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 Accepted manuscripts become the sole property of the Journal and may not be published elsewhere without the consent of the Journal.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 4, December 2015

CONTENTS

CONTENTS

CONTINUED

ESC CONGRESS

ROME 2016

27 – 31 August

e s c

c s ə

european stroke conference

ESC 2016

25th EUROPEAN STROKE CONFERENCE

13–15 APRIL 2016

VENICE (ITALY)

www.eurostroke.eu

International Journal of Biomedicine 5(4) (2015) 179-183

ORIGINAL ARTICLE

Infection and Immunity

INTERNATIONAL JOURNAL OF BIOMEDICINE

Duration of Preservation of Antibodies to the Flu Virus in the Mother-Child Pairs during the Vaccination of Women Depending on the Trimester of Pregnancy

Mikhail P. Kostinov[1], PhD, ScD; Alexander P. Cherdantsev[2], PhD, ScD; Anna D. Shmitko[1]; Dmitry V. Pakhomov[1]*, PhD; Evgenia O. Demina[1]

[1] *The I.I. Mechnikov Scientific-Research Institute of Vaccine and Sera, RAS; Moscow, Russia*
[2] *Ulyanovsk State University; Ulyanovsk, Russia*

Abstract

Vaccination against flu in the second and third trimesters of pregnancy with the use of Agrippal S1 is effective and meets the CPMP criteria. In our study, one month after vaccination, there was progress in the production of post-vaccination antibodies in protective values in pregnant women vaccinated in the third trimester of gestation. In neonates whose mothers had been vaccinated in the last trimester of pregnancy, there were also higher levels of protective antibodies to the flu A virus strains. Three months after childbirth, there were no significant differences in protective values of antibodies to all the strains of the flu virus in women of the compared groups. At the same time, in children born to women vaccinated in the third trimester of pregnancy, there was a significantly higher concentration of protective antibodies during the same period. Six months later, there were no transplacental antibodies in the protective values (≥1:40) among the observed children. Comparison of the levels of protective titers of antibodies to the three strains of the flu virus in the mother-infant pairs, in all the periods of observation of the children, showed significantly lower values.**(Int J Biomed. 2015;5(4):179-183.)**

Keywords: *flu virus; vaccination of pregnant women; neonates; antibody response; hemagglutination-inhibition antibody.*

Introduction

Vaccination of women against flu during pregnancy is safe and highly effective. Nevertheless, the immune response to the vaccine with regard to the trimester of gestation is poorly understood, both for the women and in regards to the passive transfer of maternal antibodies to the neonates. It can be assumed that the level and duration of preservation of the maternal post-vaccination immunity in infants are in direct proportion to their original values in mothers with normal physiological pregnancy. Vaccination in the third trimester of gestation is accompanied by a 63% decrease in the incidence of flu in neonates, and antibody titer values ≥1:40 may persist for up to 20 weeks of life [1-3]. It is also known that the best effect of maternal vaccination is observed within 42 to 50 days after birth, which coincides with the half-life of transplacental antibodies to influenza virus in the infant [2]. There is a direct relation between maternal vaccination and the reduction of the flu incidence in infants during the first 2 to 3 months of life, and a 92% reduction in hospitalization of children during the first 6 months [4]. Therefore, we can conclude that vaccination during pregnancy leads to a synthesis of specific antibodies that may protect not only the vaccinated women, but also their babies during the first 3 to 5 months of life.

The aim of the study was to evaluate the levels of antibodies to the flu virus in the mother-infant pairs, taking into account the trimester of vaccination of pregnant women.

Materials and Methods

We observed 48 mother-infant pairs, which were vaccinated once with Agrippal S1 (Novartis Vaccines and Diagnostic, Italy) against flu during pregnancy in the seasons of 2010–2011 and 2011–2012.

Group 1 women (n=27) and Group 2 women (n=21) were vaccinated in the second and third trimesters of pregnancy, respectively. Observation of the women was carried out both

***Corresponding author**: Dmitry V. Pakhomov, PhD. Laboratory of vaccination and immunotherapy of allergic diseases, the I.I. Mechnikov Scientific-Research Institute of Vaccine and Sera, RAS; Moscow, Russia. E-mail: dm_pachomov@mail.ru*

during pregnancy and after childbirth, including their infants under 6 months of age. The study was carried out in accordance with the requirements of the protocol of the Ministry of Health of the Russian Federation (MoH RF) of October 2, 2009 No. 808n on the Approval of the Order of Rendering Obstetric-Gynecologic Assistance [5]. Before the vaccination, after the receipt of the informed consent of women to participate in the clinical study, a general laboratory examination was conducted (blood test, biochemical parameters, and urine test). In the absence of deviations from the normal values and in compliance with the study inclusion criteria, pregnant women were assigned to receive a vaccination against flu.

The study was conducted in accordance with established protocols, meeting the national standard of the Russian Federation [6,7]. The vaccination was conducted in compliance with the ethical standards and recommendations of the WHO and MoH RF [7,8]. The study was a single-center, prospective, randomized, open-label, comparative, parallel-group study of pregnant women and children.

Women subject to vaccination and follow-up were selected in strict compliance with the individual registration card, which was reviewed and approved by the Ethics Committee at the Institute of Medicine, Environment and Physical Education of the Ulyanovsk State University.

Over 42/87.5% children born to women vaccinated during pregnancy were observed. Studying the peculiarities of the early postnatal period, we also complied with the ethical requirements for conducting biomedical research on children. In drawing up the order and number of the studied parameters, we used the provisions set forth in the Order No.370 of MoH RF [9].

To evaluate the immunogenicity of the vaccine against flu in women, we conducted blood sampling before 1 and 3 months (in case of immunization in the second trimester of pregnancy) after vaccination.

Blood sampling was carried out in the mother-infant pairs on days 2 to 3 after childbirth, and at 3 and 6 months of follow-up. Serum level of the hemagglutination-inhibition antibody (HAI-Ab) was determined in HAI reaction. The antigenic activity of the vaccine was assessed in accordance with the criteria adopted by the Committee for Proprietary Medicinal Products (CPMP) [10]:

1). *Seroprotection level:* Percentage of vaccinated persons with the serum HAI-Ab titer higher than 1:40 on the 21st day after vaccination (should be higher than70%).

2). *Seroconversion rate or immunological activity of the vaccine:* Percentage of vaccinated persons among all the immunoprotective people with a 4-fold or more increase in HAI-Ab titer between baseline and by day 21 after vaccination (should be higher than 40%).

3). *Seroconversion factor or geometric mean fold rise* as the geometric mean of the within-subject ratios of the post-vaccination reciprocal HAI titer to the day 1 reciprocal HAI titer: geometric mean HAI antibody titers (GMTs). The ratio of GMTs before and after vaccination (GMTR) should be higher than 2.5-fold.

According to these recommendations, the vaccine is considered to be sufficiently immunogenic and effective if it meets at least one of these three criteria.

Women with HAI-Ab titer higher than 1:20 but less than 1:40 were classified as seropositive (reacting to the vaccine with a low level of the formation of specific immunity).

Statistical analysis of the obtained results was carried out using the methods of descriptive statistics (Thompson rule). The reliability of differences in the quantitative indicators of the obtained data was calculated using the non-parametric Wilcoxon test for unrelated samples (W). The sign was considered to be reliable at the values of $P<0.05$ with a confidence interval of 0.95. We used the AtteStat 10.10.2 software package.

Results

The clinical analysis of the course of the post-vaccination period in the vaccinated women in the second and third trimesters of pregnancy showed an absence of unusual events, as well as no impact of vaccination on the obstetric history.

The clinical assessment of the state of children at birth was carried out using the Apgar scale. It was discovered that 8 to 9 points were recorded in 22/84.6% children. A transiently reduced level of total Apgar scores (4 to 6 points), recovering to fifth minute up to 8 to 9 points, was discovered in 15.4%. All the children had the physical harmony of age-related, weight-height parameters; at the same time, we did not register significant differences in these parameters in the compared groups.

During the 6 months of life among children born to mothers of Group 1, we registered 2/8.7% cases of respiratory infection; as for the children born to mothers of Group 2, we registered 1/5.3% case ($P>0.05$). In other words, the incidence of the acute respiratory infection of a non-flu nature was quite similar in the observed groups of infants.

One month after the vaccination in both groups of the pregnant women, the indicators of the 4-fold increase in the titers of antibodies to the flu viruses (seroconversion rate) were generally in line with the CPMP criteria (Table 1). The exception was the indicators for the A/H3N2/ strain for women vaccinated in the second trimester of pregnancy (30.4%). At the same time, a similar parameter in the vaccinated women in the third trimester had high values (72.7%). GMTR also corresponded to the CPMP criteria with a more pronounced level for the flu viruses of the A/H3N2/ strain (9.1) and B strain (7.5) in pregnant women vaccinated in the third trimester of gestation (Table 1).

Table 1.

Seroconversion rate and factor in pregnant women depending on the trimester of vaccination against flu

Parameter	Group 1			Group 2		
	A/H1N1/v	A/H3N2/	B	A/H1N1/v	A/H3N2/	B
Seroconversion rate (%)	65.2	30.4	52.2	72.7	72.7°	81.8°
Seroconversion factor	7.9	3.4	5.2	8.5	9.1°	7.5*

** - $P<0.05$; °- $P<0.01$ — between the trimesters of vaccination*

Table 2.

Level of seropositivity and seroprotection in the mother-child pairs depending on the trimester of vaccination of pregnant women against flu

	Terms of observation	Group 1						Group 2					
		A/H1N1/v		A/H3N2/		B		A/H1N1/v		A/H3N2/		B	
		Mother n=27	Child n=23	Mother n=27	Child n=23	Mother n=27	Child n=23	Mother n=21	Child n=19	Mother n=21	Child n=19	Mother n=21	Child n=19
Seropositivity level (%) (Ab titer ≥1:20)	Baseline	16.0		24.0		64.0		18.2		27.3		63.6	
	1 month after the vaccination	78.3		68.2		100		90.5 *		95.2 **		100	
	3 months after the vaccination	78.6		67.1		100		--		--		--	
	2 to 3 days after the childbirth/ neonates	78.9	63.2	68.4	68.4	94.7	84.2	90.5 *	84.2	85.7 **	73.7	100	89.5
	3 months after the childbirth/ 3 months of life	80.0	47.4 ″	65.0	52.6	85.0	57.9 ′	85.7	78.9	81.0 *	57.9 ′	95.2	63.2 ′
	6 months after the childbirth/ 6 months of life	75.0	30.0 ″/^^	60.0	20.0 ″/^^	85.0 °	35.0 ″/^	71.4 °	26.3 ″	61.9 °/′	31.6 ″	81.0 °/′	36.8 ″
Seroprotection level (%) (Ab titer ≥1:40)	Baseline	16.0		20.0		52.0		18.2		18.2		45.5	
	1 month after the vaccination	73.9		50.0		91.0		90.5 *		90.5 **		100	
	3 months after the vaccination	74.3		66.2		100		--		--		--	
	2 to 3 days after the childbirth/ neonates	78.9	38.8 ″	63.2	36.8 ″	84.2	63.2 ′	85.7 *	68.4 **/′	76.2 **	52.6 **/′	95.2	73.7 ′
	3 months after the childbirth/ 3 months of life	65.0	15.8 ″/^^	50.0	10.5 ″/^^	80.0	26.3 ″/^^	71.4	26.3 */″/^^	66.7	36.8 **/″/^	76.2	42.1 *″/^
	6 months after the childbirth/ 6 months of life	55.0 °/″	0.0 ^^	30.0 °/″	0.0 ^^	70.0 °	0.0 ^^	66.6 °	0.0 ^^	52.4 */°°/′	0.0 ^^	66.7 °°/′	0.0 ^^

**- P<0.05, **- P<0.01 — between the trimesters of vaccination; '- P<0.05, "- P<0.01 — between the mother-child groups; °- P<0.05, °°- P<0.01 — within the group of women, between 1 month after the vaccination and 6 months after the childbirth; '- P<0.05, "- P<0.01 — within the group of women, between days 2 to 3 after the childbirth and 6 months after the childbirth ; ^- P<0.05, ^^- P<0.01 — within the group of children, in comparison with the baseline data at childbirth .*

Assessing the seroprotection level in the dynamics after the vaccination, we can note that in women vaccinated against flu in the third trimester, the immune response to all the virus strains corresponded to the recommended CPMP criteria (Table 2). In pregnant women vaccinated in the second trimester against the A/H3N2/strain, a level of HAI-Ab within ≥1:40 was registered in 50%. Subsequently (3 months after vaccination), this value slightly increased (66.2%), *P>0.05*. Observation of women for 9 months in Group 1 and 7 months in Group 2 after the vaccination showed similar changes in the levels of HAI-Ab, with a significant reduction in those levels 6 months after childbirth. At the same time, the antibodies in the protective values to the tested strains of the flu virus in vaccinated women in the second and third trimesters were registered in 30%-70% and 52.4%-66.7% of cases. Moreover, by the mentioned date, significant differences in more pronounced loss of immune protection were identified only on the A/H3N2/ strain in pregnant women vaccinated in the third trimester.

By days 2 to 3 after childbirth, in children born to mothers of Group 1, HAI-Ab in the protective values to different strains of the flu virus were registered in 36.8% to 63.2% of cases, while in children born to mothers of Group 2, these indicators constituted 52.6% to 73.7% of persons.High HAI-Ab values to all the strains of the flu virus in children of Group 2 were observed after 3 months. However, 6 months later in children of the observed groups we did not find any protective values of specific antibodies. Comparing the levels of seroprotection to the flu viruses in the mother-infant pairs, we can note that in children, antibodies having protective values at all the stages of the study were registered at a lower level than in their mothers, regardless of the trimester of vaccination.

The study of dynamics of the post-vaccination level of HAI-Ab showed that one month after the vaccination in all the clinical groups there was a significant increase in GMTs, with high GMTR in the third trimester, especially against the A/ H3N2/ (Table 3). In pregnant women of Group 1, GMTs were in the same values 3 months after the vaccination (*P>0.05*). By days 2 to 3 after childbirth (3 to 6 months after vaccination, in the second and third trimesters), significant differences in this parameter between the groups of women were identified only for the A/H3N2/, with the higher being those women who were vaccinated in the third trimester. Six months after childbirth in both groups, the level of GMTs reduced gradually.

In neonates on days 2 to 3 of life, GMTs were significantly lower than in their mothers, regardless of when the mothers were vaccinated. However, in children born to women of Group 2, the GMTs to the A/H1N1/ and A/H3N2/ flu strains were higher than in infants born to mothers of Group 1. The same feature can be traced against the A/H3N2/ at 3 months after childbirth. At the sixth month of life, there were no significant differences in GMTs.

Table 3.

