in 1643 by Marco Severino.[4] SIT was described a century later, in 1797, by the British pathologist Matthew Baillie.[5] There is also a variant with the position of the heart in the left half of the chest (levocardia) with transposition of the abdominal cavity, this phenomenon occurs in approximately 1:22,000 cases, and is called incomplete situs inversus.[6] With the transposition of the internal organs with levocardia, heart defects are observed in 95% of cases.[7]

Transposition of the internal organs with levocardia, or dextrocardia without transposition, are much more dangerous congenital defects than SIT. Dextrocardia (with the normal arrangement of the internal organs) is also found in some people with Patau syndrome (Trisomy 13).[8]

In the domestic literature, we did not find publications on anatomical resection of the lung in SIT patients. Considering all of the above, we considered it necessary to provide a description of the clinical case of the right upper lobectomy at SIT.

Case presentation

A 70-year-old man was admitted to the surgical thoracic department of our Clinical Hospital, complaining of a rare cough. The patient's medical history showed that in 2010, a total hemithyroidectomy was performed for a malignant disease of the thyroid gland. During the dynamic examination in December 2017, the progression of the process was revealed—cervical lymph node metastases, a tumor in the upper lobe of the right lung (Fig. 1-3).

In January 2018, a total thyroidectomy with cervical lymphadenectomy was performed. In a remote preparation,

***Corresponding author**: Prof. Alexei L. Charyshkin, PhD, ScD, Head of the Faculty Surgery Department, Institute of Medicine, Ecology and Physical Education, Ulyanovsk State University. Ulyanovsk, the Russian Federation. E-mail: charyshkin@yandex.ru*

MTS of thyroid cancer were found. On 17 April 2018, radioiodine treatment was performed and a thoracic surgeon was recommended for radical treatment of the right lung tumor.

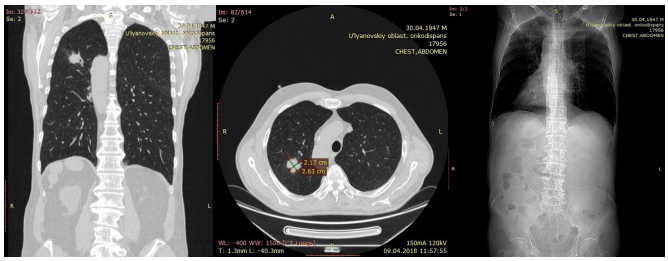


Fig. 1-3. *MSCT: a tumor in the upper lobe of the right lung.*

After the preoperative additional examination, the planned operation (05.31.2018) was performed: Right thoracotomy, upper lobectomy, systemic lymph node dissection (Fig.4). Intraoperatively: the right-sided position of the heart and aorta. In the upper lobe of the right lung, a formation with a contraction of the pleura was detected (3×3 cm in size), mediastinal lymph nodes were not enlarged. A standard upper lobectomy with systemic lymph node dissection was performed. The postoperative period was uneventful, the wound healed by first intention. Drains were removed on the third day. The duration of the operation was 96 minutes, the blood loss was minimal.

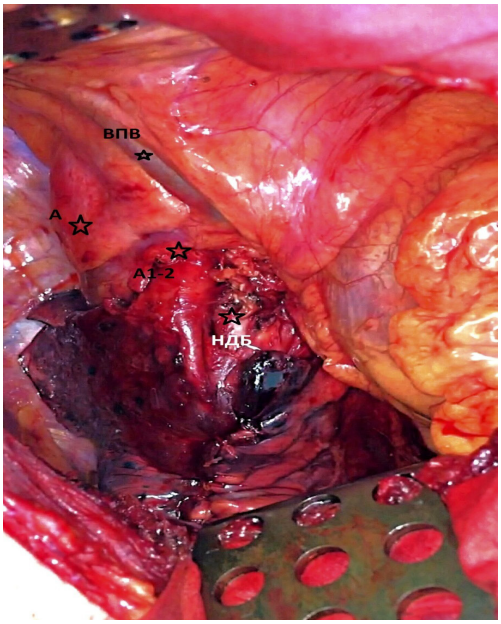


Fig. 4. *Intraoperatively photo (A - aorta, A1-2 – segmental artery, НДБ- lower lobe bronchus).*

Histological examination: papillary cancer (morphology characteristic of thyroid cancer and primary lung adenocarcinoma). All groups of lymph nodes of the lung root and mediastinum were without a tumor lesion.

Immunohistochemical examination: TTF1-positive reaction; CDX2, Tireoglobulin, CK20-negative reaction. Given the immunophenotype and morphology, more data are available for primary papillary adenocarcinoma of the lung (pT1aN0R0) (Figure 5-7.)

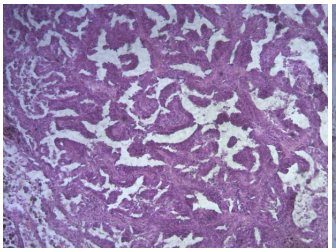


Fig. 5. *Primary papillary adenocarcinoma. H&E, ×100*

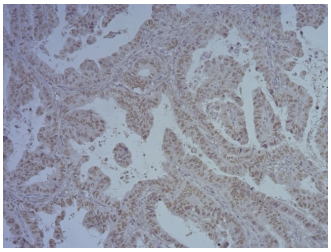


Fig. 6. *Immunohistochemistry: TTF1-positive reaction, ×200*

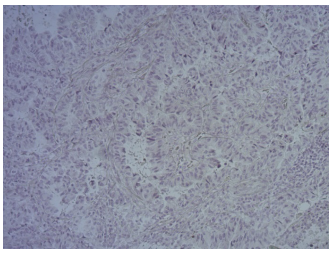


Fig. 7. *Immunohistochemistry: TgAb-negative result, ×200*

Thus, the clinical diagnosis: Multiple primary metachronous cancer.

