IJBM

INTERNATIONAL JOURNAL OF BIOMEDICINE

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 Accepted manuscripts become the sole property of the Journal and may not be published elsewhere without the consent of the Journal.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

www.ijbm.org

Volume 5, Issue 2, June 2015

CONTENTS

CLINICAL RESEARCH

CONTENTS

CONTINUED

2015 COSTEM

The 3rd International Congress on

CONTROVERSIES IN STEM CELL TRANSPLANTATION AND CELLULAR THERAPIES

BERLIN, GERMANY • OCTOBER 22-24, 2015

Sponsorship & Exhibition *Prospectus*

Last update: September 28, 2014

www.comtecmed.com/costem

Hellenic Society for Neuroscience

Israel Society for Neuroscience

Serbian Neuroscience Society

FENS Featured

Federation of European Neuroscience Societies

Regional Meeting 2015

Nourishing Neuroscience in the Cradle of Culture

Congress Secretariat:

ets Events & Travel Solutions

E.T.S. Events & Travel Solutions S.A.
154 El. Venizelou Str
N. Smyrni, Athens, Greece, 171 22
Tel.: +30 210 98 80 032, Fax.: +30 210 98 81 303
E-mail: ets@events.gr, ets@otenet.gr
Website: www.events.gr

7-10 October 2015

Thessaloniki, Greece

www.ffrm2015.com

International Journal of BioMedicine 5(2) (2015) 55-59

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

Effect of Cytochrome P450 2C19 681G>A Polymorphism on Premature Coronary Heart Disease

Zhong-Hai Chi, Yuan-Yuan Li, Juan Li, Zheng Zhang*, Li Jia

The Affiliated Hospital of Medical College Qingdao University
Qingdao, China

Abstract

Objective: The aim of our study was to evaluate the association between cytochrome P450 2C19 (CYP2C19) 681G>A polymorphisms and the age of development of coronary heart disease. Additionally, the study might find some biological indicators at the gene level that could help to predict and evaluate the risk of coronary heart disease in time.

Methods: This study included 352 individuals with coronary heart disease. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to identify CYP2C19 681G>A. The objects were divided into wild type (GG or homozygous *CYP2C19*1* wild-type) and mutant type (GA/AA or the mutant *CYP2C19*2* allele) on the basis of genotype. The association between CYP2C19 gene polymorphism and the age of onset of coronary heart disease was assessed by multivariate linear regression analysis.

Results: There was a significant association in the age of onset between the two groups (t=3.398, *P=0.001*), which was 58.51±12.72 years in the wild type and 53.95±11.63 years in the mutant group. The frequency of CYP2C19 681A (*CYP2C19*2* allele) was 0.32, 0.268, 0.227 in different age groups, which was significantly different (χ^2=10.745, *P=0.005*) in different groups, as well as in the genotype. The result, assessed by multiple linear regression, showed that genotype, smoking, obesity, and hyperlipidemia affect the age of onset of coronary heart disease (β was -0.167, 0.156, 0.155, and 0.112, *P<0.05*), and mutually affect the age of onset of coronary heart disease.

Conclusion*:* The study suggests that *CYP2C19* 681G>A gene polymorphism may be one of the risk factors in susceptibility to early onset of coronary heart disease, but not an independent factor because other factors may play a synergistic role.

Key words: *CYP2C19 681G>A polymorphism; premature coronary heart disease; age of onset.*

Introduction

In recent years, coronary heart disease (CHD) has caused a rise in mortality and morbidity, and serious harm to human life and health. Moreover, the trend in morbidity has been gradually toward younger people. The incidence of coronary heart disease is closely related to genetic and environmental factors, particularly premature CHD (age of onset <55 years in males, and <65 years in females) but is more likely to be influenced by genetic factors. At present, a large number of clinical and statistical studies have indicated that (CYP2C19) 681G>A polymorphism can affect drug metabolism associated with CHD and the prognosis for patients with the disease. However, research focusing on the effects of the incidence of CHD are still rare. Therefore, this study, with the onset age as the breakthrough point, was to investigate the correlation between (CYP2C19) 681G>A polymorphism and CHD and further explore the role of CYP2C19 gene polymorphism normality in CHD.

As one of the key microsomal mixed function oxidases, the Cytochrome P450 enzymes (CYPs) play an important role in oxidation, peroxidation, and reduction of endogenous physiological substances, such as steroid hormones and cholesterol metabolism, as well as in maintaining cardiovascular homeostasis. The polymorphic P450 (CYP) enzyme superfamily is the most important system involved in the biotransformation of many endogenous and exogenous substances including drugs, toxins, and carcinogens. Genotyping for CYP polymorphisms provides important genetic information that help to understand the effects of xenobiotics on human body. Its high gene polymorphism is one of the earliest pharmacogenetics research objects [1].

***Corresponding author:** Zheng Zhang, Graduate student from Medical College Qingdao University; Qingdao, China. E-mail: 163zhangz@163.com*

While CYP2 is the largest family, of which CYP2C19 is a member, its genetic polymorphism is the gene encoding mutation causing the enzyme's abnormal activity, and has visible differences in different populations. Even in different ethnic groups in China, there are obvious differences between the incidence of wild type and mutant-type alleles [2]. Now, a number of studies have found that CYP2C19 gene polymorphism is significantly associated with the risk and prognosis of coronary heart disease. Ecan et al. [3] found that heterozygous CYP2C19*3 allele frequency in patients with coronary artery stenosis is significantly higher than in the control group (10.2% and 5.6%, respectively), suggesting that the mutation can significantly increase the risk of CHD.

Methods

Study population

Three hundred and fifty-two patients with coronary heart disease (according to The European Society of Cardiology Pocket Guide) were enrolled in the study from January 2013 to August 2014, all of whom were from the Han nationality in northern China. Individuals with the following conditions were removed from this study: serious heart disease, chronic hepatic or kidney disease, malignant tumors, acute and chronic infection, and recent major surgery. This research had the approval of the ethics committee and subjects signed informed consent.

Study protocol

Peripheral venous blood was drawn from each patient after fasting for at least 10 hours, and was tested for serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) in half an hour. The tests were implemented by a Roche Hitachi 7600 automatic biochemistry analyzer in our hospital's biochemical laboratory. Professionals measured the height and weight of all subjects, calculating BMI=body weight (kg) /[height (m)]^2. Overweight means a body mass index >25kg/m^2, according to the standard diagnosis that was announced by WHO in 1997. All subjects needed to provide medical history, including smoking, history of diabetes, hypertension, and hyperlipidemia. Diabetes means a fasting blood glucose >7.0mmol/L and (or) postprandial blood glucose ≥11.1mmol/L or having a history of hypoglycemic treatment. Hypertension means systolic pressure≥140mmHg and (or) diastolic blood pressure 90mmHg or having a history of antihypertensive treatment. Any one of the following conditions, or receiving lipid-lowering therapy, can be diagnosed as hyperlipidemia: TC≥5.72mmol/L, TG≥1.70mmol/L, LDL-C≥3.64mmol/L, and HDL-C≥0.91mmol/L. According to the smoking standardization recommendations issued by WHO in 1984, smoking means that one smokes 1 cigarette or more per day and has done so for more than 1 year.

Genetic analysis

For our study, 2 to 3 ml of peripheral venous blood was drawn from every individual. The CYP2C19 genotype was detected by a BaiO-BE gene chip detector, a BE-2.0 biological chip reading instrument, PCR amplification detection, and finally the result was automatically determined by the Array Doctor analysis software (genotype was completed by Shanghai United gene company). The main detection of the CYP2C19 gene was the 681st locus on the fifth exon. The population could be divided into the wild type group (GG) and the mutant group (GA/AA) according to the different genotypes.

Statistical analysis was performed using SPSS v. 17.0 (SPSS, Inc., Chicago, IL). Statistical analysis was performed using the SPSS 13.0 software package (SPSS, Chicago, IL, USA). The genotype frequency distribution was tested for Hardy-Weinberg equilibrium (HWE) with a chi-square test. Differences of continuous variables with a normal distribution (presented as mean ± SD) between the two groups were calculated using the independent-sample t-test. Two-tailed P values <0.05 were considered statistically significant. The relevance between CYP2C19 gene polymorphism, as well as other risk factors and coronary heart disease was tested by multiple linear regression analysis.

Result

1. The gene distribution of the study population conformed to the Hardy-Weinberg equilibrium [4] (χ^2=0.735, P=0.392), which means the study population was typical.

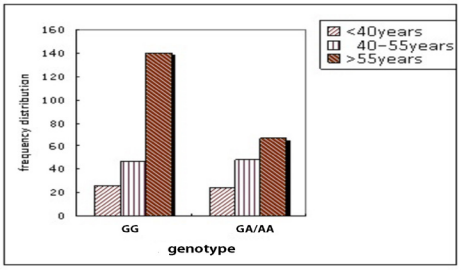
2. The clinical characteristics of the study individuals are summarized in Table 1. A chi-square test showed that the clinical characteristics, such as gender, diabetes, hypertension, hyperlipemia, overweight, and smoking, did not differ significantly between patients with different genotypes. A t-test showed that there was no significant difference in age between the two groups.

Table 1. The clinical characteristics of the study individuals

Variables	GA/AA group (n=139)	GG group (n=213)	statistics	*P*
Gender (male), n	89	130	0.321	0.653
BMI>25kg/m^2, n	73	106	0.255	0.663
Smoking habit, n	72	104	0.297	0.663
Hyperlipidemia, n	26	24	3.818	0.061
Hypertension, n	86	146	1.667	0.207
Diabetes, n	76	99	2.261	0.156
Age, years	66.30±8.63	67.11±8.53	0.867	0.387

3. The age of onset of the individuals is summarized in Graph 1 and Tables 2 and 3. According to the study of Framingham, premature coronary heart disease was identified as CHD that occurs in males under 55 years of age and in females under 66 years of age. Graph 1 shows that there is a significantly different genotype distribution of the CYP2C19 681G>A polymorphism in different groups with different ages of onset. The frequency of the CYP2C19 GA/AA

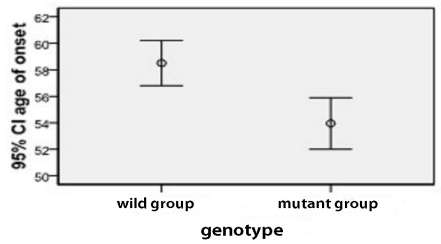
genotype was 0.480, 0.503 in patients with CHD who had an earlier age of onset and 0.324 in patients with CHD who had a later age of onset. A significant difference in the CYP2C19 681G>A genotype was observed in the different groups as shown in Table 2 (χ^2=10.753, *P=0.005*). The allele frequencies of the CYP2C19 A allele in patients with onset at <40, 40 to 55, and >55 years were 0.320, 0.268 and 0.227, respectively. And there was a significant difference between the different groups (χ^2=10.745, *P=0.005*) as shown in Table 2. The t-test showed that the age of onset in the mutant group was less than in the other group, 53.95±11.63 years and 58.51±12.72 years, respectively, as shown in Graph 2 (*t*=3.398, *P=0.001)*. And the difference was significant between the wild type and mutant groups as shown in Graph 2. Furthermore, after adjustment for traditional risk factors of CHD, CYP2C19 681A was significantly associated with the age of onset of patients with CHD by multiple linear regression analysis (*F*=5.966, *P=0.000*) (Table 3), as well as smoking, overweight and hyperlipidemia, and β was 0.167, 0.156, 0.155, and 0.122. All of the risk factors can lead to an earlier onset of CHD.



Graph 1. *Genotype distribution of the CYP2C19 G681A polymorphism in different groups*

Table 2. Genotype distribution of the CYP2C19 G681A polymorphism in different groups

Gene	<40 years	40 -55 years	>55 years	X^2	*P*
Group					
GA/AA	24(48.0%)	48(50.5%)	67(32.4%)	10.753	0.005
GG	26(52.0%)	26(52.0%)	140(67.7%)		
Genotype					
AA	8(16.0%)	3(3.2%)	10(4.8%)		
GA	16(32.0%)	45(47.4%)	57(27.5%)	8.647	0.013
GG	26(52.0%)	47(49.5%)	140(67.7%)		
Allele					
A	0.32	0.268	0.227	10.745	0.005
G	0.68	0.732	0.773		



Graph 2. *The difference* of age of onset with different genotype (* t=3.398, P=0.001)*

Table 3. Multiple linear regression analysis of determinants of CHD in individuals

	t	β	*P*
Genotype	-3.244	-0.167	0.001
BMI>25kg/m^2	2.345	0.155	0.020
Smoking	2.007	0.156	0.046
Hyperlipidemia	2.174	0.112	0.030
Diabetes	-0.835	-0.071	0.404
Hypertension	0.936	0.048	0.350
Constant	8.85	44.557	0.000

Discussion

CHD is one of the most serious diseases worldwide, endangering human health, and is a multifactorial and polygenic disease [5]. In recent years, the morbidity and mortality resulting from CHD has increased year by year. Moreover, its incidence rate among young patients is increasing. As estimated, there were 7.3 million deaths worldwide owing to CHD in 2001 [6]. With the development of molecular biological technology, more and more genetic research focuses on the pathogenesis of CHD. Studies have reported that a variety of genes are closely associated with CHD and other cardiovascular diseases, including the endothelial nitric oxide synthase gene, the angiotensin converting enzyme gene, and apolipoprotein E (APOE) [7-9]. A series of studies on genome-wide associations of CHD are ongoing, which are important to an understanding of the molecular and genetic pathogenesis of CHD, as well as individual susceptibility to CHD. These studies are also important for achieving targeted or individual therapies so as to prevent the occurrence of CHD and improve the survival rates of patients with the disease. Multiple studies have shown that cytochrome P450, a super family of cysteinate-haem enzymes, plays an important role in the onset, progression, and prognosis of CHD [10]. Some researchers have shown that CYP2C9*1/*3 may be associated with premature coronary heart disease in Chinese Han males; similarly, CYP2J2*7 may be associated with premature coronary heart disease in Chinese Han women [11]. Some studies [12] have estimated that CYP3A4*1G/*1G may increase the risk of CHD in Chinese Han females. A large number of studies [13-14] in the relevant field from home and abroad have shown that CYP2C19*1/*2, *1/*3 with only one effective allele leads to enzyme activity reduction. Thus, the residual platelet aggregation rate in patients with CYP2C19*2 or *3 alleles is higher than that of the wild homozygous type. Furthermore, anti-platelet drug efficacy in patients with CYP2C19*2 and *3 alleles is poorer, which can increase the incidence of adverse cardiovascular events [3].

With age of onset in patients with CHD as a focal point, our study observed the genotype distribution of the CYP2C19 681G>A polymorphism. The goal of this study was to evaluate the association of CYP2C19 polymorphism with CHD. The study showed that the frequency of the CYP2C19 GA/AA genotype was significantly higher in patients with earlier onset

than in those with later onset (χ^2=8.647, *P<0.001*): 0.48 in the <40 group, 0.505 in the 40 to 55 group, and 0.324 in the >55 group. In addition, the frequency of mutant allele was also significantly different in groups with the same 3 different ages of onset (χ^2=10.745, *P=0.005*): 0.32, 0.268 and 0.227, respectively, as shown in Table 2. Graph 1 demonstrates that the distribution of earlier age of onset was different in patients with different CYP2C19 genotypes, and was bigger in the mutant group than in the wild type group. These results reveal that the *CYP2C19* 681G>A polymorphism might be associated with age of onset of CHD. Furthermore, the t-test in Graph 2 shows that the age of onset in patients with different CYP2C19 genotypes was significantly different (*t*=3.398, *P=0.001*): 53.95±11.63 years in the wild type group and 58.51±12.72 years in the mutant group. After further analysis of the data, multiple linear regression found that genotype, smoking, overweight and hyperlipidemia are correlated with the age of onset of CHD (β=-0.167, 0.156, 0.155, and 0.112; *P=0.001, 0.046, 0.020, 0.030*); *CYP2C19* 681G>A had the greatest effect on the age of onset, which indicates that CYP2C19 gene polymorphism might not be an independent risk factor for CHD; rather, there might be several reasons why CYP2C19 gene polymorphism is associated with CHD. Some studies have indicated that certain CYP enzymes play an important role in CHD progression, which is likely related to their character as major regulators of L-type calcium channels and cardiomyocyte contractility [15]. CYP2C19 and CYP2A1 are related to elevation of pro-inflammatory cytokines in heart failure [16]. CYP may also influence the pathogenesis of cardiac hypertrophy by enhancing the production of ROS [17]. In recent years, some scholars [18] have researched the effect of CYP450 family gene polymorphism in the metabolism pathways of arachidonic acid. They found that the CYP2 family expressed in the cardiovascular system and CYP2C19 is the key enzyme in the metabolism of arachidonic acid, which is relevant to endothelium-dependent vasodilation and plays an important role in regulation of the cardiovascular system. It is speculated that *CYP2C19* 681G>A polymorphism can impact the pathogenesis of CHD by reducing the enzyme activity. In addition, Giusti B found that the CYP2C19 gene polymorphism is relevant to platelet hyper reactivity in a study involving 1419 patients with CHD. This gene polymorphism causes the individual difference in endogenous and exogenous drug metabolism, and even some medical conditions such as cancer and birth defects, by leading the change in CYP2C19 protease activity [19]. Some documents [20] reported that having first-degree relatives with a history of CHD not only increases the risk of CHD, but also influences the onset of CHD. Our study suggests that gene polymorphism is more likely to affect young rather than old patients and is affecting more young patients, which is consistent with some other previous studies [21]. Our study could provide new targets and ideas for the effective prevention and treatment of coronary heart disease.

In addition, our study also found that conventional risk factors, including, overweight, smoking and hyperlipidemia, are associated with the age of onset of CHD, as shown in Table 3, which is consistent with current research. The increase in coagulation factor activity in overweight patients can lead to enhanced platelet aggregation, which may be a possible cause of CHD in advance of the onset age. Smoking can cause vascular endothelial cell injury, which can result in coronary artery spasm or inflammation, even the formation of atherosclerotic plaque and CHD. Previous studies have demonstrated that lipid metabolic disorder is an independent risk factor for CHD and that the apolipoprotein E (APOE) gene is a susceptible CHD gene. A rise in triglyceride levels can increase risk of CHD by putting an individual in a hypercoagulable state. Our study shows that diabetes and hypertension are not relevant to the onset age of CHD, which is not consistent with previous studies [22]. We considered that the point of our study was the age of onset of CHD, but hypertension and diabetes are age-related diseases, which have less influence on patients with early age of onset.

Some research has shown that there are other types of mutations of CYP2C19, but we did not investigate that due to the low frequency of occurrence in the East Asian populations. Although other members of the CYP superfamily have been well studied regarding the onset, progression, and prognosis of CHD [17], little information is available about CYP2C19 specifically contributing to the pathogenesis of CHD, a subject that needs to be further studied. Since the study was a retrospective study, and subject to geographical constraints, we only provided the *CYP2C19* 681G>A genotype distribution of Han populations in North China. What is more, age of onset is affected by many factors, which need more epidemiological studies in different areas and races with larger samples to estimate the relationship between *CYP2C19* 681G>A polymorphism and CHD.

In conclusion, we demonstrated a significant association between *CYP2C19* 681G>A polymorphism and age of onset of CHD in Han populations in North China, and the result also indicate that *CYP2C19* 681G>A may be a gene polymorphism that is susceptible to CHD in the North Chinese Han population. Thus, we can improve the prevention and treatment of CHD by aiming at the susceptibility gene. For the conventional risks, including smoking, overweight and hyperlipidemia, we suggest that regular health examinations are essential for all individuals and that more attention be given to strengthening the primary prevention of CHD [23].

Competing interests

The authors declare that they have no competing interests.

References

1. Zhou X, Zhang JZ, Qi HZ, et al. Progress in cardiovascular drug metabolic enzyme and pharmacogenetic studies. China Pharm 2012; 23(26):2476-2479.
2. Shu Y, Zhou HH. Individual and ethnic differences in CYP2C19 activity in Chinese populations. Acta Pharmacol Sin 2000; 21(3):193-9.
3. Ercan B, Ayaz L, Çiçek D, Tamer L.. Role of CYP2C9 and CYP2C19 polymorphisms in patients with atherosclerosis. Cell Biochem Funct 2008; 26 (3):309-13.
4. Motulsky V. Human genetics. The third edition. Beijing;

People's Medical Publishing House 1999:749-750.
5. Gaziano TA, Bitton A, Anand S, Abrahams-Gessel S, Murphy A. Growing epidemic of coronary heart disease in low- and middle- income countries. Curr Probl Cardiol 2010; 35(2):72–115.
6. Kastelein JJ, van Leuven SI, Burgess L, Evans GW, Kuivenhoven JA, Barter PJ, et al. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. N Eng1 J Med 2007; 356(16): 1620-30.
7. Zhou B, Wu KX, Li Y, et al. Analysis of 236 cases of coronary angiography in patients with clinical background information. Chin J Prac Diag Treat,2008; 22(11):872-3.
8. Han Y, Xu W, Zhang W, Liu N, Ji Y.. T-786C polymorphism in the endothelial nitric oxide synthase gene is associated with increased risk of coronary artery disease in a Chinese population. Pharmacology 2010; 85(4):211–6.
9. Kolovou GD, Anagnostopoulou KK. Apolipoprotein E polymorphism, age and coronary heart disease. Ageing Res Rev2007; 6(2): 94–108.
10. Elbekai RH, EI-Kadi AO. Cytochrome P450 enzymes:central players in cardiovascular health and disease. Pharmacol Ther 2006; 112(2):564-87.
11. Zhan TY, Shi Lei, Zhao SJ, et al. The correlation between the CYP2J2* 7 gene polymorphism and coronary heart disease in Chinese Han population. Guangdong Med J 2011; 32(12):1543-5.
12. He BX, Shi L, Qiu J, Tao L, Li R, Yang L, Zhao SJ. A functional polymorphism in the CYP3A4 gene is associated with increased risk of coronary heart disease in the Chinese Han population. Basic Clin Pharmacol Toxicol 2011;108(3): 208-13.
13. Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 2006; 108(7):2244-7.
14. Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. J Am Coll Cardiol 2008; 51(20):1925-34.
15. Xiao YF, Huang L, Morgan JP. Cytochrome P450: a novel system modulating Ca2+ channels and contraction in mammalian heart cells. J Physiol 1998;508(Pt3):777−92.
16. Frye RF, Schneider VM, Frye CS, Feldman AM. Plasma levels of TNF-alpha and IL-6 are inversely related to cytochrome P450 dependent drug metabolism in patients with congestive heart failure. J Card Fail 2002; 8(5):315−9.
17. Elbekai RH, El-Kadi AO. Cytochrome P450 enzymes: central players in cardiovascular health and disease. Pharmacol Ther, 2006, 112(2):564–87.
18. Bièche I, Narjoz C, Asselah T, Vacher S, Marcellin P, Lidereau R, et al. Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1, CYP2 and CYP3 families in 22 different human tissues. Pharmacogenet Genomics, 2007 ; 17(9):731-42.
19. Wang Q, Zhang GJ, Kang XX. Effects of CYP2C19 gene polymorphisms on clopidogrel metabolism [J/CD]. Chin J Clin (Electronic Edition). 2013;7(21):9746-9.
20. Kral BG, Becker DM, Vaidya D, Yanek LR, Becker LC. Severity of inducible myocardial ischemia predicts incident acute coronary syndromes in asymptomatic individuals with a family hospital of premature coronary artery disease. J Nucl Cardiol 2012; 19(1):28-36.
21. Chu YR, Chu ZH, Zhu YL, Research on the relationship between apolipoprotein E gene polymorphism and early-onset coronary heart disease. Prog Mod Biomed 2007; 7(2):244-6.
22. Sun JG, Zhang XL, Hu JP. Sexual difference of clinical characteristics in premature coronary heart disease patients. Anhui Med J 2011; 32(2):213-5.
23. Yang JL, Yang FY. Risk factors for coronary heart disease and Prevention. PJCCPVD 2010; 18(7):1014-5.

International Journal of BioMedicine 5(2) (2015) 60-64

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

Relationship between the Levels of MMP-9, TIMP-1, and Zinc in Biological Samples of Patients with Carotid Atherosclerosis

Zakhro A. Usmanova

Tashkent Institute of Postgraduate Medical Education
Tashkent, Uzbekistan

Abstract

The aim our study was to evaluate the levels of zinc in blood serum, hair, and specimens of carotid artery atherosclerotic plaques (CAAPs) and their relationship to levels of MMP-9 and TIMP-1 in the serum of patients with stable and unstable CAAPs.

Material and Methods: The study included 73 patients (55 men and 18 women) aged from 46 to 88 years (mean age 65.96±1.07 years) with CAAPs. The control group consisted of 10 healthy subjects of similar age and gender. The patients were divided into two groups depending on their atherosclerotic plaque stability according to prior duplex ultrasonography. Group 1 consisted of 45 patients with stable atherosclerotic plaque, and Group 2 included 28 patients with unstable AP. Patients with hemodynamically significant carotid stenosis and unstable atherosclerotic plaques underwent carotid endarterectomy. The serum concentration of MMP-9 and TIMP-1 was determined using the standard test systems for immunoassay. Quantitative determination of the zinc level in hair and atherosclerotic plaque was carried out by optical emission spectrometry; the serum Zn was determined colorimetrically.

***Results*:** The serum levels of MMP-9 and TIMP-1 were significantly higher in Group 2 compared to Group1 and the control group. The index of MMP-9/TIMP-1 was 1.6 times higher in Group 2 compared to the control group. The level of Zn in serum and hair was not significantly different between Groups 1 and 2. However, Zn levels in unstable atherosclerotic plaque were lower than in the control group. Reducing the concentration of zinc in the hair was accompanied by a decrease in zinc level in atherosclerotic plaque specimens. With the progression of atherosclerosis and increasing the intima-media thickness of the common carotid artery, the level of zinc in serum and atherosclerotic plaques decreased. Increasing the serum concentration of MMP-9 was accompanied by decreasing the zinc level in atherosclerotic plaque. High serum concentration of MMP-9 and TIMP-1, and MMP-9/TIMP-1 imbalance testify to CAAP instability.

Keywords: *carotid atherosclerosis; atherosclerotic plaque; matrix metalloproteinase-9 (MMP-9); TIMP metallopeptidase inhibitor 1; zinc.*

Introduction

The instability of atherosclerotic plaque (AP) is the main cause of myocardial infarction (MI) and stroke [1]. Carotid artery AP (CAAP) proneness to rupture is an independent risk factor of acute coronary syndrome and MI [2]. The vulnerability of the atherosclerotic plaques depends on many factors, including endothelial function, presence of inflammatory cells, cytokine production, smooth muscle cells contents, and cell death (including necrosis and apoptosis) [3]. Vulnerable APs have a thin fibrous cap, a large lipid-rich necrotic core, and increased plaque inflammation with the elevated expression of matrix metalloproteinases (MMPs).

The activity of MMPs in various tissues is tightly regulated by endogenous tissue inhibitors of MMPs (TIMPs). Alteration of the fine physiological balance between MMPs and TIMPs may contribute to the pathophysiology of atherosclerosis [4]. MMPs are zinc-containing enzymes that are implicated in degradation and remodeling of components of the extracellular matrix. Increased levels of MMPs, and in particular of MMP-9 (gelatinase B), have been associated with several pathological inflammatory conditions, vascular diseases, and the instability of carotid atherosclerosis [5-8]. The catalytic domain of all MMPs contains a Zn2+ ion coordinated by a tris(histidine) motif; the Zn2+ ion is critical for both substrate binding and cleavage [9-11]. There is evidence that zinc blocks calcium and its several actions on

***Corresponding author:** Zakhro A. Usmanova. Tashkent Institute of Postgraduate Medical Education; Tashkent, Uzbekistan*
E-mail: zahro.usmanova@yandex.ru

atherogenesis. Increased amounts of cytotoxic cytokines, such as TNF-alpha, IL-beta and IL-8, often produced in the elderly, are blocked by high-dose zinc [12]. It has been established that there is a relationship between zinc deficiency in different biosubstrates and the development of obesity, type 2 diabetes mellitus, hypertension, coronary heart disease (CHD), and atherosclerosis.

We aimed to study the levels of zinc in blood serum, hair, and specimens of CAAPs and their relationship to levels of MMP-9 and TIMP-1 in the serum of patients with stable and unstable CAAPs.

Material and Methods

The study was approved by the Tashkent Institute of Postgraduate Medical Education Ethics Committee. The study included 73 patients (55 men and 18 women) aged from 46 to 88 years (mean age 65.96±1.07 years) with CAAPs. The control group consisted of 10 healthy subjects of similar age and gender. Written informed consent was obtained from each patient. All patients were examined by a neurologist, cardiologist and vascular surgeon. The patients were divided into two groups depending on their AP stability according to prior duplex ultrasonography. Group 1 consisted of 45 patients with stable AP, and Group 2 included 28 patients with unstable AP.