GMTs in the mother-child pairs depending on the trimester of vaccination of pregnant women against flu

Terms of observation	Group 1						Group 2					
	A/H1N1/v		A/H3N2/		B		A/H1N1/v		A/H3N2/		B	
	Mother n=27	Child n=23	Mother n=27	Child n=23	Mother n=27	Child n=23	Mother n=21	Child n=19	Mother n=21	Child n=19	Mother n=21	Child n=19
Baseline	8.47 ±0.24		8.47 ±0.28		24.28 ±0.29		10.00 ±0.40		11.34 ±0.54		21.30 ±0.28	
1 month after the vaccination	66.77 ±0.41		29.19 ±0.40		124.35 ±0.29		85.20 ±0.62 *		102.93 ±0.62 **		160.0 ±0.23 *	
3 months after the vaccination	56.57 ±0.55		27.04 ±0.48		99.02 ±0.29		--		--		--	
2 to 3 days after childbirth/ neonates	48.00 ±0.39	18.59 ±0.35 ″	26.78 ±0.37	16.66 ±0.29 ′	69.14 ±0.32	30.98 ±0.29 ″	42.60 ±0.31	29.19 ±0.30 */′	54.81 ±0.62 **	33.11 ±0.56 **/′	66.22 ±0.30	37.03 ±0.39 ″
3 months after childbirth/3 months of life	34.82 ±0.41	12.44 ±0.29 ″/^	19.32 ±0.33	11.57 ±0.26 ′/^	44.38 ±0.31	16.66 ±0.22 ″/^^	33.11 ±0.38	17.63 ±0.23 ″/^	51.47 ±0.61 **	20.00 ±0.36 */″/^	45.37 ±0.40	20.00 ±0.37 ′/^
6 months after childbirth/6 months of life	29.28 ±0.39 °°/′	8.41 ±0.20 ″/^^	16.25 ±0.31 °/′	7.32 ±0.18 ″/^^	38.64 ±0.30 °°/′	9.33 ±0.20 ″/^^	25.73 ±0.41 °°/′	8.28 ±0.21 ″/^^	31.09 ±0.53 */°°/′	9.39 ±0.28 ″/^^	37.56 ±0.49 °°/′	10.80 ±0.31 ″/^^

**- P<0.05, **- P<0.01 — between the trimesters of vaccination; '- P<0.05, ″- P<0.01 — between the mother-child groups; °- P<0.05, °°- P<0.01 — within the group of women, between 1 month after the vaccination and 6 months after childbirth; '- P<0.05 — within the group of women, between days 2 to 3 after childbirth and 6 months after childbirth ; ^- P<0.05,^^- P<0.01 — within the group of children, in comparison with the baseline data at childbirth.*

Dynamic observation of the vaccinated pregnant women makes it possible to note that the seroconversion rate and factor of the vaccine strains of the flu virus meet the CPMP criteria, with higher values among women in the third trimester. Differences are more pronounced in children whose mothers were vaccinated in the second trimester: antibodies to the A/H1N1/ strain have the value of 36.8%, and antibodies to flu B have the value of 63.2%, which was different from the same indicators in children born to women vaccinated in the third trimester – 68.4% (*P<0.01*) and 73.7% (*P<0.05*). There was a significant reduction in the level of transplacental antibodies that have protective values in both groups of children. By 3 months of age in Group1, the protective level of HAI-Ab to the flu A virus strains was observed in 10.5% to 15.8% of children and to the flu B virus it was observed in 26.3% of children, against 26.3% to 36.8% and 42.1% for the same strains for children in Group 2 (*P<0.05*). Six months later, there were no protective HAI-Ab titers in the observed children. In this period, the immune protection against the flu viruses in women vaccinated in the second trimester was 30% to 55% to the flu A strains and 70% to the flu B strains against 52.4% to 66.6% and 66.7% in the group vaccinated in the third trimester. Significant differences between the groups in terms of the seroprotection level 6 months after childbirth were observed only for the A/H3N2/ (*P<0.05*).

Discussion

The discovered differences in the production of specific antibodies to the flu viruses, depending on the trimester of vaccination, are not related to pregnancy disorders. This can be confirmed by the state of infants and children during the first months of life, which reflects the physiological development of infants and the lack of aggravated infectious history.

Despite the existing features in the formation of the immune protection against influenza, in both groups of pregnant women the ratio of increase in the protective values of antibodies (seroconversion rate and factor) met the CPMP criteria. At the same time, it should be noted that after the vaccination there was an increase by 1.5 to 3 times in the number of antibodies to the pandemic strain of A/H1N1/v of the flu virus. Vaccination with a subunit vaccine in the third trimester of pregnancy was accompanied by a more pronounced production of specific antibodies in comparison with vaccination at the earlier stages of gestation.

The study of serum showed that women who were vaccinated in the third trimester of pregnancy have a more pronounced seroprotection level in comparison with those who were vaccinated in the second trimester. Subsequently, 3 to 6 months after childbirth, the seroprotection level in late-vaccinated women in the mother-child pairs was higher than in the group of those vaccinated earlier. Regardless of the time of vaccination, 6 months after childbirth there were no antibodies in protective titers in any child. However, if we take into account that the level of specific antibodies ≥1:20 may also have conditionally protective values, 6 months later they are found in 20% to 35% of children in Group 1 and in 26.3% to 36.8% of children in Group 2 with no significant difference depending on the time of vaccination during gestation.

GMTs in pregnant women vaccinated in the third trimester of gestation were higher than for women vaccinated in the second trimester. A significant decline in this value was observed 6 months after childbirth. Moreover, although the women in Group I were vaccinated 3 months earlier than those in Group 2, significant differences in GMTs disappeared within 6 months after childbirth (there were differences in GMTs

only for the A/H3N2/, where this indicator in all the periods of observation was higher in women vaccinated at the later stages of pregnancy). In infants born to mothers vaccinated in the third trimester of pregnancy, there were more pronounced values of the GMTs to the strains of the flu A virus on day 2 to 3 of life, i.e. there was a direct correlation between the number of specific antibodies in children and the level of antibodies in their mothers. Three months later in children there were the same tendencies in the distribution of antibodies between the two groups to different strains of the flu virus during the overall decrease in the values of GMTs.

Conclusion

Thus, vaccination of women against flu with a subunit preparation of Agrippal S1 in both the second and third trimester of pregnancy has a sufficiently high efficacy. Vaccination of pregnant women in the third trimester, as opposed to the vaccination at the early stages of gestation, is accompanied by the increased production of HAI-Ab with a more pronounced transplacental transfer of maternal antibodies to the fetus. At the same time, the level of protection against all the strains of influenza virus in children born to mothers vaccinated in the third trimester of pregnancy was equal, on average, to 64.9%. However, during the vaccination of pregnant women in the second trimester, this indicator in children was equal to only 46.3%. Three months later, the number of children with protective values of antibodies decreased, on average, by 2 times, and for all the strains the decrease was 17.5% in Group 1 and 35.1% in Group 2.

It is important to discuss the issue of the significance of HAI-Ab seropositive titers (≥1:20) in the anti-infective protection against the flu virus, because in children of this age these titers are still determined on average, for all the strains of the virus, in 28.3% of children in Group 1 and 31.6% of children in Group 2. Perhaps, their presence in the body of a child somehow affects the child's resistance to the infection; at the same time, there is as yet no evidence for this assumption.

References

1. Zaman K, Roy E, Arifeen SE, Rahman M, Raqib R, Wilson E, et al. Effectiveness of maternal influenza immunization in mothers and infants. N Eng J Med. 2008;359(15):1555–64.
2. Steinhoff MC, Omer SB, Roy E, Arifeen SE, Raqib R, Altaye M, et al. Influenza immunization in pregnancy – antibody responses in mothers and infants. N Eng J Med. 2010;362(17)):1644-6.
3. Zuccotti G, Pogliani L, Pariani E, Amendola A, Zanetti A. Transplacental antibody transfer following maternal immunization with a pandemic 2009 influenza A(H1N1) MF59-adjuvanted vaccine. JAMA. 2010;304(21):2360-1.
4. Eick AA, Uyeki TM, Klimov A, Hall H, Reid R, Santosham M, et al. Maternal influenza vaccination and effect of influenza virus infection in young infants. Arch Pediatr Adolesc Med 2011;165(2):104-11.
5. Order of the Ministry of Health and Social Development of the RF of October 2, 2009 No. 808n. On the Approval of the Order of Rendering Obstetric-Gynecologic Assistance. [Electronic resource]. http://www.bestpravo.ru/federalnoje/hj-dokumenty/l4v.htm.
6. Botina AV, Medvedeva TG, Neznanov NG. Ethical aspects of medical researches with the participation of women of reproductive age. Good Clinical Practice 2003;3:43-46.
7. Good Clinical Practice. National Standard of the Russian Federation, GOSTR 52379-2005/ Moscow, 2005. [Electronic resource]. http://www.medtran.ru/rus/trials/gost/52379-2005.htm
8. Recommendations on the Organization and Implementation of Vaccination of Pregnant Women and Maternity Patients against the Flu A Virus (H1N1): Informational letter No. 15-4/3108-07 of December 11, 2009 [Electronic resource]. http://www.rosminzdrav.ru/docs/mzsr/letters/161.
9. Order of the Ministry of Health and Social Development of the RF of April 28, 2007 No.307. On the Standard of Dispensary (Routine) Observation over a Child during the First Year of Life. http://www.garant.ru/products/ipo/prime/doc/4084665/.
10. Note for guidance on harmonization of requirements for influenza vaccines (CPMP/BWP/214/96). http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf.

International Journal of Biomedicine 5(4) (2015) 184-187

ORIGINAL ARTICLE

Infection and Immunity

INTERNATIONAL JOURNAL OF BIOMEDICINE

Specific and Non-Specific Factors of Humoral Immunity as Markers for Pregnancy Loss in Women with Cytomegalovirus Infection

Irina A. Andrievskaya, PhD, ScD; Michael T. Lucenko, PhD, ScD*; Olga P. Babenko, PhD

Far Eastern Scientific Center of Physiology and Pathology of Respiration, Siberian Branch of Russian Academy of Sciences Blagoveshchensk, the Russian Federation

Abstract

The aim of this study was to estimate the changes in humoral immunity and their association with complications of pregnancy (spontaneous abortions, threatened miscarriage, preterm birth) depending on the gestational age and recurrence of cytomegalovirus infection (CMVI).

A direct relationship between the frequency of detection of an anti-CMV IgG antibody titer of 1:1600 and the prevalence of acute respiratory disease during pregnancy has been identified. We found an imbalance in the production of the non-specific antibodies (an increase in the blood levels of total IgM and a decrease in IgA and IgG levels) in the subgroup of women with relapsed CMVI at 6 to 8 weeks of gestation and spontaneous abortion, as well as in the subgroup of women with relapsed CMVI at 15 to 21 weeks of gestation and the risk of the late miscarriage, compared to those with relapsed CMVI at 9 to 14 weeks and 22 to 32 weeks of gestation. An increase in blood levels of total IgM and IgG and a decrease in IgA level was identified in the subgroup of women with relapsed CMVI at 9 to14 weeks of gestation and a threatened abortion, as well as in the subgroup of women with relapsed CMVI at 22 to 32 weeks of gestation and preterm birth. The obtained data of the imbalance in the primary and secondary immune response in CMV-seropositive pregnant women during relapsed CMVI indicate disturbances in the systemic and local intercellular interactions of immunocompetent cells, which lead to an imbalance in the production of antibodies involved in the elimination of viral agents and to the development of a systemic inflammatory response that complicates the course of pregnancy. CMVI relapse at 7 to 8 weeks of gestation is associated with reproductive losses; a risk for threatened miscarriage, threatened premature labor, and retrochorial hematoma increases significantly with CMVI relapse in the more remote gestational age. **(Int J Biomed. 2015;5(4):184-187.)**

Keywords: *cytomegalovirus infection; humoral immunity; spontaneous abortions; threatened miscarriage; preterm birth.*

Introduction

Cytomegalovirus (CMV) is an extremely common virus that can infect almost anyone; about 60%–80% of women of childbearing age have antibodies to CMV [1,2]. According to the International Classification, CMV belongs to the type 5 of Human Herpesviruses (HHV) - CMV/HHV-5. CMV has the ability to remain latent within the body over long periods. CMV activation is associated with immunosuppression and hormonal changes [1].

Among the factors of physiological immunosuppression which contribute to the spread of cytomegalovirus infection (CMVI) and its reactivation, the first place belongs to pregnancy [3,4]. This circumstance predetermines a special interest in the problem of CMVI during pregnancy [5,6]. Physiological immunodeficiency that occurs during pregnancy creates an increased risk of manifestation and recurrence of CMVI in pregnant women. The virus causes significant disturbances in the regulation of immune response, which are based on its property to cause immunosuppression [7,8]. Intracellular persistence of the virus contributes to the long latency of infection [9,10]. The intracellular location protects the virus from the action of specific antibodies. Changes in humoral immunity and their association with pregnancy, depending on the gestational age and recurrence of CMVI, have not been

***Corresponding author**: Prof. Michael T. Lucenko, PhD, ScD, Academician of RAS, Head of Far Eastern Scientific Center of Physiology and Pathology of Respiration SB of RAS, Blagoveshchensk, Russia. E-mail: Lucencomt@mail.ru*

fully disclosed. In particular, there is no consensus regarding the high titer antibodies to CMV in pregnant women. Some authors believe that a high antibody titer has an adverse prognostic significance for the fetus, while other researchers believe that these antibodies penetrate the placenta and protect the fetus from infection [7,11].

The aim of this study was to estimate the changes in humoral immunity and their association with complications of pregnancy (spontaneous abortions, threatened miscarriage, premature birth) depending on the gestational age and recurrence of CMVI.

Materials and Methods

This prospective study (between 2011 and 2013) included 165 CMV-seropositive pregnant women (the study group) and 50 CMV-seronegative pregnant women (the control group). The mean age of pregnant women in the study group was 24.6±0.4 years and 23.5±0.5 years in the control group ($P>0.05$).

Inclusion criteria for the study group were a relapse of CMVI identified by molecular biological and serological methods, as well as herpes virus infection (HHV-1,2) remission during the entire gestation period.

Exclusion criteria were primary CMVI, an aggravation of other inflammatory diseases of extragenital localization and sexually transmitted infections, genetic/endocrinological reasons for complications of pregnancy, cervical incompetence, and the presence of uterine pathology (malformations, hypoplasia of uterus, intrauterine adhesions).