1. Thyroid cancer (pT2N0M0), operation 2010. Progression of 12.2017—MTS to cervical lymph nodes, operation in 01.2018.
2. Lung cancer of the right upper lobe (pT1aN0M0).

Discussion

Most of the published papers on surgical intervention in patients with SIT are presented mainly about the abdominal organs (cholecystectomy, appendectomy).[9] There are isolated reports of SIT cases during operations on the colon,[10] stomach,[11] esophagus [12] and the heart;[13] there is a publication about lung transplantation.[14] Currently, the presence of a solitary neoplasm in the lung is an indication for its anatomical resection. It should be borne in mind that the presence of SIT does not guarantee the standard vascular and bronchial anatomy.[15] When planning an operation in patients with SIT, the available vascular or bronchial anatomical features should always be taken into account at the preoperative stage, which promotes a safe surgery. In our case, the preoperative MSCT showed the presence of two main arterial trunks going to the upper lobe: A1-2 and A3,4,5. Endoscopic ultrasonography (EUS) and endobronchial ultrasonography (EBUS) can be used as additional methods for specifying diagnosis.[16]

Subotich et al. considered that preoperative angiography should be performed on all patients with SIT to study the vascular anatomy of the lung, due to the high possibility of the presence of vascular anomalies.[17] We believe that a 3D diagnostic CT and MRI will be useful in a diagnostic search.

In the present case, MSCT was sufficient to obtain a complete picture of the vascular bed and the bronchial tree.

Our report is the first described case in the domestic literature of SIT in a patient with multiple primary metachronous cancer and is the 29th case in the available literature since 1952, when a patient with SIT had primary lung cancer and successful surgical treatment.

SIT is a complex and extremely rare clinical situation for the surgeon. It is necessary to know the patient's exact topographic anatomy, which will avoid intraoperative complications. In all such patients, it is advisable to use all available methods of imaging and diagnosis in the preoperative period, to clarify the anatomical structure of the vascular bed and bronchial tree.

Competing Interests

The authors declare that they have no competing interests.

References

1. Wójcik J, Grodzki T, Bielewicz M, Wojtyś M, Kubisa B, Pieróg J, Wójcik N. Lung cancer in situs inversus totalis (SIT)--literature review. Adv Med Sci. 2013;58(1):1-8. doi: 10.2478/v10039-012-0083-x.
2. Reimer TV, Mursalimova VF, Meteleva AV. [Family case of Kartagener syndrome]. Vestnik Orenburg State University. 2011;16(135):331-2. [Article in Russian].
3. Bondarenko IA, Chesnakova TV. [Situs Viscerus Inversus Totalis (Clinical Case)]. Radiology — Practice. 2014;(2):57-63. [Article in Russian].
4. Maria Conforti. Surgery, medicine and natural philosophy in the library of Marco Aurelio Severino (1580-1956). Bruniana & Campanelliana.2004;10(2):283-298.
5. Kaleda VI, Glyantsev SP. [Transposition of the great arteries: The first description by M. Baillie in 1797]. Heart and Vessels Diseases in Children. 2010;(4): 51-55. [Article in Russian].
6. Wang X, Shi Y, Zeng S, Zhou J, Zhou J, Yuan H, et al. Comparing levocardia and dextrocardia in fetuses with heterotaxy syndrome: prenatal features, clinical significance and outcomes. BMC Pregnancy Childbirth. 2017;17(1):393. doi: 10.1186/s12884-017-1579-y.
7. Del Prete A, Cavaliere C, Di Pietto F, De Ritis R. Situs inversus with levocardia, infrahepatic interruption of the inferior vena cava, and azygos continuation: a case report. Surg Radiol Anat. 2016;38(3):369-71. doi: 10.1007/s00276-015-1454-3.
8. Gainazarova AS, Ibragimova TM, Kaliev RR. [Clinical case of a combination of dextrocardia and cardiac arrhythmias]. Bulletin of KSM A named after I.K. Akhunbaev. 2016;(4):35-37. [Article in Russian].
9. Tacchino R, Greco F, Matera D. Single-incision laparoscopic cholecystectomy: surgery without a visible scar. Surg Endosc. 2009;23(4):896-9. doi: 10.1007/s00464-008-0147-y.
10. Kim HJ, Choi GS, Park JS, Lim KH, Jang YS, Park SY, Jun SH. Laparoscopic right hemicolectomy with D3 lymph node dissection for a patient with situs inversus totalis: report of a case. Surg Today. 2011;41(11):1538-42. doi: 10.1007/s00595-010-4530-7.
11. Dibirov MD, Fomin VS. [Laparoscopy-assisted gastrectomy for gastric carcinoma in a patient with complete situs inversus.]. Moscow Surgical Journal. 2017;53(1):34-37. [Article in Russian].
12. Yoshida T, Usui S, Inoue H, Kudo SE. The management of esophageal cancer with situs inversus totalis by simultaneous hand-assisted laparoscopic gastric mobilization and thoracoscopic esophagectomy. J Laparoendosc Adv Surg Tech A. 2004;14(6):384-9.
13. Iino K, Watanabe G, Ishikawa N, Tomita S. Total endoscopic robotic atrial septal defect repair in a patient with dextrocardia and situs inversus totalis. Interact Cardiovasc Thorac Surg. 2012;14(4):476-7. doi: 10.1093/icvts/ivr103.
14. Macchiarini P, Chapelier A, Vouhé P, Cerrina J, Ladurie FL, Parquin F, et al. Double lung transplantation in situs inversus with Kartagener's syndrome. Paris-Sud University Lung Transplant Group. J Thorac Cardiovasc Surg. 1994;108(1):86-91.
15. Safdie FM, Sanchez MV, Sarkaria IS. Prevention and management of intraoperative crisis in VATS and open chest surgery: how to avoid emergency conversion. J Vis Surg. 2017;3:87. doi: 10.21037/jovs.2017.05.02.
16. Yoshida M, Hino H, Machida H, Hatakeyama N, Okano Y, Iwahara Y, et al. Video-assisted thoracic surgery lobectomy for lung cancer in a patient with complete situs inversus. Gen Thorac Cardiovasc Surg. 2013;61(3):155-9. doi: 10.1007/s11748-012-0114-3.
17. Subotich D, Mandarich D, Katchar V, Bulajich B, Drndarski B. Lung resection for primary bronchial carcinoma in a patient with complete situs inversus. Clin Anat. 2006;19(4):358-62.