Exclusion criteria were acute myocardial infarction, cardiomyopathy, acute myocarditis, pericarditis, acute ischemic stroke, malignant tumors, diffuse connective tissue diseases, acute infectious disease, pulmonary fibrosis and severe chronic obstructive pulmonary disease, left ventricular ejection fraction less than 45%.

Carotid duplex ultrasonography (CUS)

All participants underwent a color duplex sonography of the entire extracranial carotid system using a duplex scanner HD3 (Phillips, The Netherlands) and a linear transducer (5.0-10.0 MHz). The calculation of the degree of stenosis (DS) was performed in the zone of maximum narrowing of the artery lumen. We investigated the common carotid artery (CCA), internal carotid artery (ICA), and carotid bifurcations for the presence of AP. The intima-media thickness of CCA (IMT-CCA) was measured both at a standard point (1cm before the carotid bifurcation) and in AP (maximum IMT). IMT-CCA >1.4mm was evaluated as AP, and from 1.0 to 1.3mm was determined as thickened intima. We also studied the blood flow velocity, as well as the nature, type, surface, length and location of the AP. All detected APs were evaluated as stable (calcified, homogeneous and heterogeneous) or unstable (with a rough surface, with ulceration and/or bleeding).

Carotid artery specimens

Group 2 patients with hemodynamically significant carotid stenosis and unstable APs underwent carotid endarterectomy (CEE) at the Tashkent Medical Academy. All samples were obtained immediately after CEE for determination of zinc level. Normal post-mortem carotid arteries (10 specimens) were used as control tissue.

MMP-9 and TIMP-1 quantification

In all patients, after CUS, blood was taken from the cubital vein in the morning on an empty stomach after a 12-hour overnight fast. All venous blood samples were immediately centrifuged and serums were frozen at -20°C. The serum concentration of MMP-9 and TIMP-1 was determined using the standard test systems for immunoassay (Bender-Medsystems GmbH, Austria) on the spectrophotometer Plate Reader (Hospitex Diagnostics, Italy).

Quantification of zinc in serum, AP, and hair

The serum Zn was determined colorimetrically by using a computerized, biochemical, automatic analyzer Mindray BS-200 (China) and kit «Zinc-Vital» (Vital Development Corporation, Russia). Quantitative determination of the zinc level in hair and AP was carried out by optical emission spectrometry with an inductively coupled argon plasma analyzer Optima 2100 DV (Perkin Elmer, USA).

Serum lipid profile

The total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were determined in the venous blood using automatic analyzer Mindray BS-200 (China).

Results were statistically processed. The mean (M) and standard error of the mean (SEM) were deduced. For data with normal distribution, inter-group comparisons were performed using Student's t-test. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups (for nonparametric data). 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

Both groups were comparable in frequency of hypertension, hyperlipidemia, obesity, type 2 diabetes, coronary heart disease, previous MI, and age. In Group 1, chronic cerebral ischemia (CCI) stage I, II and IV was found in 35(77.8%), 1(2.2%), and 9(20%) patients, respectively. In Group 2, we found only the advanced CCI stages III and IV in 13(46.4%) and 15(53.6%) patients, respectively. In Group 1, the number of men was 1.3 times less than in Group 2. In Croup 2, the incidence of stroke (57.1%) was 2.6 times higher than in Group 1(22.2%) (Table 1).

Differences in lipid profile were not significant (Table 2), probably because in Group 2 patients, it was 2.5 times more likely that statins were prescribed (78.6% vs. 31.1%).

The level of Zn in serum and hair was not significantly different between Groups 1 and 2. However, Zn levels in unstable AP were lower than in the control group (Table 3).

The serum level of MMP-9 was 2.7 times higher in Group 2 compared to Group1 and the control group (539.03±57.1 ng/ml vs. 200.9±8.5 and 197.42±10.4 ng/ml, respectively, $P<0.001$).

Table 1.
Characteristics of the patient groups

Variable	Group 1 (n=45)	Group 2 (n=28)
Age, years	67.9±1.5	62.8±1.2
Male/Female	30/15 (67%/33%)	25/3 (89%/11%)*
Hypertension	42 (93.3%)	26 (92.9%)
Hyperlipidemia	22 (48.9%)	15 (53.6%)
Obesity	19 (42.2%)	7 (25%)
Diabetes	14 (31.1%)	14 (50%)
CHD	36 (80%)	26 (92%)
History of MI	6 (13.3%)	4 (14.3%)
History of stroke	10 (22.2%)	16 (57.1%)*
Body mass index	27.7±0.59	27.2±0.79
IMT CCA, mm	0.99±0.04	1.2±0.05*
DCAS, %	44.2±3.6	75.4±2.9*

**p<0.05. IMT CCA – intima-media thickness of the common carotid artery, DCAS - degree of carotid artery stenosis.*

Table 2.
Serum lipid profile of patients in the study groups

Parameters	Control group	Group 1	Group 2
TC, mmol/l	5±0.5	5.2±0.16	4.6±0.18
TG, mmol/l	1.7±0.12	1.8±0.12	2.2±0.29
HDL, mmol/l	1.06±0.13	0.94±0.02	1.04±0.05
LDL, mmol/l	3.8±0.4	3.7±0.12	3.2±0.15

Table 3.
The levels of Zn in serum, scalp hair, and AP

Biosubstrates	Control group	Group 1	Group 2
Serum, mmol/l	18.3±4.16	13.7±1.4	15.9±0.62
Scalp hair, mg/g	218.65±84.23	220.5±17.07	212.5±17.01
AP, mg/g	148.55±15.3	-	82.8±16.8*

**p<0.05.*

The serum level of TIMP-1 was twice as high in Group 2 compared to Group 1 (2,410.2±123.8 ng/ml and 1,180.98±47.98 ng/ml, respectively, p<0.001). A significant difference was also found between Group 2 and the control group (*P<0.001*). In Group 2, we found an increase in serum TIMP-1 level of about 2 times in comparison with the control group. However, we did not find statistically significant differences between Group 1 and the control group (*P>0.05*).

The index of MMP-9/TIMP-1 was 1.6 times higher in Group 2 (0.24±0.03) compared to the control group (0.15±0.02) (*P<0.02*). Significant differences were not found between Group 1 and 2, or between Group 1 and the control group (Table 4).

Correlational analysis revealed a moderate positive correlation between DS with the serum levels of MMP-9 and TIMP-1 (r=0.47 and r=0.53, respectively, *P<0.001*) (Fig.1,2). We also found a significant positive correlation between MMP-9 and TIMP-1(r=0.58, *P<0.001*) (Fig.3).

Table 4.
Serum levels of MMP-9 and TIMP-1, and MMP-9/TIMP-1 ratio

Para-meters	Control group	Group 1	Group 2	P (c-1)	P (c-2)	P (1-2)
MMP-9 (ng/ml)	197.42±10.4	200.9±8.5	539.03±57.1	*>0.05*	*<0.001*	*<0.001*
TIMP-1 (ng/ml)	1192.5±61.5	1181.0±48.0	2410.2±123.8	*>0.05*	*<0.001*	*<0.001*
MMP-9/ TIMP-1	0.15±0.02	0.18±0.009	0.24±0.03	*>0.05*	*<0.02*	*>0.05*

P (c-1) – between Group 1 and the control group; P (c-2) – between Group 2 and the control group; P (1-2) - between Group 1 and Group 2.

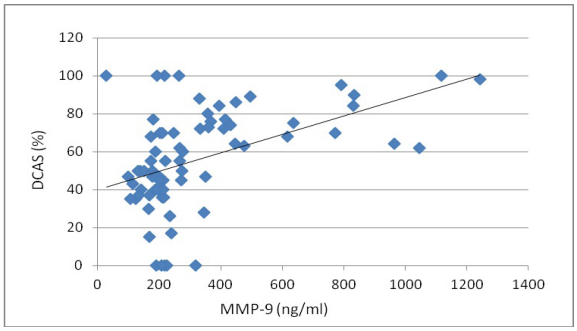


Figure 1. *The correlation between MMP-9 and DCAS (n=73, r=0.47; P<0.001)*

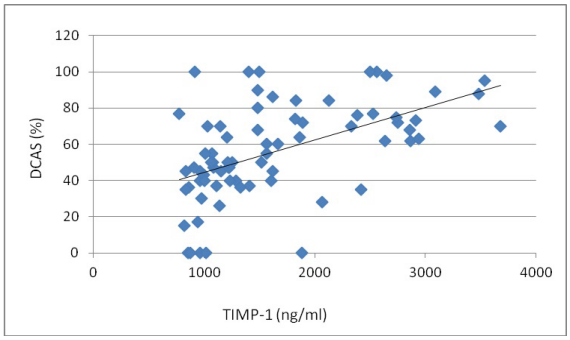


Figure 2. *The correlation between TIMP-1 and DCAS (n=73, r=0.53; P<0.001)*

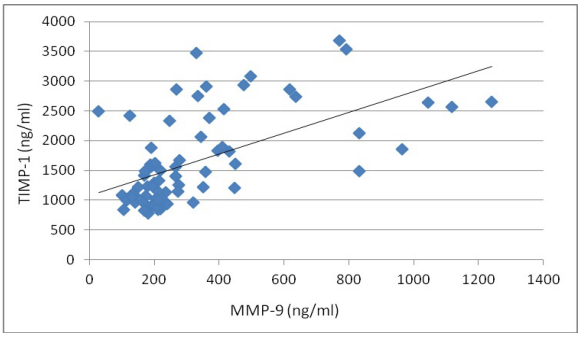


Figure 3. *The correlation between MMP-9 and TIMP-1 (n=73, r=0.58; p<0.001)*

Our results revealed no significant association of serum MMP-9 and TIMP-1 with age (r=-0.16 and r=-0.11, respectively, *P>0.05*).

We found a weak inverse correlation between the levels of serum zinc and its concentration in hair (r=-0.21*; P=0.07*), a positive correlation between serum zinc and its concentration in the APs (r=0.21*; P=0.07*), a weak positive correlation between zinc in the hair and its level in the APs (r=0.23*; P<0.05*), and a negative correlation between serum zinc level and IMT CCA(r=-0.24*; P<0.05*).

Concentration of zinc in bio-substrates also correlated with serum levels of MMP-9 and TIMP-1. We observed a weak negative correlation between serum MMP-9 and the levels of zinc in the APs (r=-0.25*; P<0.05*). The relationships between other parameters were not statistically significant.

Discussion

The results obtained show the increased serum concentrations of MMP-9 and TIMP-1 in patients with unstable AP. This result confirms the role MMP-9 and TIMP-1 play in CAAP destabilization and as predictive markers of CAAP phenotype. Similar results were obtained by other authors [5-8, 13-14]. Furthermore, we found a significant positive correlation between serum levels of MMP-9/TIMP-1 and the degree of carotid stenosis, which is consistent with the findings of some other researchers [4, 14-17].

TIMP regulates strictly the activity of MMP-9[17]. We noted a significant positive correlation between serum MMP-9 and TIMP-1. The increased level of serum TIMP-1 in parallel with the serum level of MMP-9 can be explained as a compensatory response aimed at containing the activity of MMP-9 and the progression of atherosclerosis [18]. MMPs and TIMPs levels in early post-MI period may serve as estimates of post-MI cardiac damage and remodeling. MMP-9 and TIMP-1 correlate with echocardiographic parameters of left ventricular (LV) dysfunction after acute MI and may identify patients at risk of subsequent LV remodeling and adverse prognosis [19]. However, some researchers have not found an association between MMP-9 and TIMP-1 [16]. The index of MMP-9/TIMP-1 is used to evaluate the balance between MMP-9 and its inhibitor. According to Cheng et al. [20], in healthy individuals this ratio was 0.11±0.03. In our study, this ratio was higher in all patients (0.18±0.009 in Group 1 and 0.24±0.03 in Group 2) than in the control group (0.15±0.02). In a patient with an embologenic fresh thrombus on the AP surface, this ratio increased even up to 0.47 (3 times higher than in the control group). These results indicate an imbalance towards MMP-9.

We found a significant negative correlation between IMT CCA and serum zinc, which corresponds to the results reported by other authors [21]. Existing data are contradictory regarding the negative or protective effects of zinc in the development of atherosclerosis [22-24]. In study of Stadler et al. [25], elevated levels of zinc (about 6-fold) were detected in advanced lesions compared to healthy tissue or early lesions. They found the highly-significant positive correlations between zinc and calcium in samples of carotid endarterectomy (r=0.895; *P=0.0001*). The reported protective effect of zinc accumulation is proposed to be associated with lesion calcification. It is known that highly fibrotic, calcified AP is less prone to rupture than lipid-rich, matrix-poor AP. In people with high levels of zinc, the decreased extent of cardiovascular complications may be due to calcium accumulation in a fibrous cap and hence the decreased propensity to AP ruptures.

Although there are many emerging biomarkers of inflammation and progression of atherosclerosis, their clinical utility remains unclear. Current evidence still favors the need for further investigation into the mechanisms through which these biomarkers may exert prognostic impact in patients with atherosclerotic lesions [26].

Conclusions

•High serum concentration of MMP-9 and TIMP-1, and MMP-9/TIMP-1 imbalance testify to CAAP instability.

•Increasing the serum concentration of MMP-9 is accompanied by decreasing the zinc level in AP.

•With the progression of atherosclerosis and increasing IMT-CCA, the level of zinc in serum and APs decreases

•Reducing the concentration of zinc in the hair is accompanied by a decrease in zinc level in AP specimens.

Acknowledgments

I thank the surgeons of the Tashkent Medical Academy especially to Drs. Ravshan D. Sunnatov and Abdurasul A. Yulbarisov for the provision of the artery samples. I am grateful to Prof. Abdumalik N. Aripov and Prof. Gulnora A. Rozikhodjaeva for the advice.

References

1. Newby AC. Metalloproteinases and vulnerable atherosclerotic plaques. Trends Cardiovasc Med 2007; 17:253–8.
2. Gaĭgalaĭte V, Ozheraĭtene V, Kalibatene D, Laurikenas K, Sabaliauskene Z. Association between structure of atherosclerotic plaques in carotid arteries and myocardial infarction. Kardiologiia 2013; 53(9):21-5. [Article in Russian].
3. Heo SH, Cho CH, Kim HO, Jo YH, Yoon KS, Lee JH, et al. Plaque rupture is a determinant of vascular events in carotid artery atherosclerotic disease: involvement of matrix metalloproteinases 2 and 9. J Clin Neurol 2011; 7(2):69-76.
4. Sapienza P, di Marzo L, Borrelli V, Sterpetti AV, Mingoli A, Cresti S, et al. Metalloproteinases and their inhibitors are markers of plaque instability. Surgery 2005;37(3):355-63.
5. Loftus IM, Naylor AR, Bell PR, Thompson MM. Plasma MMP-9 - a marker of carotid plaque instability. Eur J Vasc Endovasc Surg 2001;21(1):17-21.
6. Eldrup N, Grønholdt ML, Sillesen H, Nordestgaard BG. Elevated matrix metalloproteinase-9 associated with stroke or cardiovascular death in patients with carotid stenosis. Circulation 2006; 114(17):1847-54.
7. Zhou Z, Li X, Yang B, Jiang D. Relationship between lysophosphatidic acid and matrix metalloproteinase-9 plasma concentrations and carotid atheromatous plaque stability

in patients with cerebral infarction. J Int Med Res 2014; 42(3):669-676.
8. Silvello D, Narvaes LB, Albuquerque LC, Forgiarini LF, Meurer L, Martinelli NC, et al. Serum levels and polymorphisms of matrix metalloproteinases (MMPs) in carotid artery atherosclerosis: higher MMP-9 levels are associated with plaque vulnerability. Biomarkers 2014; 19(1):49-55.
9. Zitka O, Kukacka J, Krizkova S, Huska D, Adam V, Masarik M, et al. Matrix metalloproteinases. Curr Med Chem 2010;17(31):3751-68.
10. Jacobsen FE1, Lewis JA, Cohen SM. The design of inhibitors for medicinally relevant metalloproteins. ChemMedChem 2007; 2(2):152-71.
11. Lukacova V, Zhang Y, Mackov M, Baricic P, Raha S, Calvo JA, et al. Similarity of binding sites of human matrix metalloproteinases. J Biol Chem 2004; 279(14):14194-200.
12. Eby GA, Halcomb WW. High-dose zinc to terminate angina pectoris: a review and hypothesis for action by ICAM inhibition. Med Hypotheses 2006; 66(1):169-72.
13. Tan C, Liu Y, Li W, Deng F, Liu X, Wang X, et al. Associations of matrix metalloproteinase-9 and monocyte chemoattractant protein-1 concentrations with carotid atherosclerosis, based on measurements of plaque and intimaemedia thickness. Atherosclerosis 2014; 232(1):199-203.
14. Alvarez B, Ruiz C, Chacón P, Alvarez-Sabin J, Matas M. Serum values of metalloproteinase-2 and metalloproteinase-9 as related to unstable plaque and inflammatory cells in patients with greater than 70% carotid artery stenosis. J Vasc Surg 2004; 40(3):469-75.
15. Beaudeux JL, Giral P, Bruckert E, Bernard M, Foglietti MJ, Chapman MJ. Serum matrix metalloproteinase-3 and tissue inhibitor of metalloproteinases-1 as potential markers of carotid atherosclerosis in infraclinical hyperlipidemia. Atherosclerosis 2003; 169(1):139-46.
16. Gaubatz JW, Ballantyne CM, Wasserman BA, He M, Chambless LE, Boerwinkle E, et al. Association of circulating matrix metalloproteinases with carotid artery characteristics: the Atherosclerosis Risk in Communities Carotid MRI Study. Arterioscler Thromb Vasc Biol 2010; 30(5):1034–42.
17. Romero JR, Vasan RS, Beiser AS, Polak JF, Benjamin EJ, Wolf PA, et al. Association of carotid artery atherosclerosis with circulating biomarkers of extracellular matrix remodeling: the Framingham Offspring Study. J Stroke Cerebrovasc Dis 2008; 17(6):412-417.
18. Golovkin AS, Matveeva VG, Grigor'ev EV, Baĭrakova IuV, Shukevich DL, Velikanova EA, et al. Postoperative dynamic changes in matrix metalloproteinase levels in patients with coronary artery bypass graft procedure complications. Kardiologiia 2012; 52(9):4-7. [Article in Russian]
19. Kelly D, Khan SQ, Thompson M, Cockerill G, Ng LL, Samani N, et al. Plasma tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9: novel indicators of left ventricular remodelling and prognosis after acute myocardial infarction. Eur Heart J 2008; 29(17):2116-24.
20. Cheng M, Hashmi S, Mao X, Zeng QT. Relationships of adiponectin and matrix metalloproteinase-9 to tissue inhibitor of metalloproteinase-1 ratio with coronary plaque morphology in patients with acute coronary syndrome. Can J Cardiol 2008; 24(5):385-90.
21. Ari E, Kaya Y, Demir H, et al. The correlation of serum trace elements and heavy metals with carotid artery atherosclerosis in maintenance hemodialysis patients. Biol Trace Elem Res 2011; 144(1-3):351-9.
22. Beattie JH, Kwun IS. Is zinc deficiency a risk factor for atherosclerosis? Br J Nutr 2004; 91(2):177–81.
23. Hughes S, Samman S. The effect of zinc supplementation in humans on plasma lipids, antioxidant status and thrombogenesis. J Am Coll Nutr 2006; 25(4):285–91.
24. Vasto S, Mocchegiani E, Candore G, Listì F, Colonna-Romano G, Lio D, et al. Inflammation, genes and zinc in ageing and age-related diseases. Biogerontology 2006; 7(5-6):315–27.
25. Stadler N, Stanley N, Heeneman S, et al. Accumulation of zinc in human atherosclerotic lesions correlates with calcium levels but does not protect against protein oxidation. Arterioscler Thromb Vasc Biol 2008; 28(5):1024-30.
26. Doo Sun Sim, Youngkeun Ahn. Novel inflammatory biomarkers in acute coronary syndrome. Korean J Intern Med 2013; 28(2):156-8.

International Journal of BioMedicine 5(2) (2015) 65-70

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

Disturbances in Metabolism of Phenylalanine and Tyrosine as an Important Factor in the Etiology and Pathogenesis of Psychoneurological Disorders Associated with Liver Diseases

Vadim P. Komov[1]; Sergey E. Khalchitsky[2]; Michael V. Dubina[3]

[1] *Saint Petersburg State Chemical Pharmaceutical Academy*
[2] *Russian Research Center of Radiology and Surgical Technologies*
[3] *Saint Petersburg Academic University*
St. Petersburg, Russia

Abstract

Derangements of phenylalanine and tyrosine metabolism are an important factor in the etiology and pathogenesis of psychoneurological disorders. These disorders are particularly pronounced with monogenic hereditary diseases. In this work, we investigated similar disturbances in widespread and socially significant diseases – viral hepatitis and chronic alcoholism – which are accompanied by liver damage. We found serious derangements in the metabolism of phenylalanine, tyrosine and their derivatives with these diseases and concluded that such derangements make an essential contribution to the development of psychoneurological disorders in the studied pathology. In conclusion, this paper proposes recommendations for correction of such derangements and normalization of the patients' condition.

Keywords: *phenylalanine; tyrosine; liver pathology; psychoneurological disorders.*

Introduction

Phenylalanine (Phe) and tyrosine (Tyr) are the aromatic amino acids (AAA) that play an important role in neurotransmitter biosynthesis. Normally the concentration of phenylalanine in blood does not exceed 2 mg/100ml. When there are hereditary defects of the phenylalanine hydroxylase gene and other genes participating in Phe metabolism, there is a full or partial block of Phe transformation into tyrosine; thus, synthesized protein in whole or in part is not able to catalyze the transformation of Phe into Tyr. As a result, the concentration of Phe in the blood of patients with phenylketonuria (PKU)/ hyperphenylalaninemia (HPA) reaches up to 20 to 30 mg/100ml. These elevated concentrations lead to the activation of alternative ways of Phe metabolism and the formation of a number of products that are toxic to the organism, such as phenylpyruvic, phenyllactic, and phenylacetic acids. Toxic products of phenylalanine disintegration have a negative influence, primarily on the central nervous system (CNS). Patients with HPA have mental retardation, spasms, microcephaly, psychiatric disorders, etc. [1-4].

Except for hereditary disturbances of Phe metabolism, HPA often arises when various nonhereditary pathologies take place, in particular the diseases related to liver pathology. It is well known that the main enzyme of Phe biotransformation is phenylalanine hydroxylase (PAH) and that it is generally expressed in the liver; so any complicated diseases related to liver pathology can lead to disturbance of this enzyme functioning and, as a result, to HPA.

In the study of viral hepatitis (VH) and other liver damage, the special attention has been focused on the pathogenesis of hepatic encephalopathy (HE), the emergence mechanism which is still being discussed; but the majority of researchers do not consider the imbalance of AAA and nitrogen to be the main reason for HE emergence [5-7]. It should be noted that in 8% to15% of cases, the reason for HE emergence, accompanying acute hepatic failure (AHF), remains obscure, and the general lethality is 80% among patients who do not receive a liver transplant [8]. This

***Corresponding author:** Sergey E. Khalchitsky, PhD, Senior Research Scientist, Russian Research Center of Radiology and Surgical Technologies; St. Petersburg, Russia.*
E-mail: s_khalchitski@mail.ru

operation considerably reduces the intensity of HE symptoms (behavior and consciousness disorders, neuromuscular disorders etc.), ie, the pathology of metabolic processes in a liver is the trigger for HE emergence not only with VH but also with other hepatitides.

Disturbances of Phe, Tyr, and Trp metabolism are established in hereditary [9,10] as well as in exogenous liver damages [8,11]. The activity of enzymes participating in Tyr and Trp transformations is modified, which distorts the metabolism of catecholamines [12,13]. Genetically determined activity of phenylalanine, tyrosine, tryptophan hydroxylases and their cofactor tetrhydrobiopterin, as well as other enzymes of these amino acids' metabolism, determines various forms of VH progress and the development of psychoneurological disorders with hepatic insufficiency, depending on the genetic constitution of each patient. Among the variety of biochemical disturbances with alcoholism, pathology of biogenic amine metabolism plays a special role. A special emphasis is put on the disturbance of the synthesis and metabolism of AAA (Phe, Tyr, and Trp). A change in the metabolism of these acids leads to an imbalance in the CNS neurotransmitters: adrenaline, noradrenaline, dopamine, and serotonin. Derangements in the metabolism of these neurotransmitters are considered by S. Mukherjee et al. [14] to be a cause of mental and neurological disorders with alcoholism.

Currently, no consensus has been reached concerning the mechanism of alcohol influence on the metabolism of catecholamines. The available data are devoted generally to the studies of Tyr and Trp metabolism. Meanwhile, studying the PAH-system of alcoholics' livers is extremely important for understanding the pathogenetic mechanisms that disturb the metabolism of biogenic amines, and such study provides valuable information about the causes of psychiatric and behavioral disorders, as well as neurological disorders, with alcoholism. About 90% of the ethanol a person consumes is metabolized in the liver [15], which causes harsh disorders of metabolic processes in this organ with chronic alcoholism (CA) [16]. In the livers of patients suffering from alcoholism, necrotic patches appear and chronic hepatitis and cirrhosis are developed [15]. Similar morphological disturbances found in patients with viral hepatitis are accompanied by a decrease in the activity of the liver PAH-system [17]. Possibly with CA, phenylalanine-4-hydroxylase (EC 1.14.3.1.) activity, as it is the main component of the liver PAH system, also decreases because the Phe concentration in the blood of alcoholics increases [18]. However, no study has yet been carried out about the condition of the liver PAH- system and the metabolism of phenylalanine with CA in the acute period of alcoholic intoxication with expressed mental disturbances. We observed patients with liver diseases, such as VH and CA, in an effort to estimate Phe and Tyr metabolism and the influence of metabolic derangements on the development of psychoneurological disorders.

The aim of the this research was to find the severity of the Phe and Tyr metabolism disturbances and their influence on the genesis of psychoneurological disorders for patients suffering from VH and CA and to find out the dependence of these derangements on the severity of the pathological process.

Material and Methods

To analyze Phe and Tyr metabolism disturbances and to estimate the condition of the liver PAH-system at VH, we studied 80 patients aged from 16 to 70 years with an acute form of hepatitis A and B. The diagnosis was made on the basis of the detection of viral hepatitis markers (anti-HAV IgM, anti-HAV IgG for hepatitis A and anti-HBs, anti-HBe and anti-HBc for hepatitis B) using enzyme immunoassay. The severity of the disease course was estimated from the degree of intoxication, a hemorrhagic syndrome, and by laboratory criteria and other clinical indicators. In particular, cases considered as a mild form of the disease were without intoxication, but with small jaundice, serum bilirubin<85μmol/l, the prothrombin ratio (70-60%) within norm, and increased activity of transaminases by 5 to 10 times.

Clinical cases considered as moderate-to-severe forms of disease included the obvious symptoms of intoxication, an expressed jaundice, bilirubinemia within 85 to 200μmol/l, a low prothrombin ratio, and increased activity of transaminases by 10 to 15 times. The severe form of the disease included vividly expressed intoxication, obvious jaundice, manifestations of a hemorrhagic syndrome, the phenomena of AHF and HE symptoms, prothrombin ratio<60%, neutrophilic leukocytosis, and hypoalbuminemia. Out of 80 adult patients, a severe form of VH was detected in 42 patients, a moderate-to-severe form of VH in 28 patients, and a mild form of VH in 10 patients.

We determined the serum concentration of Phe, Tyr, epinephrine, norepinephrine, and serotonin, as well as the urinary excretion of phenylpyruvic acid (PPA), ortho-hydroxyphenylacetic acid (OHPAA), homogentisic acid (HGA), epinephrine, and norepinephrine. All patients underwent blood and urine tests at the peak of the disease, at the regression of clinical manifestations, and during recovery. The serum level of Phe was determined by the fluorimetric method [17] with using a 5•10-3 M solution of L-leucyl-L-alanine. The concentration of tyrosine was also determined by the fluorimetric method [17], and the level of PPA in urine was carried out according to I.A. Bulycheva et al. [19].

To estimate the activity of the liver PAH system, 16 patients (12 with a severe form and 4 with a moderate-to-severe form of VH) were selected for oral administration of Phe in a dose of 0.1 g/kg. The control group consisted of 20 healthy people aged from 17 to 35 years. The peroral loading test was carried out on an empty stomach; the blood test was carried out before the loading and after 1, 2, 4 and 6 hours. Blood samples were taken from an elbow vein. Serum levels of Phe, Tyr, and catecholamines were determined. Excretion of PPA, OHPAA, HGA, epinephrine, and norepinephrine in daily urine were measured before, during, and after 24 hours of Phe load.