Blood samples (5 ml) were collected from the ulnar vein in standard vacuum tubes with EDTA to obtain the samples of mononuclear cells. For serological tests, we used blood that does not contain anticoagulants. Mononuclear cell isolation for PCR was carried out with Ficoll-Urografin (d-1,077g/ml) ("DNA-Technology", Moscow). The verification of CMV, the definition of type-specific antibody, the total immunoglobulin (IgA, IgG, IgM), and the avidity index were determined by ELISA on the spectrophotometer "Stat Fax 2100" (USA) using the sets of CC "Vector-Best" (Novosibirsk, Russia). CMV DNA was detected by PCR on the machine DT-96 using sets of "DNA-Technology" (Moscow, Russia). The relapse of CMVI was diagnosed in a comprehensive study of the peripheral blood to check for the presence of IgM or a four-fold or more increase in the IgG antibody titer in paired serum in the dynamics after 10 days, an avidity index > 65%, and the presence of CMV DNA in samples of blood, urine, buccal epithelium, and cervical mucosa.

The clinical relapse of CMVI was manifested by acute respiratory disease. HHV infection type 1 and 2 in the stage of stable remission (anti-HHV-1,2 IgG antibody titer of 1:800, an avidity index > 65%) was found in all patients at the first examination.

In the study group, in the first phase, the patients were divided into subgroups according to the course of pregnancy. Subgroup 1 included patients with reproductive losses (n=15); Subgroup 2 included patients without reproductive losses (n=150). At the second stage, we formed the sub-subgroups within subgroups, taking into account the timing of CMVI relapse during gestation. In particular, the relapse of CMVI occurred at 7 and 8 weeks of gestation among all patients of Subgroup 1. Subgroup 2 was divided into the following sub-subgroups: Subgroup 2a (n=50) with CMVI relapse at 9 and 14 weeks of gestation, Subgroup 2b (n=50) at 15 and 21 weeks of gestation, and Subgroup 2c (n=50) at 22 and 32 weeks of gestation.

The study was conducted in the laboratory of pathogenesis and regenerative processes of the respiratory system in non-specific lung diseases and the department of pregnancy pathology of the Far Eastern Scientific Center of Physiology and Pathology of Respiration SB RAS (FESPPR) in line with the requirements of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (2008). The study was approved by the Far Eastern Scientific Center of Physiology and Pathology of Respiration Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using a statistical software package, Statistica 6.0. The mean (M) and standard deviation (SD) were calculated. Analysis of the distribution of values obtained was performed using the Kolmogorov-Smirnov test. 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). The statistical significance of differences between distributions was estimated by Fischer's t-test for angular transformed proportions. A probability value of $P<0.05$ was considered statistically significant.

Results

In the study group, the total frequency of all complications of the pregnancy was 180%, which was 4.3 times higher than in the control group. In all pregnant women of Subgroup 1, pregnancy was interrupted due to spontaneous miscarriage. In subgroup 2a, threatened abortion was diagnosed in 60% of pregnant women; it was significantly greater than in Subgroups 2b ($P<0.01$) and 2c ($P<0.001$). In the subgroup 2b, threatened abortion was observed in 68%, which was significantly greater than in Subgroups 2a ($P<0.001$) and 2c ($P<0.001$). In Subgroup 2c, a threatened preterm birth was diagnosed in 56% of pregnant women, i.e., 3.6 times *higher* than in the control group ($P<0.001$). Retrochorial hematoma was seen in 24% of pregnant women in the study group, with a significantly higher frequency in Subgroup 2a compared to Subgroup 2b ($P<0.05$) and Subgroup 2c ($P<0.01$). Primary placental insufficiency was diagnosed in 12.7% of pregnant women in the study group, while it was not found in the control group. In the study group, chronic placental insufficiency, retrochorial hematoma, and chorion previa were diagnosed in 53.3%, 24%, and 12.7% of participants, respectively.

Analysis of the parameters of specific immunity revealed the following features. In the study group, among

CMV-seropositive women, the frequency of the anti-CMV IgG antibody titer of 1:1600 associated with IgM was 71.3%, while an anti-CMV IgG antibody titer of 1:800 was detected in 28.7% of cases.

In Subgroup 1, the anti-CMV IgG antibody titer of 1:1600 was detected in 80% of cases, while the anti-CMV IgG antibody titer of 1:800 was detected in 20% of cases. In subgroups 2a, 2 b and 2c, the anti-CMV IgG antibody titer of 1:1600 was detected in 62%, 76% and 78% of cases, respectively, while the anti-CMV IgG antibody titer of 1:800 was detected in 38%, 24% and 22% of cases, respectively.

We found a direct relationship between the anti-CMV IgG antibody titer of 1:1600 and acute viral respiratory infection during pregnancy (*P<0.01*), clinical manifestations of which were diagnosed in 74% of cases. An asymptomatic course of CMVI was observed in 26% of cases.

Based on these data, we can conclude that a high level of anti-CMV IgG antibody titer may be a diagnostic sign of the intensity of the infection process, and the absence of a reduction in the level of antibody titer is a sign of persisting viremia during pregnancy.

Analysis of the parameters of non-specific immunity also revealed the following features (Table 1). Serum IgA level was significantly less in Subgroup 1 compared to the control group and Subgroups 2a, 2b, and 2c. In addition, the level of total IgG was significantly higher in Subgroup 1 *than in the control group*, and the level of total IgM was significantly higher than in the control group and Subgroup 2a.

Table 1.

Parameters of humoral immunity in pregnant women with CMVI

Serum Ig	The study group			
	Subgroup 1	Subgroup 2a	Subgroup 2b	Subgroup 2c
IgA	0.94±0.06 2.40±0.11 *P<0.001,* *P1,P2,P3<0.01*	1.05±0.05 2.40±0.10 *P<0.001* *P4, P5<0.01*	1.12±0.07 2.30±0.08 *P<0.001,* *P6<0.01*	1.25±0.06 2.20±0.09 *P<0.001*
IgG	14.0±0.41 10.1±0.09 *P, P1, P2,* *P3<0.001*	18.00±0.25 12.40±0.11 *P<0.001,* *P5<0.01*	16.70±0.74 13.60±0.11 *P, P6<0.001,* *P4<0.01*	20.0±0.52 14.1±0.12 *P<0.001*
IgM	2.70±0.07 1.22±0.06 *P, P1<0.001* *P3<0.01*	2.30±0.05 1.20±0.05 *P<0.001*	2.80±0.09 1.12±0.07 *P<0.001* *P2,P4,P6<0.01*	2.40±0.04 1.09±0.03 *P<0.001* *P5<0.01*

Numerator – values of the indexes in the study group; denominator – values of the indexes in the control group. P- between Subgroups (1,2a,2b,2c) and the control group; P1 – between Subgroup 1 and Subgroup 2a; P2- between Subgroup 1 and Subgroup 2b; P3 - between Subgroup 1 and Subgroup 2c; P4 - between Subgroup 2a and Subgroup 2b; P5 - between Subgroup 2a and Subgroup 2c; P6 - between Subgroup 2b and Subgroup 2c.

In Subgroup 2a, serum IgA level was significantly less compared to the control group and Subgroups 2b and 2c, but higher than in Subgroup 1. Serum level of total IgM in CMV-seropositive women was 1.9 times higher in Subgroup 2a than in the control group, but slightly less compared to Subgroup 1, Subgroups 2b and 2c.

In subgroup 2b, the serum IgA level was less compared to the control group (2 times) and Subgroup 2c. The serum level of total IgM was 2.5 times greater in Subgroup 2b than in the control group and was also higher compared to Subgroups 2a and 2c. The serum level of total IgG was also higher in Subgroup 2b *t*han in the control group and Subgroup 1, but less than in Subgroups 2a and 2c.

In Subgroup 2c among CMV-seropositive women, the serum IgA level was 1.8 times less compared to the control group, but higher compared to Subgroups 1,2a, and 2b. The serum level of total IgM was 2.2 times greater in Subgroup 2c than in the control group, but less compared to Subgroups 2a and 2c. The serum level of total IgG was higher in Subgroup 2c than in all Subgroups.

Discussion

As we know, CMV persisting in lymphoid organs reveals an activity under the influence of unfavorable factors and conditions that disturb homeostasis [12]. Pregnancy is a powerful factor causing neuroendocrine and immune changes [13,14]. An increase in production of anti-CMV antibodies and non-specific antibodies (IgA, IgG, IgM) by the activated B cells is a manifestation of the immune response during CMVI exacerbation in pregnant women [15].

IgM is the earliest factor of phylogenetic and ontogenetic development. IgM production provides the first line of defense in the immune response. Therefore, the detected increase in serum level of total IgM in all subgroups of the CMV-seropositive pregnant women is a sign of an early inflammatory response during CMVI relapse.

IgG forms the basic line of specific immunological mechanisms of antiviral defense. The ambiguity of the results regarding the content of total IgG in the blood of CMV-seropositive pregnant women, namely low levels in Subgroups 1 and 2b compared to Subgroups 2a and 2c in conjunction with a high level of total IgM, indicates a disturbance in immunoreactivity of the B cells. It should be noted that IgM and IgG are directly involved in the formation of circulating immune complexes (CICs), while increased CIC levels are associated with damaging tissue and vessel walls [16]. In the case of an inadequate response of the immune system, CICs circulate a long time in the blood and are deposited in various organs and tissues. The filter membrane and areas with strong turbulent flow of blood are the most likely sites for CIC fixation. These biological filters are the placentas and kidneys of pregnant women [17,18]. CIC accumulation in the blood is accompanied by the development of rheological disorders and leads to the formation of retrochorial hematomas and the blockage of uteroplacental and fetoplacental circulation. Thromboembolic damage to the placenta and trophoblast caused by dysfunction of vascular endothelium and blood platelets can result in the death of the embryo (fetus). In our early studies, we revealed disturbances in the gestational trophoblast transformation in chorionic villi during CMVI [19], as well as a delay in mesenchyma differentiation towards the formation of vascular endothelium (the reduced villus vascularization) and the degenerative changes in the chorionic

structures (fibrosis, necrosis of the villous stroma).

According to other authors, a clinically significant placental insufficiency is associated with the presence of fixed immune complexes in the basal membrane of chorionic villi, the syncytiotrophoblast, the basal membrane of endothelium of villus vessels, and the chorionic plate. These abnormalities are accompanied by focal destruction of the marked structures, a massive fibrinoid deposition, and lymphocytic infiltration in the area of damage with the development of circulatory disorders and necrotic processes in surrounding tissues [17,18].

In determining the main immunoglobulins in the blood of CMV-seropositive pregnant women, we revealed a significant reduction in IgA level in association with gestational age. It should be noted that the main function of IgA is neutralization of toxins, an activation of phagocytosis and the complement system, and formation of the antiviral defense for mother and fetus.

In conclusion, the obtained data of the imbalance in the primary and secondary immune response in CMV-seropositive pregnant women during relapsed CMVI indicate disturbances in the systemic and local intercellular interactions of immunocompetent cells, which lead to an imbalance in the production of antibodies involved in the elimination of viral agents and to the development of a systemic inflammatory response that complicates the course of pregnancy. CMVI relapse at 7 to 8 weeks of gestation is associated with reproductive losses; a risk for threatened miscarriage, threatened premature labor, and retrochorial hematoma increases significantly with CMVI relapse in the more remote gestational age.

Competing interests

The authors declare that they have no competing interests.

References

1. Kulakov VI, Gurtovoiy BL, Ordzhonikidze NV. Cytomegalovirus infection in obstetrics. M.: GEOTAR-Media, 2001. [in Russian].
2. Orekhov KV, MV Golubeva, Barycheva LY. Congenital cytomegalovirus infection. Detskie Infektsii 2004; 1:49-55. [in Russian].
3. Alekseeva ML, Ekimov AN, Ekimova VE, Ivanec Yu, Kolod'ko VG. The approaches to the diagnosis of cytomegalovirus infection in obstetrics and neonatology. Problemy Reproduktsii. 2010; 5: 52-56. [in Russian].
4. Vladimirova NYu, Nikitin VG, Chizhova GV. Planning for pregnancy in women with asymptomatic CMV infection. Dal'ne-Vostochniy Zhurnal Infect Pathol. 2012; 21:71-73. [in Russian].
5. Grigorieva EA, Moscow IA, Atyasheva LN, Nikiforova NN. Perinatal cytomegalovirus infection (clinical and laboratory parallels). Epidemiologia Infect Boleznei. 2003;1:40-42. [in Russian].
6. Ozhegov AM, Maltsev SV, Myakishev LS. Clinical and immunological characteristics of active CMV infection with co-infection in children of the first year of life. Pediatriia. 2001; 2:26-31. [in Russian].
7. Asrankulova D, Rizopulu AP, Kurbanov DD. The immune status and anti-inflammatory cytokines in pregnant women with acute cytomegalovirus infection. Zh Microbiol Epidemiol Immunol. 2004;4:84-86. [in Russian].
8. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus and newborn infant. Clin Microbiol Rev. 2002; 15(4): 680-715.
9. Dolgikh TI, Dalmatov VV, Gashina EA, Stasenko VL, Kosykh NI, Kmito NL, Pakhalkova EV. Epidemiological aspects of cytomegalovirus infection in babies aged under one. Zh Mikrobiol Epidemiol Immunobiol. 2004;(5):25-30. [in Russian].
10. Kisteneva LB. Cytomegalovirus infection as a problem of perinatal pathology: classification, clinical presentation, differential diagnosis, treatment, and prevention. Ross Vest Perinatol Pediatrii. 2003; 5:52-56. [in Russian].
11. Sidelnikova VM, Dadalyan DG, Van'ko LV, Sukhih GT. Cytomegalovirus infection in women with recurrent miscarriage. Akush Ginekol (Mosk).1996;4:21-24. [in Russian].
12. Klimov VA. Infectious diseases and pregnancy. M.: MEDpress; 2009. [in Russian].
13. Andrievskaya IA. Morphofunctional characteristics of the placenta and disorders in metabolism of hormones and bioactive substances in pregnant women with herpes virus infection. Abstract of PhD thesis. Irkutsk , 2004. [in Russian].
14. Lucenko MT, Solovieva AS, Andrievskaya IA. Mechanisms of changes in immune system of pregnant women with herpes viral infection. Novosibirsk- Blagoveshchensk: Publishing House of ASMA; 2007. [in Russian].
15. Moskalev AV, Sboychakov VB. Infectious Immunology. In: Lobzina YuV, editor. SPb.: Foliant; 2006. [in Russian].
16. Konstantinova NA. Immune complexes and tissue damage. M.: Medicine; 1996. [in Russian].
17. Gabelova KA, Arutyunyan AV, Zubzhitskaya LI, et al. Fixed immune complexes and NO-synthase activity in the placenta during preeclampsia. Vestnik Ross Assotsiatsii Akush Ginekol. 2000; 1:22-24. [in Russian].
18. Starostina TA, Belokrinitskiĭ DV, Kudriashova NM, Razmakhnina NI, Goloshchapova OV, Seredina TA. Circulating immune complexes and immunoglobulins A, M and G in the mother-fetus system in normal pregnancy and gestosis. Akush Ginekol (Mosk). 1992; 1: 19-22. [in Russian].
19. Babenko OP. Morphofunctional characteristics of the placenta during exacerbation of cytomegalovirus infection in the early stages of gestation. Bull Physiol Pathol Resp (SB RAMS) 2013; 47:72-76. [in Russian].