International Journal of Biomedicine 9(2) (2019) 179-181
http://dx.doi.org/10.21103/Article9(2)_ShC1

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

Electrophysiological Effects of a New Antiarrhythmic Drug Aksaritmin

Ravshanbek D. Kurbanov, PhD, ScD; Nodir U. Zakirov, PhD, ScD; Oybek S. Salaev*

Republican Specialized Center of Cardiology, Tashkent, Uzbekistan

Abstract

The purpose of this report was to study the pharmacodynamics of aksaritmin and its effect on cardiac electrophysiological parameters in patients with various heart arrhythmias.

Materials and Methods: Fifty-one patients with various heart arrhythmias aged between 18 and 60 years (mean age of 38.4±11.6 years) were examined. The effect of aksaritmin on cardiac electrophysiological parameters was studied using 12 lead ECG, transesophageal electrophysiological study (TES) and intracardiac electrophysiological study (EPS). Effects of aksaritmin (25-50 mg per os) were studied in an acute drug test (ADT) (3 hours after the start of testing) and during the course of treatment (on the fifth day). TES was performed on patients with paroxysmal tachycardias. Aksaritmin was used once at a dose of 50 mg per os, and all indicators were measured 3 hours after patients took the drug. In TES, we studied the sinus-node recovery time (SRT), the Wenckebach point, ERP of the atrioventricular node (ERP-AVN) and accessory atrioventricular connection (ERP-AAVC). The effect of the drug in intracardiac EPS was studied 3 hours after patients were given a single dose of aksaritmin (50 mg) per os. All parameters were measured according to the standard EP protocol.

Results: The action of aksaritmin begins 45-60 minutes after the drug is taken and reaches a maximum after 3-4 hours; effects last an average of 8 hours, which allows one to prescribe aksaritmin 3 times a day. The drug in ADT and during the course of treatment increases HR by 8.1% and 4.9%, respectively. Aksaritmin slows down the conduction of impulses via the atria, AV node and His-Purkinje system, and does not affect ventricular ERP. Accordingly, on ECG, the duration of PQ interval and QRS complex is significantly longer, while the duration of the QTc interval does not change. Aksaritmin prolongs ERP-AVN in the retrograde direction by 8.1% and completely blocks anterograde conduction via the AAV pathway in WPW patients. (**International Journal of Biomedicine. 2019;9(2):179-181.**)

Key Words: aksaritmin • pharmacodynamics • electrophysiological parameters • effective refractory period

Abbreviations

AAD, antiarrhythmic drugs; **AAE**, antiarrhythmic effect; **ADT**, acute drug test; **ERP**, effective refractory period; **EPP**, electrophysiological parameters; **EPS**, electrophysiological study; **HR**, heart rate; **PVCs**, premature ventricular contractions; **PSVCs**, premature supraventricular contractions; **SE**, side effects; **TES,** transesophageal electrophysiological study; **WPW**, Wolff-Parkinson-White syndrome; **WP**, Wenckebach point.

Introduction

As is known, the algorithm for prescribing AAD is primarily based on the safety of the drug in a particular heart disease, and then the effectiveness of the drug in a given heart arrhythmia.[1] Unfortunately, any AAD has side effects that also reduce the patient's quality of life. In this regard, it seems relevant to create new AAD, which not only eliminate the arrhythmia itself, but also have minimal SE. One of the drugs [2,3] successfully used in patients with heart arrhythmias, without or with minimal organic heart disease, is allapinin. However, many studies have identified dose-dependent central nervous system side effects (dizziness, pressure in the head, diplopia) of allapinin (from 18% to 65%), which limit its scope and require discontinuation of the drug in up to 10% of cases.[4]

***Corresponding author:** Dr. Oybek Salaev. Republican Specialized Center of Cardiology, Tashkent, Uzbekistan. E-mail: os.salaev@gmail.com*

The researchers at the Institute of the Chemistry of Plant Substances (ICPS) of the Academy of Sciences of the Republic of Uzbekistan have created a new AAD—aksaritmin. Aksaritmin, as well as allapinin, is obtained from the roots of Aconitum septentrionale. The technology of its production is simpler, and the economic cost is 1.5-2 times lower than that of allapinin. On the basis of IASD, experimental studies on various laboratory animals were conducted, which showed high efficacy and safety of the drug in reproducible ventricular and supraventricular arrhythmias.[5,6] The Republican Specialized Center of Cardiology continues to study the clinical and electrophysiological properties of aksaritmin in patients with various heart arrhythmias. The purpose of this report was to study the pharmacodynamics of aksaritmin and its effect on cardiac EPP in patients with various heart arrhythmias.