To estimate PAH activity, a direct determination of enzyme activity in the liver bioptates of 5 VH patients was carried out. Bioptates (mass from 21 to 159 mg) were quickly processed; PAH activity was defined in supernatant liquid [17] using tetrahydrobiopterin (2-amino-4-hydroxy-6,7-dimethyl-tetrahydrobiopterin) (BH4) as a cofactor. The control group

consisted of 11 people aged 25 to 30 years without liver pathology.

For identification of biochemical disturbances in the metabolism of Phe and Tyr, 34 CA patients, males aged between 25 and 48 years, were examined. Among these patients, 12 men were in their first day of hospitalization with the evident alcoholic abstinence syndrome; the remaining patients had undergone at least a 30-day treatment with complete abstinence from alcohol. The vast majority of patients had stage 2 alcoholism. The stages of alcoholism were estimated according to Goffman's classification [20]. The control group consisted of 20 healthy volunteers, males aged 21 to 35 years.

For identification of mutations in the PAH gene, 156 CA patients (120 men and 36 women between 25 and 70 years) were examined. Alcoholism in stages 2 and 3, complicated with psychoneurological disorders and cases of delirium tremens, was marked in the anamnesis. The control group consisted of 417 volunteers of the same age.

DNA was extracted from peripheral blood leukocytes using "DNA-sorb" diagnostic sets produced by the DNA-technology company (Russia) according to the manufacturer's protocol. The R408W mutation leading to a sharp decrease in PAH activity was analyzed by PCR/RFLP. A PKU-408 diagnostic set produced by the Center for Molecular Genetics (Russia) was used.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki. It was approved by the local Ethics Committees. Written informed consent was obtained from all participants.

Results were statistically processed using the software package Statistica 6.0. Group comparisons with respect to categorical variables were performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5.

Result and Discussion

Table 1 shows the serum levels of Phe and Tyr as well as concentrations of PPA, epinephrine, and norepinephrine in the urine of VH patients. These data demonstrate an increase in serum Phe level, which is accompanied by increased urinary excretion of PPA. The increase of serum Phe concentration depends directly on the severity of the pathological process.

Table 2 shows the results of Phe load in VH patients with the severe form of disease at the peak of manifestation. The results of Phe load in the patients with VH and AHF are close to the changes observed in patients who are heterozygous for the R408W mutation, which is characterized by a decrease in PAH activity. Therefore, it is reasonable to assume that psychoneurological symptoms in VH patients, to a certain extent, may be caused by disturbances in Phe and Tyr metabolism and accumulation of toxic metabolites as a result of reduction in PAH activity (Table 3).

Phe load revealed a reduction in hepatic PAH activity in VH patients. The biochemical disturbances in Phe metabolism found with VH were also characteristic for PKU patients. Our data are consistent with the results received for Phe load in the heterozygous carriers of the R408W mutation, when depression of the PAH system is genetically caused [21].

Before Phe load, the elevated serum content of amino acids and catecholamines was associated with the severity of VH. After Phe load, the serum level of amino acid increased in all patients; 6 hours after loading, the serum level of this acid also exceeded the initial level. The increase in serum Tyr concentration was rather small. Urinary excretion of epinephrine and norepinephrine did not change significantly.

For the proof of disturbance of PAH activity in the liver, we determined the enzyme activity in liver bioptates of 5 VH patients (Table 3) and found that the hepatic PAH activity was reduced by various degrees. PAH activity in the liver of patients with a mild form of VH at the peak of the disease and in the regression stage (patients #1 and #4; Table 3) was from 55.2 to 50.0 micromoles of Tyr/g protein/hour, respectively, and 18.8, 23.6,30.6 (mean 24.3±3.4 micromoles of Tyr/g protein/hour) (patients #2, #3 and #5; Table 3) in patients with a moderate-to-severe form VH at the peak of disease and in the regression stage, respectively. The serum Phe level also increased in these patients. It is interesting to note that the enzyme activity was reduced more in patients with a high serum Phe level. Furthermore, the more severe form of the disease was accompanied by a pronounced decrease in PAH activity. Thus, the direct estimation of enzyme activity in the livers of patients with various clinical manifestations showed that the severity of disease is associated with the severity of disorders in the metabolism of Phe and Tyr.

Table 1. Concentration of Phe and Tyr in the blood serum; PPA, epinephrine and norepinephrine in daily urine of VH patients

Group	Stage of disease	Phe	Tyr	PPA	Epinephrine	Norepinephrine
		(mg/100 ml)		(mg)	(μg)	
Moderate-to-severe form of VH	Peak of disease	2.9±0.53	1.8±0.18	108.0±13.5	7.8±0.70	37.0±9.6
	P	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.4*
	Regression stage	2.1±0.36	1.7±0.20	40.0±8.2	6.5±0.34	44.7±2.8
	P	*<0.04*	*<0.01*	*<0.01*	*<0.05*	*<0.01*
Severe form of VH	Peak of disease	4.5±0.55	2.5±0.17	160±15.6	8.8±0.85	48.2±12.8
	P	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*<0.2*
	Regression stage	2.3±0.16	1.7±0.16	65.0±9.35	6.9±0.49	45.0±3.7
	P	*<0.05*	*<0.01*	*<0.01*	*<0.05*	*<0.01*
Control group		1.8±0.17	1.14±0.03	0	5.2±0.71	28.8±4.48

P - vs. control group

Table 2.

Concentration (mg/100ml) of Phe and Tyr in the blood serum of VH patients with the severe form of disease after Phe load

Time after Phe load (hours)	Control		Patients	
	Phe	Tyr	Phe	Tyr
0	1.8±0.17	1.14±0.003	3.86±0.65	1.97±0.193
P			*<0.01*	*<0.01*
1	10.7±0.88	1.53±0.12	10.35±1.05	2.08±0.19
P			*<0.8*	*<0.02*
2	6.6±1.66	1.61±0.12	9.52±0.80	2.14±0.22
P			*<0.2*	*<0.05*
4	2.2±0.17	1.65±0.29	8.4±0.86	2.17±0.18
P			*<0.01*	*<0.02*
6	1.7±0.15	1.51±0.13	7.1±0.67	2.23±0.15
P			*<0.01*	*<0.01*

Table 3.

Liver PAH activity (µmol of Tyr/g protein/hour), the levels of Phe and Tyr in the blood serum (mg/100 ml), and PPA excretion in daily urine (mg/100 ml) in VH patients

Patients	Age	Severity of disease	Stage of disease	Phe	Tyr	PPA	Liver PAH activity	
							+cofactor	-cofactor
1. S.V.	42	Mild	Peak	2.0	0.89	0.2	55.2	-
2. C.G.	47	Moderate	Peak	3.1	1.52	5.3	23.6	2.6
3. S.E.	67	Moderate	Peak	3.5	2.4	2.8	18.8	2.0
4. S.A.	26	Mild	Regression	2.8	1.16	1.3	50.0	-
5. P.V.	44	Moderate	Peak	3.0	1.23	0.5	30.6	-

Investigating PAH activity, we established that the activity of the enzyme makes 0.7% to 13% and even less to total absence in PKU patients [22], 43% for heterozygous carriers [23], and 44% for VH patients in comparison with data for people without any liver pathology.

Comparing data of Phe load with data for PAH activity in livers of VH patients; it is possible to conclude that the disturbance in reaction of Phe hydroxylation with VH is caused by the decrease in PAH activity in the liver. Inasmuch as PAH activity is normalized ambiguously with various forms of VH during the recovery period, this activity can serve as one of the indicators of the existence and intensity the pathological process, as well as the efficiency of treatment.

The insufficiency of liver function in AAA metabolism may be considered as the main factor among the major factors responsible for HE [5,8], which causes an imbalance of biogenic amines that, penetrating through the hematoencephalic barrier, disturb the function of neurons. This disturbance leads to the emergence of mental and neurologic disorders [8].

The study of catecholamine metabolism with liver disease showed the role of "false mediators" in the development of hepatic insufficiency. It is believed that impairment of consciousness among patients with hepatic insufficiency is associated with a reduction in the formation of neurochemical transmitters, such as norepinephrine and dopamine. Studies of monoamines in the brains of patients who died from hepatic coma have revealed a decrease in the level of dopamine and an increase in the concentration of serotonin in all areas of brain [8].

With VH, an increase in the activity of bacterial amino acid decarboxylases causes the formation of substances like tyramine and octopamine with a structure similar to norepinephrine; consequently, these substances can collect in the nervous system, replacing norepinephrine. These false mediators disturb the neurochemical transfer in the brain and cause the HE syndrome. The introduction of DOPA, which is the predecessor of norepinephrine and easily penetrates into the brain, causes a strengthened formation of norepinephrine in the brain and replaces the false mediators.

The accumulation of substances relating to phenolic compounds plays an important role in AHF pathogenesis. It has been found that a toxic effect is produced by free phenol and AAA, as well as by their metabolites – the phenolic compounds that were increased in the blood, cerebrospinal fluid, and urine of patients with the severe forms of VH and hepatic coma [8].

Direct determination of PAH activity in liver bioptates of VH patients has shown a decrease in enzyme activity. Currently, more than 500 various mutations in the PAH gene have been discovered, and the frequency of the mutant alleles is 1:50 in the general population [24]. The frequency of complications of AHF with hepatic coma is 1:40 to 1:100 in patients. It is quite probable that AHF and coma with VH develop in patients who are heterozygous for PAH gene mutation, while the homozygous patients for the wild-type gene undergo VH in mild forms or act as virus carriers.

All these data were typical for our patients. The severity of psychoneurological symptoms (emotional instability, dizziness, the slow thinking, disorientation, tremors of hands, the confused consciousness, and psychomotor excitement) was correlated with the severity of phenylalanine and tyrosine metabolism disorders, and the severity decreased with normalization of these indicators.

Thus, the data we obtained confirm disturbances of AAA metabolism and a decrease in PAH activity by 44% in VH patients with the moderate-to-severe and severe forms of disease. These data also reveal disturbances of hydroxylation of Phe to Tyr. PAH-system dysfunction is the trigger for development of Tyr and Trp metabolism disorders that are accompanied by HE during development of AHF. Inasmuch as PAH activity is normalized ambiguously with various forms of VH during the recovery period, this activity can serve as one of the indicators of the existence and intensity the pathological process, as well as the efficiency of treatment. In this state, the inclusion of tetrahydrobiopterin in the treatment scheme is appropriate since it leads to the normalization of AAA metabolism.

Table 4 shows the serum levels of Phe and Tyr and their metabolites in urine of patients suffering from alcoholism and in those of the control group. It can be seen that, in comparison with the control group, patients suffering from alcoholism had an increased serum Phe level, while the Tyr level was decreased.

The changes in Phe/Tyr ratio are especially vivid. The most expressed disturbances in the content of blood amino

acids were revealed in patients with the abstinence syndrome. As for the patients in the remission period, despite their abstention from alcohol for a month or more, when practically all clinical indicators are normalized, the Phe level remained elevated, while Tyr content was decreased, in comparison with the control group. The urine test showed that the Phe and Tyr metabolism of patients with alcoholism was drastically changed. Disturbances of Phe metabolism in patients with the alcoholic abstinence syndrome lead to a pathological increase in PPA excretion, which is absent in the urine of healthy people and patients with alcoholism in the remission stage. The excessive urinary excretion of Phe was detected both in the abstinence syndrome and in the remission stage. Decreased excretion of HGA, a metabolite of Tyr, indicates the disturbances in the metabolism of Tyr.

Table 4.

Serum levels of Phe and Tyr, Phe/Tyr ratio and excretion of Phe, PPA and HGA in daily urine of patients with chronic alcoholism (mg/100ml)

Study samples	Indicator	Patients with chronic alcoholism		Control
		abstinence syndrome	remission stage	
Serum	Phe	2.43±0.16*	1.96±0.05	1.80±0.17
	Tyr	0.96±0.09*	0.83±0.018*	1.14±0.03
	Phe/Tyr	2.53	2.36	1.58
Urine	PPA	5.7±0.6*	0	0
	HGA	8.2±0.6*	8.6±1.47	10.4±0.03
	Phe	2.83±0.16*	2.1±0.11	1.9±0.23

**P <0.01 vs. control*

The disorders in Phe metabolism found with alcoholism are similar to the disorders with VH, and also to the results received at Phe load in heterozygous carriers for the R408W mutation of the PAH gene, which is associated with genetically determined reduction in PAH activity (PAH activity was less than 50% in comparison with the healthy people).

Especially pronounced metabolic disorders in Phe and Tyr metabolism have been found in patients with abstinence syndrome. In these patients, serum Phe concentration was much higher, and the level of Tyr was lower, in comparison with the control group. The urinary excretion of PPA was increased, and the HGA excretion was decreased, which showed the disturbance of Tyr metabolism with chronic alcoholism.

The data obtained allow us to assume that there is an interrelation between the severity of mental disturbances associated with alcoholic intoxication and the severity of PAH-system disorders as well as Phe and Tyr metabolism distortion. The decrease in the activity of the liver PAH-system can lead to accumulation of Phe and its pathological derivatives in the brain tissues, which causes disorders of biogenic amine metabolism in CNS.

We also assumed that patients with CA, especially those who had *delirium tremens* in the anamnesis, may have mutations in the PAH gene, ie, these patients may be heterozygous carriers of the R408W mutation. For this purpose, the group of patients with alcoholism (stages 2 and 3) was tested for R408W mutation typical for PKU.

According to several studies, the average frequency of the heterozygous carriage of PKU in various populations is 1:40 to 1:200. When we examined the control group for the R408W mutation, which is typical for 65% to 70% of PKU patients, our experimental data have been consistent with data of other researchers; ie, the frequency of PKU heterozygous carriage of the R408W mutation was 2.39%.

In patients with CA, the frequency of heterozygous carriage of the R408W mutation was much higher: Among 156 patients with CA, 11 people had a heterozygous state of 7.05% (*P<0.02*).

Because the number of PKU heterozygous carriers was clearly higher among patients with CA, we assume that the disturbance of the hepatic PAH activity contributes to the development of alcoholic illness and *delirium tremens*. One of the possible mechanisms for such a phenomenon is the congenital disorder in metabolism of CNS neurotransmitters in PKU heterozygous carriers, which manifests in psychological discomfort, emotional instability, and a tendency toward deviant behavior. Alcohol intake, on the one hand, temporarily softens such symptomology, and on the other, accelerates the process of alcoholization and the phenomena of mental and physical dependence, and leads further to a heavier somatic and psychoneurological frustration in this group of patients.

Competing interests

The authors declare that they have no competing interests.

References

1. Brumm VL, Bilder D, Waisbren SE. Psychiatric symptoms and disorders in phenylketonuria. Mol Genet Metab 2010; 99 Suppl 1:S59-63.
2. Huttenlocher PR. The neuropathology of phenylketonuria: human and animal studies. Eur J Pediatr 2000; 159 Suppl 2:S102–6.
3. Moyle JJ, Fox AM, Arthur M, Bynevelt M, Burnett JR. Meta-analysis of neuropsychological symptoms of adolescents and adults with PKU. Neuropsychol Rev 2007; 17(2):91–101.
4. Perez-Duenas B, Pujol J, Soriano-Mas C, Ortiz H, Artuch R, Vilaseca MA, et al. Global and regional volume changes in the brains of patients with phenylketonuria. Neurology 2006; 66(7):1074–8.
5. Cash WJ, McConville P, McDermott E, McCormick PA, Callender ME, McDougall NI. Current concepts in the assessment and treatment of hepatic encephalopathy. Q J Med 2010; 103(1):9–16.
6. Dejong CH, van de Poll MC, Soeters PB, Jalan R, Olde Damink SW. Aromatic amino acid metabolism during liver failure. J Nutr 2007;137(6 Suppl 1):1579S-1585S.
7. Toris GT, Bikis CN, Tsouroufl is GS, Theocharis SE. Hepatic encephalopathy: an updated approach from pathogenesis to treatment. Med Sci Monit 2011; 17(2):RA53-63.
8. Ivashkin VT. *Liver and Biliary System Diseases.* Manual for doctors. M.: Publishing house «M-Vesti», 2005. [Manual in Russian].
9. Maier KP. Hepatitis – Hepatitisfolgen. Praxis der Diagnostik, Therapie und Prophylaxe akuter und chronischer Lebererkrankungen. Hans Huber Verlag, 6. Aufl. 2010. [Book in German].

10. Shaposhnikov AM, Khal'chitskiĭ SE, Shvarts EI. Disorders of phenylalanine and tyrosine metabolism in Down's syndrome. Vopr Med Khim 1979; 25(1):15-9.[Article in Russian]
11. Dooley JS, Lok A, Burroughs AK, Heathcote J, editors. Sherlock's Diseases of the Liver and Biliary System. Wiley-Blackwell 12th Edition; 2011.
12. Hörtnagl H, Lochs H, Kleinberger G, Hackl JM, Hammerle AF, Binder H, Wewalka F. Plasma catecholamines in hepatic coma and liver cirrhosis: role of octopamine. Klin Wochenschr 1981; 59(20):1159-64.
13. Krauns P, Ruge W. Plasma catecholamine levels in liver disease. Z Gastroenterol 1985; 23(2):64-73. [Article in German].
14. Mukherjee S, Das SK, Vaidyanathan K, Vasudevan DM. Consequences of alcohol consumption on neurotransmitters – an overview. Curr Neurovasc Res 2008; 5(4):266-72.
15. Sherlock S. Alcoholic liver disease. Lancet 1995; 345(8944):227-9.
16. Ehomoto N., Takase S., Takada N, Takada A. Alcoholic liver disease in heterozygotes of mutant and normal aldehyde dehydrogenase-2 genes. Hepatology 1991; 13(6):1071-5.
17. Shaposhnikov AM, Khal'chitskiĭ S.E. Patochemistry of phenylalanine, tyrosine, tryptophan metabolism and activity of liver phenylalanine hydroxylase in viral hepatitis. Natur Tech Science 2007; 2: 137-154 [Article in Russian].
18. Mukherjee S, Vaidyanathan K, Vasudevan DM, Das SK. Role of plasma amino acids and GABA in alcoholic and non-alcoholic fatty liver disease-a pilot study. Indian J Clin Biochem 2010; 25(1):37-42.
19. Bulycheva I.A., Khalchitsky S.E. Diagnostic importance of phenylpyruvic acid definition in urine in alcoholic disease. Medical sciences 2010; 2:40-49 [Article in Russian].
20. Gofman AG. *Clinical Narcology*. Moscow: Miclosh, 2003, 23-34 [Book in Russian].
21. Westwood A and Raine DN. Heterozygote detection in phenylketonuria. Measurement of discriminatory ability and interpretation of the phenylalanine loading test by determination of the heterozygote likelihood ratio. J Med Genet 1975; 12(4):327–333.
22. Shaposhnikov AM, Barashnev IuI, Khal'chitskiĭ SE, Korneĭchuk VV, Okat'ev VS. Phenylalanine hydroxylase activity in the liver in children with the classic form of phenylketonuria._Vopr Okhr Materin Det 1978;23(6):42-7. [Article in Russian]
23. Shaposhnikov AM. *B*iochemical manifestations of genetic heterogeneity in human hereditary enzymopathy. Diss Doctor Med *Sci*. Leningrad, 1975. [Dissertation in Russian].
24. Williams RA, Mamotte CD, Burnett JR. Phenylketonuria: an inborn error of phenylalanine metabolism. Clin Biochem Rev 2008; 29(1):31–41.

International Journal of BioMedicine 5(2) (2015) 71-75

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

Approach to Diagnosis and Treatment of Allergy to *Alternaria alternata* in Patients with Chronic Obstructive Pulmonary Disease and Perennial Allergic Rhinitis

Alexander P. Nazarenko*; G.I. Nazarenko; A.G. Kuznetsov

The P.L. Shupyk National Medical Academy of Postgraduate Education (NMAPE);
Clinic of Immunology and Allergology "Forpost"
Kyiv, Ukraine

Abstract

The aim of our study was to determine the degree of sensitization to *Alternaria alternata* in patients with chronic obstructive pulmonary disease (COPD) and perennial allergic rhinitis (PAR), and evaluate the effectiveness of allergen-specific immunotherapy (SIT) in patients sensitized to *Alternaria alternata*.

Material and Methods: All patients were divided into two groups. Group 1 included 130 patients (53 women and 77 men) with COPD aged from 24 to 50 years. Group 2 included 162 patients (90 women and 72 men) with PAR aged from 15 to 46 years. The allergen-specific IgE to fungi of the genera: *Alternaria alternata, Aspergillus fumigatus, and Penicillinum notatum*, as well as the allergen-specific serum IgG antibodies to Alt a 1 of *Alternaria alternata* were determined. To perform subcutaneous SIT, we used purified major Alt a 1 allergen of *Alternaria alternate.*

Results: Our study showed that specific IgE antibodies to Alt a 1 were found in 38% and 44.6% patients with COPD and PAR, respectively. SIT induced the IgG response against Alt a 1. The concentration of specific IgG antibodies to Alt a 1 increased approximately 8-fold in COPD patients and 15-fold in PAR patients after 8 months of treatment.

Keywords: *Chronic obstructive pulmonary disease (COPD); perennial allergic rhinitis (PAR); Alternaria alternate; allergen-specific immunotherapy (SIT).*

Introduction

Respiratory allergies are increasing worldwide. People are exposed to aeroallergens in various settings, both at home and at work. Fungi are ubiquitous airborne allergens and are important causes of human diseases, especially in the upper and lower respiratory tracts. It is estimated that approximately 2-6% of the general population in developed countries is allergic to fungi [1]. Mostly sensitivity is detected to genera of *Alternaria alternata, Cladosporium herbarum, Aspergillus fumigatus, Penicillium spp and Fusarium. Alternaria alternata* is one of the most important allergenic molds found in Europe [2]. *Alternaria alternata* is one of the most common outdoor molds, but also has been found in the indoor environment. Dampness and mold problems have been reported to occur in 20% to 50% of modern homes [3-5].

Alternaria alternata is known to be a problem in allergic disease. Studies have shown that up to 70 % of mold-allergic patients have skin test reactivity to Alternaria *alternata*. Allergy to fungi often appears as type I immediate, IgE-mediated hypersensitivity, which manifests various allergic diseases, such as bronchial asthma, most types of sinusitis, allergic rhinitis, and pollinosis. Many studies showed that increased serum total IgE is also a sensitive marker for chronic obstructive pulmonary disease (COPD) patients with higher smoking index, longer duration of illness, more severe lung function impairment [6-9]. COPD is characterized by persistent airflow limitation, and is a major cause of morbidity and mortality worldwide. COPD is a heterogeneous disease and can be classified into different "phenotypes" [10]. A recent study of Jamieson et al. [11] showed that there was an

***Corresponding author:** Alexander P. Nazarenko, Director of Clinic of Immunology and Allergology "Forpost"; Kyiv, Ukraine. E-mail: director@forpost.ua*

"allergic phenotype" of COPD, which accounted for 21% or 30% by allergy history (doctor-diagnosed hay fever or allergic symptoms) or allergy testing (increased allergen-specific IgE) in two cohorts.

A high level of IgE antibodies is one of the risk factors for development of COPD according to Standards for the diagnosis and treatment of patients with COPD (Table 1) [12].

Table 1. Risk factors for chronic obstructive pulmonary disease

Host factors	Exposures
Genetic factors Sex Airway hyperreactivity, IgE and asthma	Smoking Socio-economic status Occupation Environmental pollution Perinatal events and childhood illness Recurrent bronchopulmonary infections Diet

Study of G. Rohde et al. [13] showed elevated IgE antibodies directed against *S. aureus enterotoxins* (SAE) in the serum of patients with COPD. Production of IgE to SAE is of non-atopic origin, but rather reflects the superantigen activity on B- and T-cells. This data indicate an immunological reaction to superantigens as a possible trigger of chronic inflammation in COPD, which needs further study.

In addition, sensitization to molds is also a reason for development of perennial allergic rhinitis (PAR) [3,5,14]. Therefore, the investigation of sensitization to infectious agents including molds is the actual problem in the study of the pathogenesis of COPD and PAR.

The use of allergen-specific immunotherapy (SIT) to treat the mold sensitized patients one of the most debated aspects. The use of purified protein allergens takes a particular place, which implies determination of their components and evaluation immunotherapy by determining the specific antibodies to the purified allergens.

In this regard, the aim of our study was to determine the degree of sensitization to *Alternaria alternata* in patients with COPD and PAR, and evaluate the effectiveness of SIT in patients sensitized to *Alternaria alternata*.

Material and Methods

All patients were divided into two groups. Group 1 included 130 patients (53 women and 77 men) with COPD aged from 24 to 50 years. The differential diagnosis of COPD was performed according to standard criteria [14]. Group 2 included 162 patients (90 women and 72 men) with PAR aged from 15 to 46 years. PAR diagnosis was verified according to standards of diagnosis based on the clinical manifestation of the disease, allergic anamnesis, and data of the quantitative level of allergen-specific IgE. The control group constituted 20 healthy, age-matched, randomly selected persons.

The study samples were the blood serum of patients. The allergen-specific IgE to fungi of the genera: *Alternaria alternata, Aspergillus fumigatus, and Penicillinum notatum*, as well as the allergen-specific serum IgG antibodies to the protein Alt a 1 of *Alternaria alternata* were determined. The investigation of allergen-specific serum IgE and IgG antibodies was performed by immunofluorescence method using ImmunoCAP system («Phadia AB», Sweden).

To perform *subcutaneous* SIT, we used purified major Alt a 1 allergen of *Alternaria alternata* of «Diater» (Spain) production. The injections were performed in the presence a physician and in clinic that was equipped to manage possible life-threatening reactions. An assessment of the patient's current health status was made before the administration of immunotherapy injections to determine whether there have been any recent changes in the patient's health that may require modifying or withholding treatment (e.g., exacerbation of allergy symptoms). The *subcutaneous* injections of an allergen-containing extract were carried out according to the scheme.

During the build-up phase, the injections were performed weekly during the month:

Concentration #1: 0.1ml, then 0.2ml after 30 minutes;

Concentration #2: 0.4ml, then 0.4ml after 30 minutes;

Concentration #3: 0.1ml, then 0.2ml after 30 minutes;

Concentration #4: 0.4 ml and 0.4 ml after 30 minutes or 0.8ml in one stage.

During the maintenance phase, the injection of 0.8 ml of allergen-containing extract was performed monthly during 7 months; the total number of injections was 8. The treatment continued for a period of 8 months.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki. It was approved by the local Ethics Committees. Written informed consent was obtained from all participants.

Results were statistically processed using the *software* package Statistica 6.1 for Windows. The mean (M) and standard error of the mean (SEM) were deduced. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. *P* values of <0.05 were considered statistically significant.

Results and Discussion

We found the increased serum total IgE in COPD/PAR patients: 68.9±11.4/185.4±28.3 kU/l vs. 16.4±5.7 kU/l in the control group ($P<0.01$). The presence of specific IgE antibodies to fungal allergens was detected in 42.2% of COPD patients. The sensitization to *Alternaria alternata* and *Aspergillus fumigatus* was detected most frequently (Fig.1).

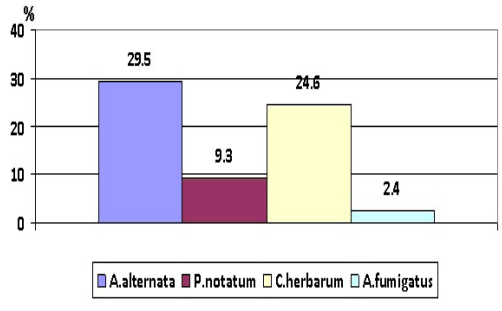


Fig.1. *The frequency of allergen specific IgE antibodies to molds in COPD patients*

The next step in our study was to determine the serum level of allergen-specific IgE antibodies in PAR patients. The analysis of the spectrum of sensitization in PAR patients showed that the sensitization to the indoor allergens, especially to dust mites and epidermal allergens of pets was identified most frequently. In particular, sensitization to the indoor allergens was detected in 75.6% of patients, among which the dust mites were dominated; sensitization to fungal allergens was detected in 24.4% of patients. Mostly sensitivity was detected to genera of *Alternaria alternate* (60%), *Penicillium notatum* (8%), Cladosporium herbarum (13%), and *Aspergillus fumigates* (19%), Fig.2.