International Journal of Biomedicine 5(4) (2015) 188-191

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Influence of Lipid Transport System Gene Polymorphism on the Dyslipidemia and Coronary Lesions in Patients with Unstable Angina

Aziz S. Eshpulatov; S.U. Hashimov; Alexander B. Shek, PhD, ScD; Ravshanbek D. Kurbanov, PhD, ScD

The Republican Specialized Center of Cardiology
Tashkent, Uzbekistan

Abstract

The purpose of this study was to identify the features of lipid metabolism and coronary lesions in view of the combined carrier of the ε4 allele of APOE ε2/ε3/ε4 polymorphism and the S2 allele of APOC3SstI polymorphism in UA patients.

Materials and Methods: The study included 141 Uzbek patients with UA class IIB (Braunwald E. et al., 1989) who had coronary atherosclerosis of varying degrees, according to coronary angiography. The control group consisted of 50 healthy, age-matched, randomly selected Uzbek persons without clinical and instrumental signs of CHD according to the exercise test. Coronary angiography was performed using Allura CV-20 (Philips, Netherlands). Genotyping of the APOE (ε2/ε3/ε4) gene polymorphism and the APOC3 (SstI) gene polymorphism was performed by the PCR-RFLP method.

Results: Our analysis revealed a significant prevalence of carriers of the S2 allele of APOC3SstI polymorphism and carriers of the ε4 allele of APOE ε2/ε3/ε4 polymorphism among UA patients compared to healthy ethnic Uzbeks. A combination of these two "damaging" alleles was observed in 26.2% of UA patients, which was accompanied by significantly higher blood levels of TC and LDL-C ($P<0.05$), a higher APOB/APOAI ratio ($P<0.05$), and a lower level of HDL-C ($P<0.05$). According to coronary angiography, a three-vessel lesion and more significantly predominated among these UA patients (OR: 2.25, 95% CI 1.05-4.84, $\chi 2$ 3.66, $P<0.05$). **(Int J Biomed. 2015;5(4):188-191.)**

Keywords: *APOC3 SstI gene polymorphism; APOE (ε2/ε3/ε4) gene polymorphism; unstable angina; dyslipidemia; coronary lesions.*

Abbreviations

CHD, coronary heart disease; **TC**, total cholesterol; **TG**, triglycerides; **LDL-C**, low density lipoprotein cholesterol; **HDL-C**, high density lipoprotein cholesterol; **AI**, atherogenic index; **(APOAI),** apolipoprotein A-I; **(APOB)**, apolipoprotein B; **APOC3**, apolipoprotein C-III; **APOE,** apolipoprotein E; **LP(a)**, lipoprotein(a); **FG,** fasting glucose.

Introduction

Multiple genetic, experimental, clinical, and epidemiological studies clearly indicate the primary role of the lipid metabolism in the development and progression of atherosclerosis and coronary heart disease (CHD). In this connection, of great interest is the study of genes involved in the regulation of transport and metabolism in blood plasma lipids [1-3]. Among the genes under consideration for involvement in the risk of CHD, most important are genes encoding apolipoproteins A, B, C, E [4-9], whose polymorphic variants have been studied in detail, including in our studies. Earlier we have shown a high incidence of coronary revascularization within one year in patients with coronary artery disease who were carriers of the ε4 allele of the APOE gene [10-11], as well as a reliable relationship between the carriage of the S2 allele of APOC3SstI polymorphism, an elevated plasma TG level, and a high frequency of coronary artery lesions [12]. The above-mentioned data determined our interest in studying the effect of the combined carriage of "damaging" alleles of these genes on lipid metabolism and the degree of coronary lesions in patients with unstable angina (UA).

***Corresponding author**: Aziz S. Eshpulatov. The Republican Specialized Center of Cardiology, Tashkent, Uzbekistan. E-mail: dr.azizbek84@mail.ru*

The purpose of this study was to identify the features of lipid metabolism and coronary lesions in view of the combined carrier of the ε4 allele of APOE ε2/ε3/ε4 polymorphism and the S2 allele of APOC3SstI polymorphism in UA patients.

Materials and Methods

The study included 141 Uzbek patients with UA class IIB (Braunwald E. et al., 1989) who had coronary atherosclerosis of varying degrees, according to coronary angiography. The control group consisted of 50 healthy, age-matched, randomly selected Uzbek persons without clinical and instrumental signs of CHD according to the exercise test.

Exclusion criteria were myocardial infarction (MI) within previous 3 months, diabetes mellitus requiring insulin treatment, arterial hypertension (BP>159/99 mmHg), hypotension (blood pressure <100/60 mmHg), atrial fibrillation and life-threatening ventricular arrhythmias, valvular heart disease, long time treatment with lipid-lowering drugs and ACE inhibitors, chronic heart failure (NYHA FC>II), chronic renal and hepatic failure.

All patients underwent the following examinations: assessment of traditional risk factors (high blood pressure, smoking, body mass index, diabetes), physical examination; clinical and biochemical laboratory methods, 12-lead ECG, echocardiography and assessment of the thickness of the intima-media of the carotid arteries (IMT), treadmill test, and coronary angiography.

Blood samples were obtained in the morning after a 12h overnight fast. TC, LDL-C, HDL-C, TG, ALT, AST, CPK, APOAI, APOB, LP(a), hsCRP, fibrinogen, fasting glucose , ESR, WBC were determined in plasma using "Daytona» analyzer (RANDOX, Ireland).

Coronary angiography was performed using Allura CV-20 (Philips, Netherlands). To assess the degree of narrowing of vessels, a visual assessment was used with the following characteristics: normal coronary artery, changing contours of artery without determining the degree of stenosis, narrowing < 50% of the luminal diameter, narrowing of 51–75%, 76–95%, 95-99% (subtotal occlusion), and 100% (total occlusion). Narrowing of the luminal diameter >50% was considered significant, < 50% hemodynamically insignificant.

We isolated DNA from whole blood using a set of Diatom™ DNA Prep 200. Genotyping of the APOE (ε2/ε3/ε4) gene polymorphism and the APOC3 (SstI) gene polymorphism was performed by the PCR-RFLP method using Applied Biosystems Geneamp PCR *S*ystems 2700 and 9700 (USA) at the Institute of Genetics and Plant Experimental Biology and the Republican Specialized Center of Cardiology. PCR amplification was carried using "SibEnzim" kits (Russia).

The sequence primers for APOC3 gene were used according to AR Bandegi et al. [13].

ApoC3 F: 5 '- GGT GAC CGA TGG CTT CAG TTC CCT GA-3'

ApoC3 R: 5'-CAG AAG GTG GAT AGA GCG CTG GCC T-3 '.

PCR products were digested with 15 units of SstI enzyme for a minimum of 3 h at 37°C. The resulting fragments were separated according to their size by electrophoresis on 8% nondenaturing polyacrylamide gel. The alleles lacking the restriction site were designated as S1, while those containing the SstI site were designated as S2.

The sequence primers for APOE gene were used according to P.R. Wenham [14].

ApoE F: 5'-TCCAAGGAGCTGCAGGCGGCGCA3 '

ApoE R: 5'-ACAGAATTCGCCCCGGCCTGGTACACTGCCA3 '

The amplified product was digested with Hhal restriction enzyme. Each of the isoforms was distinguished by a Nigue combination of Hhal fragment sizes that enabled unambiguous typing of all homo-zygotic and heterozygotic combinations.

ε3/ε 3 (91, 48 bp), ε3/ε2 (91, 81, 48 bp) and ε3/ε 4 (91, 72, 48 bp) alleles were genotyped according to restriction fragments.

Basic therapy included anticoagulants (heparin or enoxaparine sodium) in the acute period (100%), antiplatelet agents (100%), beta-blockers (bisoprolol, 100%), if necessary, nitrates (95%) and ACE inhibitors (95%).

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the Republican Specialized Center of Cardiology Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using a statistical software package, Statistica 6.0. The mean (M) and standard deviation (SD) were calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test and F-test. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups (for nonparametric data). Chi square (χ^2) or Fischer's exact test (two sided) was used to compare the association between the genotypes and alleles in relation to the cases, and test for deviation of genotype distribution from Hardy–Weinberg equilibrium. The odds ratio (OR) and their 95% confidence intervals (CI) were calculated to estimate the strength of the association. A probability value of $P<0.05$ was considered statistically significant.

Results

The observed frequency of genotypes of APOE ε2/ε3/ε4 polymorphism and APOC3SstI polymorphism was in Hardy–Weinberg equilibrium. There were significantly more carriers of the ε4 allele of APOE ε2/ε3/ε4 polymorphism among UA patients (61.7%) than in the control group (12%) (Table 1).

Table 1.

Carrier state of the ε4 allele of APOE ε2/ε3/ε4 polymorphism and the S2 allele of APOC3SstI polymorphism among UA patients and healthy persons of Uzbek nationality

Gene polymorphism		UA patients (n=141)	Healthy persons (n=50)	*P*
APOEε2/ε3/ε4 polymorphism	Carriers of ε4 allele	87 (61.7%)	6 (12%)	*P<0.001* (OR 11.82; 95%CI: 4.7-29.6; χ2=34.5)
	Non-carriers of ε4 allele	54 (38.3%)	44 (88%)	
APOC3SstI polymorphism	Carriers of S2 allele	51 (36.2%)	9 (18%)	*P<0.05* (OR 2.58; 95%CI: 1.2-5.7; χ2=4.8)
	Non-carriers of S2 allele	90 (63.8%)	41 (82%)	

Among UA patients, a plasma level of APOB in ε4 allele carriers was significantly higher (113.9±26.8 mg/dl) than in non-carriers (100.3±20.9 mg/dl, *P<0.05*); additionally, the trend towards higher values of TC and LDL-C was observed in ε4 allele carriers. This confirms the potentially high atherogenic properties of dyslipidemia in ε4 allele carriers.

There were also significantly more carriers of the S2 allele of APOC3SstI polymorphism among UA patients (36.2%) than in the control group (18%),Table 1. S2 allele carriers have a significantly higher blood level of TG than do non-carriers (261.2±113.7 mg/dL vs. 225.8±87.3 mg/dL; *P<0.05*). At the same time, the average levels of TC, LDL-C, HDL-C, APOAI, APOB, and the ratio of APOB/APOAI did not differ between two groups.

In the next stage of the analysis, we joined the carriers of the ε4 allele of APOE ε2/ε3/ε4 polymorphism and carriers of the S2 allele of APOC3SstI polymorphism into Group 1 (n=37/26.2%). The remaining patients (104/73.8%) constituted Group 2. As shown in Table 2, Group 1 patients had significantly higher TC and LDL-C blood levels than did those in Group 2 (238.0±54.3 mg/dL vs. 220.6±39.8, 154.7±51.5 mg/dL vs. 138.8±37.6 mg/dL; *P<0.05* in both cases). At the same time, Group 1 patients had significantly lower levels of HDL-C versus Group 2 patients (34.1±6.3 mg/dL and 36.8±6.8 mg/dL, respectively, P<0.05). These changes in lipid levels resulted in a significantly higher level of AI in Group 1 patients (6.2±2.0 vs. 5.2±1.4 in Group 2; *P<0.05*). At the same time, there was no difference in the average level of blood TG between the two groups. Group 1 patients had slightly higher plasma levels of APOB and lower levels of APOAI than did Group 2 patients, which resulted in a significantly higher APOB/APOAI ratio: 0.9±0.3 vs. 0.8±0.2, *P<0.05* (Table. 2).

Table 2.

Comparative evaluation of the initial blood biochemical parameters in view of the combined carrier of the ε4 allele of APOE ε2/ε3/ε4 polymorphism and the S2 allele of APOC3SstI polymorphism in UA patients

Values	Group 1 (n=37)	Group 2 (n=104)	*P*
TC, mg/dL	238.0±54.3	220.6±39.8	*P<0.05*
TG, mg/dL	245.9±95.0	234.7±93.7	*P>0.05*
LDL-Cl, mg/dL	154.7±51.5	138.8±37.6	*P<0.05*
HDL-C, mg/dL	34.1±6.3	36.8±6.8	*P<0.05*
VLDL-C, mg/dL	49.2±19.0	46.9±18.7	*P>0.05*
AI, relative units	6.2±2.0	5.2±1.4	*P<0.05*
FG, mmol/L	5.8±1.7	5.8±1.3	*P>0.05*
APOA-I, mg/dL	133.8±21.3	139.1±22.2	*P>0.05*
APOB, mg/dl	115.0±24.9	107.0±25.7	*P>0.05*
APOB/APOA-I	0.9±0.3	0.8±0.2	*P<0.05*
LP (a), mg/dL	30.1±22.8	34.7±35.5	*P>0.05*

According to the severity of coronary lesions, patients were divided into subgroups with one, two, and three or more multivessel lesions (Table 3). In the most severe subgroup, patients with a three-vessel lesion and more, the patients of Group 1 (59.5%) significantly predominated compared to the patients of Group 2 (39.4%) (OR=2.25, 95%CI: 1.05-4.84; $\chi 2$=3.66, *P<0.05*).

Table 3.

Comparative evaluation of the coronary angiography data in the studied patient groups in view of the combined carrier of the ε4 allele of APOE ε2/ε3/ε4 polymorphism and the S2 allele of APOC3SstI polymorphism

Variable	Group 1 (n=37)	Group 2 (n=104)	*P*
Stenosing coronary atherosclerosis	36 (97.3%)	89 (85.6%)	*P>0.05*
one-vessel lesion	7 (18.9%)	24 (23.1%)	*P>0.05*
two-vessel lesion	7 (18.9%)	24 (23.1%)	*P>0.05*
three- and multi-vessel lesion	22 (59.5%)	41 (39.4)	*P<0.05*
No angiographic evidence of coronary artery stenosis	1 (2.7%)	15 (14.4%)	*P>0.05*

Discussion

One of the most effective approaches to the study of genetic mechanisms of CHD is the identification of genetic markers of the disease. Such studies provide an opportunity to establish the involvement in CHD pathogenesis of specific candidate genes and on this basis to identify the group of individuals with a high genetic risk of CHD development. A number of epidemiological studies have analyzed the impact of APOE polymorphism on cardiovascular disease. Eichner et al. [15] and Lehtinen et al.[16] suggested that ε4 allele carriers were particularly predisposed to develop coronary lesions or to possess elevated risk of CAD death. The authors suggest that this could be a consequence of lipoprotein metabolism dysfunction associated with the ε4 isoform, with elevation of total cholesterol and triglyceride levels [17].