Materials and Methods

Fifty-one patients with various heart arrhythmias aged between 18 and 60 years (mean age of 38.4±11.6 years) were examined. Of 8 patients with WPW syndrome, 4 were diagnosed with a manifest form, 2 had an intermittent form, and 2 patients had a latent form of WPW syndrome, which was diagnosed during TES. An intracardiac EPS was performed on 10 patients. Inclusion criteria were frequent PVCs and/or PSVCs, including high gradations, frequent paroxysmal supraventricular tachycardia in patients with the absence or minimal manifestations of organic heart disease. The exclusion criteria were age of patients <18 years and >60 years, acute myocardial infarction (MI), MI history, unstable angina, NYHA III-IV with LVEF<50%, wall thickness <14mm, sick sinus syndrome, second- or third-degree AV block, hepatic and renal failure and other comorbidities in the decompensated stage, pregnancy and lactation, taking other AAD.

The study protocol was reviewed and approved by the Ethics Committee of the Republican Specialized Centre of Cardiology. All participants provided the written informed consent.

The effect of aksaritmin on cardiac EPP was studied using 12 lead ECG, TES and intracardiac EPS (n=10).

Effects of aksaritmin (25-50 mg per os) using 12 lead ECG were studied in an ADT (3 hours after the start of testing) and during the course of treatment (on the fifth day).

TES was performed on patients with paroxysmal tachycardias. Aksaritmin was used once at a dose of 50 mg per os, and all indicators were measured 3 hours after patients took the drug. In TES, we studied the sinus-node recovery time (SRT), WP, the ERP of the atrioventricular node (ERP-AVN), and the ERP of accessory atrioventricular connection (ERP-AAVC).

The effect of the drug in intracardiac EPS was studied 3 hours after patients were given a single dose of aksaritmin (50 mg) per os. All parameters were measured according to the standard EP protocol with assessment by such indicators as P-wave duration, RR, PQ, QRS, QT, QTc, PA, AH, HV, spike H duration, anterograde and retrograde WP (AWB/RWB), anterograde and retrograde ERPs of the atrioventricular node (AERP-AVN/RERP-AVN), ERP of the right ventricle (ERP-RV), and the right and left atrial ERPs (ERP-RA, ERP-LA).

The statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA).

Results and Discussion

The pharmacodynamics of aksaritmin was studied in 51 patients of both sexes with consistently frequent PVCs or PSVCs. Of the 8 patients taking aksaritmin at a dose of 12.5 mg, only one patient experienced a positive AAE with suppression of PVCs by more than 90%. At the same time, AAE of aksaritmin began after 60 minutes and lasted up to 8 hours.

When prescribing aksaritmin at a dose of 25 mg, 10(43.5%) of the 23 patients achieved AAE, which consisted in a decrease in the number of PVCs/PSVCs by an average of 80% for more than 8 hours, while in 5(21.7%) patients there was complete suppression of PVCs/PSVCs. When prescribing aksaritmin at a dose of 50 mg, positive AAE was observed in 15(75%) of 20 patients, with complete suppression of arrhythmias observed in 5(25%) patients. In another 10(50%) patients, the number of PVCs/PSVCs decreased by more than 70%, but in one patient, the effect was short-lived (5 hours).

In general, AAE of aksaritmin lasted from 5 to 12 hours (average of 8.1±1.2 h), which allows us to conclude that the drug can be administered 3 times a day and in some patients, 4 times a day.

ECG data

In ADT and course of treatment, aksaritmin increased HR by 8.1% (P<0.05) and 4.9% (P>0.05), respectively. Aksaritmin in ADT significantly increased the PQ interval by 9.4% (P<0.05), while during the course of treatment, this indicator increased slightly less—by 7.3% (P<0.05). The width of the QRS complex significantly increased by 10.8% (P<0.05) in ADT and by 7.8% (P<0.05) on the fifth day of the course of treatment. Changes in the QTc interval while patients were taking the drug were statistically insignificant.

TES data

EPP were studied in patients with WPW syndrome with atrioventricular reciprocating tachycardia (AVRT) and orthodromic atrioventricular reciprocating tachycardia (OAVRT). Studies were conducted before and 5 days after drug taking. In this report, we present only the effect of the drug on the heart ERP.

We found that aksaritmin shortened the duration of the cardiac cycle from 747 msec to 693 msec (7.2%). In patients with normal sinus-node function, aksaritmin reliably shortened the duration of the cardiac cycle, and at the same time, it practically did not change SRT. Aksaritmin did not affect such an indicator as WP, while it insignificantly shortened ERP-AAVC by 5% (from 321±44 msec to 305±42 msec). In patients with a latent form of WPW syndrome with OAVRT (n=2) and typical AVRT (n=11), in the absence of a significant effect on anterograde AV conduction, the drug prevented paroxysm induction in 7 patients with AVRT, which indicates blocking of the retrograde knee of the re-entry circle (conduction via the accessory pathway or via the fast AV nodal pathway).