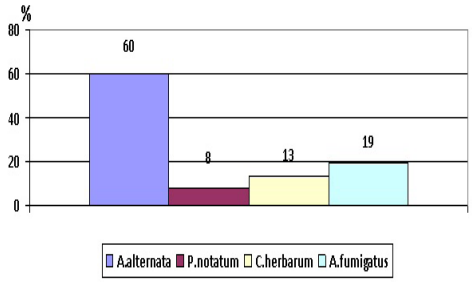


Fig. 2. *The frequency of allergen specific IgE antibodies to molds in PAR patients*

Because of a high percentage of sensitization to molds, SIT was conducted in COPD/PAR patients sensitized to *Alternaria alternata*. To determine the genuine sensitization to *Alternaria alternata*, we identified specific IgE antibodies to Alt a 1, the major *Alternaria* allergen. Currently, total of 11 allergenic extracts of *Alternaria alternata* are identified, although only one of them is the most common. Alt a 1 causes the production of specific IgE antibodies in more than 90% of *Alternaria alternata* - sensitized patients [2,4,15]. Our study showed that specific IgE antibodies to Alt a 1 were found in 38% of patients with COPD; the genuine sensitization to *Alternaria alternata* was not confirmed in 4.2% of cases, and levels of specific IgE antibodies to Alt a 1 were within normal limits.

Studies suggest that before starting SIT need to identify a causative protein that causes allergic reactions and, accordingly, determine the presence of sensitization to the major allergens and exclude the minor components which cause cross-reactivity.

All patients sensitized to Alt a 1 underwent SIT with using a highly purified Alt a 1 produced by «Diater» (Spain). The treatment continued for a period of 8 months. Currently in Europe, the quality of commercial fungal extracts is unstable. In this regard, the purified mold allergens have a great interest, since the purified allergens can be produced in the appropriate conditions of purity and stability for each batch and, therefore, can be a fully standardized diagnostic material. Control and effectiveness of the treatment was determined by assessing the patient's condition. The presence of chronic cough and dyspnea on exertion in COPD patients, as well as nasal congestion and rhinorrhea in PAR patients were registered. In addition, the serum levels of specific anti- Alt a 1 IgE and IgG antibodies were monitored. As is known, the specific IgG antibodies block the development of allergic reactions, and, according to recent studies, the blocking antibodies appear in form of IgG4 and IgG1-antibodies [15].

Figure 3 shows the results obtained in patients of Group 1. At the end of the 1st month of treatment, we observed a slight improvement in clinical status of patients (Fig.3a). Chronic cough decreased only in 1% of cases, and dyspnea on exertion in 3% of cases. However, at the end of the 4th month of SIT, chronic cough already mentioned only in 62% of patients, which was 25% less than its initial value. Dyspnea on exertion was observed in 36% of patients, which was 17% less than its initial value. At the end of therapy, morning cough persisted in only 17% of patients, dyspnea on exertion in 12% of patients.

The serum level of specific IgE antibodies continued to increase by 6% (on average) per month until the end of five months of treatment (Fig.3b). Thus, before SIT, the serum level of specific IgE antibodies to Alt a 1 was 85.4±4.9 kU/l in COPD patients, and at the end of 5 months of treatment it increased up to 110±13.2 kU/l *(P<0.01)*. Before SIT, the serum level of specific IgG antibodies to Alt a1 was 2.1±0.2kU/l, and at the end of 5 months of treatment it increased by almost 3 times and reached 5.6±1.2 kU/l. At the end of 6 months of treatment, we observed a decrease in the level of specific IgE antibodies, and his level was 26.7±8.2 kU/l *(P<0.01)* to SIT completion, while the level of specific IgG antibodies continued to rise, and at the end of treatment it was 15.9±4.3kU/l *(P<0.01)*, which was about 8 times higher as compared with baseline values (Fig.3c).

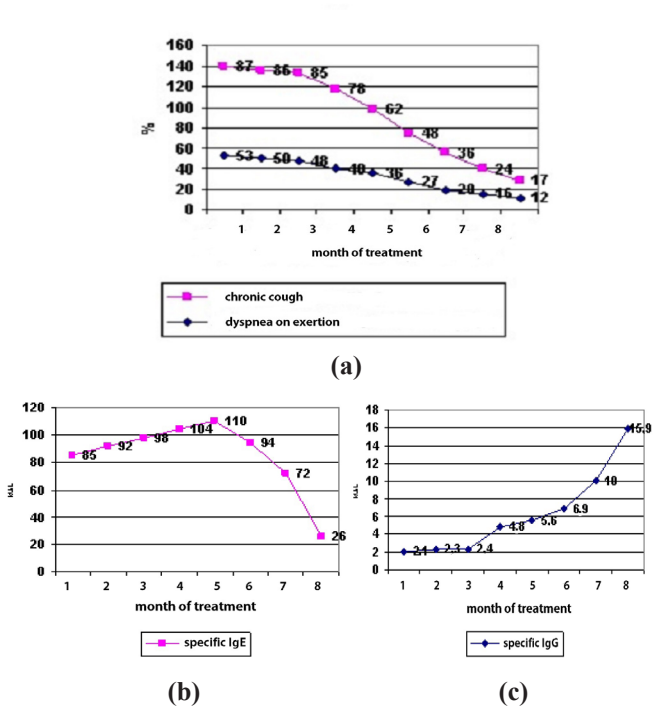


Fig.3. *Dynamics of clinical manifestations (a), the level of specific IgE (b) and IgG (c) antibodies to Alt a 1 during SIT in COPD patients*

Specific IgE antibodies to Alt a 1 were found in 44.6% of Group 2 patients; the genuine sensitization to *Alternaria*

alternata was not confirmed in 5.4% of cases. Control and effectiveness of the treatment was determined by assessing the clinical manifestation and the serum levels of specific anti-Alt a1 IgE and IgG antibodies were also monitored. Figure 4 shows the results obtained in patients of Group 2. At the end of the 3rd month of SIT, nasal congestion remained in 52% of PAR patients, which was 32% less than its initial value; rhinorrhea decreased to 25%, which was 30% less than its initial value (Fig.4a). During the next three months of treatment, we have defined a significant reduction in complaints. After 6 months of treatment, nasal congestion and rhinorrhea persisted only in 20% and 10% of PAR patients, respectively. At the end of SIT, number of patients with signs of clinical improvement continued to increase. At the end of the 8th month of treatment, nasal congestion persisted only in 15% of patients and was less pronounced; the less pronounced rhinorrhea was only in 5% of patients.

The serum level of specific IgE antibodies to Alt a 1 significantly increased before the end of the 5th month of SIT (Fig.4b); at the same time the level of specific IgG antibodies has changed a little in this period of observation. At the end of the 5th month of SIT, it was noted an increase in the level of specific IgG antibodies to Alt a 1, which was associated with the reduced level of specific IgE antibodies (Fig.4c). At the end of treatment, the level of specific IgG antibodies was 29.7±8.2 kU/l *(P<0.01)*, while the level of specific IgE antibodies decreased to 58±12.4 kU/l *(P<0.01)*.

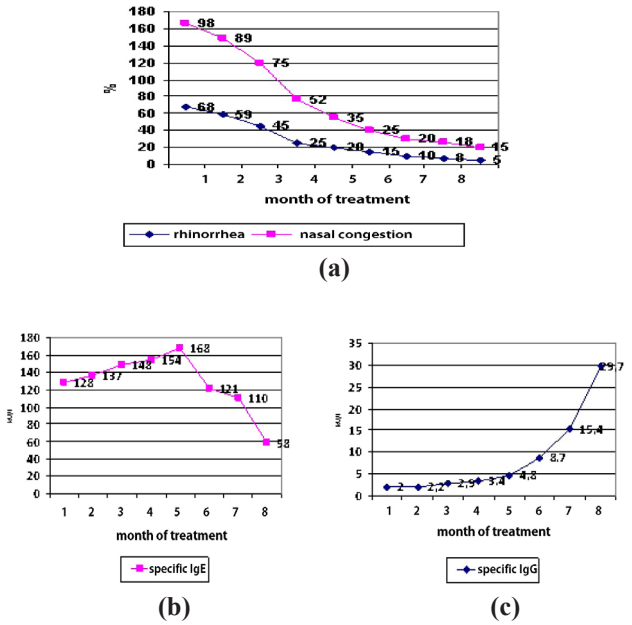


Fig.4. *Dynamics of clinical manifestations (a), the level of specific IgE (b) and IgG (c) antibodies to Alt a 1 during SIT in PAR patients*

Thus, a significant improvement in the clinical condition of patients during SIT was noted in both patient groups. In COPD patients, reduction of chronic cough and dyspnea on exertion was detected in 70% and 41%, respectively. Among PAR patients, a disappearance of nasal congestion and rhinorrhea was observed in 83% and 63%, respectively. The treatment induced the IgG response against Alt a 1. The concentration of specific IgG antibodies to Alt a 1 increased approximately 8-fold in COPD patients and 15-fold in PAR patients after 8 months of treatment. The mechanism of action of SIT is not definitively established, but it is known that the result of treatment-induced changes is the normalization of the immunological response. Immunologic changes that occur during allergen-specific immunotherapy are complex and not completely understood. However, successful immunotherapy has been associated with a shift from T helper cell type-2 (Th2) immune responses, which are associated with the development of atopic conditions, to Th1 immune responses [16]. It is also associated with the production of T regulatory cells that produce the anti-inflammatory cytokine, IL-10, amongst others such as transforming growth factor (TGF)-beta. IL-10 has been shown to reduce levels of allergen-specific IgE antibodies, increase levels of IgG (blocking) antibodies that play a role in secondary immune responses, and reduce the release of pro-inflammatory cytokines from mast cells, eosinophils and T cells [16-19]. Furthermore, there is an assumption that the allergen-specific IgG antibodies have the ability to reduce the early response to allergen, blocking the activation of Fcε-dependent mast cells and releasing the carriers of perforin [2,13].

Thus, our results indicate a positive effect of SIT on the clinical condition of COPD/PAR patients sensitized to *Alternaria alternata*. The clinical improvement was accompanied by a pronounced response of specific IgG antibodies to Alt a 1. However, research surrounding the mechanisms of immunotherapy is still ongoing and will help further elucidate how this form of therapy exerts its beneficial effects in patients sensitized to Alt a 1.

Competing interests

The authors declare that they have no competing interests.

References

1. Żukiewicz-Sobczak WA. The role of fungi in allergic diseases. Postepy Dermatol Alergol 2013; 30(1):42–45.
2. Asturias JA, Ibarrola I, Ferrer A, Andreu C, Lopez-Pascual E, Quiralte J, et al. Diagnosis of Alternaria alternata sensitization with natural and recombinant Alt a 1allergens. J Allergy Clin Immunol 2005; 115:1210-7.
3. Andersson M, Downs S, Mitakakis T, Leuppi J, Marks G. Natural exposure to Alternaria spores induces allergic rhinitis symptoms in sensitized children. Pediatr Allergy Immunol 2003; 14(2):100-5.
4. Aden E, Weber B, Bossert J, Teppke M, Frank E, Wahl R et al. Standardization of Alternaria alternata: Extraction and quantification of Alt a 1 by using an mAb-based 2-site binding assay. J Allergy Clin Immunol 1999; 104:128-35.
5. Mokhtari Amirmajdi M, Mokhtari Amirmajdi NA, Eftekharzadeh Mashhadi I, Jabari Azad F, Tavakol Afshari J, Shakeri MTIran. Alternaria in patients with allergic rhinitis. J Allergy Asthma Immunol 2011; 10(3):221-6.
6. Samaha HMS, Elsaid AR, NasrEldin E. Total serum IgE level in COPD patients. Egypt J of Chest Diseas Tubercul 2015; doi:10.1016/j.ejcdt.2015.02.005
7. Jamieson DB, Matsui EC, Belli A, McCormack MC,

Peng E, Pierre-Louis S, et al. Effects of allergic phenotype on respiratory symptoms and exacerbations in patients with chronic obstructive pulmonary disease Am. J. Respir. Crit. Care Med., 188 (2013), pp. 187–192
8. Bourdin A, Serre I, Flamme H, Vic P, Neveu D, Aubas P, et al.Can endobronchial biopsy analysis be recommended to discriminate between asthma and COPD in routine practice? Thorax, 59 (2004), pp. 488–493.
9. Jin J, Liu X, and Sun Y. The prevalence of increased serum IgE and Aspergillus sensitization in patients with COPD and their association with symptoms and lung function. Respiratory Research 2014, 15:130. doi:10.1186/s12931-014-0130-1
10. Han MK, Agusti A, Calverley PM, Celli BR, Criner G, Curtis JL, et al. Chronic obstructive pulmonary disease phenotypes: the future of COPD**.** *Am J Respir Crit Care Med* 2010, **182:**598-604.
11. Jamieson DB, Matsui EC, Belli A, McCormack MC, Peng E, Pierre-Louis S, Curtin-Brosnan J, Breysse PN, Diette GB, Hansel NN: Effects of allergic phenotype on respiratory symptoms and exacerbations in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 201*3*, 188:187-192.
12. Celli BR, MacNee W, and committee members. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 2004; 23: 932–946.
13. Rohde G, Gevaert P, Holtappels G, Borg I, Wiethege A, Arinir U et al. Increased IgE-antibodies to Staphylococcus aureusenterotoxins in patients with COPD. Respiratory Medicine 2004; 98:858–64.
14. Cortellini G, Spadolini I, Patella V, Fabri E, Santucci A, Severino M. Sublingual immunotherapy for *Alternaria*-induced allergic rhinitis: a randomized placebo-controlled trial. Ann of Allergy, Asthma and Immunol 2010; 105(5):382-86.
15. Wachholz P.A, Durham S.R. Mehanisms of immunotherapy: IgG revizited. Cur Opin Allergy Clin Immun 2004; 4(4):313-18.
16. Moote W, Kim H. Allergen-specific immunotherapy. Allergy Asthma Clin Immunol. 2011; 7(Suppl 1): S5. doi: 10.1186/1710-1492-7-S1-S5.
17. Frew AJ. Allergen immunotherapy. J Allergy Clin Immunol 2010; 125 (2 Suppl 2):S306–13.
18. Canadian Society of Allergy and Clinical Immunology. Immunotherapy Manual. Fall. 2010.
19. Allergen immunotherapy: a practice parameter second update. Joint Task Force on Practice Parameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunology. J Allergy Clin Immunol 2007; 120(3 Suppl):S25–85.

International Journal of BioMedicine 5(2) (2015) 76-78

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

Serum Hepcidin Evaluation in Patients with Chronic Dialysis

V. Manolov[1], D. Yonova[2], E. Vazelov[2], B. Bogov[3], M. Velizarova[1], B. Atanasova[4], V. Vasilev[4], K. Tzatchev[4], I. Bogov[5]

[1]*Department of Medical Genetics, Medical University, Sofia, Bulgaria*
[2] *Clinical Center of Dialysis, Medical University, Sofia, Bulgaria*
[3] *Department of Clinical Nephrology, Medical University, Sofia, Bulgaria*
[4] *Department of Clinical Laboratory and Clinical Immunology, Medical University, Sofia, Bulgaria*
[5] *National Cardiological Hospital,Sofia, Bulgaria*

Abstract

The aim of our study was to quantify serum hepcidin levels in Bulgarian patients with chronic kidney disease (CKD). Expected high values of peptide hormones might provide a new therapeutic choice for anemia of chronic disease. We looked for correlation between serum hepcidin levels and some iron metabolism parameters in CKD patients.

Results: The sandwich ELISA is highly specific for hepcidin-25. We found statistically significant differences in serum hepcidin levels in patients of the control group, with CKD stages II to IV and CKD stage V (on chronic dialysis): 12.7±8.7μg/L, 90.74±21.1μg/L, and 282.49±81.1μg/L, respectively. Significant correlation between serum hepcidin and transferrin saturation (r=0.340, $P<0.05$) was found in the group of patients with CKD stage V.

Conclusion: The use of 2 monoclonal antibodies in a sandwich ELISA format provides a reliable, reproducible, and inexpensive method for measuring serum concentrations of the bioactive form of hepcidin in Bulgarian laboratory practice.

Key words: *Hepcidin; anemia of chronic disease; chronic kidney disease (CKD); hemodialysis.*

Introduction

Maintaining a balance of iron in the body is critical to the state of health. Identifying hepcidin as a key regulator of iron dramatically improves our understanding of the molecular control mechanisms of homeostasis and allows a more detailed understanding of the pathophysiology in clinical disorders. Recent studies highlight the role of hepcidin as a useful diagnostic tool and therapeutic target in various diseases with impaired iron exchange. Hepcidin is a 25-aminoacid, cysteine-rich, iron-regulating peptide. Hepcidin quantification in human serum provides new topics for the pathogenesis of disorders of iron homeostasis and their treatment.

Hepcidin regulates systemic iron homeostasis—absorption in the duodenum, recycling demolition erythrocytes, controlled release from landfills in hepatocytes. It is a peptide which is synthesized by the liver in response to a series of signals according to the needs of the body for iron. The biological action of hepcidin is mediated by its binding to the receptor ferroportin, which is the only known exporter of iron present in the duodenum, macrophages, hepatocytes, or placenta. Hepcidin binds to the receptor in general complex, is internalized, and unlocks the lysosomal degradation ferroportin structure [1-4].

Anemia of chronic disease (ACD), also known as anemia of inflammation, is the most common type of anemia in hospitalized patients worldwide. It is observed in patients with acute or chronic inflammatory diseases, infections, cancer, rheumatoid arthritis, chronic kidney disease (CKD), organ failure, and trauma [1]. Anemia is usually mild or moderate, often without clear changes in the morphological characteristics of red blood cells. The pathogenesis is associated with induced hepcidin synthesis due to the catalytic effect of the inflammatory cytokines, of which the most important is interleukin-6 (IL-6). High hepcidin levels are cause for reduced intestinal absorption of iron and difficult release of the macrophages, leading to subsequent hypoferremia and suppressed erythropoesis.

***Corresponding author:** Victor Manolov, MD. Department of Medical Genetics, Medical University, Sofia, Bulgaria E-mail: victhedoc2@yahoo.com*

The aim of our study was to quantify serum hepcidin levels in Bulgarian patients with CKD. Expected high values of peptide hormones might provide a new therapeutic choice for anemia of chronic disease. We looked for correlation between serum hepcidin levels and some iron metabolism parameters in CKD patients.

Material and Methods

This study included 55 healthy volunteers and 60 patients (27 men (mean age 38.9±9.7) and 28 women (mean age 41.3±8.3)) with CKD stages II to V, including 32 CKD patients on chronic dialysis. Staging of the disease was performed using eGFR as recommended by the CKD-EPI Creatinine Equation. All enrolled subjects filled out informed consent according to the Helsinki Declaration (Directive 2001/20/EC).

The study analyzed serum from individuals included in the study. All samples were stored at -70° C until determination of serum hepcidin. Characterization of iron metabolism parameters was determined using an automatic biochemical analyzer Cobas Integra 400 (of Roche Diagnostics) and Advia 2120 hematology analyzer (Siemens Healthcare Diagnostics). We used the verified ELISA method to quantify serum hepcidin levels [5,6]. Data were analyzed by the Student t-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 and Discussion

We found statistically significant differences in serum hepcidin levels in patients with CKD stages II to IV and CKD stage V (on chronic dialysis) versus the control group: 90.74±21.1μg/L, 282.49±81.1μg/L, and 12.7±8.7μg/L, respectively ($P<0.01$). We looked for a correlation between the analyzed parameters for the iron deficiency in CKD and hepcidin (Table 1). Significant correlation between serum hepcidin and transferrin saturation (r=0.340, $P<0.05$) was found in the group of patients with CKD stage V.

Table 1.
Correlation between hepcidin and the measured parameters

Parameter	Hepcidin			
	CKD (stages II to IV)		CKD (stage V)	
	r	*P*	r	*P*
PCR	0.155	< *0.05*	n.a.	*n.a.*
eGFR	-0.297	< *0.05*	-0.083	< *0.05*
MCV (fl)	0.139	> *0.5*	0.372	< *0.05*
Iron (μmol/l)	0.088	< *0.05*	0.204	< *0.05*
TSAT (%)	0.156	< *0.05*	0.340	< *0.05*
TIBC (μmol/l)	-0.089	< *0.05*	-0.235	< *0.05*
Reticulocytes (%)	0.012	< *0.05*	-0.268	< *0.05*

PCR – protein to creatinine ratio; MCV – middle cell volume; TSAT – transferrin saturation; TIBC – total iron binding capacity.

Development of erythropoesis stimulating agents (ESA) such as erithropetin allows effective therapy in patients with CKD. The optimal target hemoglobin concentration is subjected to various discussions; during the treatment a significant number of patients become resistant to erythropoesis stimulating agents. Soon after its discovery, hepcidin was closely associated with CKD because of its role in advancing homeostasis imbalance between iron and resistance to ESA [7,8]. As such, hepcidin was described as a marker that could be used to predict response to the ESA, and lead clinicians in conducting therapy with the ESA and intravenous iron. Later this role was well recognized in practice.

ACD is an acquired disorder of iron homeostasis. This condition is associated with infection, organ failure, severe trauma, or other causes of inflammation. Anemia is usually mild or moderate; the erythrocytes cannot show typical characteristics of iron deficiency. This type of anemia is caused by induced hepcidin synthesis due to the action of interleukin-6 and other cytokines. According to some authors, normocytic erythrocytes in anemia of chronic diseases are the result of the action of two opposite effects: on the one hand, insufficient iron, and on the other, a tendency to macrocytosis that is still unknown in detail but is probably due to abnormal folate homeostasis in response to inflammation [9]. There are currently no laboratory tests that can fully distinguish ACD of iron deficiency anemia (IDA) [10,11]. Differentiation between those two states is often achieved by a combination study of various biochemical markers of iron metabolism. ACD is a heterogeneous disease that is typically characterized by normocytic anemia, alterations in the erythropoietic response, low serum iron, lower transferrin saturation, and iron accumulation in macrophages [1]. It is important to note that the functional iron deficiency in ACD is different from the actual iron deficiency in IDA, where the iron is exhausted due to the circulation and macrophages [12]. Some studies over the past 10 years have shown that functional iron deficiency in ACD may be due to increased levels of the regulatory hormone hepcidin.

Pre-clinical and clinical studies have shown that hepcidin antagonists, such as antibodies against hepcidin, antibodies to cytokine receptors, and the stabilizing hypoxia inducible factor (HIF), can lead to a reduction in hepcidin over-expression and can correct the pathological changes in the iron status [13-15]. BMP-2 administration increases hepcidin expression and decreases serum iron levels in vivo [13]. In *Hfe*$^{-/-}$mice, supraphysiologic doses of exogenous BMP6 improved hepcidin deficiency, reduced serum iron, and redistributed tissue iron to appropriate storage sites [16]. In order to suppress tumor angiogenesis HIF antagonists are used in malignancies [17]. Blocking of HIF could increase the concentration of hepcidin in conditions of iron overload.

New knowledge on the role of hepcidin in the development of anemia in CKD can significantly contribute to the correct choice of therapeutic approach [18].

Conclusion

Implementation in clinical laboratory practice as a routine method for the study of plasma concentrations of

hepcidin is a step forward in the treatment of impaired iron homeostasis. It would allow improving the differentiation between iron-deficiency anemia and anemia of chronic disease. The use of 2 monoclonal antibodies in a sandwich ELISA format provides a reliable, reproducible, and inexpensive method for measuring serum concentrations of the bioactive form of hepcidin in Bulgarian laboratory practice.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

This study was conducted with the support of the Medical University of Sofia - Grant № 10/2013.

References

1. Sun CC, Vaja V, Babitt JL, Lin HY. Targeting the hepcidin-ferroportin axis to develop new treatment strategies for anemia of chronic disease and anemia of inflammation. Am J Hematol 2012; 87(4):392-400.
2. Ganz T, Nemeth E. Iron sequestration and anemia of inflammation. Semin Hematol 2009; 46(4):387–393.
3. Ganz T. Hepcidin and iron regulation, 10 years later. Blood 2011; 117(17):4425–33.
4. Ramey G, Deschemin JC, Durel B, Canonne-Hergaux F, Nicolas G, Vaulont S.. Hepcidin targets ferroportin for degradation in hepatocytes. Haematologica 2010; 95(3):501–4.
5. Manolov V, Atanasova B, Velizarova M et al. Hepcidin evaluation in biological fluids. Medical Review 2013; XLIX(2): 41-46.
6. Manolov V, Atanasova B, Velizarova M, Vasilev V, Tzatchev K. Serum hepcidin levels in Bulgarian population. Clin Lab 2014; 60(12):2001-6.
7. Nicolas G, Viatte L, Bennoun M, Beaumont C, Kahn A, Vaulont S. Hepcidin, a new iron regulatory peptide. Blood Cells Mol Dis 2002;29(3):327–35.
8. Swinkels DW, Wetzels JF. Hepcidin: a new tool in the management of anaemia in patients with chronic kidney disease? Nephrol Dial Transplant 2008;23(8):2450–3.
9. Andrews NC. Forging a field: the golden age off iron biology. Blood 2008; 112(2):219-30
10. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. Cell 2010; 142(1):24 –38.
11. Kroot JJ, Tjalsma H, Fleming RE, Swinkels DW. Hepcidin in human iron disorders: diagnostic implications. Clin Chem 2011; 57(12):1650-69.
12. Theurl I, Aigner E, Theurl M, Nairz M, Seifert M, Schroll A, et al. Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. Blood 2009;113(21):5277–86.
13. Babitt JL, Huang FW, Xia Y, Sidis Y, Andrews NC, Lin HY.. Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. J Clin Invest 2007;117(7):1933-9.
14. Sasu BJ1, Cooke KS, Arvedson TL, Plewa C, Ellison AR, Sheng J, et al. Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammationinduced anemia. Blood 2010;115(17):3616-24.
15. Fatih N, Camberlein E, Island ML, Corlu A, Abgueguen E, Détivaud L, et al. Natural and synthetic STAT3 inhibitors reduce hepcidin expression in differentiated mouse hepatocytes expressing the active phosphorylated STAT3 form. J Mol Med (Berl) 2010; 88(5):477-86.
16. Corradini E, Schmidt PJ, Meynard D, Garuti C, Montosi G, Chen S, et al. BMP6 treatment compensates for the molecular defect and ameliorates hemochromatosis in Hfe knockout mice. Gastroenterology 2010;139(5):1721-9.
17. Semenza GL. Development of novel therapeutic strategies that target HIF-1. Expert Opin Ther Targets 2006;10(2):267-80.
18. Yonova D, Vazelov E, Manolov V, et al. Diagnostic tools of hepcidin in dialysis patients. Neph Dial Transpl (Bulg) 2013; 19(3): 22-7.

International Journal of BioMedicine 5(2) (2015) 79-83

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

Objective Assessment of the Severity of Patients Suffering from Fall from Height with Combined Injuries of the Abdominal Parenchymal Organs

Abdukhakim Khadjibaev, PhD, ScD; Pulat Sultanov*

Republican Scientific Center of Emergency Medicine
Tashkent, Uzbekistan

Abstract

In recent years, fall from a height (FFH) has been a relatively frequent cause of injury and death in the urban environment. ***The purpose of this study*** was to optimize the risk stratification of FFH victims with combined injuries of the abdominal organs by using Injury Severity Score (ISS) scale. The study included 111 patients (aged between 15 and 80 years) injured by FFH. All the falls were accidental and occurred mainly among males (82%). The height of the fall ranged from 2 to 5 meters. Combined injuries were found in 98 patients and isolated injuries in 13 patients. The combination of the 6 injured body regions was identified in 5 patients, 5 regions in 17, 4 in 35, 3 in 23, and 2 in 18. The abdomen trauma was most commonly associated with the following injured body regions: head and neck-chest-extremities and pelvis (13.3%), head and neck-chest-extremities (12.2%), and head and neck-chest-pelvis (9.2%). Among the combined injuries of the abdomen, ruptures of parenchymal organs (liver, spleen and kidneys) were predominant. To assess the severity of the injury, the ISS scale was applied. The injuries of abdominal parenchymal organs were evaluated according to the AAST (American Association for the Surgery of Trauma) classification. Comparative analysis of the assessment of the severity of a patient's condition according to the traditional scale and the ISS scale showed that the ISS scale promotes the active and timely detection of the extremely severe and terminal condition in patients with injuries due to FFH with combined trauma of the abdominal organs. Objective assessment of the severity of trauma and the dominant injury region allows determining the optimal treatment algorithm and predicting the outcome of the injury.

Keywords: *fall from a height; combined injuries; abdominal trauma*, *Injury Severity Score; severity of a patient's condition.*

Introduction

In recent years, fall from a height (FFH) has been a relatively frequent cause of injury and death in the urban environment [1,2]. Injuries due to FFH are characterized by severe associated injuries of different organs and systems, including severe abdominal trauma. The high frequency and prevalence of multiple injuries of abdominal organs determine the importance of this problem in emergency medicine [3-7]. The combination of abdominal trauma with injuries of other anatomical regions complicates the severity of injured patients and diagnosis of injuries that worsen the prognosis [4,5,8]. At equal severity of the injury and pathophysiological changes, the severity of a patient's condition depends on the functional reserves and adaptive capacities of the organism. In this regard, an objective assessment of the severity of a patient's condition presents certain difficulties [3,9-11].