Schiele et al. [18], studying northern European populations with higher cholesterol levels and higher CVD mortality, have also shown higher ε4 allele presence. Hixson et al. [19] and Ilveskoski et al. [20], studying vascular necropsy alterations, showed more atherosclerotic lesions in ε4 allele carriers, independent of total cholesterol levels. On the other hand, according to L.M. Lima et al. angiographic studies did not evidence higher CHD risk in ε4 allele carriers [21]. In the MONICA study [22], a ε4 allele frequency elevation of 0.01 was associated with a rise in CHD death of 24.5 per 100,000 patients. In a 4S study [9], ε4 allele carriers presented an 80% higher death risk after myocardial infarction, in comparison with other patients. Lahoz et al. [23] concluded that ε2 and ε4 allele presences were associated with higher cardiovascular risk in men. The authors argued that the ε4 allele determines higher risk partly due to the lipid alterations that it brings on.

The relationship between the SstI polymorphism of APOC3 and an increased risk of coronary artery disease was confirmed by genetic analysis in the framework of the classic Framingham study [24]. According to our study, a significant prevalence of S2 allele carriers of APOC3SstI polymorphism among UA patients, compared to healthy ethnic Uzbeks, also indicates the presence of an association between this polymorphic gene marker and the risk of CHD progression. The combined carriage of two "damaging" alleles (S2 and

ε4) is one of the factors that increase the risk of atherogenic dyslipidemia and coronary stenosis, which can be a useful additional marker for the assessment of cardiovascular risk and indications for coronary angiography.

Conclusion

Our analysis revealed a significant prevalence of carriers of the S2 allele of APOC3SstI polymorphism and carriers of the ε4 allele of APOE ε2/ε3/ε4 polymorphism among UA patients compared to healthy ethnic Uzbeks. A combination of these two "damaging" alleles was observed in 26.2% of UA patients, which was accompanied by significantly higher blood levels of TC and LDL-C *(P<0.05)*, a higher APOB/APOAI ratio *(P<0.05)*, and a lower level of HDL-C (P<0.05). According to coronary angiography, a three-vessel lesion and more significantly predominated among these UA patients (OR=2.25, 95%CI: 1.05-4.84; $\chi 2$=3.66, *P<0.05*).

Competing interests

The authors declare that they have no competing interests.

References

1. Vinogradova SV. The role of apolipoprotein E gene polymorphism in the development of atherosclerosis (review). Medical Genetics. 2006; 2:3-10 [Article in Russian].
2. Song Y. Stampfer MJ. Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Int Med. 2004;141(2):137-47.
3. Davignon J, Cohn JS, Mabile L, Bernier L. Apolipoprotein E and atherosclerosis: insight from animal and human studies. Clin Chim Acta. 1999; 286:115-43.
4. de Andrade M, Thandi I, Brown S, Gotto A Jr, Patsch W, Boerwinkle E. Relationship of the apolipoprotein E polymorphism with carotid artery atherosclerosis. Am J Hum Genet. 1995; 56:1379-90.
5. Wang CS, McConathy WJ, Kloer HU, Alaupovic P. Modulation of lipoprotein lipase activity by apolipoproteins. J Clin Invest. 1985; 75:384-90.
6. Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex B, Stampfer MJ, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. Circulation. 2000;102:1886-92.
7. Alaupovic P, Mack WJ, Knight-Gibson C, Hodis HN. The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. Arterioscler Thromb Vasc Biol. 1997;17(4):715-22.
8. Tsai MY, Ordovas JM. APOC3 mutation, serum triglyceride concentrations, and coronary heart disease. Clin Chem. 2009; 55(7):1274-6.
9. Gerdes LU, Gerdes C, Kervinen K, Savolainen M, Klausen IC, Hansen PS, et al. The apolipoprotein epsilon4 allele determines prognosis and the effect on prognosis of simvastatin in survivors of myocardial infarction: a substudy of the Scandinavian simvastatin survival study. Circulation. 2000; 101(12):1366-71.
10. Bekmetova FM, Kan LE, Hoshimov SU. Kurbanov RD. Clinical value distribution of polymorphism of the lipidtransport systems genes in patients with unstable angina and coronary heart disease in family history. Eurasian Heart J. 2013; 2:51-59. [Article in Russian].
11. Eshpulatov AS, Hashimov SU, Bekmetova FM, Shek AB, Kurbanov RD. Influence of the SstI polymorphism in the apolipoprotein C3 gene on the dyslipidemia and coronary lesions in patients with unstable angina. Int J Biomed. 2014; 4(4, Suppl1):15-9.
12. Eshpulatov AS, Hashimov SU, Bekmetova FM, Shek AB, Kurbanov RD. Distribution of gene APO CIII polymorphism in unstable angina patients with three-vessel coronary arteries lesion. Kardioangiologia i Revmatologia. 2014; 7:23-29. [Article in Russian].
13. Bandegi AR, Firoozrai M, Akbari Eidgahi MR, Kokhaei P. SstI Polymorphism of the Apolipoprotein CIII Gene in Iranian Hyperlipidemic Patients: A Study in Semnan Province. Iran J Basic Med Sci. 2011;14(6):506-13.
14. Wenham PR, Price WH, Blundell G. Apolipoprotein E genotyping by one-stage PCR. Lancet.1991;337(8750):1158–9.
15. Eichner JE, Kuller LH, Orchard TJ, Grandits GA, McCallum LM, Ferrell RE, et al. Relation of apolipoprotein E phenotype to myocardial infarction and mortality from coronary artery disease. Am J Cardiol. 1993;71(2):160-5.
16. Lehtinen S, Lehtimaki T, Sisto T, Salenius JP, Nikkila M, Jokela H, et al. Apolipoprotein E polymorphism, serum lipids, myocardial infarction and severity of angiographically verified coronary artery disease in men and women. Atherosclerosis. 1995;114(1):83–91.
17. Utermann G. Apolipoprotein Epolymorphism in health and disease. Am Heart J. 1987;113(2 Pt 2):433–40.
18. Schiele F, De Bacquer D, Vincent-Viry M, Beisiegel U, Enholm C, Evans A, et al. Apolipoprotein E serum concentration and polymorphism in six European countries: the ApoEurope Project. Atherosclerosis. 2000;152(2):475–88.
19. Hixson JE. Apolipoprotein E polymorphisms affect atherosclerosis in young males. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Arterioscler Thromb. 1991;11(5):1237–44.
20. Ilveskoski E, Perola M, Lehtimaki T, Laippala P, Savolainen V, Pajarinen J, et al. Age-dependent association of apolipoprotein E genotype with coronary and aortic atherosclerosis in middle-aged men: an autopsy study. Circulation. 1999;100(6):608–13.
21. Lima LM, Carvalho Md, Ferreira CN, Fernandes AP, Neto CP, Garcia JC, et al. Atheromatosis extent in coronary artery disease is not correlated with apolipoprotein-E polymorphism and its plasma levels, but associated with cognitive decline. Curr Alzheimer Res. 2010;7(6):556–63.
22. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol. 1988;41(2):105–14.
23. Lahoz C, Schaefer EJ, Cupples LA, Wilson PW, Levy D, Osgood D, et al. Apolipoprotein E genotype and cardiovascular disease in the Framingham Heart Study. Atherosclerosis. 2001;154(3):529–37.
24. Russo GT, Meigs JB, Cupples LA, Demissie S, Otvos JD, Wilson PW, et al. Association of the Sst-I polymorphism at the APOC3 gene locus with variations in lipid levels, lipoprotein subclass profiles and coronary heart disease risk: the Framingham offspring study. Atherosclerosis. 2001; 158(1):173-81.

International Journal of Biomedicine 5(4) (2015) 192-194

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Diastolic Heart Function and Myocardial Electrical Instability in Patients with Q-wave Myocardial Infarction

Ravshanbek D. Kurbanov, PhD, ScD; A. A. Kilichev;
Guzal U. Mullabaeva, PhD*

The Republican Specialized Center of Cardiology, Tashkent, Uzbekistan

Abstract

The study included 131 male patients between the ages of 30 and 69 (51.9±9.13 years) with primary Q-wave myocardial infarction (Q-MI). All patients underwent clinical examination, including a physical examination, medical history, ECG in 12 conventional leads, echocardiography, and 24-hour ECG monitoring from the 10th to the 14th day of MI. The progression of left ventricular diastolic dysfunction in Q-MI patients is associated with a longer history of coronary heart disease and arterial hypertension. With worsening diastolic dysfunction, a marked decrease in LV systolic function is revealed. The severe diastolic dysfunction in Q-MI patients is closely associated with myocardial electrical instability. **(Int J Biomed. 2015;5(4):192-194.)**

Key words: *diastolic heart function; Q-wave myocardial infarction; 24-hour ECG monitoring; myocardial electrical instability.*

Abbreviations

LVEDD, left ventricular end-diastolic dimension; **LVESD**, left ventricular end-systolic dimension; **LVEDV**, left ventricular end-diastolic volume; **LVESV**, left ventricular end-systolic volume; **LAESV,** left atrium end-systolic volume; **IVST**, interventricular septal thickness; **LVPWT**, left ventricular posterior wall thickness; **LVM**, left ventricular mass; **EF**, ejection fraction; **PVCs**, premature ventricular contractions; **VA**, ventricular arrhythmias; **PE**, peak early filling velocity; **PA**, peak atria1 filling velocity; **EPIA**, early post-infarction angina.

Introduction

The main problem of patients with coronary heart disease (CHD), especially after myocardial infarction (MI) and development of left ventricular remodeling, is preventing reinfarction, congestive heart failure, and cardiac arrhythmias[1,2].

The relationship between pro-arrhythmic indicators and systolic dysfunction is well defined, but the role of diastolic dysfunction (DD) in the formation of myocardial electrical instability remains unclear [3]. It is necessary to clarify the role played by formation of the arrhythmogenic mechanisms of ventricular arrhythmias in CHD patients, depending on the degree of left ventricular dysfunction [4].

***Corresponding author**: Guzal U. Mullabaeva, PhD. The Republican Specialized Center of Cardiology, Tashkent, Uzbekistan. E-mail: anchomed@gmail.com*

Materials and Methods

The study included 131 male patients between the ages of 30 and 69 (51.9±9.13 years) with primary Q-MI. The study was approved by the Republican Specialized Center of Cardiology Ethics Committee. Written informed consent was obtained from each patient.

The treatment of acute MI was carried out in accordance with recommendations for the Management of Patients with ST-Elevation Myocardial Infarction and included thrombolytic therapy, early administration of beta-blockers, antiplatelet agents, anticoagulants, nitrates, statins, ACE inhibitors, and loop diuretics. All patients underwent clinical examination, including a physical examination, medical history, ECG in 12 conventional leads, echocardiography, and 24-hour ECG monitoring on the 10th through the 14th days of MI.

Echocardiography and Doppler sonography study carried out on the unit «Sonoline Versa Pro» by standard methods using the recommendations of the American Society

of echocardiography [5]. The following parameters were measured and calculated: IVST, LVPWT, LVEDD, LVESD, LVEF, LVEVD, LVESV, and LVM (LVM was calculated using the formula R.Devereux [6]). Further analysis was indexed to body surface area indicators: LVEDVI, LVESVI, IVSI, LA ESVI, and LVMI.

As an indication, to the greatest extent reflecting the process of cardiac remodeling, RWT was calculated by the formula (IVS+PW)/LVEDD A value of ≥0.45 showed an increase in RWT. The diastolic function was evaluated by Doppler echocardiography. A decrease in PE/PA ratio less than 1.0 was considered as a sign of DD.

To characterize the PVCs, the B. Lown and M. Wolf classification (1971) and the prognostic classification of J.Bigger (1984) were used. Hourly qualitative and quantitative assessment of PVCs was performed in accordance with the Lown-Wolf gradation: Class 0 – absence of PVCs, Class I – rare monomorphic PVCs; Class II – frequent single PVCs; Class III – polymorphic (polytopic) PVCs; Class IVA – paired PVCs; Class IVB – group PVCs; Class V – early PVCs, R/T phenomena. After MI, according to the J.Bigger classification, PVCs>10 per hour, pair and group PVCs are potentially hazardous ventricular arrhythmias. Follow-up was 24 months.

The obtained data were processed using computer software Microsoft Excel, STATISTICA 6 and Biostat. *T*he mean (M) and standard deviation (SD) were calculated. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated using logistic regression. Group comparisons with respect to categorical variables are performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. Correlations were examined using regression analysis and Spearman rank correlation coefficient. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

To assess the relationship between LV systolic dysfunction and LV diastolic dysfunction, patients were divided into 2 groups (Table 1): a group with preserved LV systolic function (EF≥50%) and a group with a reduced EF (EF<50%). Analysis of the prevalence of different types of LV diastolic dysfunction showed that severe diastolic dysfunction (pseudonormal and restrictive types) was significantly more frequent in patients with reduced LV systolic function [7,8].

Table 1.

Prevalence of LV diastolic dysfunction in patients with different LV contractility

Diastolic dysfunction	LV EF >50%	LV EF <50%	*P*
Impaired relaxation	36/80(45%)	44/80(55%)	*0.27*
Pseudonormal filling	15/42(35.7%)	27/42 (64.3%)	*0.016*
Restrictive filling	4/25(16%)	21/25(84%)	*0.00001*

All patients were divided into 3 groups, depending on the severity of LV diastolic dysfunction: Group 1 (n=80) included patients with impaired relaxation of LV (type 1), Group 2 (n=42) included patients with pseudonormal filling (type 2), and Group 3 (n=25) included patients with restrictive filling (type 3).

Analysis of clinical and anamnestic indicators (Table 2) showed that patients with severe LV diastolic dysfunction were more likely to have anterior localization of MI (72% vs. 38% and 66.6%, respectively, Groups 1 and 2). Arterial hypertension (AH) history with the same frequency was found in all comparisons, but it should be noted that the duration of AH was significantly higher in Group 3 with LV diastolic dysfunction type 3 (9.5 versus 4.9 and 6.5 years in Group 1 and 2, respectively, $P<0.05$). Diabetes mellitus (DM) with a significant frequency prevailed in Group 3 patients (20% vs. 7.5%, 7.1% in Group 1 and 2, respectively). A similar trend can be seen in relation to CHD duration before the MI onset: 10.8 years in Group 3 with severe LV diastolic dysfunction compared with 4.1 and 6.5 years in Groups 1 and 2, respectively ($P<0.05$). Significant differences were also identified in relation to body mass index (BMI). BMI was 30.5 kg/m^2 in Group 3 vs. 27.1 kg/m^2 and 27.7 kg/m^2 in Groups 1 and 2, respectively.