In patients with induced tachycardia with repeated TES, the drug lengthened the VA interval by 9.4% and AV by 10.4%, both in AVRT and orthodromic tachycardia, which indicates that the impulse is slowed down as retrograde (the fast AV nodal pathway and accessory pathway) and antegrade (via AV connection). Due to the slowing down of the impulse in inducing persistent paroxysmal tachycardia, the A-A interval was significantly longer, by 6.6%, which indicated a decrease in the frequency of tachycardia, despite the fact that the drug was not effective in preventing a recurrence of tachycardia.

It should be noted that in two patients with an intermittent form of WPW syndrome on the daily ESG monitoring and frequent atrial stimulation, there were no signs of pre-excitation, i.e. aksaritmin also completely blocked anterograde conduction via the AAV pathway.

Intracardiac EPS data

It was established that aksaritmin in a dose of 50 mg slowed down the rate of impulses in various parts of the cardiac conduction system. The speed of the pulses in the atria (RA interval) slowed down from 22.9 msec to 27.7 msec (P <0.05). The AV nodal conduction time (AH interval) was also lengthened reliably by 8.2%. The His-Purkinje system conduction time slowed down significantly by 21.8%. As a result, the PQ interval was prolonged from 144 msec to 160.5 msec (P<0.05) and the QRS duration from 80.7 msec to 87.4 msec (P<0.05).

The duration of ERP-AVN was insignificantly reduced in the anterograde direction, while in the retrograde direction ERP-AVN was prolonged by 8.1% (P<0.05). The drug significantly reduced the duration of the refractory period of the His-Purkinje system, but practically did not change ERP-RV. On the ECG, respectively, the duration of the QTc interval was practically unchanged.

The effect of aksaritmin on atrial ERP in the studied patients was non-unidirectional. Thus, ERP-RA increased in 2 patients, shortened in one patient, and in 7 patients remained unchanged.

ERP-LA was shortened in 5 patients, lengthened in 3 patients and did not change in 2 patients. At the same time, the average indices of atrial ERP were not statistically significantly changed.

It should be emphasized that in our study, we measured the ERP-RA only in the upper section, which may explain the results obtained by the different directions of the drug's action. It should also be noted that the drug was studied in a small amount—only in 10 patients—which also plays an important role in the statistical evaluation of research results.

On the whole, according to 12 lead ECG and EPS data, the effects of aksaritmin coincide with the effects of AAD class IC (the Vaughan Williams classification). They are characterized by a pronounced negative effect on the rate of impulses in the atrial and ventricular myocardium, as well as in the specialized intraventricular conduction system of the heart. At the same time, the duration of refractory periods varies slightly.

Conclusions:

- The action of aksaritmin begins 45-60 minutes after the drug is taken and reaches a maximum after 3-4 hours; effects last an average of 8 hours, which allows one to prescribe aksaritmin 3 times a day.
- Aksaritmin in ADT and during the course of treatment increases HR by 8.1% and 4.9%, respectively.
- Aksaritmin slows down the conduction of impulses via the atria, AV node and His-Purkinje system, and does not affect ventricular ERP. Accordingly, on ECG, the duration of PQ interval and QRS complex is significantly longer, while the duration of the QTc interval does not change.
- Aksaritmin prolongs ERP-AVN in the retrograde direction by 8.1% and completely blocks anterograde conduction via the AAV pathway in WPW patients.

Competing Interests

The authors declare that they have no competing interests.

References

1. Wann LS, Curtis AB, January CT, Ellenbogen KA, Lowe JE, Estes NA 3rd; 2006 Writing Committee Members, Jacobs AK, Anderson JL, Albert N, Hochman JS, Buller CE, Kushner FG, et al.; ACCF/AHA Task Force Members. ACCF/AHA/HRS focused update on the management of patients with atrial fibrillation (updating the 2006 Guideline); a report of the American College of Cardiology Foundation/ American Heart Association Task Force on Practice Guidelines. Circulation. 2011;123(1):104-23. doi: 10.1161/CIR.0b013e3181fa3cf4.
2. Sokolov SF, Bakalov SA, Mironova NA, Rogova MM, Malkina TA, Golitsyn SP. [Efficiency and safety of allapinin in short- and long-term treatment of patients with normal heart and ventricular premature beats]. Kardiologiia. 2014;54(1):20-6.[Article in Russian].
3. Melnik MG. Restoration and maintenance of sinus rhythm with combinations of antiarrhythmic drugs in patients with atrial fibrillation. Abstract of PhD Thesis. Krasnodar; 2006. [In Russian].
4. Abdalla A, Mazur NA, Fofanova TV. [Pharmacodynamics of allapinin and its possible adverse effects]. Kardiologiia. 1989;29(7):29-32. [Article in Russian].
5. Jahangirov FN, Kasimov KR. [Preclinical toxicological study of the amount of alkaloids from Aconitum Septentrionale (Axarmin)]. Infektsiia, Immunitet, Farmakologiia. 2009;(1):26-30. [Article in Russian].
6. Jahangirov FN, Kasimov KR. [The study of acute toxicity and antiarrhythmic activity of the amount of alkaloids from Aconitum septentrionale (Axarmin)]. Farmatsevticheskii Vestnik Uzbekistana. 2009;(2):67-70. [Article in Russian].