A traditional classification of the severity of a patient's condition lacks clear criteria and unity of interpretation [12]. This classification is widely used in everyday practice and describes a patient's condition as satisfactory, moderate, severe, extremely severe or terminal. To assess the severity of a patient's condition, various scales and indices are suggested, which are based on a score of the clinical symptoms or laboratory indicators [11-13]. An objective assessment of a patient's condition at admission to hospital allows maximum avoidance of diagnostic and tactical mistakes, and improves the accuracy of decisions [9,14,15]. At the same time, a number of authors have noted that the integrated systems are not reliable and have suggested possible ways to improve them.

***Corresponding author:** Pulat Sultanov. Republican Scientific Center of Emergency Medicine Tashkent, Uzbekistan E-mail: sultanovp@bk.ru*

Thus, the MFS-AC scale (MFS - military field surgery, A - admission, C - condition) developed by EK Gumanenko et al [16] is focused on the clinical signs. Calculations are based on a score of 12 of the most important and easily identifiable clinical signs. Among these are the color of the skin and condition of the respiratory system, central nervous system, circulatory system, and gastrointestinal tract, as well as the magnitude of blood loss. In many countries, an assessment of the severity of a patient's condition at admission is performed by using the ISS (Injury Severity Score). This scale best meets the requirements of emergency surgery and scientific analysis. The ISS scale is easy to use and requires no additional equipment. Among anatomical severity scores, the ISS scale created by Baker et al. in 1974 [10], has been considered for over 40 years to be the gold standard to classify trauma injuries, both blunt and penetrating [3,4,10,11,16]. The ISS is based upon the Abbreviated Injury Scale (AIS). AIS is one of the most common anatomic scales for traumatic injuries. The first version of the scale was published in 1969 [17] with major updates in 1976, 1980, 1985, 1990, 1998, 2005, and 2008. The AIS is a consensus-derived, anatomically based system of grading injuries on an ordinal scale ranging from 1 (minor injury) to 6 (lethal injury) [18]. The ISS is obtained by summing the square value of the 3 highest AIS scores, identifying the severity of patients and enabling stratification of them. Six body regions are defined, as follows: head and neck, face, chest, abdomen, extremities (including pelvis), and external structures. Only one injury per body region is allowed. The ISS ranges from 1 to 75, and an ISS of 75 is assigned to anyone with AIS of 6.

Despite the numerous advantages of the ISS, it has some limitations [19,20]. The most obvious limitation is its inability to account for multiple injuries to the same body region; therefore, the ISS scale is rarely used to assess the severity of injury in victims of FFH with combined injuries of the abdominal organs.

The purpose of this study is to optimize the risk stratification of FFH victims with combined injuries of the abdominal organs by using ISS.

Material and Methods

The study included 111 patients (aged between 15 and 80 years, mean age 34.46±5.92 yrs) injured by FFH. All the falls were accidental and occurred mainly among males (82%). The height of the fall ranged from 2 to 5 meters. All patients were treated in the Republican Scientific Center of Emergency Medicine (RSCEM) between 2010 and 2013. The study was approved by the RSCEM Ethics Committee. Written informed consent was obtained from each patient.

Combined injuries were found in 98 patients and isolated injuries in 13 patients. The combination of the 6 injured body regions was identified in 5 patients, 5 regions in 17, 4 in 35, 3 in 23, and 2 in 18. The abdomen trauma was most commonly associated with the following injured body regions: head and neck-chest-extremities and pelvis (13.3%), head and neck-chest-extremities (12.2%), head and neck-chest-pelvis (9.2%), head and neck-chest (9.2%), head and neck-chest – extremities and pelvis-spine (5.1%), chest (5.1%), head and neck-chest–spine (4.1%), pelvis (4.1%), head and neck-extremities and pelvis (3.1%), head and neck- extremities (3.1%), chest-pelvis –spine (3.1%), and chest–spine (3.1%). The remaining 25 percent included other rare combinations.

Among the combined injuries of the abdomen, ruptures of parenchymal organs were predominant (Table 1). Spleen injuries were observed in 30 (27%) of cases, liver injuries in 28 (25.2%) cases, and combined injuries of spleen and liver were observed in 6 (5.4%) cases. Isolated injuries of spleen and liver occurred in 17 (15.3%) and 14 (12.6%) cases, respectively. In other cases, the traumatic injuries of abdominal parenchymal organs (liver, spleen and kidneys) had a multiple character. Traumatic injuries of hollow organs were observed in 12(12.8%) patients. Retroperitoneal hematoma occurred in 19(17.1%) cases.

To assess the severity of the injury, the ISS scale was applied [10]. The injuries of abdominal parenchymal organs were evaluated according to the AAST (American Association for the Surgery of Trauma) classification [21]. According to this classification, the following injuries were diagnosed: Grade I splenic injury was in 1(3%) patient, Grade II in 2(7%) patients, Grade III in 11(37%) patients, and Grade IV in 16(53%) patients. Grade V splenic injury was not identified. Grade I hepatic injury was observed in 5 patients, Grade II in 8 patients, grade III in 11 patients, and grade IV in 4 patients. Grade V hepatic injury was not identified. The distribution of patients into groups depending on the degree of injury of parenchymal organs was carried out based on the data of clinical-instrumental methods of investigation and intraoperative findings. An ISS score was obtained by summing the square value of the 3 highest AIS scores: from 1 (minor injury) to 6 (lethal injury). Major trauma was defined using an ISS threshold of 13.

Statistical analysis was performed using the SPSS 10.0 software package (SPSS, Chicago, IL, USA). The mean (M) and standard error of the mean (SEM) were deduced. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. We also used the Chi-square test to compare observed data. P values of <0.05 were considered statistically significant.

Results and Discussion

Assessment of the severity of a patient's condition according to the traditional scale and the ISS scale is presented in Table 2. The severity of the injuries depended on the combination of the anatomical regions involved. In our studies, the isolated injuries of the abdominal organs were mainly assessed as satisfactory and moderate severity; when the involvement of other anatomical regions increased, the number of patients with a severe, extremely severe and terminal condition also increased. The maximum score, ISS of 75, was assigned to 4 patients; 2 of them died within the first hour. The severity of these patients with traumatic cerebral injury had an AIS score of 6. The remaining 2 patients died on the first day; in these patients, we found a dominant combination of three anatomical regions with AIS of 5, which resulted in an ISS of 75.

Table 1.
The frequency of the abdomen trauma in FFH victims

№	Combined Injuries of the Abdominal Organs	Abs	%
1.	Contusion and hematoma of anterior abdominal wall	41	36.9
2.	Injuries of internal organs. Hemoperitoneum.	3	2.7
3.	Splenic capsular tear	1	0.9
4.	Splenic capsular tear + hepatic hematoma	1	0.9
5.	Splenic rupture	17	15.3
6.	Splenic rupture + retroperitoneal hematoma	1	0.9
7.	Splenic rupture + kidney rupture	1	0.9
8.	Splenic rupture + kidney rupture + retroperitoneal hematoma	1	0.9
9.	Splenic rupture + kidney rupture + rupture of the pancreatic capsule + retroperitoneal hematoma	1	0.9
10.	Splenic rupture + hepatic rupture/hepatic capsular tear	2	1.8
11.	Splenic rupture + hepatic rupture + retroperitoneal hematoma	1	0.9
12.	Splenic rupture + hepatic rupture + rupture of the small-intestinal mesentery + retroperitoneal hematoma	1	0.9
13.	Splenic rupture + gallbladder avulsion + retroperitoneal hematoma	1	0.9
14.	Splenic rupture + bladder rupture + deserosation of colon + retroperitoneal hematoma	1	0.9
15.	Splenic rupture + ovarian apoplexy + deserosation of colon + retroperitoneal hematoma	1	0.9
16.	Hepatic hematoma	2	1.8
17.	Hepatic capsular tear	2	1.8
18.	Hepatic rupture	10	9.0
19.	Hepatic rupture + retroperitoneal hematoma	2	1.8
20.	Hepatic rupture + contusion of the pancreatic head	1	0.9
21.	Hepatic rupture + contusion of the pancreatic head + hepatoduodenal ligament hematoma	1	0.9
22.	Hepatic rupture + ovarian apoplexy + deserosation of colon	1	0.9
23.	Hepatic rupture + rupture of the small intestine + diffuse serofibrinous peritonitis	1	0.9
24.	Hepatic rupture + rupture of the small intestine + tear in the stomach wall + diffuse serofibrinous peritonitis	1	0.9
25.	Kidney rupture + colon rupture + diffuse serofibrinous peritonitis	1	0.9
26.	Rupture of the small Intestine + diffuse serofibrinous peritonitis	2	1.8
27.	Rupture of the small Intestine + rupture of the small-intestinal mesentery + rupture of the pancreatic capsule + hepatic capsular tear + tear in the stomach wall + deserosation of colon + diffuse serofibrinous peritonitis	1	0.9
28.	Rupture of the small-intestinal mesentery + retroperitoneal hematoma	1	0.9
29.	Rupture of the small-intestinal mesentery + contusion of the pancreatic head + retroperitoneal hematoma	1	0.9
30.	Gastrointestinal ligament hematoma + retroperitoneal hematoma	1	0.9
31.	Retroperitoneal hematoma	6	5.4
32.	Penetrating abdominal trauma without injuries of abdominal organs	1	0.9
33.	Penetrating abdominal trauma with injuries of colon	1	0.9
34.	Penetrating abdominal trauma with injuries of the small intestine	1	0.9
Total		111	100

Table 2.
The severity of a patient's condition with combined injuries according to the traditional scale and the ISS scale

Traditional scale			ISS scale		
Severity of a patient's condition	total (n=98)	death rate	ISS range	total (n=98)	death rate
Satisfactory	1.0%	0	< 13 points	18.4%*	0
Moderate severity	22.4%	0	14-21 points	17.3%	0
Severe	62.2%	17.3%	22-32 points	30.6%*	7.1%*
Extremely severe	8.2%	7.1%	33-46 points	21.4%*	12.2%*
Terminal	6.1%	6.1%	46 -66 points	8.2%*	7.1%
Lethal			75 points	4.1%*	4.1%*
Total	100.0%	30.5%	Total	100.0%	30.5%

P<0.05 vs Traditional scale.

Table 3.
Types of surgical procedures on abdominal organs in FFH victims

№	Types of surgical procedures	Abs	%
1.	Laparoscopic microwave coagulation for hepatic rupture	2	3.4
2.	Laparoscopic microwave coagulation for splenic rupture	1	1.7
3.	Laparoscopic appendectomy	1	1.7
4.	Laparotomy, splenectomy	17	28.8
5.	Laparotomy, splenectomy + cholecystectomy	1	1.7
6.	Laparotomy, splenectomy + coagulation / suturing the hepatic rupture	3	5.1
7.	Laparotomy, splenectomy + suturing the kidney rupture	1	1.7
8.	Laparotomy, splenectomy + nephrectomy	2	3.4
9.	Laparotomy, splenectomy + suturing the bladder rupture + suturing the colon rupture	1	1.7
10.	Laparotomy, splenectomy + coagulation for hepatic rupture + suturing the small-intestinal mesentery rupture	1	1.7
11.	Laparotomy, splenectomy + suturing the colon rupture + suturing the ovarian rupture	1	1.7
12.	Laparotomy, APC the splenic rupture	1	1.7
13.	coagulation for splenic capsular tear + coagulation for hepatic rupture	1	1.7
14.	Laparotomy, suturing and/or coagulation for hepatic rupture	12	20.3
15.	Laparotomy, suturing the hepatic rupture + removal of retroperitoneal hematoma	1	1.7
16.	Laparotomy, removal of retroperitoneal hematoma, inspection of the retroperitoneal area	1	1.7
17.	laparotomy, suturing the small-intestinal/colon mesentery rupture	2	3.4
18.	Laparotomy, suturing the colon rupture	1	1.7
19.	Laparotomy, suturing the colon rupture + suturing the ovarian rupture + coagulation for hepatic rupture	1	1.7
20.	Laparotomy, coagulation for hepatic rupture, side-to-side entero-entero anastomosis and sigmastoma (due to co-morbidity, a pelvic tumor)	1	1.7
21.	Laparotomy, resection of the part of the descending colon with end-to-end colo-colonic anastomosis on the metallic frame + suturing the left kidney rupture with nephrostomy	1	1.7
22.	Laparotomy, suturing the small-intestinal wall and mesentery rupture, suturing the stomach tear and sigmoid colon + coagulation for the tears of the left hepatic lobe	1	1.7
23.	Laparotomy, suturing the small intestine rupture	2	3.4
24.	laparotomy, suturing the hepatic rupture + suturing the small intestine rupture	1	1.7
25.	Laparotomy, suturing the mesentery defect and deserosation of the small intestine part	1	1.7
26.	Laparotomy, suturing the tears of the anterior and posterior walls of the stomach + coagulation for the hepatic rupture + suturing the small intestine rupture	1	1.7
Total		59	100

We found differences in the number of patients with varying degrees of severity determined by the traditional grading scale and the ISS scale. These differences were most significant in groups of patients whose condition at admission was determined as moderate, severe, and extremely severe. Thus, the number of patients with combined injuries and a high degree of severity on the ISS scale was 2 times less than the traditional classification, but the number of patients with an extremely severe and terminal condition was 4.15 and 12.35 times greater, respectively. At the same time, the patients admitted with fatal injuries according to the traditional scale were not allocated separately, whereas, 4(4.1%) patients were given the highest ISS score (75). As shown in Table 2, the severe condition according to the traditional scale was identified in 64 patients and extremely severe and terminal condition in only 8 and 6 patients, respectively. According to the ISS scale, among the admitted patients, the severe condition was determined in 30 patients, extremely severe condition in 21 patients, terminal condition in 8 patients, and a lethal injury in 4 patients. We found a direct correlation between the clinical outcome and severity of the condition at admission. No deaths have been noted among the patients with satisfactory and moderate severity of condition at admission. In the patient group with a severe condition, according to the ISS scale the number of deaths was 2.4 times less than the same group according to the traditional scale. Conversely, in the patient group with the extremely severe and terminal condition according the ISS scale, the number of deaths was 1.72 and 1.16 times greater, respectively.

Active surgical tactics were used in 94 cases: a diagnostic laparoscopy was performed in 35 cases, laparoscopic surgery in 4 patients, conversion to laparotomy in 27 patients, and the initial laparotomy in 28 patients (Table 3). Three (2.7%) patients with Grade I or II splenic injury were subjected to argon plasma coagulation (APC); splenectomy was performed at Grade III or IV in 27(24.3%) patients. Electrocoagulation

and/or APC were performed at Grade II hepatic injury in 34.8% cases; suturing the liver rupture in combination with coagulation was carried out at Grade III or IV hepatic injury. The ruptures and tears of the hollow organs were sutured in 12(12.8%) cases; suturing the kidney rupture and nephrostomy was performed on 2 patients, nephrectomy in 2 patients. The diagnostic and treatment procedures in patients with injuries due to FFH with the combined trauma of the abdominal organs were carried out with the participation of surgeons of different specialties. The choice of treatment policy was determined by the severity of a patient's condition, features of injury (the combined or multiple injuries), the severity of the injuries of the abdominal parenchymal organs, and a number of other factors. Overall mortality was 30.5%. Causes of deaths were pneumonia, cerebral edema, and multiple organ failure.

In conclusion, the use of the ISS scale optimizes the analysis of clinical material. Comparative analysis of the assessment of the severity of a patient's condition according to the traditional scale and the ISS scale showed that the ISS scale promotes the active and timely detection of the extremely severe and terminal condition in patients with injuries due to FFH with combined trauma of the abdominal organs. Objective assessment of the severity of trauma and the dominant injury region allows determining the optimal treatment algorithm and predicting the outcome of the injury.

Competing interests

The authors declare that they have no competing interests.

References

1. Khadjibaev A Sultanov P. Katatravma: Problems and Perspectives. Bulletin of Emergency Medicine 2013; 4: 83-88. [Article in Russian].
2. Dickinson A1, Roberts M, Kumar A, Weaver A, Lockey DJ. Falls from height: injury and mortality. J R Army Med Corps 2012; 158(2):123-7.
3. Abakumov M, Lebedev N, Malyarchuk V. Abdominal injury with an associated trauma. Moscow: Meditsina; 2005. [in Russian].
4. Ermolova A Khubutiya M, Abakumov M. Abdominal trauma. St. Petersburg: Vidar- M; 2010. [in Russian].
5. Alekseev V, Ivanov V, Alekseev S, Vanyukov V. Objective assessment of the severity of injury in victims with splenic injury. Bulletin of Surgery 2013; 1: 50-54. [Article in Russian].
6. Puzanov S, Alishikhov A, Rutenberg G, Bogdanov D. The usefulness of laparoscopy in traumatic injuries of the abdominal cavity. Endoscopic Surgery 2014; 2: 14-17. [Article in Russian].
7. Longo WE, Baker CC, McMillen MA, Modlin IM, Degutis LC, Zucker KA. Nonoperative management of adult splenic trauma. Criteria for successful outcome. Ann Surg 1989; 210(5):626-9.
8. Bagnenko S. Combined mechanical trauma. St. Petersburg, 2005. [in Russian].
9. Nathens AB, Cryer HG, Fildes J. The American College of Surgeons Trauma Quality Improvement Program. *Surg Clin North Am* Apr 2012;92(2):441-54
10. Baker SP, O'Neill B, Haddon WJr, Long WB. The Injury Severity Score; a method for describing patients with multiple injuries and evaluating emergency care. J Trauma 1974; 14(3):187-96.
11. Boyd CR, Tolson MA, Copes WS. Evaluating trauma care: the TRISS method.Trauma Score and the Injury Severity Score. J Trauma 1987; 27(4):370-8.
12. Malinin D, Bosco O. Methods of objective assessment of the severity of injuries and their practical application (Guidelines). Volgograd, 2008. [Article in Russian].
13. Brivet F. Scoring system and severe acute pancreatitis. Crit Care Med 2000; 28(8):3124-5.
14. Svetukhin AM, Zviagin AA, Slepnev SIu. Systems of objective evaluation of patients' severity status. Part II. Khirurgiia (Mosk) 2002; 10: 60-9. [Article in Russian].
15. Moreno R, Morais P. Outcome prediction in intensive care: results of prospective, multicentre, Portuguese study. Intensive Care Med 1997; 23(2):177-86.
16. Gumanenko E, Boyarintsev B, Suprun T, et al. Objective evaluation of the severity of injuries. St. Petersburg: Military Medical Academy, 1999. [in Russian].
17. John D. States: The Abbreviated and the Comprehensive Research Injury Scales. In: STAPP Car Crash Journal. 13, Society of Automotive Engineers, Inc., New York 1969, ISSN 1532-8546, S. 282–294, LCCN 67-22372.
18. Committee on Medical Aspects of Automotive Safety. Rating the severity of tissue damage, I. The abbreviated scale. JAMA 1971; 215(2):277-80.
19. Kaida A, Petruk J, Sevcik W, Latoszek K, Ohinmaa A, Jacobs P , et al. Investigating the impact of lowering the Injury Severity Score cutoff for major trauma in pediatrics. Acad Emerg Med. 2004;11:513.
20. Aharonson-Daniel L, Giveon A, Stein M; Israel Trauma Group (ITG), Peleg K. Different AIS triplets: different mortality predictions in identical ISS and NISS. J Trauma 2006;61(3):711–7.
21. Moore EE1, Shackford SR, Pachter HL, McAninch JW, Browner BD, Champion HR, et al. Organ injury scaling: spleen, liver, and kidney. J Trauma 1989; 29(12):1664-6.

International Journal of BioMedicine 5(2) (2015) 84-86

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

Neuroendoscopic Intervention for Deep Midline Brain Tumors with Secondary Occlusive Hydrocephalus

Ulugbek M. Asadullaev, PhD

Republican Research Center of Neurosurgery
Tashkent, Uzbekistan

Abstract

This article analyzes the results of a clinical examination of 102 patients (78/76.47% men and 24/23.53% women) with a brain tumor (BT) complicated with a secondary obstructive hydrocephalus (SOH). All the patients were divided into 3 groups according to the type of surgery. Group 1 included 38(37.2%) patients who underwent Torkildsen's ventriculocisternostomy. Group 2 consisted of 34(33.3%) patients who underwent endoscopic third ventriculocisternostomy (ETV) with simultaneous endoscopic tumor removal. Group 3 included 30 (29.4%) patients who underwent a two-stage intervention: ETV in the first stage, and the endoscopic tumor removal in the second stage. The distinct advantages of EVT with tumor removal in the second stage of the operation were revealed.

Keywords: *brain tumor; secondary obstructive hydrocephalus; endoscopic third ventriculocisternostomy (ETV).*

Introduction

Surgeries of deep midline brain tumors (BTs) remain one of the most difficult sections of clinical neurosurgery [1,2]. Characteristic features of BT with secondary obstructive hydrocephalus (SOH) and the bright manifestation of hydrocephalus, so-called brain dropsy, require the solution of two important issues. First of all, elimination of a progressive manifestation of the hypertension-hydrocephalic syndrome (HHS) and SOH; second, microsurgical BT removal [2-4]. Traditional methods of surgical correction of HHS caused by occlusion of the cerebrospinal fluid (CSF) pathways (Torkildsen's ventriculocisternostomy (VCS), ventriculoperitoneostomy, ventriculoatriostomy) have a number of contraindications and are quite traumatic. Marked interventions often contribute to the development of severe complications, such as infection and occlusion of shunts, particularly in decompensated patients [1,3]. An alternative to these interventions for SON elimination in BT patients is endoscopic third ventriculocisternostomy (ETV), which contributes to the creation of new the CSF pathway, that provide drainage of excess CSF to subarachnoidal space via cisterns [5-8]. The bypass surgery is mainly performed by leading neurosurgeons, as is ETV with simultaneous tumor removal. It should be noted that tumor removal at the second stage after SOH elimination, on the background of absence of the cerebral symptoms and relatively satisfactory patient's condition, is performed significantly less frequently, which is not always justified.

The purpose of this study was to compare the results of one- and two-stage surgical procedures after EVT and Torkildsen's ventriculocisternostomy (VCS) in patients with midline brain tumors complicated with SOH.

Material and Methods

The study included 102 patients (78/76.47% men and 24/23.53% women) with BT complicated with SOH treated in the Republican Research Centre of Neurosurgery (RRCN). All the patients were admitted with severe clinical symptoms of secondary hydrocephalus. Age of the patients ranged from 1.5 to 50 years: from 1.5 to 5 years in 4 (3.9%) cases, from 6 to 15 years in 44(43.1%) cases, from 16 to 25 years in 25(24.5%) cases, from 26 to 35 years in 19(18.6%) cases, and from 36 to 50 years in 10(9.8%) cases.

All the patients were divided into 3 groups according to the type of surgery. Group 1 included 38(37.2%) patients who underwent VCS of Torkildsen. Group 2 consisted of 34(33.3%) patients who underwent ETV with simultaneous endoscopic

***Corresponding author:** Ulugbek M. Asadullaev, PhD. Republican Research Centre of Neurosurgery, Tashkent, Uzbekistan E-mail: asadullaevu@gmail.com*

tumor removal. Group 3 included 30 (29.4%) patients who underwent a two-stage intervention: ETV in the first stage, and the endoscopic tumor removal in the second stage; the second step was performed 5–35 days after elimination of SOH symptoms and stabilization of the patient's condition.

ETV was performed using video endosurgical complex "Aesculap" (Germany) and a set of standard microinstruments. Indication for ETV was the presence of open access to inferior part of the third ventricle. At the occlusion of the foramen of Monroe by BT in the anterior and middle regions of the third ventricle, the performance of EVT in most cases was impossible, and we performed VCS of Torkildsen. The length of the catamnestic observation time was from 3 to 12 months.

The clinical condition of the patients was assessed according to standard physical examination to determine neurological status. The severity of cerebral symptoms was determined by the complaints related to obstructive hydrocephalus: headaches, dizziness, nausea, uncontrollable vomiting, bradycardia, etc., as well as the results of a neuro-ophthalmic examination and MRI. The ophthalmological exam included in-depth study of the structures of the eye fundus. The severity of papilledema was assessed according to accepted standards of the Frisen grading scale. Severity of hydrocephalus was assessed in view of the state and sizes of the ventricles, the state of the subarachnoid space, and the degree of the periventricular edema according to MRI data. The results of surgery were assessed by the dynamics of clinical symptoms associated with regression of hydrocephalus, papilledema, and MRI signs of lesion. Before surgery, the condition of the ventricular system was assessed by neuroimaging techniques; all cerebral cisterns (interpeduncular, prepontine) were evaluated. The study was approved by the RRCN. Written informed consent was obtained from each patient. Statistical analysis was performed using the statistical software «Statistica». The difference was considered reliable when $P<0.05$.

Results and Discussion

For the development of SOH, BT location and proximity to the CSF pathway had a paramount importance in comparison with the size and histological structure of BT. Among the 102 patients, severe hyrocephalic symptoms were observed in 81(79.4%) patients. An extremely critical condition, diffuse headaches, dizziness, nausea, vomiting, Bruns syndrome, bradycardia, and severe papilledema with retinal hemorrhage were observed in 21(20.58%) patients. According to fundoscopy, Stage 1 of papilledema was detected in 38(37.2%) patients, stage 2 in 43(42.25%) patients, and stages 3–4 with signs of optic atrophy in 21(20.6%) patients. Moderate and severe bradycardia was detected in 36(76.6%) patients. Ventriculomegaly with symptoms of periventricular edema on CT and/or MRI brain imaging was determined in all patients.

The choice of surgical investigation was determined according to the level of the occlusion. Table 1 shows the distribution of patients according to the level of occlusion and surgery method. Comparative analysis of the clinical results of different methods of surgical interventions based on mortality, clinical deterioration, and clinical improvement is shown in Table 2. The distinct advantages of EVT according to all aspects should be noted; in addition, Group 3 patients who underwent tumor removal in the second stage of the operation were characterized by a significant advantage in comparison with the Group 1 patients. Patients of Group 3, after the endoscopic intervention, were in satisfactory condition and quickly recovered from anesthesia. Symptomatic relief of hydrocephalus was achieved in all patients. Furthermore, after surgery we observed in all patients a clear tendency to reduction of cerebral symptoms, a statistically significant decrease of diffuse headaches in 22(73.3%) patients, and the presence of local pain in the occipital region in 8(26.6%). After EVT, moderate dizziness persisted, but nausea and vomiting completely stopped. On the second and third days after the first stage of the intervention, a regression of disorders in the CSF circulation, according to CT and MRI data, was observed in most patients. Periventricular edema disappeared in all patients of Group 3 and a significant change in the size of the subarachnoid space was observed in 25(83.3%); reduction in the size of the ventricular system ("Flow void" phenomenon) in the area the anterior regions of the third ventricle and the stoma occurred in 14(46.76%) patients.

Table 1.

Distribution of patients according to the cause and the level of occlusion

The causes of SOD	The posterior third ventricle region and aqueduct of Sylvius		Posterior fossa region (fourth ventricle, worm, cerebellar hemisphere)		Amount	
	Abs	%	Abs	%	Abs	%
Tumors of the pineal region and quadrigemina	6	28.6	15	71.4	21	20.6
Posterior fossa tumor	10	12.3	71	87.6	81	79.4
Total	16	15.7	86	84.3	102	100

Table 2.

Outcomes of surgical treatment in the postoperative period in accordance with the method of surgical treatment

Method of surgery	Clinical improvement		Clinical deterioration		Mortality		Amount	
	Abs	%	Abs	%	Abs	%	Abs	%
VCS of Torkildsen	15	39.5	14	36.8	9	23.8	38	37.2
ETV with simultaneous endoscopic tumor removal	21	61.8	8	23.5	5	14.7	34	33.3
ETV with endoscopic tumor removal in the second stage	22	73.3*	5	16.7*	3	10.0*	30	29.4
Total	58	56.9	27	26.5	17	16.7	102	100

**- $P<0.05$ versus VCS of Torkildsen*

Conclusion

ETV is the best neurosurgical technique and should be considered as the initial treatment for BT complicated with SOH. ETV excludes mechanical complications and lowers the risk of biological complications, which are characteristic for drainage operations. EVT leads to successful outcomes by ensuring an adequate internal drainage of the cerebrospinal fluid and maximum recovery of the CSF circulation. EVT with endoscopic tumor removal in the second stage of the operation, compared with the VCS of Torkildsen, which is accompanied by significant damage to the brain structure, provides additional benefits to normalize the clinical status on the background of the absence of cerebral symptoms.