Table 2.

Comparative characteristics of groups with different types of LV diastolic dysfunction

Variable	Group 1	Group 2	Group 3
Anterior MI	31/38%	28/66.6%*	18/72%^
Posterior MI	49/61.2%	14/33.4%*	7/28%^
AH	72/90%	32/76.2%	20/80%
DM	6/7.5%	3/7.1%	3/20%^,Δ
Aneurism	24/30%	11/26.2%	13/52%
Thrombolysis	11/13.7%	5/12%	3/12%
EPIA	30/37.5%	17/40.1%	10/40%
BMI, kg/m^2	27.1+3.8	27.7+3.7	30.5+4.9^,Δ
RWT	0.40+0.10	0.34+0.09	0.35+0.10
LVMI, g/m^2	133.4+37.7	132.3+34.5	141.6+38.4
IVSIdiast, cm/m^2	1.11+0.29	0.97+0.27	1.0472+0.37
LV EDVI, ml/m^2	74.4+19.9	89.9+28.7*	93.8+36.3Δ
LV ESVI, ml/m^2	38.5+16.4	48.97+20.38	50.9+23.47Δ
LA ESVI, ml/m^2	38.7±1.9	44.8±2.6	52.0±2.4^,Δ
LVEF, %	49.2+11.6	46.08+9.33	38.4+12.06^,Δ

** - P <0.05 between Groups 1 and 2; ^ - P <0.05 between Groups 2 and 3; Δ - P <0.05 between Groups 1 and 3*

Analysis of the geometry and contractility of the left chambers of the heart showed that the LV volume indicators (the indexed EDV and ESV) in patients of Groups 2 and 3 were significantly higher compared with Group 1 patients. In particular, the indexed EDV and ESV were higher by 20% and 27% in Group 2 vs. Group 1, and by 26% and 32% in Group 3 vs. Group 1, respectively ($P<0.05$) [9-11].

Group 3 patients experienced an increase in dilatation of the left chambers and LVM, a decrease in LV contractility, and significant differences in the index of contractile function of the left atrium. The indexed ESV of the left atrium was

significantly different compared to this parameter in Groups 1 and 2 ($P<0.05$).

According to 24-hour ECG monitoring, VAs, including the potentially malignant forms, were detected in 81/57% and 54/36.7% patients, respectively, on the 12th through the 14th days of MI. In Q-MI patients, the comparative analysis of the structure of the ectopic activity, depending on the type of LV diastolic dysfunction, identified a high grade of VA, according to the J.Bigger classification, in 55.6%, 81.2% and 78.5% of cases in Groups 1, 2, and 3, respectively. A similar trend was observed in relation to PVCs, according to the Lown-Wolf classification (Table 3). In particular, Class I was detected in 11/50%, 5/26.3%) and 1/7.7% patients in Groups 1, 2, and 3, respectively; Class II in 4/18.2%, 2/21.1%, and 2/15.4% patients in Groups 1, 2, and 3, respectively; Class III in 6/27.3%, 7/36.8%, and 4/30.8% patients in Groups 1, 2, and 3, respectively. Class IV+V was detected in 1/4.5%, 3/15.8%, and 6/46.2% patients in Groups 1, 2, and 3, respectively. We did not observe significant differences in the registration of Class II and Class III PVCs between the groups of patients with type 2 and type 3 LV diastolic dysfunction, but complex forms of PBCs were often identified in Group 3 patients with the restrictive type of diastolic dysfunction vs. Group 2 patients with a pseudonormal type of diastolic dysfunction ($P<0.05$).

Table 3.

PVCs in patients with different types of LV diastolic dysfunction

PVCs	Group 1	Group 2	Group 3
Number of patients with PVCs	22	19	13
Class I, n/%	11/50%	5/26.3%	1/7.7%*
Class II, n/%	4/18.2%	4/21.1%	2/15.4%
Class III, n/%	6/27.3%	7/36.8%	4/30.8%
Class IV+V, n/%	1/4.5%	3/15.8%	6/46.2%*^

**- P<0.05 between Groups 1 and 3; ^-P<0.05 between Groups 2 and 3.*

In conclusion: The progression of LV diastolic dysfunction in Q-MI patients is associated with a longer history of CHD and hypertension. With worsening diastolic dysfunction, a marked decrease in LV systolic function is revealed. The severe diastolic dysfunction in Q-MI patients is closely associated with myocardial electrical instability.

References

1. Boldueva SA, Shabrov AV, Nesterko AO, Leonova IA, Burak TIa, Samokhvalova MV, et al. Factors affecting development of sudden death and risk stratification of patients after myocardial infarction. Kardiologiia 2006; 46(6):64-5. [Article in Russian].
2. Zipes DP, Camm AJ, Borggrefe M, Buxton AE, Chaitman B, Fromer M, Gregoratos G, et al. ACC/AHA/ESC Guidelines for Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death. J Am Coll Cardiology 2006; 48(5):247-346.
3. Arbolishvili GN1, Mareev VI, Orlova IA, Belenkov IN. Heart Rate Variability in Chronic Heart Failure and Its Role in Prognosis of the Disease. Kardiologiia 2006; 46(12):4-11. [Article in Russian].
4. Meta-Analysis Research Group in Echocardiography (MeRGE) AMI Collaborators, Møller JE, Whalley GA, Dini FL, Doughty RN, Gamble GD, Klein AL, et al. Independent prognostic importance of a restrictive left ventricular filling pattern after myocardial infarction: an individual patient meta-analysis: Meta-Analysis Research Group in Echocardiography acute myocardial infarction. Circulation 2008; 117(20): 2591-8.
5. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Eur J Echocardiogr 2009; 10(2):165-93.
6. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. Circulation 1977; 55(4):613–8
7. Kapel'ko VI. Diastolic dysfunction. Kardiologiia 2011; 51(1):79-90. [Article in Russian].
8. Somaratne JB, Whalley GA, Poppe KK, Gamble GD, Doughty RN. Pseudonormal mitral filling is associated with similarly poor prognosis as restrictive filling in patients with heart failure and coronary heart disease: a systematic review and meta-analysis of prospective studies. J Am Soc Echocardiogr 2009; 22(5):494–8.
9. Kindermann M, Reil JC, Pieske B, van Veldhuisen DJ, Böhm M. Heart failure with normal left ventricular ejection fraction: what is the evidence? Trends Cardiovasc Med. 2008; 18(8):280-92.
10. Sohn DW. Heart failure due to abnormal filling function of the heart. J Cardiol 2011; 57(2):148-59.
11. Andersen NH, Karlsen FM, Gerdes JC, Kaltoft A, Bøttcher M, Sloth E, et al. Diastolic dysfunction after an acute myocardial infarction in patients with antecedent hypertension. J Am. Soc. Echocardiogr 2008; 21(2):171-7.

International Journal of Biomedicine 5(4) (2015) 195-197

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Main Predictors of Sudden Cardiac Death in Patients with Q-Wave Myocardial Infarction

Ravshanbek D Kurbanov, PhD, ScD; Guzal U. Mullabaeva, PhD; Anvar A Kilichev

The Republican Specialized Center of Cardiology, Tashkent, Uzbekistan

Abstract

The study included 131 patients (mean age 51.9±9.13 year) with Q-wave myocardial infarction (Q-MI). All patients underwent echocardiography and 24-hour ECG monitoring on the 10th through the 14th days of MI. Treatment included thrombolytic therapy, early administration of beta-blockers, antiplatelet agents, anticoagulants, statins, ACE inhibitors, if needed - antiarrhythmics and aldosterone antagonists. Follow-up was 24 months. During the observation period, of the 131 study patients 17(13.0%) died suddenly. Our study suggests that the high risk of sudden cardiac death (in the first 2 years after MI) in patients with Q-MI is associated with anterior localization, early pathological left ventricular remodeling, low myocardial contractility, and development of AHF high Killip classes in the early period of MI, as well as the identification of high heart rate at rest, frequent premature ventricular contractions (mainly polymorphic), systolic dysfunction in the early stages of observation (on the 10th through the 14th days), and older age of patients. **(Int J Biomed. 2015;5(4):195-197.)**

Keywords: *sudden cardiac death; myocardial infarction; premature ventricular contractions; 24-hour ECG monitoring.*

Introduction

The problem of sudden cardiac death (SCD) is particularly relevant in patients after a myocardial infarction (post-MI patients), since this patient population is particularly vulnerable to the development of fatal ventricular rhythm disorders [1-3]. During the first year after MI, 3% to 6% of patients die, the majority of them suddenly [4]. Because this event develops very quickly, within an hour of the onset of symptoms, and treatment interventions are often too late, the main approach to solving this problem is the prevention of SCD. Premature ventricular contractions (PVCs) are one of the predictors of SCD [5,6].

The aim of the study was to evaluate the incidence of PVCs at the 10th through the 14th days after Q-wave myocardial infarction (Q-MI) and its relationship with the SCD development.

Materials and Methods

We examined 131 (mean age 51.9±9.13 years) male patients with primary Q-MI. The study was approved by the Republican Specialized Center of Cardiology Ethics Committee. Written *i*nformed consent was obtained from each patient. The treatment of acute MI was carried out in accordance with recommendations for the Management of Patients with ST-Elevation Myocardial Infarction and included thrombolytic therapy, early administration of beta-blockers, antiplatelet agents, anticoagulants, nitrates, statins, ACE inhibitors, if needed - antiarrhythmics and aldosterone antagonists.

All patients underwent echocardiography and 24-hour ECG monitoring on the 10th through the 14th days of MI. To characterize the premature ventricular contractions (PVCs), the B. Lown and M. Wolf classification (1971) and the prognostic classification of J. Bigger (1984) were used. Hourly qualitative and quantitative assessment of PVCs was performed in accordance with the Lown-Wolf gradation: Class 0 – absence of PVCs, Class I – rare monomorphic PVCs; Class II – frequent single PVCs; Class III – polymorphic (polytopic) PVCs; Class IVA – paired PVCs; Class IVB – group PVCs; Class V – early PVCs, R/T phenomena. After MI, according to the J. Bigger classification, PVCs>10 per hour, pair and group PVCs are potentially hazardous ventricular arrhythmias. Follow-up was 24 months.

The obtained data were processed using computer software Microsoft Excel, STATISTICA 6 and Biostat. *T*he mean (M) and standard deviation (SD) were calculated. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated using logistic regression. Group comparisons with

respect to categorical variables are performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. Correlations were examined using regression analysis and Spearman rank correlation coefficient. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

During the observation period, of the 131 study *patients* 17/13.0% died suddenly. In particular, 8/47.1% patients died in the first 6 months, 5/29.4% during the first year, and 4/23.5% patients died after 1 year from the onset of the disease. Our data are consistent with the view of S.H. Hohnloser [7] that 10% to 20% of patients die within one year, while more than 50% of deaths occur within the first 3 to 6 months after acute MI. Clinical characteristics of the dead (Group 1) and surviving (Group 2) patients are presented in Table 1.

Table 1.

Baseline clinical characteristics of patients

Variable	Patients (n)	Group 1 (n=17)	Group 2 (n=114)	OR	95% CI	*P*
Age, year		52.6±9.2	51.7±0.14			*0.71*
Anterior MI	72	15/88.2%	67/58.8%	5.26	1.15-24.1	*0.04*
Inferior MI	49	2/11.8%	47/41.2%			
DM	10	3/17.6%	7/6.1%	3.28	0.76-14.1	*0.24*
EPIA	52	8/47.1%	44/38.6%	1.41	0.51-3.94	*0.69*
AH	111	12/70.6%	99/86.8%	0.36	0.11-1.18	*0.17*
Aneurism	43	11/64.7%	32/28.1%	4.70	1.60-13.8	*0.006*
Thrombolytic	16	2/11.8%	14/12.3%	0.95	0.20-4.67	*0.74*
AHF Killip I-II	77	13/76.5%	64/56.1%	2.54	0.78-8.26	*0.16*
AHF Killip III-IV	10	4/23.5%	6/5.3%	5.54	1.38-22.2	*0.03*
LVEF,%		39.6±8.8	49.2±12.3			*0.002*
LVEF<50%	77	15/88.2	62/54.4	6.29	1.38-28.8	*0.02*

Polymorphic (polytopic) PVCs and frequent monomorphic PVCs were detected more frequently in Group 1 compared to Group 2: 58.8% vs. 13.2% (OR=9.43; 95% CI: 3.11-28.6; *P<0.0001*) and 35.3% vs. 12.3% (OR=3.90; 95% CI: 1.25-12.2; *P=0.04*), respectively, (Fig. 1).

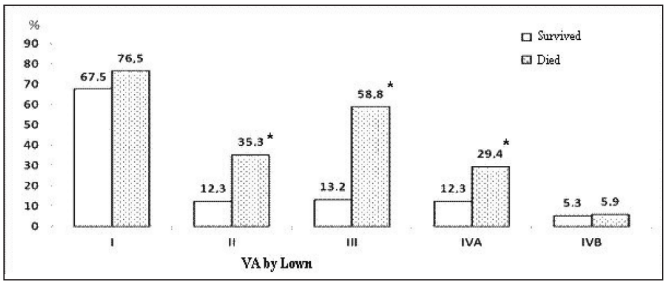

Fig. 1. *The frequency of PVCs in the two groups*

It should be noted that other high-grade PVCs were found more frequently in Group 1 than in Group 2 (OR=2.56; 95% CI 0.85-7.75; *P=0.17*), but the differences did not reach a statistically significant level. In Group 1, we revealed a positive correlation between age and PVCs (*r=0.74; P=0.001*), heart rate (HR) per min (*r=0.84; P=0.0001*), PVCs>10/hour (*r=0.34; P=0.04*), and frequent PVCs (*r=0.82; P=0.0001*). A negative correlation was found between the left ventricular ejection fraction (LVEF) and PVCs (*r=-0.51; P=0.04*), HR per min (*r=-0.70; P=0.002*), PVCs>10/h (*r=-0.57; P=0.04*), and frequent PVCs (*r=-0.46; P=0.05*) (Fig. 2). Significant correlations in Group 2 were not found.