International Journal of Biomedicine 9(2) (2019) 182-184
http://dx.doi.org/10.21103/Article9(2)_ShC2

SHORT
COMMUNICATION

INTERNATIONAL
JOURNAL
OF BIOMEDICINE

Computed Tomography Imaging of Degenerative Disease in the Lumbar Spine

V. A. Malakhanov, PhD[1,2,3]; P. V. Seliverstov, PhD, ScD[1*]; Ya. A. Lubashev, PhD, ScD[4]; V. A. Ratnikov, PhD, ScD[5]

[1]Irkutsk Scientific Center of Surgery and Traumatology, Russia
[2]Irkutsk Municipal Clinical Hospital №1, Russia
[3]Road Clinical Hospital at Irkutsk-Passenger Station of JSCo "RZD", Russia
[4]Branch Clinical Diagnostic Center of PJSC Gazprom, Moscow, Russia
[5]Clinical Hospital № 122 named after L.G. Sokolov under the Federal Biomedical Agency of the Russian Federation, St. Petersburg, Russia

Abstract

The degenerative spinal lesions are one of the most frequent causes of lumbar pain syndrome. Diagnosing them is difficult due to poor correlation between radiological data and clinical symptoms. Computed tomography is one of the key modalities in the diagnosis of degenerative disc disease. (**International Journal of Biomedicine. 2019;9(2):182-184.**)

Key Words: lumbar spine • degenerative spine • computed tomography • lumbar stenosis.

Introduction

Osteochondrosis is the most commonly encountered form of degenerative spinal lesion with a primary degeneration of nucleus pulposus as a base. Hyaline plates and adjacent areas of vertebral bodies become injured because of lack of nucleus pulposus elasticity, which leads to fissures and ruptures of the hyaline lamellae (Schmorl's nodes) and the annulus fibrosus (disc protrusions and herniations). Other components of the spinal motion segment (vertebral bodies, ligaments, articulations) eventually get involved in this pathological process, and then osteosclerosis develops in adjacent bone parts and the body height decreases. The articular surfaces become displaced, so the subluxation in facet joints gradually appears, and osteoarthrosis develops.[1,2]

Degenerative changes finally lead to spinal stenosis with pain syndrome in its debut. There are central and lateral (foraminal) forms of spinal stenosis: relative – over 12 mm and absolute – under 12 mm. Many researchers describe the incompatibility between the imaging and clinical signs. In cases of relative stenosis there are no clinical findings, but minimal intervertebral protrusion could produce significant pain with myelopathy.[3]

Spinal computed tomography (CT) can provide assessment of details of the configuration of the bone canal's walls; can prove the fact of presence of disc herniation; and can define its dimensions, the diameter of vertebral and foraminal canals.[4] The cause of the lumbar canal's stenosis in most patients with degenerative spinal disease is the segmental degenerative instability related to intervertebral destruction.[5]

Materials and Methods

Fifty patients had lumbar spine CT (Toshiba Aquilion One 64) studies at the Radiology Department of Road Clinical Hospital in the period from August 2018 to February 2019. All patients (mean age of 48±12.0 years), predominantly able-bodied men between 40 and 59 years of age, had chronic lumbar pain and neurogenic intermittent claudication.

***Corresponding author:** Pavel V. Seliverstov, MD. Irkutsk Scientific Center of Surgery and Traumatology. Irkutsk, Russia. E-mail: pavv2001@gmail.com*

Results and Discussion

All 50 patients with neurological symptoms of osteochondrosis had degenerative spinal CT changes (Table 1).

Table 1.

CT signs of osteochondrosis in the lumbar spine

Pathological process	Patients	
	n	%
Spondylosis	38	76
Spondyloarthrosis	33	66
Dystrophic stenosis of vertebral canal	4	8
Spondylolisthesis	3	6
Osteophyte in vertebral canal	5	10
Hypertrophy of facet joint	3	6

CT showed a decrease in the intervertebral disc height, a vacuum phenomenon, a disc protrusion, a lordosis alignment, a vertebral displacement (Fig.1), an ossification of posterior longitudinal ligament (Fig.2), and a subchondral osteosclerosis.

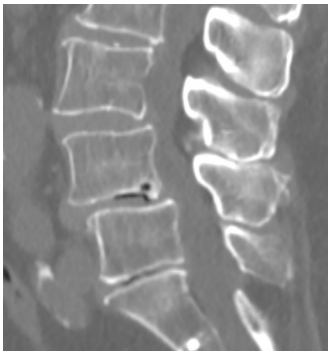


Fig. 1. *CT, sagittal view. Spondylolisthesis at the L3–L4 level, grade 1.*

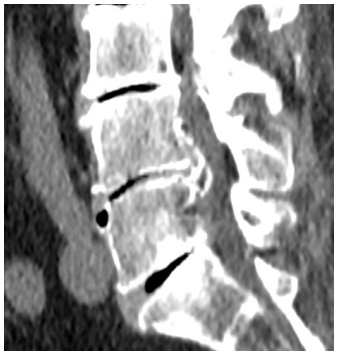


Fig. 2. *CT, sagittal view. Ossification of posterior longitudinal ligament at the L4–L5 level.*

The Schmorl's nodes were visualized in 75% of patients as an intracorporal intrusion of discs with a hyperdense rim. Osteoarthrosis often induces a decrease of lateral recess (average diameter >5 mm). Further progression of degenerative process leads to load increase to facet joints with their deformation. The main radiological signs of spondyloarthrosis were a decrease of the articular space height, subchondral epiphyseal osteosclerosis, formation of osteophytes, the vacuum phenomenon and subchondral cysts (Fig.3).