References

1. Asadullayev UM. The choice of methods for treatment hydrocephalus at brain tumors and the clinical outcomes. Neurol 2012; 2:28-30. [Article in Russian].
2. Konovalov AN1, Pitskhelauri DI, Shishkina LV, Kopachev DN, Sanikidze AZ, Gavriushin AV, et al. Intraparenchymal brainstem schwannomas: report of three cases and literature review. Zh Vopr Neirokhir Im N N Burdenko 2013; 77(2):35-43. [Article in Russian].
3. Omarov AD, Kopachev DN, Sanikidze AZ, Pitskhelauri DI, Panshin GA, Datsenko PV, et al. Treatment for hydrocephalus of the neoplastic etiology. State of the problem. Bulletin of the Russian Scientific Center of Radiology 2011,11. [Article in Russian]
4. Sanikidze AZ Microsurgical ventriculocisternostomy for surgery of deeply and mid-located brain tumors. Abstract of PhD Thesis. Moscow; 2014.
5. Gaab MR, Schroeder HW. Neuroendoscopic approach to intraventricular lesions. J Neurosurg 1998; 88(3):496-505.
6. Teo C, Young R 2nd. Endoscopic management of hydrocephalus secondary to tumors of the posterior third ventricle. Neurosurg Focus 1999; 7(4):e2.
7. Stachura K, Grzywna E, Kwinta BM, Moskała MM. Endoscopic third ventriculostomy - effectiveness of the procedure for obstructive hydrocephalus with different etiology in adults. Wideochir Inne Tech Malo Inwazyjne 2014; 9(4):586-95.
8. Hader WJ, Brooks BL, Partlo L, Hamilton M. Neuropsychological outcome after endoscopic third ventriculostomy. Can J Neurol Sci 2014; 41(6):729-34.

International Journal of BioMedicine 5(2) (2015) 87-90

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

Comparison of Chromatographic Methods for Determination of the Major Metabolites of Catecholamines and Serotonin

Ilgar S. Mamedov[1], PhD; Irina V. Zolkina[2]*, PhD; Pavel B. Glagovsky[1]; Vladimir S. Sukhorukov[1], PhD, ScD

[1]*Russian State Medical University, Moscow, Russia*
[2] *Research Clinical Institute of Pediatrics, Moscow, Russia*

Abstract

In the present article, we set out a new high-tech method for determining the major metabolites of catecholamines and serotonin in urine. We then discuss the effects of these substances and the value of their main metabolites in normal and various pathological conditions, and show the efficiency of applying this technique in the laboratory diagnosis of several diseases.

Keywords: *catecholamines; serotonin; homovanillic acid; vanillylmandelic acid; 5-hydroxyindolacetic acid; GC-MS.*

Introduction

Catecholamines are a group of aromatic amines, including adrenaline (epinephrine), noradrenaline (norepinephrine), and dopamine, which are synthesized in the adrenal medulla, the sympathetic nervous system, and the brain and act as neurotransmitters and hormones. A significant increase in the level of catecholamines is detected in tumor diseases such as pheochromocytoma, neuroblastoma, and ganglioneuroma, as well as in hypertension, cardiomyopathy, schizophrenia, and manic-depressive disorders [1-4].

Adrenaline and noradrenaline are formed from dopamine and act on the heart muscle, helping the body cope with stress [5,6]. Identification of these compounds is necessary in clinical practice mainly for diagnostic pheochromocytoma (adrenal tissue tumor) and the differential diagnosis of arterial hypertension [3,7]. According to different reviews and statistics, pheochromocytomas account for approximately 0.05% to 0.6% of patients with any degree of sustained hypertension [8-10]. Pheochromocytomas are rare catecholamine-secreting tumors that arise from chromaffin tissue within the adrenal medulla and extra-adrenal sites. These neuroendocrine tumors are characterized by an increase in blood catecholamine concentration of 10 to 100 times with preferred secretion of norepinephrine. In essential hypertension, the levels of these compounds in the blood are at the upper limit of normal or increased by 1.5 to 2 times [3]. Furthermore, there is a 10-fold increase in plasma epinephrine during stress. However, catecholamines are rapidly eliminated from the blood; therefore, for diagnostic purposes it is advisable to examine the urine.

Investigation of catecholamine levels in the blood and urinary excretion is important not only for the diagnosis of the disease, but also to monitor the effectiveness of treatment; thus, the re-increased excretion of catecholamines after radical removal of the tumor may be indicative of its recurrence. Separate determination of epinephrine and norepinephrine in urine allows one to get an indication of the possible location of the tumor: an increased excretion of epinephrine indicates a tumor of the adrenal medulla, and an increased excretion of norepinephrine indicates extra-adrenal tumor. According to the results, the ratio of catecholamines and their metabolites in urine makes it possible to determine the degree of the tumor's "maturation." [7].

A reduced concentration of catecholamines in the urine is marked with a decrease in the filtration capacity of the kidneys, collagenoses, and acute leukemia (especially in children) [11]. Determination of dopamine and the metabolites of epinephrine and norepinephrine is particularly important to confirm the diagnosis of neuroblastoma in children, the third most common neoplasm in older children [12]. The increased excretion of catecholamines and their major metabolites with the urine (homovanillic (HVA) and vanillylmandelic (VMA) acid) is used as a screening test [12]. Among all patients

***Corresponding author:** Irina V. Zolkina, PhD. Research Clinical Institute of Pediatrics, Moscow, Russia. E-mail: izolkina81@gmail.com*

examined with an identified neuroblastoma at the early stages, the survival rate was 97% in contrast to 45% in children who were diagnosed clinically.

Serotonin is a monoamine neurotransmitter. Approximately 85% to 90% of the human body's total serotonin is located in the enterochromaffin cells in the gastrointestinal tract, where it is used to regulate intestinal movements. The remainder is synthesized in serotonergic neurons of the central nervous system, where it has various functions. Serotonin secreted from the enterochromaffin cells eventually finds its way out of tissues into the blood. There, it is actively taken up by blood platelets, which store it. When the platelets bind to a clot, they release serotonin, where it serves as a vasoconstrictor and helps to regulate hemostasis and blood clotting. Serotonin is metabolized mainly to 5-hydroxyindolacetic acid (5-HIAA), chiefly by the liver, and excreted by the kidneys. In clinical practice, the determination of serotonin in the blood is particularly informative in carcinoid tumors (a type of neuroendocrine tumor) of the stomach, colon, and lung, when its concentration is increased 5 to 10 times [13]. Determination of elevated levels of the serotonin metabolite 5-HIAA in the urine confirms the presence of a tumor. In typical cases, the diagnosis of the carcinoid syndrome of flushing, which is characterized by short rushes of blood to the face and upper body, diarrhea, development of endocardial fibrosis, is not difficult. But sometimes, in severe hypertension and non-noticeable other symptoms, it is mistaken for a hypertensive crisis. Such attacks are also observed in mastocytosis, as well as in thyroid cancer in women [14].

Another promising application of a thin chromatographic analysis of metabolites of catecholamines and serotonin is the evaluation of the vegetative status in children. Because, catecholamines, serotonin and their metabolites are sensitive and specific biochemical markers for the diagnosis of various diseases, including childhood, the development of new methods for the analysis of these compounds is important for fundamental medicine as well as for practical medicine.

Methods for determining the levels of metabolites of catecholamines and serotonin in the urine should be very sensitive and accurate, allowing one to identify and trace the dynamics of changes in these values. Such methods of their joint determination as HPLC and GC-MS meet these requirements [15]. For this purpose, ELISA is not sufficiently informative.

The objective of this research was to study the possibility of applying the new algorithm to diagnose various diseases by determining levels of metabolites of catecholamines and serotonin in urine using new high-tech methods and evaluating the effectiveness of the joint determination of these compounds.

Methods

The study included 100 children aged from 2 to 16 years who were hospitalized in clinical departments (nephrology, genetics, and psycho-neurology) of the Research Clinical Institute of Pediatrics. Written informed consent was obtained from the child's parents.

The study samples were daily urine of patients. After collection of daily urine, about 100 ml from the total urine volume were selected in a plastic container with indication of diuresis. We used 6N of hydrochloric acid as the preservative. Samples were frozen at -20° C for prolonged storage, because term storage of samples in a refrigerator at +2-+8° C after acidification cannot exceed 5 days. Selected biomaterial was analyzed by gas and high performance liquid chromatography. Gas chromatography was combined with mass spectrometric detection (GC-MS). Sample preparation processes included selective solvent extraction of catecholamines and serotonin metabolites, and concentration of extractant and derivatization at 80°C. Methyltestosterone was used as the internal standard.

Determination of metabolites of epinephrine, norepinephrine, serotonin and dopamine (HVA, VMA, and 5-HIAA) was performed by a GC-MS system equipped with an autoinjector AOC--20i (Russian State Medical University, Department of Clinical Laboratory Diagnostics). For each sample, we obtained a chromatogram, in which a concentration of the metabolite in mg/day was determined via a computer data processing system, CLASS-VPTM Chromatography Data System, for gas chromatography-mass spectrometer. All data were subjected to statistical analysis. A chromatogram of urine obtained by GC-MS is shown in Figure 1.

High performance liquid chromatography (HPLC)

In the analysis, HVA, VMA, and 5-HIAA were used as the standards. Iso-vanillylmandelic acid served as an internal standard. All compounds were purchased from Sigma-Aldrich. Standard solutions of test compounds at the concentrations of 100mg/l were prepared by diluting hydrochloric acid at 10 to 3M and stored at 4°C. In this state, they were stable during 6 months. Working solutions were prepared from 10-fold diluted standard solutions.

Analysis of the samples was performed by the liquid chromatography tandem mass spectrometer Agilent Technologies 6410 Triple Quad LC/MS with autosampler, consisting of a double gradient pump Agilent 1200 series, vacuum degasser, and the analytical column Zorbax Eclipse XDB8-C18 (4.0h150 mm; particle size 5m) equipped with a temperature controller.

The data obtained were analyzed using Mass Hunter software for the analysis and identification of the chromatographic peaks. Ultimately, the concentration of a certain metabolite in the urine was calculated in mg/day. Figure 2 shows the chromatogram of urine obtained by HPLC.

Results

The high-tech methods of GC-MS and HPLC-MS for the joint determination of metabolites of catecholamines and serotonin in the urine showed the effectiveness of these methods as a diagnostic algorithm, which can be recommended as methods of screening for early detection of a number of neoplastic diseases in children. The methods described in this article are highly sensitive, specific, rapid and not very expensive, which allows them to be used in laboratory practice of medical institutions.

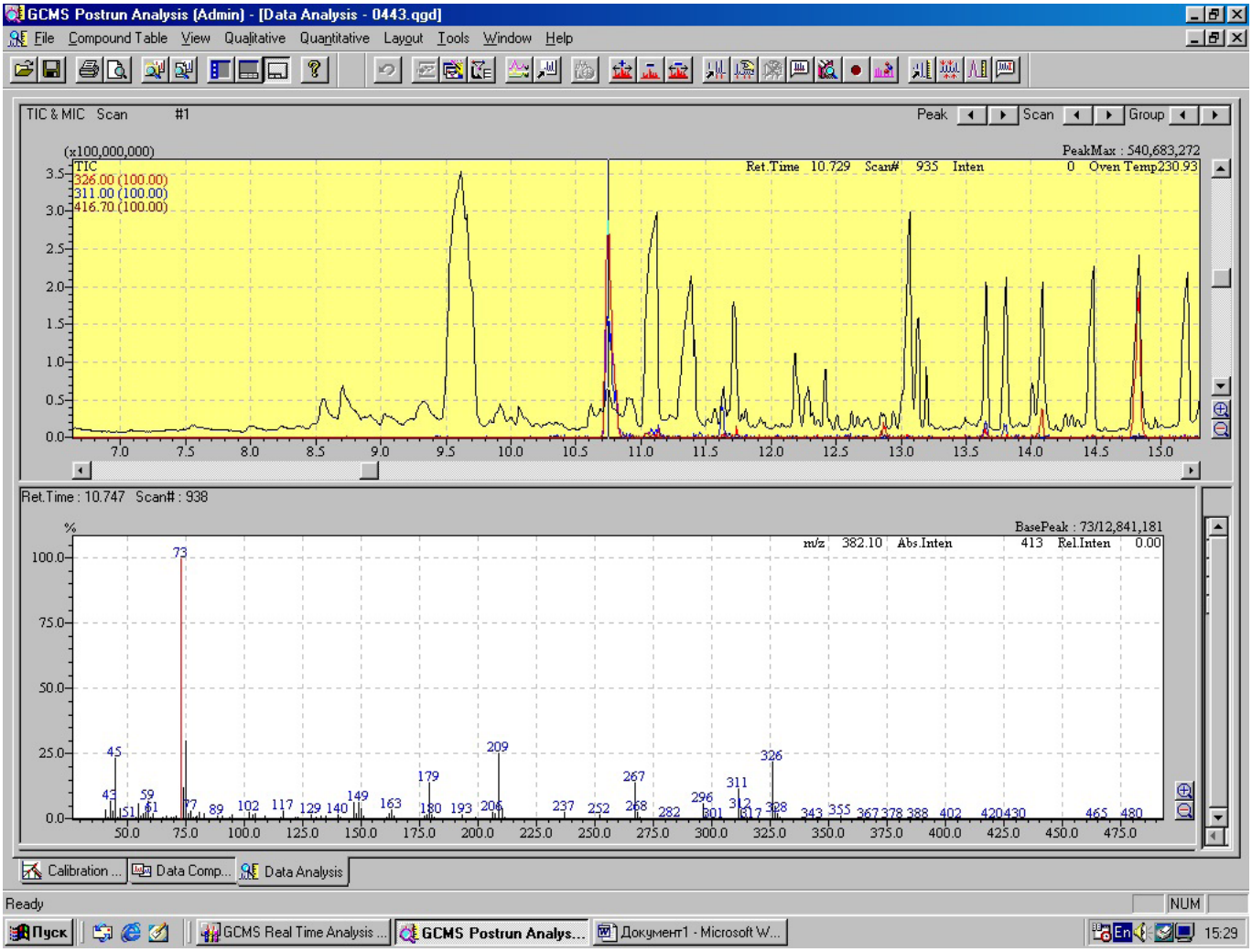


Fig. 1. The chromatogram of urine obtained by GC-MS

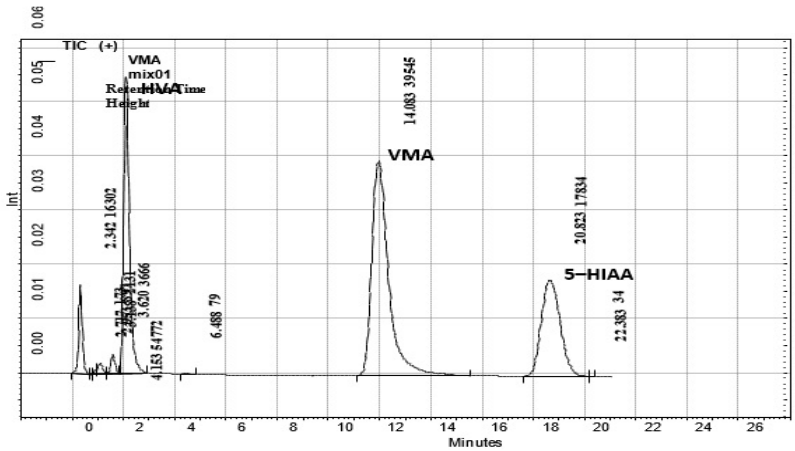


Fig. 2. The chromatogram of urine obtained by HPLC

Chromatographic analysis of the catecholamine and serotonin metabolites was performed in 100 children with suspected various diseases. Based on analysis of the patients' urine, the reference values of catecholamines, serotonin and their main metabolites were determined (Table 1). Diagnosis of neuroblastoma in children was confirmed in 3 cases, when the levels of VMA and HVA in urine were above the age norm, on average 3 times higher. Diagnosis of pheochromocytoma was confirmed in 5 children, and in 3 cases this disease was diagnosed at early stages in the subclinical phase.

Table 1.

The reference values of catecholamines, serotonin and their major metabolites obtained on the basis of urine analysis by HPLC-MS

Analytes	Value, nmol/day	Value, µg/day
Norepinephrine	< 570	< 97
Epinephrine	< 150	< 27
Dopamine	< 3,240	< 500
VMA	< 33.0	< 6.6
HVA	< 38.0	< 6.9
5–HIAA	10.5 – 47.1	2.0 – 9.0

Discussion

Thanks to the joint research of the Laboratory of General Pathology of RCIP and the Department of Clinical Laboratory Diagnostics of RNRMU, the modern diagnostic algorithm for simultaneous determination of metabolites of catecholamines and serotonin (HVA, VMA, and 5-HIAA) in the urine was designed by GC-MS. It should be noted that the GC-MS method proved to be more convenient for the final processing of results (chromatograms) of patients, because in the software there is an option comparing the obtained peaks with an embedded database of analytes by molecular weight of the substances, which makes the GC-Ms method more accurate and specific, and minimizes the analytical error that can be obtained during the chromatographic separation of substances by liquid chromatography. The results lead us to make a choice in favor of GC-MS, as the most sensitive method. The technique developed reduces analysis time and allows one to analyze up to 100 samples per day. Thus, it is possible to successfully use this method to screen for the presence of neuroblastoma, pheochromocytoma, carcinoid syndrome, and other diseases.

Conclusions

- A comparative study of the chromatographic methods (HPLC, GC-MS) showed that GC-MS is the most accurate method of determining the major metabolites of catecholamines and serotonin.
- A new modern diagnostic algorithm for jointly identifying the major metabolites of catecholamines and serotonin in urine was developed on the base of GC-MS.
- The reference values of catecholamines, serotonin, and their major metabolites in the urine were determined.
- The study showed that certain levels of the main metabolites of catecholamines and serotonin (HVA, VMA, and 5-HIAA) in the urine could possibly be used as diagnostic markers in clinical and laboratory practice.

Competing interests

The authors declare that they have no competing interests.

References

1. Tkachenko BI, Pyatina VF. *Human Physiology Compendium*. 4th ed. Samara: Samara Printing House, 2002.
2. Kushner B.H. Neuroblastoma: a disease requiring a multitude of imaging studies. J Nucl Med 2004; 45(7):1172–88.
3. Reisch N, Peczkowska M, Januszewicz A, Neumann HP. Pheochromocytoma: presentation, diagnosis and treatment. J Hypertens 2006; 24(12):2331–9.
4. Kjeldsen SE, Neubig RR, Weder AB, Zweifler AJ. The hypertension-coronary heart disease dilemma: the catecholamine-blood platelet connection. J Hypertens 1989; 7(11):851-60.
5. Litschka-Schimpf G, Manzl G, Schimpf A, Weiss M, Eberspächer H, Weicker H. Influence of different experimental recreation treatments on sympathoadrenergic and metabolic regulation mechanisms in repeated exercises. Int J Sports Med 1988; Suppl 2:S146–50.
6. Zumárraga M, Dávila R, Basterreche N, Arrue A, Goienetxea B, Zamalloa MI, et al. Catechol O-methyltransferase and monoamine oxidase A genotypes, and plasma catecholamine metabolites in bipolar and schizophrenic patients. Neurochem Int 2010; 56(6–7):774–9.
7. Barron J. Phaeochromocytoma: diagnostic challenges for biochemical screening and diagnosis. J Clin Pathol 2010; 63(8):669–674.
8. Manger WM, Gifford RW. Pheochromocytoma. J Clin Hypertens (Greenwich) 2002;4(1):62–72.
9. Bravo EL, Tagle R. Pheochromocytoma: state-of-the-art and future prospects. Endocr Rev 2003;24(4):539–53.
10. Omura M, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T. Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. Hypertens Res 2004; 27(3):193–202.
11. Silverstein FS, Hutchinson RJ, Johnston MV. Cerebrospinal fluid biogenic amine metabolites in children during treatment for acute lymphocytic leukemia. Pediatr Res 1986; 20(4):285–91.
12. Izbicki T, Izbicka E, Mazur J. Prognostic significance of biochemical heterogeneity of catecholaminergic clones in neuroblastoma. J Pediatr Surg 2006; 41(9):1506–12.
13. Sukhareva GV, Khomeriki SG. Carcinoid and carcinoid syndrome. Eksp Klin Gastroenterol 2008; 3:110–9. [Article in Russian].
14. Korse CM1, Bonfrer JM, Aaronson NK, Hart AA, Taal BG. Chromogranin A as an alternative to 5-hydroxyindoleacetic acid in the evaluation of symptoms during treatment of patients with neuroendocrine tumors. Neuroendocrinology 2009; 89(3):296–301.
15. Mamedov IS, Glagovsky PB, Zolkina IV, Sukhorukov VS, Vasin VI. A new method of chromatographic analysis of the metabolites of catecholamines and serotonin in the laboratory diagnosis of various diseases. Vopr Biol Med Farm Khim 2014; 7:59-63. [Article in Russian].

International Journal of BioMedicine 5(2) (2015) 91-94

INTERNATIONAL JOURNAL OF BIOMEDICINE

MODERN MEDICAL EQUIPMENT

Validity of Point-of-Care Testing Mission Plus in Detecting Anemia

Noor Ani Ahmad*, MBBS, MPH; S Maria Awaluddin, MD; Rahama Samad, RN; Noraida Mohd Kasim, RN; Muslimah Yusof, RN; Mohd Aznuddin Abd Razak, BSc; Chan Ying Ying, MMedSc; Norhafizah Sahril, BSc

Institute for Public Health, Ministry of Health Malaysia, Kuala Lumpur, Malaysia

Abstract

Background: Point-of-care testing, POCT, was widely used to assess hemoglobin status before proceeding with the confirmation test. Our study aimed to assess the validity of Mission® Plus Hb in detecting hemoglobin levels in a general population compared with a standard laboratory hematology analyser as the gold standard.

Methods: Two types of samples, capillary and venous blood, were collected from all respondents by trained nurses. Both blood samples were tested using Mission® Plus while the remaining venous blood in EDTA test tubes was sent to the reference laboratory.

Results: A total of 622 respondents participated in this study, 75% of them females. The mean Hb tested from capillary blood using Mission® Plus Hb was 11.80±2.02 g/dl. For venous blood, the mean Hb concentrations using Mission® Plus Hb and Sysmex XE-2100 hematology analyser were 12.16±1.84 g/dl and 13.07±1.87 g/dl, respectively. Sensitivity and specificity in detecting anemia from venous samples were 98.8% and 73.4% respectively. Positive Predictive Value (PPV) was 58.5%, while Negative Predictive Value (NPV) was 99.4%.

Conclusion: The findings of moderate PPV should alert the programme managers to the importance of a confirmatory test following screening using Mission® Plus Hb.

Keywords*: anemia; point-of-care testing (POCT); Mission® Plus Hb.*

Introduction

Anemia affects populations of both developed and developing countries. In 2008, WHO estimated that anemia affected 1.62 million people worldwide, with estimated prevalence of 30.2% among non-pregnant women above 15 years of age [1]. In the Western Pacific region, it is estimated that 21.5% of non-pregnant women are anaemic [1]. In Malaysia, there is a lack of data on the national prevalence of anemia among the general population of non-pregnant women. A regression-based estimate by WHO reported that 30.1% of non-pregnant women aged 15 to 49.9 years of age were anaemic [1]. Several local studies estimated that the prevalence ranges from 17.2% [2] among non-pregnant women in interior Sarawak to 34.6% to 42.3% among pregnant women [3,4]. Anemia is considered to be a moderate public health problem when the prevalence is more than 20% [1].

Various methods have been used to detect anemia in the population at risk. The methods range from hemoglobin colour scale, Sahli technique, copper-sulphate method, HaemoCue, and automated haematology analysers [5]. HemoCue was considered to be the method of choice for POCT due to its reliability, portability, and ease of use [6-9]. But taking into account the economic implication, a less costly but still reliable POCT should be looked into to be used in a nationwide survey to estimate the prevalence of anaemia in Malaysia. In Malaysia, assessment of hemoglobin level using Mission® Plus Hb has been used in several primary-care settings without proper validation. As it is, a scientific validity test is urgently needed to ensure the validity of this POCT in detection of anemia.

Our study aimed to assess the validity of Mission® Plus Hb in detecting hemoglobin levels in a general population compared with a standard laboratory hematology analyser (Sysmex XE-2100) as the gold standard.

***Corresponding author: NoorAni Ahmad.** Institute for Public Health, Ministry of Health Malaysia, Jln Bangsar, Kuala Lumpur, Malaysia. E-mail: drnoorani@moh.gov.my*

Methods

This is a cross-sectional study involving outpatient respondents recruited from a suburban primary care clinic in Selangor. A minimum sample size of 600 was required, based on 30% estimated prevalence of anaemia, with 90% sensitivity and specificity, using formulae for the sensitivity and specificity studies [10]. The study was reviewed and approved by the Medical Ethics and Research Committee, Ministry of Health Malaysia (NMRR-13-797-17207). Mission® Plus Hb (Acon Laboratories, USA) provided an in-kind Hemoglobin Meter and strips for the study.

The eligible respondents voluntarily participated in this study. The eligibility criteria were that participants must be 15 years of age or older and have never been diagnosed as having any blood disorders. Written consents were taken from all respondents. They were informed of the results of their blood tests, and respondents with anemia were referred to a family physician at the clinic for further management.

Data were collected using quota sampling in July 2014. Capillary and venous blood samples were collected from all respondents by trained nurses. A drop of venous blood directly from the needle was then tested with Mission Plus and its reading was documented. The remaining venous blood was then transferred into a test tube containing potassium EDTA as anticoagulant and tested using Sysmex XE-2100 at the reference laboratory within 2 hours. Capillary blood from a similar respondent was then taken from the fingertip and directly tested using Mission Plus. For capillary testing, care was taken to remove the first drop of blood with a sterile cotton swab, and pressure on the finger that might result in hemodilution was avoided. The blood sample was taken using pipette supplied together with the strip. The blood was then dropped on the strip connected to the hemoglobin meter. Its reading was then documented. The Mission Plus used in this study was calibrated daily and the procedure was supervised by a representative from the manufacturer to minimise human error.

The hemoglobin concentration of venous blood using Mission® Plus Hb was compared to the hemoglobin concentration of venous blood using the gold standard procedure (Sysmex XE-2100). Sysmex XE-2100 was selected as the gold standard in this study based on its accuracy and precision in detecting anemia [11,12]. Venous and capillary blood readings using Mission Plus were then compared to determine the accuracy of the test using capillary blood in detecting anemia. Anemia was defined as Hb less than 12gm/dl for women above 15 years of age and Hb less than 13gm/dl for men above 15 years of age [1].

The sensitivity, specificity, predictive positive and predictive negative values were then calculated using the following formulae:

Sensitivity = TP/(TP+FN)
Specificity = TN/(TN+FP)
Positive Predictive Value, PPV = TP/(TP+FP)
Negative Predictive Value, NPV = TN/(TN+FN)
Accuracy = (TP+TN)/n,
where TP is the number of respondents correctly identified as anaemic by Mission Plus; FN is the number of respondents identified as not anaemic by Mission® Plus Hb but noted to be anaemic using Sysmex; FP is the number of participants identified as anaemic using Mission Plus but not anaemic using Sysmex; TN is the number of participants correctly observed to be not anaemic using Mission Plus; and n is the number of participants. Correlation between the results based on the Mission Plus in comparison to those tested using the gold standard were calculated. Pearson's correlation coefficient and paired t-test were used to analyse the data using IBM SPSS Statistics (IBM Corporation, New York, USA).

Results

A total of 622 respondents participated in this study, 75% of them females. Age ranged from 15 to 77 years (35.7±12.9 years). The results obtained from Mission® Plus Hb and the standard reference method are summarised in Table 1.

Table 1.
Hemoglobin (Hb) values of respondents (n=622)

Test		Hb (g/dl) mean±SD	Range (min-max)
Mission® Plus Hb	Capillary	11.80 ± 2.02	6.4-19.8
	Venous	12.16±1.84	5.5-20.1
Gold standard (reference) Sysmex XE-2100	Venous	13.07±1.87	6.9-21.3

On average, the Hb concentration using capillary and venous samples tested using Mission Plus were both lower than the level using Sysmex, a difference of -1.27 g/dl (95% CI: -1.34, -1.21) and -0.911 g/dl (95% CI: -0.96, -0.86), respectively.

Overall, 169 respondents (98.8%) were observed to be truly anaemic based on a venous sample using Mission Plus. Based on the capillary results, 166 respondents (97.1%) were identified as anaemic using Mission Plus, while five respondents were falsely identified as anaemic. Table 2 shows the sensitivity, specificity, PPV, NPV and accuracy of detecting anaemia using Mission Plus.

Table 2.
Validity of Mission Plus in detecting anemia in comparison to standard reference

Validity	Anemic* (venous)	Anemic* (capillary)
Sensitivity (95% CI)	98.8%	97.1%
Specificity (95% CI)	73.4%	62.5%
Positive Predictive Value	58.5%	49.6%
Negative Predictive Value	99.4%	98.3%
Accuracy	80.4%	72.8%

**Hb < 12 g/dl for females and Hb < 13 g/dl for males*

The Pearson's correlation coefficient between Mission® Plus Hb and standard reference was high; for capillary hemoglobin the correlation, r, was 0.909 (*P<0.001*), while for venous haemoglobin, r was 0.940 (*P<0.001*).