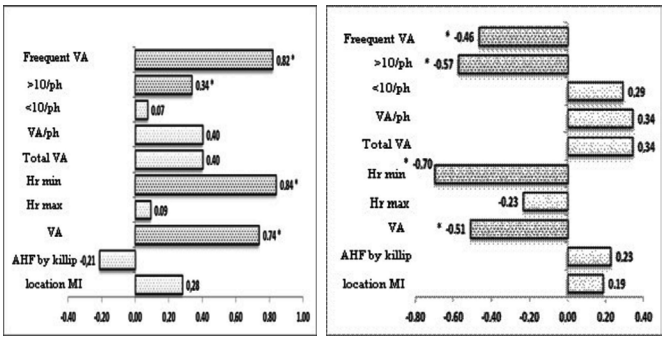

Fig. 2. *Correlation analysis between age, LVEF and indicators of VAs in Group 1*

Many studies have shown that the main electrophysiological causes of SCD are malignant rhythm disorders [8,9]. H.J. Trappe, P. Brugada et al. [10], based on the results of observations of 200 patients who underwent MI complicated with VT or VF, concluded that these types of arrhythmias can be considered as prognostically life-threatening only in cases when they are accompanied by loss of consciousness and have occurred within the first 2 months after MI onset. The mortality among these patients reaches 83% [9]. However, some experts are showing less confidence in assessing the value of PVCS as an independent factor determining the prognosis of patients with MI, considering that the survival of patients is also dependent on the state of LV and condition of the coronary arteries. Based on the above data, the aim of this study was to assess the detectability of PVCS on the 10th through the 14th days of Q-MI and analyze the survival of these patients during 24 months of follow-up. So, by the end of the observation period, out of 131 patients 114 (87.0%) survived and SCD appeared in 13.0% of cases, with most of the deaths occurring in the first year (76%). So far, there is no consensus about the prognostic significance of localization of MI. Currently, it is known that anterior localization of MI makes a significant contribution to CHF development and the frequency of re-infarction [11], but the issue about SCD is still debated. In our study, anterior Q-MI and complications such as heart failure and LV aneurysm were found significantly more often in the Group 1 patients with SCD. Absence of differences in the incidence of early post-infarction angina in the two groups was not consistent with the opinion of other researchers that the presence of residual ischemia often predicts the risk of re-infarction [4,6]. In our study, we found that resting HR was significantly higher in Group 1 compared to Group 2 (72.6±8.30 vs. 64.5±12.1, *P=0.009*). The analysis of the detectability of PVCS in the two groups showed that groups

were initially different in detection of PVCs. This corresponds to the data of GISSI-2 [12], namely, the presence of more than 10 PVBs per hour or of complex ventricular arrhythmias was significantly associated with a higher mortality risk regardless of the presence of LV dysfunction. Meanwhile, in our study the initially more expressed systolic dysfunction was detected in Group 1. In particular, LVEF was 39.6±8.81% in Group 1 vs. 49.2±12.3% in Group 2 (P=0.002). The number of patients with LVEF<50% was 88.2% in Group 1 vs. 54.4% in Group 2 (*P=0.02*). The correlation analysis also showed a strong negative relationship between LVEF and PVC frequency. According to many researchers, older age is one of the factors that determine SCD risk in post-MI patients [11]. In our study, we did not observe significant differences between the study groups, but the correlation analysis revealed a positive relationship between age and the frequency of PVCs and HR only in Group 1.

In conclusion: Based on our data it can be argued that PVCS cannot be regarded as an independent predictor of SCD. Our study suggests that the high risk of SCD (in the first 2 years after MI) in patients with Q-MI is associated with anterior localization, early pathological LV remodeling, low myocardial contractility, and development of AHF high Killip classes in the early period of MI, as well as the identification of high HR at rest, frequent PVCs (mainly polymorphic), systolic dysfunction in the early stages of observation (on the 10th through the 14th days), and older age of patients.

Competing interests

The authors declare that they have no competing interests.

References

1. Bokeria OL, Akhobekov AA. Sudden cardiac death: mechanisms and risk stratification. Ann Aritm 2012; 3:5-13. [Article in Russian].
2. Bokeria LA, Bokeria OL, Kirtbaya LN. Heart failure and sudden cardiac death. Ann Aritm 2009; 4:7-20. [Article in Russian].
3. Bokeria LA, Revishvili AS. Sudden cardiac death. M.: GEO-TAP-Media; 2011. [Manual in Russian].
4. Boldueva SA, Shabrov AV, Lebedev DS, Burak Tatyana Leonova IA, Samohvalova MV, Beetle VS, Bykov EG. Prediction and prevention of sudden cardiac death in patients with myocardial infarction. Kardiov Ter Prof 2008; 7(3):56-62. [Article in Russian].
5. Kurbanov RD. Ventricular arrhythmias. Tashkent; 2012. [Monograph in Russian].
6. Kurbanov RD, Kiyakbaev GK. Myocardial infarction. Life expectancy. Tashkent; 2001. [Monograph in Russian].
7. Goldstein S, Medendorp SV, Landis JR, Wolfe RA, LeigttonR, Ritter G, et al. Analysis of cardiac symptoms preceding cardiac arrest. Am J Cardiol 1986; 58(13):1195-8.
8. Epstein AE, DiMarco JP, Ellenbogen KA, Estes NA 3rd, Freedman RA, Gettes LS, et al. ACC/AHA/HRS 2008 Guidelines for Device-Based Therapy of Cardiac Rhythm Abnormalities: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the ACC/AHA/NASPE 2002 Guideline Update for Implantation of Cardiac Pacemakers and Antiarrhythmia Devices): developed in collaboration with the American Association for Thoracic Surgery and Society of Thoracic Surgeons. Circulation 2008; 117(21):e350-408.
9. Goldberger JJ, Buxton AE, Cain M, Costantini O, Exner DV, Knight BP, et al. Risk stratification for arrhythmic sudden cardiac death: identifying the roadblocks. Circulation 2011; 123(21): 2423-30.
10. Trappe HJ, Brugada P, Talajic M, Lezaun R, Wellens HJ. Ventricular tachycardia and ventricular fibrillation following myocardial infarct: determinants of prognosis and disease course. Z Kardiol 1988; 77(11):687-95. [Article in German].
11. Stecker EC, Vickers C, Waltz J, Socoteanu C, John BT, Mariani R, et al. Population-based analysis of sudden cardiac death with and without left ventricular systolic dysfunction: two-year findings from the Oregon Sudden Unexpected Death Study. J Am Coll Cardiol 2006; 47(6):1161-6.
12. Maggioni AP, Zuanetti G, Franzosi MG, Rovelli F, Santoro E, Staszewsky L, et al. Prevalence and prognostic significance of ventricular arrhythmias after acute myocardial infarction in the fibrinolytic era. GISSI-2 results. Circulation 1993; 87:312–22.

International Journal of BioMedicine 5(4) (2015) 198-202

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Antiremodelling Efficacy and Clinical Safety of Zofenopril in Patients with Grade 1 and 2 Hypertension

G. A. Khamidullaeva, PhD, ScD; G. J. Abdullaeva, PhD; L. Sh. Khafizova, PhD; N. Z. Srojidinova*, PhD; N. B. Tursunova, PhD; D. R. Kurbanova, PhD, ScD; N. Sh. Shakirova, PhD

The Republican Specialized Center of Cardiology, Tashkent, Uzbekistan

Abstract

Objective: to estimate the antihypertensive, antiremodelling efficacy and clinical tolerability of the monotherapy with Zofenopril in patients with Grade 1 and 2 hypertension (HT 1 and 2)

Materials and Methods: The study included 30 patients aged from 30 to 60 years with HT 1 and 2 (ESH/ESC, 2013) without severe comorbidities and cardiovascular complications. Zofenopril was prescribed as monotherapy to HT patients who had never been treated before or patients after one week of lavage from previous antihypertensive therapy, who did not reach target levels of blood pressure (BP). Before and during treatment all patients were checked on office BP using Korotkov's method and ambulatory blood pressure monitoring (ABPM). Echocardiography and Doppler sonography were carried out by standard methods using the recommendations of the American Society of Echocardiography. Intima-media thickness (IMT) of the carotid artery and brachial artery was measured by a 7.5 MHz high-resolution ultrasound. Assessment of flow-mediated dilation (FMD) of the brachial artery was used as a method of determining endothelial function.

Results: A 12-week monotherapy with Zofenopril in average daily dose of 36.0±9.54 mg showed a high antihypertensive efficacy and a good safety profile without side effects. We noted a reliable decrease in systolic BP (SBP), diastolic BP (DBP), mean BP, and pulse pressure by -19.53±5.93%, -18.64±7.18%, -19.05±6.14%, and -20.65±12.07%, respectively. Target SBP, DBP, and SBP+DBP were reached in 90%, 86.6%, and 83.3% of patients, respectively. We found a significant regression of LVH, significant improvement in volume indicators of LV echogeometry and parameters of FMD of the brachial artery, as well as a decrease in IMT of carotid and brachial arteries. Monotherapy with Zofenopril showed metabolic neutrality regarding the lipid and carbohydrate metabolism, a good safety profile without the side effects and undesired events. **(Int J Biomed. 2015;5(4):198-202.)**

Keywords*: arterial hypertension; Zofenopril**; antihypertensive efficacy; organprotective effects.*

Abbreviations

LVEDV, left ventricular end-diastolic volume; **LVESV**, left ventricular end-systolic volume; **LVM**, left ventricular mass; **LVH**, left ventricular hypertrophy; **RWT,** relative wall thickness; **IMT**, intima-media thickness; **FMD**, flow-mediated dilation.

Introduction

For cardiology, the last quarter of the twentieth century was the era of angiotensin-converting enzyme (ACE) inhibitors. Captopril is the first representative of ACE inhibitors containing the sulfhydryl group. Over that period, many random clinical studies of Captopril were conducted, and it was shown to have high antihypertensive efficacy with cardio protection for patients with myocardial infarction and congestive heart failure, as well as nephroprotection for patients with diabetes and hypertension. Captopril had an advantage of organ protection over other ACE inhibitors because of the presence of the sulfhydryl group's antioxidant and anti-atherogenic features. However, Captopril started to give way to modern ACE inhibitors, mainly because of tissue affinity, lower compounding level with ACE, and prolongation of dose, since a triple dose of Captopril is necessary to maintain daily effect.

***Corresponding author**: Nigora Z. Srojidinova, PhD. The Republican Specialized Center of Cardiology, Tashkent, Uzbekistan. E-mail: nigora5505@gmail.com*

During the last 25 years, a new sulfhydryl ACE inhibitor, Zofenopril, was synthesized and clinically tested. This drug has many distinctive characteristics compared to its predecessor, Captopril. Zofenopril calcium is a highly lipophilic ACE inhibitor, which is converted to its active form, zofenoprilat, in both blood serum and various tissues. This is the essential difference between Zofenopril and other prodrugs such as Ramipril and Enalapril, which are largely activated only in blood serum and kidneys. Zofenopril, as other representatives of this class, inhibits ACE in plasma and tissues, as well as preventing degradation of bradykinin, which is known to be associated with cardio-vascular and renal effects of the drug. One of the main differences of Zofenopril is its high lipophilic level and affinity to ACE, which contribute to fast and full penetration and accumulation of the drug in the tissues and maximum inhibition of tissue ACE. Therefore, Zofenopril is associated with such pleiotropic effects as cardioprotection and prevention of endothelial dysfunction; anti-ischemic, anti-inflammatory and antiatherogenic effects; its angiogenesis ability; and its ability to reverse development of apoptosis. The presence of the sulfhydryl group in Zofenopril gives it the ability to reduce oxidative stress, compound free radicals, increase production of nitric oxide, and provide an antiatherogenic and vasoprotective effect [1, 2]. In accordance with that, Zofenopril increases coronary circulation and reduces ischemia, which lets us characterize Zofenopril as an antihypertensive drug with cardioprotective properties [3]. Organ-protective effects of antihypertensive drugs are especially necessary for HT treatment to achieve one of the main goals of antihypertensive therapy – prevention of target-organ damage and reduction of cardiovascular risk.

Thereby, the aim of our study was to estimate the antihypertensive, antiremodelling efficacy and clinical tolerability of the monotherapy with Zofenopril in patients with Grade 1 and 2 hypertension (HT 1 and 2).

Materials and Methods

The study included 30 patients aged from 30 to 60 years (mean age 49.93±9.0) with HT 1 and 2 (ESH/ESC, 2013) [4] without severe comorbidities and cardiovascular complications (heart failure, cerebrovascular disease, myocardium infarction, diabetes mellitus). Zofenopril (Zocardis, Berlin-Chemi, MENARINI group) was prescribed as monotherapy to HT patients who had never been treated before or patients after one week of lavage from previous antihypertensive therapy, who did not reach target levels of blood pressure (BP). Before and during treatment all patients were checked on office BP using Korotkov's method and ambulatory blood pressure monitoring (ABPM) ("Registrator BR-102 plus" SCHILLER, Switzerland).

Echocardiography was carried out according to Penn Convention method [5] using «EnVisorC®». The following parameters were measured and calculated: RWT, LVEDV, LVESV, EF, and LVM. LVM was indexed to body surface area (LVMI). Left ventricular hypertrophy (LVH) was defined as LVMI of >95g/m^2 (women) and >115g/m^2 (men) [4]. The diastolic function was evaluated by Doppler echocardiography. A decrease in PE/PA (peak early filling velocity/peak atria1 filling velocity) ratio less than 1.0 was considered as a sign of diastolic dysfunction.

Intima-media thickness (IMT) of the carotid artery and brachial artery was measured by a 7.5 MHz high-resolution ultrasound (EnVisorC®). Assessment of flow-mediated dilation (FMD) of the brachial artery was used as a method of determining endothelial function [6]. The diameter of the brachial artery was measured from two dimensional ultrasound images, with the 7.5 MHz linear array transducer. In each study, scans were taken at rest and during reactive hyperemia. FMD was estimated as the percent change in the diameter relative to the baseline diameter at rest. Level of FMD ≥10% was taken as the norm threshold [6].

The total cholesterol (TC), High-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were determined in the venous blood using the A-25 Biosystems Autoanalyzer (DAYTONA) and the "RENDOX" sets. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Fridvald's formula. Fasting glucose and insulin were also determined.

After starting at 15mg/day, dosing was titrated to a maximum of 60mg/day at 4-week intervals to achieve a target blood pressure of <140/<90mmHg. The targeted lifestyle modifications were recommended for all patients. For patients with dyslipidemia, a lipid-lowering diet was also recommended.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki. It was approved by the Republican Specialized Center of Cardiology Ethics Committee. Written informed consent was obtained from all participants.

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

Results and Discussion

Among studied patients, Grade 1 HT was identified in 46.7% of cases and Grade 2 HT in 53.3% of cases. Before therapy, systolic and diastolic BP were 149.67±9.99 mmHg and 95.5±4.97 mmHg, respectively. HT duration was 5.07±4.38 years. Fifty percent of patients suffered from first or second degree obesity (body mass index >30 kg/m^2), only 5 patients had normal body weight and the rest were overweight. Overall, 60% of patients had LVH: 73.3% of cases for women, 53.3% for men. Dyslipidemia, thickening of IMT, impaired FMD, and fasting hyperglycemia were found in 70%, 56.6%, 53.3%, and 16.6% of cases, respectively. Thus, more than half of the HT patients had a high cardiovascular risk. Average daily dose of Zofenopril was 36.0±19.54 mg, which was apportioned as

follows: 10/33.3% patients took 15 mg/day; 9/30% patients, 30 mg/day; and 11/36.7% patients, 60 mg/day.