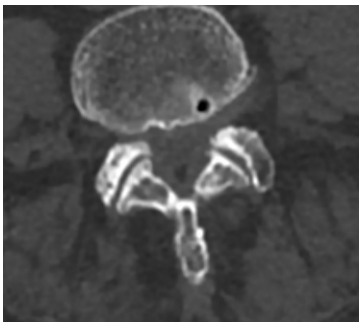


Fig. 3. *CT, axial view. Facet joints arthrosis.*

The herniation level was assessed on the axial and sagittal views; we took such a horizontal disc deformation when the diameter of herniation exceeded its width because CT could not show the continuity of the annulus fibrosus under the herniated disc. The dimensions and the localization of disc herniations are demonstrated (Table 2).

Table 2.

A frequency of different forms of disc herniations in the lumbar spine

The forms of disc herniations	Number (n=20)	
	n	%
Median	3	15
Paramedian	7	35
Posterolateral	4	20
Foraminal	3	15
Sequestrated	3	15

The disc herniations were visualized in 20(40%) patients, 4(8%) of them had 2 herniated discs (Fig.4). The majority of disc herniations were between 5 mm and 8 mm in size (75.5%), of paramedian form (35%), and occurred at L4–L5 and L5–S1 levels (Fig.5). There were sequestrated discs in 3(15%) patients.

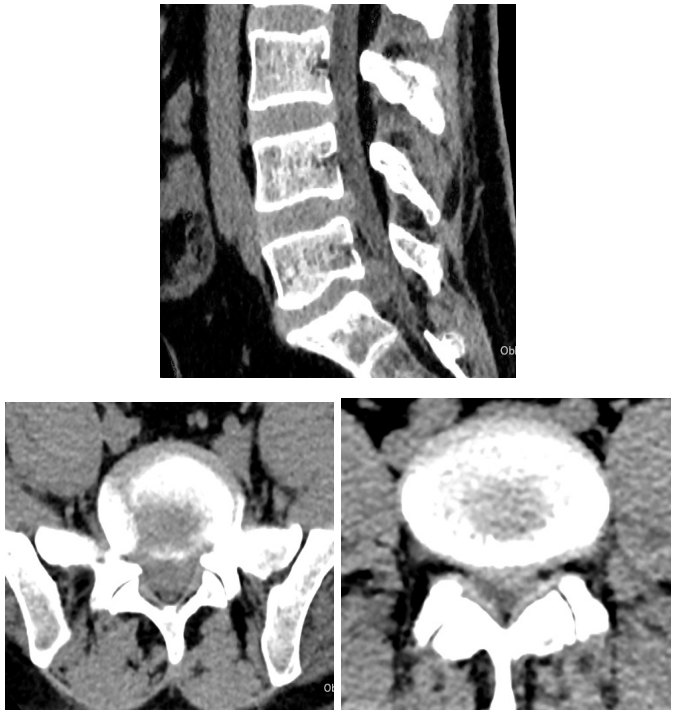


Fig. 4. *CT, sagittal and axial views. The disc herniations at L4–L5, L5–S1, spondylolisthesis L5.*

CT showed stenosis of the spinal canal in 19(38%) patients, 15-18 mm in its sagittal diameter. The causes of spinal stenosis were the posterior and posterolateral osteophytes, spondylarthrosis, spondylolisthesis, ossification of posterior longitudinal ligaments, and hypertrophy of ligamentum flavum (>5 mm).

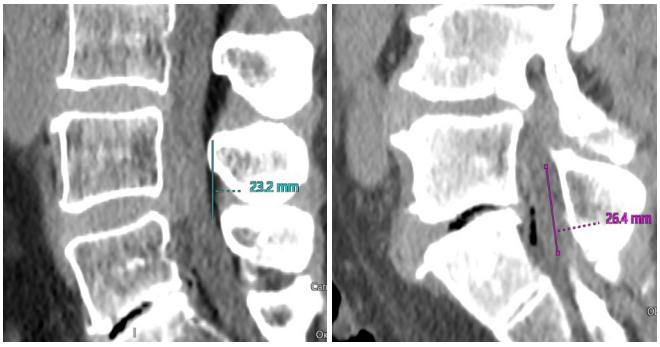


Fig. 5. *CT, sagittal view. The sequestrated disc herniations.*

Conclusion

Thus, degenerative spinal lesions are one of the most frequent causes of lumbar pain syndrome. Diagnosing them is difficult due to poor correlation between radiological data and clinical symptoms. CT is one of the key modalities in the diagnosis of degenerative disc disease; it permits us to visualize the signs of spinal stenosis. Therefore, CT should be performed on patients with chronic lumbar pain and neurogenic intermittent claudication.

Competing Interests

The authors declare that they have no competing interests.

References

1. Boos N, Aebi M. Spinal Disorders Fundamentals of Diagnosis and Treatment. Berlin: Springer Verlag; 2008. doi: 10.1007/978-3-540-69091-7.
2. Kapural L, Kim P, Deer T. Diagnosis, Management, and Treatment of Discogenic Pain. NY; Philadelphia: Elsevier Science; 2011.
3. Nouri A, Tetreault L, Singh A, Karadimas SK, Fehlings MG. Degenerative Cervical Myelopathy: Epidemiology, Genetics, and Pathogenesis. Spine (Phila Pa 1976). 2015;40(12):E675-93. doi: 10.1097/BRS.0000000000000913.
4. Van Goethem JWM, Hauwe L, Parizel PM. Spinal imaging. Diagnostic imaging of the Spine and Spinal Cord. Springer-Verlag, Berlin-Heidelberg; New York; 2007.
5. Hayashi T, Wang JC, Suzuki A. Takahashi S, Scott TP, Phan K, et al. Risk factors for missed dynamic canal stenosis in the cervical spine. Spine (Phila Pa 1976). 2014 ;39(10):812-9. doi: 10.1097/BRS.0000000000000289.