Discussion

In a community survey, the collection of venous blood samples is mostly unacceptable and the analysis of fresh blood at the standard laboratory is unfeasible. The anemia estimation using POCT allows the collection of information that may otherwise be inaccessible. In order for Mission Plus to be an acceptable POCT for haemoglobin measurement in a nationwide survey, it would have to be precise and accurate. The Ministry of Health Malaysia has produced a guideline to safeguard patient safety and management [13].

Our study found that the Hb concentrations in the capillary and venous samples tested with Mission® Plus Hb were on average lower than those recorded in the reference laboratory using venous samples. The standard for assessing the accuracy of the Hb measurement using POCT varies based on its clinical usage; it ranges from 0.5 g/dl for a perioperative blood transfusion [14] to 1.0 g/dl in various other settings [6,15]. As such, a mean difference of 1.27 g/dl for capillary and 0.91 g/dl for venous samples using Mission® Plus Hb as compared to the standard reference is considered good. The Hb estimation using Mission Plus as the screening tool in a national survey should be interpreted with caution as it may result in overestimating the prevalence of anaemia in Malaysia. The possibility that humidity may change the reagents in the Mission® Plus Hb hemoglobin meter should also be considered, as happened in a validity study of POCT HemoCue in Australia [16]. Thus, maintenance of proper storage and handling is very crucial.

The findings of lower Hb using POCT Mission® Plus Hb did not agree with findings from POCT HemoCue, which exhibited higher Hb compared to the results from the automated analyser [9,17-20]. Technical errors such as incomplete filling or bubbles in a pipette may have also influenced the results from some samples in our study.

Our study found that the ability of the POCT to detect anaemia, based on the PPV, was moderate. In addition, the specificity of the Mission Plus in detection of non-anaemia was 62.5% and 73.4% for capillary and venous samples, respectively. This figure is lower compared to a specificity of 100% using POCT HemoCue [9,17,18]. In contrast, the sensitivity of the Mission Plus for screening for anaemia was almost 100% for both capillary and venous samples. Furthermore, the correlation was also high. Thus, considering the above-mentioned values, Mission Plus is suitable to be used as a screening method for detection of anaemia in a mass population although it is necessary to emphasize a thorough training of those involved in the survey to minimize technical errors.

Unlike other POCTs that have high specificity but moderate sensitivity, we found high sensitivity with moderate specificity and accuracy of Mission Plus in the detection of anaemia. The reasons for these differences require further investigation. Further study is also required to assess the conversion factor from capillary to venous blood at different levels of anemia.

Our study has several strengths. Our samples included the whole spectrum of age groups from young adults to the elderly to avoid selection bias. We also used a blind and independent comparison between the findings using Mission® Plus Hb at a primary care centre with the measurement of haemoglobin using Sysmex XE-2100 at the reference laboratory. Our sample size was properly calculated using a specific formula and was much bigger compared to the sample size of other validity studies of a POCT. A limitation noted from this study is the lack of comparison with arterial blood samples. Other validation POCT studies have compared capillary with venous and arterial blood samples, but in our study, collection of arterial blood samples was not suitable as the study site was a primary care centre.

Implications and recommendations

A highly sensitive tool is crucial for a screening, but high specificity is also a desired criterion to avoid unnecessary false positive results. As such, a positive finding with this POCT must be followed up by a confirmatory test before starting on any treatment. This tool might not be suitable to be used in a clinical setting such as monitoring of anaemic patients. On the positive side, we support the use of this POCT for screening for anemia in a mass population due to its characteristics as a rapid, fairly accurate and less costly method compared to other POCTs. However, it is necessary to note that any POCT should not replace formal laboratory venous sampling, which still remains the gold standard for haemoglobin measurement.

Conclusion

Our study found that Mission® Plus Hb has high sensitivity and moderate specificity and is considered suitable to be used as a screening tool for assessment of hemoglobin levels in a mass population.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors would like to thank the Director General of Health Malaysia for his permission to publish this article.

We thank Dr Nazrila Hairizan and her staffs at Pandamaran Health Clinic for collecting blood samples for the study.

We acknowledge Dr Wan Hayati Wan Yusof and her staffs at Hospital Tengku Ampuan Rahimah Klang for processing and analysis of all venous blood samples. We thank Dr Tahir Aris, Director of the Institute for Public Health Malaysia for his support in conducting this study.

References

1. Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. Edit by De Benoist B, McLean E, Egli I, Cogswell M. World Health Organization, Geneva, 2008.
2. Sagin DD, Ismail G, Mohamad M, Pang EK, Sya OT. Anemia in remote interior communities in Sarawak, Malaysia.

Southeast Asian J Trop Med Public Health 2002; 33(2): 373-7.
3. Hassan R, Abdullah WZ, Nik Hussain NH. Anemia and iron status of Malay women attending an antenatal clinic In Kubang Kerian,Kelantan, Malaysia. Southeast Asian J Trop Med Public Health 2005; 36(5):1304-7.
4. Thirukkanesh S, Zahara AM. Compliance to Vitamin and Mineral Supplementation among Pregnant Women in Urban and Rural Areas in Malaysia. Pakistan J Nutrition 2010; 9(8):744-750.
5. Srivastava T, Negandhi H, Neogi SB, et al. Methods for hemoglobin estimation: a review of "What Works". J Hematol Transfus 2014; 2(3):1028.
6. Sanchis-Gomar F1, Cortell-Ballester J, Pareja-Galeano H, Banfi G, Lippi G. Hemoglobin point-of-care testing: the HemoCue system. J Lab Autom 2013; 18(3):198-205.
7. Neufeld L, García-Guerra A, Sánchez-Francia D, Newton-Sánchez O, Ramírez-Villalobos MD, Rivera-Dommarco J. Hemoglobin measured by Hemocue and a reference method in venous and capillary blood: a validation study. Salud Publica Mex 2002; 44(3):219-27.
8. Paiva Ade A, Rondó PH, Silva SS, Latorre Mdo R. Comparison between the Hemocue and an automated counter for measuring hemoglobin. Rev Saude Publica 2004; 38(4):585-7.
9. Shahshahani HJ, Meraat N, Masouri F. Evaluation of the validity of a rapid method for measuring high and low haemoglobin levels in whole blood donors. Blood Transfus 2013; 11(3):385-90.
10. Sample size calculation for sensitivity and specificity studies. http://www.docstoc.com/docs/168682724/Sample-size-for-sensitivity-_-specificity-studies-_by-Lin-Naing_ (accessed on January 5, 2015).
11. Walters J, Garrity P. Performance Evaluation of the Sysmex XE-2100 Hematology Analyzer. Lab Hematol 2000; 6:83-92.
12. Tsuruda K, Tsuji T, Usui T, et al. Evaluation and Clinical Usefulness of the Automated Hematology Analyser, Sysmex XE-2100. Sysmex J Int 1999; 9(2):129-138.
13. National Point of Care Testing: Policy and Guidelines. Departmental Policy of Pathology Services, Ministry of Health Malaysia, 2012.
14. Gehring H, Hornberger C, Dibbelt L, Rothsigkeit A, Gerlach K, Schumacher J, et al. Accuracy of point-of-care-testing (POCT) for determining haemoglobin concentrations. Acta Anaesthesiol Scand 2002; 46(8):980–6.
15. Agarwal R, Heinz T. Bedside hemoglobinometry in hemodialysis patients: lessons from point-of-care testing. ASAIO J 2001; 47(3):240-3.
16. Nguyen HT. High humidity affects HemoCue cuvette function and HemoCue haemoglobin estimation in tropical Australia. J Paediatr Child Health 2002; 38(4):427-8.
17. Mendrone A Jr1, Sabino EC, Sampaio L, Neto CA, Schreiber GB, Chamone Dde A, et al. Anemia screening in potential female blood donors: comparison of two different quantitative methods. Transfusion 2009; 49(4):662-8.
18. Akhtar K, Sherwani RK, Rahman K, Hasan J, Shahid M.Hemocue photometer: a better alternative of haemoglobin estimation in blood donors? Indian J Hematol Blood Transfus 2008; 24(4):170-2.
19. Bahadur S, Jain S, Jain M. Estimation of hemoglobin in blood donors: a comparative study using hemocue and cell counter. Transfus Apher Sci 2010; 43(2):155-7.
20. Cable RG, Steele WR, Melmed RS, Johnson B, Mast AE, Carey PM, et al. The difference between fingerstick and venous haemoglobin and hematocrit varies by sex and iron stores. Transfusion 2012; 52(5):1031-40.

International Journal of BioMedicine 5(2) (2015) 95-99

INTERNATIONAL JOURNAL OF BIOMEDICINE

EPIDEMIOLOGY

Epidemiology of Postmenopausal Osteoporosis and Related Risk Factors in Female Residents of Tashkent and Namangan (Republic of Uzbekistan)

S. I. Ismailov, L.S. Abboskhodjaeva, N.M. Alikhanova

Center for the Scientific and Clinical Study of Endocrinology, Uzbekistan Public Health Ministry Tashkent, Uzbekistan

Abstract

Our epidemiological survey is the first step in studying prevalence and risk factors of postmenopausal osteoporosis (PMO) in the Republic of Uzbekistan, aiming at development of early preventive and therapeutic measures to reduce osteoporosis-associated fractures.

Methods: We screened 1378 postmenopausal female residents of Tashkent and Namangan, two cities with the largest populations in Uzbekistan, aged from 50 to 80. The duration of the postmenopausal period was ≥1 year.

Results: According to our data, the prevalence of osteoporosis in different regions of Uzbekistan varies widely (33.5% and 51.1% in Tashkent and Namangan, respectively). The prevalence of osteoporosis increases with age from 25.6% (50 to 59 years) to 51.3% (in >70 age group) in Tashkent and from 44.0% to 80.0% in the same age groups in Namangan.

There were significantly more women in Namangan with body mass <57 kg than in Tashkent (OR 2.44; 95%CI 1.72-3.46; *P<0.00001*). We found that the number of women doing physical exercises in Tashkent was 2 times more than in Namangan (65.5% versus 36.1%, OR 3.36; 95% CI 2.64-4.27; *P<0.0001*).

Conclusion: Our research shows that osteoporosis is widely spread among women above 50 living in two big densely populated cities of Uzbekistan (Tashkent and Namangan). Low body mass and irregular physical activity, fracture history, and duration of menopause are the factors of risk in the studied cohorts of women.

Keywords: *menopause; risk factors; osteopenia; osteoporosis.*

Introduction

Postmenopausal osteoporosis (PMO) is a systemic, multifactorial skeletal disease occurring in women in postmenopause as a consequence of deficiency of sexual hormones, mainly estrogens. The disease is characterized by progressive loss of bone mass and change in microstructure, resulting in increased risk of fracture, morbidity, and mortality. PMO constitutes up to 80% to 85% of all types of osteoporosis [1-3]. Osteoporosis frequency in all skeletal sites increases with age; thus, according to WHO, osteoporosis is registered in 70% of women older than 80 years [4]. Findings from epidemiological studies in the Russian Federation demonstrated that in the age group of ≥50 years, according to WHO criteria, osteoporosis occurred in 30.5% to 33.1% of women and in 22.8% to 24.1% of men, totaling10 million people [5], which means that 1 woman out of 5 and 1 man out of 3 have osteoporosis. Similar data were published on prevalence of osteoporosis among women of the white population of North America and a number of countries of Western Europe [6-8]. Frequency of osteopenia and osteoporosis among perimenopausal female residents of Tashkent is 55.7% and 12.2%, respectively [9]. But no epidemiological studies of postmenopausal osteoporosis have been conducted in the Republic of Uzbekistan so far.

According to the U.S. Census Bureau International Database, in 2014 the population of Uzbekistan was 29 million people, 17% (4.2mln) and 3.4% (971,000) of people being ≥ 50 and ≥70 years of age, respectively. By 2050 in the face of a general population rise to 35 million people, 40% (14mln) and 12% (4.2mln) are expected to be ≥ 50 and ≥ 70 years of age, respectively. No specially designed epidemiological studies of

***Corresponding author:** Prof. Said I. Ismailov, PhD, ScD. Director of the Center for the Scientific and Clinical Study of Endocrinology, Tashkent, Uzbekistan. E-mail: ismailov.said@list.ru*

osteoporosis and osteoporotic fractures have been conducted. However, according to the Research Institute of Traumatology and Orthopedics, there are at least 30,000 people with osteoporosis and 150,000 with osteopenia in Uzbekistan. The number of patients with osteoporosis and osteopenia is predicted to increase up to 250,000 by 2020.

Our epidemiological survey is the first step in studying prevalence and risk factors of postmenopausal osteoporosis in the Republic of Uzbekistan, aiming at development of early preventive and therapeutic measures to reduce osteoporosis-associated fractures.

The work was initiated to study prevalence and various risk factors of postmenopausal osteoporosis among female residents of Tashkent and Namangan.

Materials and methods

We screened 1378 postmenopausal female residents of Tashkent and Namangan, two cities with the largest populations in Uzbekistan, aged from 50 to 80. The duration of the postmenopausal period was ≥1 year. The groups were comparable by parameters. Duration of osteoporosis and menopause ≥1 year was the inclusion criterion. Diseases affecting bone metabolism, such as hyperparathyroidism, thyrotoxicosis, Itsenko-Cushing's syndrome and disease, hypogonadism in medical history, rheumatic disorders, malabsorption syndrome, renal insufficiency, hepatic dysfunction, and malignancies, as well as prior treatment with medications affecting calcium metabolism 12 months before the study, were the exclusion criteria.

The study was conducted in accordance with the ethical principles stated in Declaration of Helsinki of 1964 (revised in Seoul in 2008). The trail is registered on *www. who.int/bulletin/archives/79(4)373;http://www.wma.net/en/30publications/10 policies /b3/*. The study was approved by the Center for the Scientific and Clinical Study of Endocrinology Ethics Committee. Written informed consent was obtained from all participants. A special questionnaire chart was developed in the Center and was filled out for each woman. The chart included demographic and anthropometric data (age, height, weight), gynecological and hormonal history (age of menarche, age of menopause, the number of children, reproductive history), private and familial history of fractures, the present way of life (physical activity, smoking, drinking, and everyday use of dairy products).

Bone mineral density (BMD) was measured by ultrasound osteodensitometry (Omnisense 8000, Sunlight, Israel).

According to clinical guidelines, diagnosis of osteoporosis or osteopenia was based on the values of a T-score, the number of standard deviations (SD) from age norm. Thus, osteoporosis was diagnosed with T-score of 2.5 SD, the parameter's range from >-2.5 SD to ≤ 1.0 SD determined osteopenia, and the value <-1.0 SD was taken as normal. Every patient filled in a card-questionnaire developed at the Center for the Scientific and Clinical Study of Endocrinology, Uzbekistan Public Health Ministry.

Results were statistically processed using Excel 2010 and the *software* package STATISTICA 6.0 (Stat Soft, 2001). Logistic regression was used to calculate OR and 95% CI. Quantitative parameters are presented as M±SD, as well as Median (*Me*) and 25th and 75th percentiles as Inter Quartile Range (IQR). We used the Chi-square test to compare observed data. *P* values of <0.05 were considered statistically significant.

Results

We screened 1378 postmenopausal women aged ≥ 50 years (mean age 57.9 ± 6.4 years, IQR 53.0 to 62.0) within the period from 05.01.2010 to 05.01.2011. Residents of Tashkent were placed into Group 1 (n=963), and residents of Namangan into Group 2 (n=415). Among the examinees 371 (26.9%) women had normal BMD (nBMD), while osteopenia and osteoporosis were diagnosed in 473(34.3%) and 534 (38.8%) examinees, respectively (Table 1).

Table 1.
Characteristics of women in Tashkent and Namangan

Variable	Tashkent n=963		Namangan n=415		Total n=1,378	
	n	%	n	%	n	%
nBMD	296	30.7	75	18.1	371	26.9
Osteopenia	345	35.8	128	30.8	473	34.3
Osteoporosis	322	33.5	212	51.1	534	38.8
Age						
50-59 year	555	57.6	327	78.8	882	64.0
60-69 year	330	34.3	68	16.4	398	28.9
≥70 year	78	8.1	20	4.8	98	7.1
Weight <57 kg	74	7.7	70	16.8	144	10.4
BMI<20.0 kg/m^2	15	1.6	11	2,7	26	1.9
Daily consumption of dairy products	208	21.6	90	21.7	298	21.6
Regular physical activity (not less than 30 min per day)	631	65.5	150	36.1	781	56.7
Cigarette smoking	26	2.7			26	1.9
Consumption coffee	52	5.4	41	9.9	93	6.7
Previous fracture	16	1.7	25	6.0	41	3.0
Age, year	59.9±6.4		55.5±5.8*		57.9±6.4	
Me: IQR	58.0; 54.0:63.0		54.0; 50.0:59.0		57.0; 53.0:62.0	
BMI	29.7±5.3		28.9±5.5*		29.5±5.4	
Me: IQR	29.3; 26.1:32.9		28.3; 25.3:32.3		28.9; 26.0:32.7	
T-score	-1.86±1.5		-2.34±1.52*		-2.00±1.54	
Me: IQR	-1.89; -2.91:-0.78		-2.60; -3.60:-1.20		-2.05; -3.10:-0.90	
Duration of menopause, year	10.8±8.2		9.4±7.1*		10.4±7.9	
Me: IQR	10.0; 4.0:16.0		8.0; 4.0:14.0		9.0; 4.0:15.0	
Weight, kg	75.3±13.8		70.0±13.7*		73.7±14.0	
Me: IQR	74.0; 65.4:84.8		69.0; 60.0:78.0		72.0; 64.0:82.0	

** - P < 0.0001*

The maximum number of women with nBMD was found in the age group of 50 to 59 years (80.6%). With ageing, a progressive reduction in the percentage of patients with normal parameters was observed: 17.5% and 1.89% in the age groups of 60 to 69 and 70 to 79 years, respectively. In women with osteopenia the tendency persists, but there were significantly fewer women aged from 50 to 59 years in the osteopenia group than in the group with nBMD (62.8% versus 80.6%; OR 0.41; 95%CI 0.30-0.56; *P<0.0001*). There were significantly more women aged from 60 to 69 (OR 2.0; 95%CI 1.43-2.79; *P<0.0001*) and from 70 to 79 (OR 4.16; 95%CI 1.82-9.47; *P<0.0001*) years in the group with osteopenia than in the group with nBMD.

In the osteoporosis group there were confidently fewer women aged from 50 to 59 (53.6%) than in the group with nBMD (OR 0.28; 95%CI 0.2-0.38; *P<0.0001*) and in the group with osteopenia (OR 0.68; 95%CI 0.53-0.88; *P<0.0001*). However, among examinees with osteoporosis there were confidently fewer women aged from 60 to 69 (36.0%) than in the group with nBMD (OR 2.64; 95%CI 1.92-3.64; *P<0.0001*) and in the osteopenia group (OR 1.32; 95%CI 1.01-1.72; *P=0.05*).

About 10.5% of women with osteoporosis belonged to the 70- to 79-year-old group; they were confidently more in number than in the group with nBMD (OR 6.09; 95%CI, 2.74-13.5; *P<0.0001*) and more, though not confidently, than in the group with osteopenia (OR 1.47; 95%CI 0.94-2.28; P=0.11). As to residency, there were confidently fewer women with nBMD in Namangan than in Tashkent (18.1% versus 30.7%, OR 0.50; 95%CI 0.37-0.66; *P<0.0001*). In addition, osteoporosis in Group 2 was found in 51.1% of examinees from Namangan versus 33.5% in those from Tashkent (OR 2.08; 95%CI 1.64-2.63; *P<0.0001*) (Fig.1).

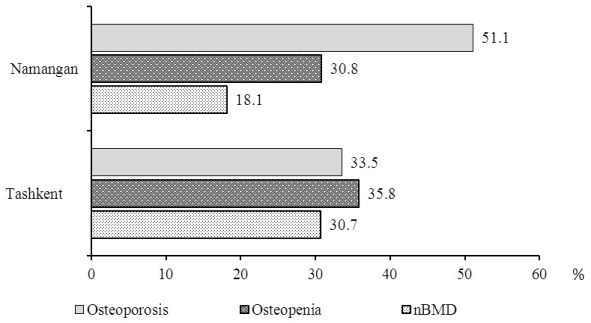


Figure 1. *Frequency of BMD disorders among residents of Tashkent and Namangan*

Namangan residents in the 50 to 59 and the 60 to 69 age groups were 1.5 and almost 5 times less than Tashkent residents, respectively. Similarly, a low frequency of nBMD could be seen in women aged from 70 to 79. Prevalence of osteopenia was found not to depend on age in Tashkent residents. The number of osteopenia cases confidently reduced among examinees from Namangan. With ageing, the number of women with osteoporosis was found to increase in both cities, but among Namangan residents the number was almost 2 times more. Thus, osteoporosis frequency was higher in the Namangan group than in the Tashkent group. In Namangan, nBMD was found in fewer female residents, and osteoporosis frequency was higher.

By means of analysis of BMD dependence on age, we found that the mean age of the examinees was 57.9±6.4 years; the Tashkentian examinees were significantly older than Namanganians (59.9±6.4 versus 55.5±5.8). This tendency was confirmed by more detailed analysis of the dependence of the degree of BMD on age. However, despite the fact that among examinees in the second group osteopenia and osteoporosis frequencies were higher, the women on average were 3 to 6 years younger than those in the first group.

There were significantly more women in Namangan with weight <57 kg than in Tashkent (OR 2.44; 95%CI 1.72-3.46; *P<0.00001*) (Fig.2.). There were more patients with low BMI in the cohort of Namangan residents with osteopenia and osteoporosis. Comparative analysis of parameters in the two cities demonstrated more women with lower limit of weight in Namangan. This seems to be the cause of higher osteopenia and osteoporosis in Group 2. We have found no substantial differences in the rate of BMI<20.0 kg/m^2 in the studied cohort of women (OR 1.72; 95%CI 0.78-3.78; *P=0.25*). Neither irregular administration of calcium nor smoking made a significant difference.

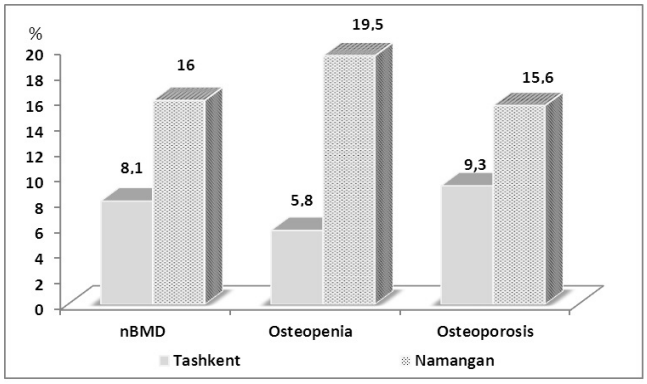


Figure 2. *The percentage of women with weight less than 57 kg depending on the bone mineral density*

Regular physical activity among women with osteoporosis was significantly rarer than in women with nBMD (48.1% versus 64.4%, OR 0.51; 95%CI 0.39-0.67; *P<0.0001*). At the same time, the number of women doing physical exercises in Tashkent was 2 times more than in Namangan (65.5% versus 36.1%, OR 3.36; 95%CI 2.64-4.27; *P<0.0001*). Tashkent female residents with osteoporosis were physically active more regularly than Namangan female residents (62.4% versus 26.4%, OR 4.63; 95%CI 3.17-6.76; *P<0.0001*). In Namangan, the percentage of physically active women in the osteoporosis group (26.4%) was confidently lower than those in nBMD group (49.3%, OR 0.37; 95%CI 0.21-0.64; *P<0.0001*) and in the osteopenia group (44.5%, OR 0.45; 95%CI 0.28-0.71; *P<0.0001*).

We studied osteoporosis frequency by duration of menopause. In Namangan, there were more women with menopause onset before 45 years of age than in Tashkent

(30.8% versus 14.2%, OR 2.69; 95%CI 2.04-3.54; *P<0.0001*). There were many more nBMD women with a menopause duration less than 5 years compared to PMO women (45.0% versus 20.8% OR 3.13; 95%CI 2.26-4.32; *P<0.0001*). It was much more frequent among residents from Tashkent (52.4% versus 15.2%, OR 6.09; 95%CI 3.98-9.32; *P<0.0001*) than from Namangan (30.1% versus 31.7%, OR 0.93; 95%CI 0.54-1.59; *P=0.89*). Osteoporosis was confidently more frequent among patients with menopause duration more than 10 years (51.7% versus 14.3%, OR 6.11; 95%CI 4.61-8.11; *P<0.0001*).

Discussion

Osteoporosis has become a major public health concern, which leads to increased rates of morbidity and mortality. Epidemiological investigations have shown that there is no country, nationality or race free from osteoporosis. According to NHANES data, there are 14 million women in the US aged more than 50 with low hip bone density. Prevalence of osteoporosis in all parts of the skeleton rises with age. According to WHO data, it exists in 70% of women more than 80 years old [10]. The disorder is being diagnosed in all age groups (from 6% in individuals >50 years to 50% among individuals >80 years) [11]. A recent study on the epidemiology of osteoporosis in the United States found a prevalence of 15.4% among women older than 50 years and a prevalence of 34.9% among women older than 80 years [12].

According to our data, the prevalence of osteoporosis in different regions of Uzbekistan varies widely (33.5% and 51.1% in Tashkent and Namangan, respectively). The prevalence of osteoporosis increases with age from 25.6% (50 to 59 years) to 51.3% (in >70 age group) in Tashkent and from 44.0% to 80.0% in in the same age groups in Namangan.

Low weight or low BMI is an indicator of low mineral density of bone tissue and a predictor of future fractures, particularly of the hip. Low BMI is <20 kg/m^2, low BMD - <57 kg [13,14]. Our data show that there were many more residents with body mass <57 kg in Namangan, than in Tashkent, but there was no difference in the number of residents with BMI<20 kg/m^2.

A low intake of calcium can lead to increased resorption of the bone matrix with demineralization and a consequent increase in fracture risk [1]. There were an equal number of women in studied cohorts using dairy products.

Persistent low physical activity is known to facilitate osteoporosis in older years. A sedentary style of life and immobilization result in rapid bone mass loss associated with accelerated bone resorption and slow bone formation [15]. Our study showed that the number of women doing physical exercises in Tashkent was 2 times more than in Namangan (65.5% versus 36.1%, OR 3.36; 95%CI 2.64-4.27; *P<0.0001*).

According to Van Geel [16,17], the risk of refracture increases if the previous fracture took place less than 5 years ago. According to our data from two densely populated regions, fractures occurred much more frequently in Namangan (6.02% versus 1.7%, OR 3.79; 95%CI 2.0-7.18; *P<0.0001*).

Menopause and its duration is the most significant osteoporosis risk factor. After the onset of menopause, bone mass loss is nearly 2% to 3% a year up to the age of 65 to 70, the rate subsequently reducing to 0.3% to 0.5% a year [18]. We found that the frequency of women with nBMD substantially decreases as the duration of menopause increases.

Conclusion

Our research shows that osteoporosis is widely spread among women above 50 living in two big densely populated cities of Uzbekistan (Tashkent and Namangan). Osteoporosis prevalence among Namangan female residents is higher than among Tashkent residents (51.1% versus 33.5%), the former being 3 to 6 years younger than the latter. Low body mass and irregular physical activity, fracture history, and duration of menopause are the factors of risk in the studied cohorts of women.

Competing interests

The authors declare that they have no competing interests.

References

1. Baccaro LF, Conde DM, Costa-Paiva L, Pinto-Neto AM. The epidemiology and management of postmenopausal osteoporosis: a viewpoint from Brazil. Clin Interv Aging 2015; 10:583–91.
2. Kim MY, Im SW, Park HM.The demographic changes of menopausal and geripausal Women in Korea. J Bone Metab 2015; 22(1):23-8.
3. Matin N, Tabatabaie O, Keshtkar A, Yazdani K, Asadi M. Development and validation of osteoporosis prescreening model for Iranian postmenopausal women. J Diabetes Metab Disord 2015; 14:12.
4. Hodgson SF, Watts NB, Bilezikian JP, Clarke BL, Gray TK, Harris DW, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the prevention and treatment of postmenopausal osteoporosis: 2001 edition, with selected updates for 2003. Endocr Pract 2003; 9(6):544-64.
5. Toroptsova NV, Nikitinskaya OA, Demin NV, Benevolenskaya LI. Prevention of post-menopausal osteoporosis: results of three-year observation. Sci Prac Rheum 2006; 5:25-32. [Article in Russian].
6. Barrett-Connor E1, Siris ES, Wehren LE, Miller PD, Abbott TA, Berger ML, et al. Osteoporosis and fracture risk in women of different ethnic groups. J Bone Miner Res 2005; 20(2):185–94
7. Cauley JA1, Palermo L, Vogt M, Ensrud KE, Ewing S, Hochberg M, et al. Prevalent vertebral fractures in black women and white women. J Bone Miner Res 2008; 23(9):1458–67.
8. Lansdown D1, Bennet B, Thiel S, Ahmed O, Dixon L, Vokes TJ. Prevalence of vertebral fractures on chest radiographs of elderly African American and Caucasian women. Osteoporos Int 2011;22(8):2365–2371
9. Kandiloytu AYu. Osteopenic syndrome in per-menopausal female residents of Tashkent, its correction. Abstract of PhD Thesis. Tashkent; 2005. [in Russian].
10. Aggarwal N1, Raveendran A, Khandelwal N, Sen RK, Thakur JS, Dhaliwal LK, et al. Prevalence and related risk factors of osteoporosis in peri- and postmenopausal Indian women. J Midlife Health 2011; 2(2):81-5.

11. Rahmani P, Morin S. Prevention of osteoporosis-related fractures among postmenopausal women and older men. CMAJ 2009; 181(11): 815-20.
12. Wright NC1, Looker AC, Saag KG, Curtis JR, Delzell ES, Randall S, et al. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. J Bone Miner Res 2014; 29(11):2520–6.
13. Brown JP, Josse RG; Scientific Advisory Council of the Osteoporosis Society of Canada. 2002 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada. CMAJ 2002;167 (10 Suppl):S1–34
14. De Laet C, Kanis JA, Oden A, et al. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporosis Int. 2005;16(11): 1330–1338.
15. Bonaiuti D, Arioli G, Diana G, Franchignoni F, Giustini A, Monticone M, et al. Rehabilitation treatment Guidelines in postmenopausal and senile Osteoporosis. Eura Medicophys 2005; 41(4):315–37.
16. van Geel TA1, Geusens PP, Nagtzaam IF, van der Voort DJ, Schreurs CM, Rinkens PE, et al. Risk factors for clinical fractures among postmenopausal women: a 10-year prospective study. Menopause Int 2007; 13(3):110-5.
17. van Geel TA, Nguyen ND, Geusens PP, Center JR, Nguyen TV, Dinant GJ, et al. Development of a simple prognostic nomogram for individualising 5-year and 10-yearabsolute risks of fracture: a population-based prospective study among postmenopausal women. Ann Rheum Dis 2011;70(1):92-7.
18. Rojinskaya LYa. *Systemic osteoporosis*. Moscow, “Mokeev” Publishing House; 2000. [in Russian].

International Journal of BioMedicine 5(2) (2015) 100-103

INTERNATIONAL JOURNAL OF BIOMEDICINE

EPIDEMIOLOGY

Structure of Congenital Heart Defects in Newborns in the Sakha Republic (Yakutia)

Tuyara I. Nelunova[1], PGS; Vyacheslav G. Chasnyk[1], PhD, ScD;
Tatiana E. Burtseva[2], PhD, ScD; Mikhail I.Tomsky[2], PhD, ScD; Evdokia D. Son[3], PhD

[1]*Saint Petersburg Medical University, St. Petersburg, Russia*
[2]*Yakutsk Research Center for Complex Medical Problems, Yakutsk, Sakha Republic, Russia*
[3]*North-Eastern Federal University Ammosov, Yakutsk, Sakha Republic, Russia*

Abstract

The results of a retrospective study of newly diagnosed cases of congenital heart defects (CHDs) among liveborn infants in the Sakha Republic (Yakutia, SR(Y)) are presented. The study was divided into two time periods: Period A (from 2002 to 2002) and Period B (from 2011 to 2013). A comparative analysis of the prevalence of CHD detection and the structure of clinical entities of CHDs among liveborn infants in Period A (42,827 liveborn infants and 186 cases of CHDs) and Period B (50,104 liveborn infants and 899 cases of CHDs) was performed. The prevalence of CHDs among newborns in Period A was 4.34 per 1,000 live births, which corresponded to the average CHD frequency in Russia. The prevalence of CHDs among newborns in Period B was 17.9 per 1,000 live births, which is significantly higher than in Period A (*P=0.0000*). The overall growth of the incidence of CHDs among newborns in period B was due to an increase in the prevalence and frequency of septal defects. The increase in the prevalence of CHDs could be due to many factors, including improvement in the quality of diagnosis in 2011–2013.

Keywords: *congenital heart defects; the Sakha Republic (Yakutia); quality of diagnosis.*

Introduction

Currently, the congenital malformations (CDF – congenital defect fetus) that occur in 4.0% to 6.0% of newborns play an important role in the structure of child morbidity, disability, and infant mortality; their contribution to the structure of a child's death in the first year of life is more than 20% [1-3]. In CDF structure, CHDs and defects of the great vessels occupy one of the first places (22% of all CDFs) and their prevalence at birth is 8 to 14 cases per 1,000 newborns in all countries [4]. Studies conducted in the USA and the UK showed that the natural course of CHD resulted in death at the end of the first year of life in more than 70% of cases. In North America, CHDs are the cause of death of 37% of infants and in Western Europe, 45% [5,6]. In the Russian Federation, the birth rate of children with CHDs is from 3.2 to 8.0 per 1,000 live births and tends to rise [5]. According to the Russian State Statistics Committee (2013), the SR(Y) has a population of 955,580 people. The population of Yakutsk is 311,900 people. In the SR(Y), in the structure of infant mortality for a number of years, CDFs have takeen second place after the "diseases specific to the neonatal period." In 2012 compared to 2010, in connection with the transition to neonatal care of infants with extremely low birth weight and registration of births from 22 weeks of pregnancy (according to WHO criteria), there was an increase in the infant mortality rate (IMR) of about 1.4 times (9.9 per 1,000 live births) and in the perinatal mortality rate (PMR) of about 1.6 times (13.7 per 1,000 live births and stillborn). In the structure of IMR, the proportion of CDF increased by about 1.5 times. The multiple malformations (33.9%) and malformation of heart and central nervous system (21.4%) retain a leading position in the structure of all congenital malformations. Congenital malformations, of which 48.3% are anomalies of the circulatory system, are the main causes of disability in children, according to the Sakha Ministry of Health (2012). Thus, a significant contribution of CHDs to the formation of perinatal and infant mortality, and disability of children, as well as insufficient data on the spread, frequency and structure

***Corresponding author:** Tatiana E. Burtseva, PhD, ScD. Deputy Director of the Yakutsk Research Center for Complex Medical Problems, Yakutsk, Sakha Republic, Russia E-mail: bourtsevat@rambler.ru*

of CHD in the SR(Y), served as the cause for this study, which was performed for the first time.

The aim of this study was to analyze the structure of CHDs and great vessels in the newborns in the SR(Y) according to the data of the Perinatal Center of the SR(Y) in periods from 2002 to 2004 and from 2011 to 2013.

Material and Methods

A retrospective study of newly diagnosed cases of CHDs among newborns was conducted on the basis of the Perinatal Center of the SR(Y) at the department of neonatal pathology, at the department of neonatal care for premature infants, and at the department of infectious diseases of newborn infants. The study included all cases of CHDs in infants born alive. The study was divided into two time periods: Period A (from 2002 to 2002) and Period B (from 2011 to 2013). Period A was considered as a control group. CHD were recorded according to ICD-10: Chapter XVII "Congenital malformations, deformations and chromosomal abnormalities", subparagraph "Congenital malformations of the circulatory system '' (Q20-Q28). Primary documentation included hospital journals and statistical cards of the inpatients. The clinical diagnosis of CHD was confirmed by Doppler echocardiography of the heart and blood vessels, ECG, X-ray, *angiographic study*, and CT angiography. Incidence rate was calculated per 1,000 live births. Written informed consent was obtained from the *child's parents*. Statistical analysis was performed using statistical software "Statistics Calculator", https://www.statpac.com/statistics-calculator/percents.htm). *P* values of *<0.05* were considered statistically significant.

Results and Discussion

According to case records, diagnosis of CHDs among newborns was registered in 186 cases for Period A and in 899 cases for Period B. A comparative analysis of the prevalence of CHD detection and the structure of clinical entities of CHDs among liveborn infants in Period A (42,827 liveborn infants and 186 cases of CHDs) and Period B (50,104 liveborn infants and 899 cases of CHDs) was performed. The results of the study are presented in Table 1. According to our data, the prevalence of CHDs among newborns in Period A was 4.34 per 1,000 live births, which corresponded to the average CHD frequency in Russia [1]. The prevalence of CHDs among newborns in Period B was 17.9 per 1,000 live births, which is significantly higher than in Period A (*P=0.0000*). The increase in the prevalence of CHDs could be due to many factors, including improvement in the quality of diagnosis in 2011–2013.

All cases of CHDs were divided into three groups: 1) septal defects: atrial septal defect (ASD), ventricular septal defect (VSD) in combination with patent ductus arteriosus (PDA), and pulmonary valve stenosis (PVS); 2) complex CHDs: pulmonary atresia (PA), tetralogy of Fallot (ToF), atrioventricular septal defect (AVSD), total anomalous pulmonary venous connection (TAPVC), transposition of the great vessels (TGV), pulmonary stenosis, Ebstein's anomaly, tricuspid atresia, single ventricle, double outlet right ventricle (DORV), truncus arteriosus, and mitral valve atresia (MVA); 3) other CHDs: patent ductus arteriosus (PDA), coarctation of the aorta (CoA), PVS, partial anomalous pulmonary venous connection (PAPVC), tricuspid valve dysplasia, and coronary artery anomalies (CAAs). We analyzed the prevalence and frequency of malformations in view of the groups and the periods of the study (Table 1).

The results showed a statistically significant increase in the prevalence of all CHDs in Period B compared to Period A (*P=0.0000*). The prevalence of septal defects increased from 3.08 (n=42.827 newborns) to 15.55 (n=50.104 newborns), of complex CHDs from 0.54 (n=42.827 newborns) to 0.92 (n=50.104 newborns), and of other CHDs from 0.61(n=50.104 newborns) to 1.48 (n=50.104 newborns). The frequency of septal defects increased from 73.66% (n=186 CHDs) to 86.65% (n=899 CHDs), and the frequency of the complex CHDs decreased from 12.37% (n=186 CHDs) to 4.67% (n=899 CHDs), in comparison with Period A (*P=0.0001*). We also noted a decrease in frequency of other CHDs from 13.97% (n=186 CHDs) to 8.68% (n=899 CHDs) in the structure of CHDs (*P=0.0259*). Thus, we concluded that the overall growth of the incidence of CHDs among newborns in period B was due to an increase in the prevalence and frequency of septal defects.

Table 1.

The structure of CHDs among newborns

CHDs (ICD-10)	Period A (2002-2004)			Period B (2011-2013)			*P*	
	Abs. number of CHDs	1)	2)	Abs. number of CHDs	3)	4)	1) vs. 3)	2) vs. 4)
Q21.0-21.1 Group of septal defects	137	3.08	73.66	779	15.55	86.65	*P=0.0000*	*P=0.0000*
Group of complex CHDs	23	0.54	12.37	46	0.92	4.67	*P=0.0000*	*P=0.0001*
Group of others CHDs	26	0.61	13.97	74	1.48	8.68	*P=0.0000*	*P=0.0259*
Total	186	4.34		899	17.94		*P=0.0000*	

1)The prevalence of CHDs per 1,000 live births±0.01, n=42,827 newborns. 2)The frequency of the individual forms of CHDs (%±0.01), n=186 CHDs. 3)The prevalence of CHDs per 1,000 live births ± 0.01, n=50,104 newborns. 4)The frequency of the individual forms of CHDs (%±0.01, n=899 CHDs.

Table 2.

Nosological forms of CHDs for 2002-2004 and 2011-2013

CHD (ICD-10)	Period A (2002-2004)			Period B (2011-2013)			*P*	
	Abs. number of CHDs	1)	2)	Abs. number of CHDs	3)	4)	1) vs. 3)	2) vs. 4)
Q21.0-21.1 VSD, ASD, VSD+PDA, PVS	137	3.08	73.66	779	15.55	86.65	*P=0.0000*	*P=0.0000*
Q25.0 PDA	8	1.19	4.30	51	1.02	5.67	*P=0.01*	*P=0.45*
Q25.1 CoA	0	0	0	21	0.42	2.34	*P=0.0000*	*P=0.04*
Q22.1 PVS	12	0.28	6.45	0	0	0	*P=0.0000*	*P=0.0000*
Q22.0 PA	2	0.05	1.08	7	0.14	0.78	*P=0.0000*	*P=0.68*
Q21.3 ToF	4	0.09	2.15	10	0.20	1.11	*P=0.0000*	*P=0.25*
Q21.0 AVSD	1	0.02	0.54	10	0.20	1.11	*P=0.0000*	*P=0.48*
Q26.2-26.3 TAPVC	1	0.02	0.54	3	0.06	0.33	*P=0.0029*	*P=0.67*
Q26.3 PAPVC	5	0.12	2.69	1	0.02	0.11	*P=0.0000*	*P=0.0000*
Q25. Pulmonary stenosis	4	0.09	2.15	4	0.08	0.45	*P=0.60*	*P=0.01*
Q20.3 TGV	2	0.05	1.08	4	0.06	0.45	*P=0.08*	*P=0.28*
Q20.1 DORV	1	0.02	0.54	2	0.04	0.22	*P=0.08*	*P=0.45*
Q20.4 Single ventricle	0	0	0	1	0.02	0.11	*P=0.0035*	*P=0.65*
Q22.5 Ebstein's anomaly	1	0.02	0.54	4	0.06	0.45	*P=0.0001*	*P=0.85*
Q22.8 Tricuspid valve dysplasia	0	0	0	1	0.02	0.11	*P=0.0035*	*P=0.65*
Q22.6 Tricuspid atresia	1	0.02	0.54	1	0.02	0.11	*P=1.00*	*P=0.21*
Q20.0 Truncus arteriosus	2	0.05	1.08	0	0	0	*P=0.0000*	*P=0.0019*
Q23.4 MVA	4	0.09	2.15	0	0	0	*P=0.0000*	*P=0.0000*
Q24.5 CAAs	1	0.02	0.54	0	0	0	*P=0.0016*	*P=0.03*
Total	186	4.34		899	17.94		*P=0.0000*	

Note: See Table 1.

We analyzed the structure of CHDs according to the nosological forms (Table 2). In Period B, there was a statistically significant increase in the prevalence and frequency of septal defects (ASD, VSD combined with PDA and PVS) from 0.32 (n=42.827 liveborn infants) to 1.55 (50.104 liveborn infants) (*P=0.0000*) and from 73.66% (n=186 CHDs) to 86.65%(n=899 CHDs) (p=0.0000), respectively, and the prevalence and frequency of PAPVC decreased from

0.012 (n=42.827 liveborn infants) to 0.002 (n=50.104 liveborn infants) (*P=0.06*) and from 2.69 (n=186 CHDs) to 0.11% (n=899 CHDs) (*P=0.0000)*, respectively. There was a lack of PVS, arteriosus, and MVA in Period B. Such abnormalities as single ventricle, hypoplastic right heart syndrome, t*ricuspid valve dysplasia*, and CAAs were detected in a few cases in both Periods A and B. Among malformations of the great vessels, CoA was not identified in Period A, while in Period B, 21 cases of CoA were identified with the prevalence of 0.42 (n=50.104 liveborn infants) and frequency of 2.34% (n=899 CHDs), which may be explained by the improvement in the quality of diagnosis, in particular, the use of cardiac catheterization with x-ray images. It was noted an increase in the prevalence of PA from 0.05 to 0.14 (*P=0.0000*), ToF from 0.09 to 0.20 (p=0.0000), and AVSD from 0.02 to 0.20 (*P=0.0000*) without statistically significant change in the share of particular defect in the structure of all CHDs in Period B.

Conclusions

• Our study revealed a statistically significant increase of the newly diagnosed CHDs among newborns: 17.9 in Period B versus 4.34 (*P=0.0000*) in Period A. The increase in the prevalence of CHDs could be due to many factors, including improvement in the quality of diagnosis in 2011–2013.

• The overall increase in the prevalence and frequency of CHDs among newborns in the Period B was due to an increase in the prevalence and frequency of septal defects.

• The identified 21 cases of CoA with the prevalence of 0.42 (liveborn infants) and frequency of 2.34% (CHDs) in Period B may be explained by the improvement in the quality of diagnosis, in particular, the use of cardiac catheterization with x-ray images.

• The prevalence of PA, ToF, and AVSD increased without a statistically significant change in the share of particular malformation in the structure of all CHDs in Period B.

• The prevalence and frequency of PAPVC decreased in the structure of all CHDs in Period B.

Competing interests

The authors declare that they have no competing interests.

References

1. Mutafian OA. Malformations and minor heart abnormalities in children and adolescents. SPb.: MAPS; 2005. [in Russian].
2. Bogantsev S.V Analysis of the structure of congenital heart defects in children. Omsk Nauch Vest 2006; 3:196-200. [in Russian].
3. Magomedova Sh M. Epidemiology of congenital heart defects in children in different climatic zones of the Republic of Dagestan. Abstract of PhD Thesis. Makhachkala; 2006. [in Russian].
4. Zeminskaya DI, Balaeva LS. Child disability. M .: Med., 2001. [in Russian].
5. Liapin VA. Socially significant pathology of the child population of the industrial center of Western Siberia. Siberia-East 2005; 3:9-11. [in Russian].
6. Rosano A, Botto L.D, Botting B, Mastroiacovo P. Infant mortality and congenital anomalies from 1950 to 1994: an international perspective. J Epidemiol. Community Health 2000; 54(9):660-6.

International Journal of BioMedicine 5(2) (2015) 104-105

INTERNATIONAL JOURNAL OF BIOMEDICINE

PERSPECTIVE

How Digital Health Technology Aids Physicians

Nik Tehrani, PhD

Argosy University, CA, USA

Abstract

There is so much health and medical information available today that physicians cannot be expected to know it all. Thus, advances in technology have become a necessity for doctors to track patient information and care, and add to patient databases for reference and to conduct research. It is important to understand the new language of digital health, such as Personal Health Record (PHR), Electronic Medical Record (EMR) and Electronic Health Record (EHR), all of which sound similar, but are not interchangeable. The ideal comprehensive IT system would empower patients, advance healthcare delivery and transform patient data into life-saving research (Kaiser, 2015). OmniFluent Health is language translation software that will allow for better patient/practitioner communication and avoid errors. Digital technology employs the use of big data that is shared, accessed, compiled and applied using analytics. However, information transfer, especially as mandated by current ethics of use of technology, has resulted into breach of patient privacy. Improved digital technology is providing the health care field with upgrades that are necessary, electronic files and health records, from mobile apps, and remote monitoring devices.

Keywords: Personal Health Record (PHR); Electronic Health Record (EHR); digital health; remote monitoring.

The embracing of health information technology by physicians has increased, which has resulted in better patient care. But with so much new medical oriented technology, it is important to understand of the language of digital health. Personal Health Record (PHR), Electronic Medical Record (EMR) and Electronic Health Record (EHR) sound quite similar, but they are not identical and are not interchangeable. Each has its own function separate from the other. An estimated 70 percent of U.S. doctors already utilize some aspect of EHR, however, only about one quarter of those subscribe to a sophisticated multifunctional system. Ideally, a comprehensive IT system should empower patients, advance healthcare delivery and transform patient data into life-saving research [1]. The new technological approaches to healthcare delivery have resulted in faster and more accurate diagnostic and monitoring, more sophisticated coordination across regions and agencies and sophisticated risk-checking procedures.

A patient's Personal Health Record (PHR) is a secure portal by which he/she can access the EMR to make and change appointments, check lab results, order prescriptions and modify personal information. The EMR is the electronic version of a patient's medical record that connects doctors and other caregivers with patient data from every point of healthcare, such as x-rays, prescriptions and MRI's. The EHR is a secure electronic database information from all of a patient's EMRs under one umbrella to avoid redundant testing and errors in prescription medication [1], and also allows doctors to create a large patient database from which they can conduct research. The EMR enables doctors to electronically exchange key clinical information with other caregivers to get the broader picture of a patient's medical history [1].

Digital technologies enable analysis of patient data to present better and quicker treatment and diagnoses. E. Lee [2] points out that Dr. Watson, a computer developed by IBM, helps medical practitioners formulate more accurate diagnoses and recommend treatment. Dr. Watson helps doctors monitor the history of their patients, refer to the latest medical studies and analyze up-to-date treatment alternatives, enhancing doctors' abilities to diagnose and monitor patient health using current information [3].

According to V. Kaptelinin [4] translation technologies are making doctors more effective because they can communicate with their patients more easily by overcoming language barriers. OmniFluent Health, a product from Science Applications International Corporation (SAIC), is translation software for all medical practitioners. The software includes a mobile application (app) which allows a practitioner to ask, for example, if a patient is allergic to a certain drug. The app translates to a language that is the patient understands, lessening the possibility of prescribing the wrong

***Corresponding author:** Nik Tehrani, PhD. Argosy University, CA, USA E-mail: nik@niktehrani.com*

medication. R. Symthe [5] points out that, in the United States, approximately forty seven million residents do not speak English fluently. Hence, clarifying the communication between patients and their doctors more easily will increase the doctor's patient knowledge and avoid errors.

E. Topol [6] indicates that digital technologies will allow medical practitioners to easily link up with each other without meeting face-to-face. Nearly one-third of healthcare professionals use exclusive healthcare mainstream social media networks where they can collaborate with colleagues and share resources online [7]. One social network tailored for physicians is Doximity. According to E. Lee [2], this platform allows doctors in the United States to collaborate online and discuss difficult cases. Doximity has 250,000 members, representing approximately 40 percent of all U.S. doctors who exchange information [7]. Most of the "HIPAA-compliant one-to-one messages and discussion forums focus on business challenges or diagnoses" [7].

G. Eyesenbach [3] maintains that medical digital technology, Sherpaa, helps patients and doctors connect via the phone or online, avoiding a trip to the emergency room. Reliable advice is rapidly provided to patients from medical specialists. V. Kaptelinin [4] points out that digital technology employs the use of big data that is shared, accessed, compiled and applied using analytics. The medical setting benefits by having a robust and efficient clinical and business decision-making platform. For instance, medical practitioners can leverage huge amounts of patient information collected from a number of sources to establish the clinical validity of particular managements and how to improve them [5]. In addition, doctors can access and share patient medical records to eliminate unnecessary medication and/or testing.

The doctor/patient experience is enhanced by technology, due to ease in cooperating with experts and physicians in innovative ways and utilizing computers that analyze patients' medical information to provide more efficient and better treatment for the patients [2]. As digital technology continues to expand the scope of medical interactions and medicine, a new revolution in the health care setting is on the horizon, such as remote health monitoring for elderly through interactive television [8] and other types of hand-held remote monitoring devices.

Digital tools have, however, been found to be incompatible with changing patient needs. Ideally, the current area of research in molecular technology, including laser guided surgeries, has fallen short of the necessary transmission of information needed to help in completing diagnostics. However, innovative diagnostics which involve extreme reliance of technology have in some instances led to misdiagnosis, leading to fatalities [4]. Further, information transfer, especially as mandated by current ethics of use of technology, has resulted into breach of patient privacy.

Improved digital technology is providing the health care field with upgrades that are necessary, electronic files and health records, from mobile apps, remote monitoring devices, and medical translation tools which help individuals to have healthier lives. All of the advancements in digital technologies are increasingly more sophisticated and are becoming a necessity. Doctors cannot avoid the rush of new advancements. And why should they? There is so much information available that physicians cannot be expected to know everything (Smythe, 2015). Digital technologies are there to help doctors work smarter and more efficiently; hence digital technology has become essential in the healthcare context.

References

1. Kaiser Permanente. (2015). Papers to pixels. Understanding the language of digital health. Retrieved from https://businessresources.kaiserpermanente.org/Global/FileLib/kp-toolkits/Kaiser_Permanente-_EMR_infographic.pdf.
2. Lee E. *5 ways Technology is Transforming Health Care.* Forbes, 2013; 15 (4):9-10
3. Eysenbach G. What is e-health? J Med Internet Res 2005; 3(2):20.
4. Kaptelinin V. *Activity theory: implications for human-computer interaction.* Cambridge, MA: MIT Press; 2008:103-116.
5. Smythe R. *Is technology dumbing down your doctor?* Forbes, 2015; 75(2): 8-10.
6. Topol E. *The creative destruction of medicine: how the digital revolution will create better health care*. New York: Basic Books; 2014:63-98.
7. Diana A. (2014). Information week healthcare. Healthcare social networks: New choices for doctors, patients. Retrieved from http://www.informationweek.com/healthcare/patient-tools/healthcare-social-networks-new-choices-for-doctors-patients/d/d-id/1234884.
8. Spinsante S, Gambi E. Remote health monitoring for elderly through interactive television. Biomedical Engineering Online 2012; 11, 54. doi: http://dx.doi.org/10.1186/1475-925X-11-54.

EuroMediCom
Promoting Science for Tomorrow

informa
exhibitions

ICAD 2015

INTERNATIONAL CONGRESS OF AESTHETIC DERMATOLOGY

BANGKOK CONVENTION CENTRE AT CENTRALWORLD

8-9-10
OCTOBER 2015

BANGKOK - THAILAND

ORGANIZED IN COOPERATION WITH THE OFFICIAL
DST - DERMATOLOGICAL SOCIETY OF THAILAND

WWW.EUROMEDICOM.COM

AMWC AMAC

Part of the **AMWC Monaco & AMAC** Series of
World Events in Global Ageing Management

IJBM

INTERNATIONAL JOURNAL OF BIOMEDICINE

Instructions for Authors

Editorial Policies

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

Manuscript Submission

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. 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.

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

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.

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

Abstract

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

Key Words

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

Text

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

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

Authorship

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

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

Acknowledgments

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

References

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

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

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

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

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

Figures and Legends

All figures should be cited in the text and numbered in order of appearance. Figures should be uploaded as individual files and named accordingly (ie, Figure 1.tiff, Figure 2.tiff). They should be saved in either tiff or eps file formats only, PowerPoint files will be sent back to the author. Color illustrations are not accepted for print publication unless the author agrees to pay all costs associated with producing color art. The cost is $150 per page of color illustrations. However, all illustrations submitted in color will be published in color online, at no cost to the author.

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. The magnification of any photograph should be omitted unless it is not generally apparent (as in an electron photograph). Legends should be double-spaced on a separate page within the manuscript, with all abbreviations and symbols appearing on the illustration described.

Tables

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

Permissions

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

Processing Fees

***Open Access Publication*:** all manuscripts submitted to IJBM will be submitted under the Open Access publishing model. In this publishing model, papers are peer-reviewed in the normal way under editorial control. When a paper is accepted for publication the author is issued an invoice for payment of a publication processing fee. Payment of this charge allows IJBM to partially recover its editorial process and production of the printed version, and development of online functionality, and provide our content at no cost to readers. IJBM charges a processing fee of $100 per printed journal page to help meet the above costs. The average length for an IJBM Journal paper is four (4) printed journal pages.

Surely, a processing fee of $100 per printed journal page does not cover the whole cost. For IJBM, the income might be from subscriptions for the printed journal, foundation and grant support, advertisements, and institutional support. Published papers appear electronically and are freely available from our website. Authors may also use their published .pdf's for any non-commercial use on their personal or non-commercial institution's website. A subscription to the printed version of IJBM remains available.

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

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

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

Page Proofs: Page proofs are sent from the Publisher electronically and must be returned within 72 hours to avoid delay of publication. All authors must sign and return the author approval and final page of Publication Agreement.

Generally peer review is complete within 3-4 weeks and the editor's decision within 7-10 days of this. It is therefore very rare to have to wait more than 6 weeks for a final decision.

AUTHOR'S CHECKLIST

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

• Cover Letter

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

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

• Manuscript, including:
– Title page
– Article title
– Short title (less than 65 characters w/ spaces)
– Authors' names, academic degrees, affiliations
– Acknowledgment of grants and other financial support
– Word count
– Number of figures and tables
– Name, address, telephone, fax, and email address of corresponding author
– All authors must disclose any financial or other relations that could lead to a conflict of interest. If a potential conflict exists, its nature should be stated for each author. When there is a stated potential conflict of interest a foot note will be added indicating the author's equity interest in or other affiliation with the identified commercial firms.
– All sources of financial support for the study should be stated including federal or state agencies, nonprofit organizations, and pharmaceutical or other commercial sources.
• Abstract
• Key words
• Text
• Acknowledgments
• References
• Table and Figure Legends
• Figures (individual tiff or eps file format)
• Tables (individual word documents)
• Permissions for the use of any previously published materials
• Disclosure Form (fax or e-mail to Editorial Office)

Advancing Toxicology Science in Developing Countries

9th
Congress of Toxicology in Developing Countries

XIX
Congresso Brasileiro de Toxicologia

NOV 07 - 10, 2015

NATAL | BRASIL