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. International Journal of Biomedicine welcomes submissions of the following types of paper: Original articles, Reviews, Perspectives, Viewpoints, and Case Reports.

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 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. Use terms from the Medical Subject Headings (MeSH) list of Index Medicus when possible.

Original articles

Original articles present the results of original research. These manuscripts should present well-rounded studies reporting innovative advances that further knowledge about a topic of importance to the fields of biology or medicine. These can be submitted as either a full-length article (no more than 6,000 words, 4 figures, 4 tables) or a Short Communication (no more than 2,500 words, 2 figures, 2 tables). An original article may be Randomized Control Trial, Controlled Clinical Trial, Experiment, Survey, and Case-control or Cohort study. Original articles should be presented in the following order: 1) Title page; 2) Abstract with keywords; 3) main text in the IMRaD format (Introduction; Materials and Methods; Results; and Discussion). 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. 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. The IMRaD format does not include a separate Conclusion section. The conclusion is built into the Discussion. More information on the structure and content of these sections can be found in the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (ICMJE Recommendations), available from www.ICMJE.org.
4) Acknowledgements; 5) Conflicts of interest; 6) References; 7) Tables (each table on a separate page, complete with title and footnotes); 8) Figure legends; 9) Figures.

Case Reports

Case reports describe an unusual disease presentation, a new treatment, a new diagnostic method, or a difficult diagnosis. The author must make it clear what the case adds to the field of medicine and include an up-to-date review of all previous cases in the field. These articles should be no more than 5,000 words with no more than 6 figures and 3 tables. Case Reports should consist of the following headings: Abstract (no more than 100 words), Introduction, Case Presentation (clinical presentation, observations, test results, and accompanying figures), Discussion, and Conclusions.

Reviews

Reviews analyze the current state of understanding on a particular subject of research in biology or medicine, the limitations of current knowledge, future directions to be pursued in research, and the overall importance of the topic. Reviews could be non-systematic (narrative) or systematic. Reviews can be submitted as a Mini-Review (no more than 2,500 words, 3 figures, and 1 table) or a long review (no more than 6,000 words, 6 figures, and 3 tables). Reviews should contain four sections: Abstract, Introduction, Topics (with headings and subheadings, and Conclusions and Outlook.

Perspectives

Perspectives are brief, evidenced-based and formally structured essays covering a wide variety of timely topics of relevance to biomedicine. Perspective articles are limited to 2,500 words and usually include ≤10 references, one figure or table. Perspectives contain four sections: Abstract, Introduction, Topics (with headings and subheadings), Conclusions and Outlook.

Viewpoints

Viewpoint articles include academic papers, which address any important topic in biomedicine from a personal perspective than standard academic writing. Maximum length is 1,200 words, ≤70 references, and 1 small table or figure.

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
- ✓ drafting the paper or revising it critically
- ✓ final approval of the paper to be published.

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 follow the standards summarized in the NLM's International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals: Sample References webpage (www.nlm.nih.gov/bsd/uniform_requirements.html) and detailed in the NLM's Citing Medicine, available from www.ncbi.nlm.nih.gov/books/NBK7256/. MEDLINE abbreviations for journal titles (www.ncbi.nlm.nih.gov/nlmcatalog/journals) should be used. 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: Serruys PW, Ormiston J, van Geuns RJ, de Bruyne B, Dudek D, Christiansen E, et al. A Polylactide Bioresorbable Scaffold Eluting Everolimus for Treatment of Coronary Stenosis: 5-Year Follow-Up. J Am Coll Cardiol. 2016;67(7):766-76. doi: 10.1016/j.jacc.2015.11.060.

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 numbered consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by Arabic numerals in parentheses and listed at the end of the article in citation order.

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. Place explanatory matter in footnotes, not in the heading. Explain all nonstandard abbreviations in footnotes, and use symbols to explain information if needed. 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, archiving and repository policies

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.

Authors are allowed to deposit their works into an Open Access Repository.

Until an electronic backup and preservation of access to the journal content is implemented, we strongly encourage authors to archive the original data sets in publicly available and permanent repositories whenever possible and appropriate.

Open Access and Processing Fees

***Open Access Publication*:** all manuscripts submitted to IJBM will be submitted under the Open Access publishing model. Readers are allowed to freely read, download, copy, distribute, print, search, or link to full texts. 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 $200 per printed page of color illustrations.

IJBM charges a processing fee of $75 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 = $300.

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= $200 × 2 color journal pages = $400

Total Charge for article = $700

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

**For non-native English writers, we also provide additional copyediting services, which include the detailed grammar check (word use, sentence structure, phrases, clauses, verb-noun use, etc.). Our fee for such service is $10 per page (roughly 500 words)*

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, subject to a selected user license. 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: Articles from IJBM website may be reproduced, in any media or format, or linked to for any commercial purpose, subject to a selected user license.

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 completed 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 e-mail 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
• Figure Legends on a separate page
• Each figure should be saved as a separate electronic file
• Tables on a separate page
• 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 **International Journal of Biomedicine.**