A 12-week monotherapy with Zofenopril showed a high antihypertensive efficacy, which was evaluated by the BP decrease (more than 15%) and achievement of target BP. We noted a reliable decrease in systolic BP (SBP), diastolic BP (DBP), mean BP (MBP), and pulse pressure (PP) by -19.53±5.93%, -18.64±7.18%, -19.05±6.14%, and -20.65±12.07%, respectively. Thus, target SBP, DBP, and SBP+DBP were reached in 90%, 86.6%, and 83.3% of patients, respectively (Table 1).

Table 1.

BP indexes during 12-week monotherapy with Zofenopril

Indexes	SBP	DBP	MBP	PP
Before treatment, mmHg	149.67 ±9.99	95.5 ±4.97	113.79 ±6.34	54.79 ±6.44
After treatment, mmHg	120.33 ±10.98*	77.67 ±7.74*	91.89 ±8.43*	42.67 ±6.4*
Level of BP decrease,%	-19.53 ±5.93%	-18.64 ±7.18%	-19.05 ±6.14%	-20.65 ±12.07%
Target BP, %	90%	86.6%	83.3%	

**- P<0.0001 – between before and after treatment*

According to ABPM data, there was a significant reduction in average daily SBP and DBP and a tendency to reduction in average night systolic and diastolic BP indexes (Table 2).

Table 2.

ABPM indexes during 12-weekly monotherapy with Zofenopril

Indexes	Before treatment	*P*	After treatment
Average 24- h SBP, mmHg	132.57±13.78	*0.036*	128.07±13.23
Average 24- h DBP, mmHg	83.16±8.9	*0.047*	79.7±8.26
Daytime SBP, mmHg	134.69±13.86	*0.041*	129.8±12.86
Daytime DBP, mmHg	85.03±8.98	*0.07*	81.63±8.62
Nighttime SBP, mmHg	125.39±15.33	*0.56*	124.07±16.07
Nighttime DBP, mmHg	76.4±8.84	*0.4*	75.07±10.05
Nocturnal MBP fall,%	8.81±6.65	*0.34*	9.07±6.93
Daytime SBP variability, mm Hg	14.09±3.11	*0.61*	13.8±2.98
Daytime DBP variability, mm Hg	12.01±3.73	*0.55*	11.58±2.49
Nighttime SBP variability, mm Hg	13.67±4.31	*0.03*	11.59±2.75
Nighttime DBP variability, mm Hg	10.21±3.61	*0.27*	9.31±2.45
Daytime SBP load, %	34.36±28.15	*0.049*	24.7±26.93
Daytime DBP load, %	32.71±23.36	*0.054*	23.69±23.02
Nighttime SBP load, %	55.18±31.08	*0.64*	52.04±32.66
Nighttime DBP load, %	40.51±31.59	*0.13*	30.76±31.15
RoR in morning SBP, mmHg/h	25.57±23.25	*0.39*	22.13±19.19
RoR in morning DBP, mmHg/h	25.52±29.2	*0.57*	20.74±21.72

The degree of nighttime BP reduction had a trend of decrease from 9.78±6.04 to 7.77±8.23% during therapy (P=0.08). During the monotherapy, we noted a normalization of the degree of nighttime BP reduction in patients with significant abnormal nocturnal BP dipping status, which shows the safety of Zofenopril and the absence of night hypotension. Overall, to the end of observation, the normal degree of nocturnal BP fall was demonstrated in 50% of patients. Results showed that the other half of patients had impaired degrees of nocturnal BP fall during the monotherapy, which requires the addition of a second drug for these patients. During the 12-week therapy with Zofenopril, we noted a reduction in daytime SBP load and DBP load. Nighttime SBP load and DBP load also decreased, but without statistical reliability. Another significant indicator was the rate of rise (RoR) in BP in the morning, because the frequency of cardiovascular complications is greater during early morning hours. During therapy, RoR in SBP and DBP in the morning did not decrease enough: RoR for SBP and DBP was normalized in 22% and 40.7% of patients, respectively.

Reaching a target level of BP in more than 80% of patients proved the organ-protective effect of Zofenopril. We found a significant regression of *LVH*, significant improvement in volume indicators of LV echogeometry and parameters of FMD of the brachial artery, as well as a decrease in IMT of carotid and brachial arteries. Regression of LVH was evaluated by the dynamics of LVMM, LVMMI and RWT of LV (Table 3). A 12-week monotherapy with Zofenopril was associated with significant decrease in LVEDV and LVMI by 9.6±5.6%. Zofenopril did not affect LV diastolic function.

Significant cardioprotective effects of Zofenopril were accompanied by expressed vasoprotection: the improvement and normalization in FMD of the brachial artery and positive dynamics in IMT of common carotid and brachial arteries (Table 3).

Table 3.

LV echogeometry and ultrasound vascular indexes during 12-week monotherapy with Zofenopril

Indexes	Before treatment	*P*	After treatment
LVEDV, ml	140.08±29.24	*0.01*	135.83±27.83
LVESV, ml	44.11±11.94	*NS*	43.6±11.23
LVM, g	231.14±73.5	*0.000*	207.94±64.14
LVMI, g/m^2	117.84±32.5	*0.000*	106.15±28.6
PE/PA	0.99±0.27	*NS*	0.97±0.26
RWT, мм	35.79±4.79	*0.000*	34.25±4.61
FMD, %	9.86±3.82	*0.000*	14.13±6.17
IMTcc, mm	1.0±0.3	*0.002*	0.91±0.24
IMTba, mm	0.58±0.11	*0.005*	0.56±0.09

IMTcc – IMT of common carotid artery; IMTba - IMT of brachial artery.

During 12-week monotherapy with Zofenopril, we did not reveal negative changes in the level of blood lipids and fasting blood glucose, which indicated the metabolic neutrality of Zofenopril (Table 4). A 12-week monotherapy with Zofenopril in average daily dose of 36±19.54 mg was

characterized with a good safety profile without side effects. All patients finished the 12-week study; compliance to therapy was high.

Table 4.

Blood lipids and fasting glucose during 12-week monotherapy with Zofenopril

Indexes	Before treatment	*P*	After treatment
TC, mg/dl	234.13±45.87	*NS*	229.93±43.88
TG, mg/dl	186.77±145.93	*NS*	178.7±138.02
HDL, mg/dl	45.97±10.68	*NS*	45.4±10.96
LDL, mg/dl	150.93±38.77	*NS*	143.2±35.9
AI	4.3±1.12	*NS*	4.35±1.46
FG, mmol/l	5.21±0.62	*NS*	5.14±0.51

AI – atherogenic index; FG -fasting glucose.

Discussion

In many clinical studies, Zofenopril in a daily dose from 30 to 60 mg showed high antihypertensive efficacy in patients with HT 1 and 2, which was not inferior when compared with Candesartan, Enalapril, Amlodipin, and Atenolol [7-9]. Many studies have demonstrated a smooth 24-hour antihypertensive effect of Zofenopril with normalization of daily BP profile. Decrease of BP in therapeutic diapason does not influence cerebral circulation, which remains on the necessary level even after reduced BP. Our research demonstrated a high antihypertensive efficacy, reaching the target level of BP in 83.3% of Grades 1 and 2 hypertensive patients. We revealed a significant normalization in daily, daytime and nighttime BP profile without the hypotonic episodes. The results obtained also showed the possibility of LVH regression, improvement and normalization of endothelial function, and reduction in IMT of the carotid and brachial arteries, which is associated with a positive effect on the prognosis of HT patients on the background of therapy with Zofenopril.

As is known, antecedent hypertension represents a risk factor for adverse outcomes in survivors of acute myocardial infarction (AMI). The results of SMILE study [10] suggest that treatment with Zofenopril started within 24 h of the onset of anterior AMI could be highly beneficial in patients with a history of HBP.

In SMILE-4 study, the efficacy of Zofenopril 60mg and acetylsalicylic acid (ASA) 100mg versus Ramipril 10mg and ASA was compared in patients with AMI complicated by left ventricular dysfunction, classified according to a history of hypertension. This retrospective analysis of the SMILE-4 study confirmed the good efficacy of Zofenopril and ASA in the prevention of long-term cardiovascular outcomes. The superiority of Zofenopril versus Ramipril was particularly evident in patients with isolated systolic hypertension (n=131, 0.48 (0.23-0.99), $P=0.045$) [11].

Zofenopril proved to be very effective in patients with coronary artery disease and myocardial infarction, thanks to its unique effective mechanism of action for improving blood pressure control, left ventricular function and myocardial ischemia burden, as well as angiotensin-converting enzyme inhibition. The SMILE project involved more than 3,500 patients with coronary artery disease and demonstrated that Zofenopril treatment may reduce mortality and morbidity in patients with myocardial infarction, also when combined with acetyl salicylic acid and to a greater extent than Lisinopril and Ramipril. In addition, the results of the SMILE-ISCHEMIA study have demonstrated an interesting anti-ischemic effect of Zofenopril, and these properties largely contribute to the overall clinical benefit of the drug. The effects of Zofenopril on blood pressure control and cardiovascular protection clearly support its primary role for prevention and treatment of cardiovascular diseases [12].

According to our results, we can draw some ***conclusions***:

•The 12-week monotherapy with Zofenopril showed a high antihypertensive efficacy with a decrease in mean BP of 19% and achievement of the target BP in 83% patients with HT 1 and 2.

•According to ABPM, a full normalization of daily BP profile without the episodes of nightly hypotension was found in 50% of patients with HT 1 and 2.

•Antiremodeling efficacy of a 12-week monotherapy with Zofenopril was characterized by LVH regression with LVMI reduction by 9.6%, normalization in FMD of the brachial artery in 83.3% of patients, as well as a significant decrease in IMT of common carotid and brachial arteries.

•Monotherapy with Zofenopril showed metabolic neutrality regarding the lipid and carbohydrate metabolism, a good safety profile without the side effects and undesired events.

Competing interests

The authors declare that they have no competing interests.

References

1. Pasini AF, Garbin U, Nava MC. Stranieri C, Pellegrini M, Boccioletti V, et al. Effect of sulfhydryl and non-sulfhydryl angiotensin-converting enzyme inhibitors on endothelial function in essential hypertensive patients. Am J Hypertens. 2007; 20(4):443-50.
2. Evangelista S, Manzini S. Antioxidant and cardiopritective properties of the sulfhydryl angiotensin-converting enzyme inhibitor zofenopril. J Int Med Res. 2005; 33(1):42-54.
3. Ambrosioni E. Defining the role of zofenopril in the management of hypertension and ischemic heart disorders. Am J Cardiovasc Drugs. 2007; 7(1):17-24.
4. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34(28):2159-219.
5. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. Circulation. 1977;55(4):613-8.
6. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of

atherosclerosis. Lancet.1992; 340(8828):1111-1115.
7. Nilsson P. Antihypertensive efficacy of zofenopril compared with atenolol in patients with mild to moderate hypertension. Blood Press Suppl. 2007;2:25-30.
8. Mallion JM. An evaluation of the initial and long-term antihypertensive efficacy of zofenopril compared with enalapril in mild to moderate hypertension. Blood Press Suppl. 2007;2:13-8.
9. Napoli C, Bruzzese G, Ignarro LJ, Crimi E, de Nigris F, Williams-Ignarro S, et al. Long-term treatment with sulfhydryl angiotensin-converting enzyme inhibition reduces carotid intima-media thickening and improves the nitric oxide/oxidative stress pathways in newly diagnosed patients with mild to moderate primary hypertension. Am Heart J. 2008;156(6):1154.e1-8.
10. Borghi C, Bacchelli S, Esposti DD, Bignamini A, Magnani B, Ambrosioni E. Effects of the administration of an angiotensin-converting enzyme inhibitor during the acute phase of myocardial infarction in patients with arterial hypertension. SMILE Study Investigators. Survival of Myocardial Infarction Long-term Evaluation. Am J Hypertens. 1999;12(7):665-72.
11. Borghi C, Ambrosioni E, Omboni S, Cicero AF, Bacchelli S, Esposti DD,et al. Zofenopril and ramipril and acetylsalicylic acid in postmyocardial infarction patients with left ventricular systolic dysfunction: a retrospective analysis in hypertensive patients of the SMILE-4 Study. J Hypertens. 2013;31(6):1256-64.
12. 12. Borghi C1, Bacchelli S, Degli Esposti D. Long-term clinical experience with zofenopril. Expert Rev Cardiovasc Ther. 2012;10(8):973-82

International Journal of Biomedicine 5(4) (2015) 203-206

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Effectiveness of Personalized Therapy in Elderly Patients with Isolated Systolic Hypertension

E.A. Kartashova, PhD[1]; I.V. Sarvilina, PhD, ScD[2]

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

Abstract

The purpose of this study was the development of personalized modes of therapy in elderly patients with isolated systolic hypertension (ISH).

The study included 306 persons divided into two groups: Group 1 (Control) included 150 elderly persons without arterial hypertension (AH), and Group 2 included 256 elderly patients (early old-age pension, between 65 and 74 years) with ISH (ESH/ESC,2013) according to the inclusion/exclusion criteria. All patients of Group 2 were divided into three subgroups depending on the combination of drugs at the beginning of the study. Group 2a (n=53) received amlodipine (5 mg/day) and indapamide-retard (1.5 mg/day), Group 2b (n=53) received valsartan (80 mg/day) and indapamide-retard (1.5 mg/day), and Group 2c (n=50) received amlodipine (5 mg/day) and valsartan (80 mg/day). The duration of therapy was 5.2 years.

At the stage of data collection and screening, we applied standard methods for identification of ISH and secondary hypertension. Molecular phenotyping of blood serum was performed with methods of proteomics. We obtained the data of the molecular interactions and functional features of proteins from the STRING 10.0 database.

Proteomic analysis contributes to the development of a personalized mode treatment in ISH patients, which is the safest and most efficient: 135 ISH patients switched to the administration of the amlodipine+valsartan combination. **(Int J Biomed. 2015;5(4):203-206.)**

Keywords: *isolated systolic hypertension; personalized therapy; proteomics; bioinformatics; molecular interactions*.

Introduction

The purpose of this study was the development of personalized modes of therapy in elderly patients with isolated systolic hypertension (ISH).

Currently, arterial hypertension (AH) is often defined as accelerated aging. Aging has a pronounced effect on the cardiovascular system, largely involving vessels (vascular aging). Aging is associated with the development of the remodeling processes in the cardiovascular system [1-3]. Experimental and clinical trials have shown that myocardial contractility and stiffness of vascular walls are vulnerable to age-related changes [3]. A molecular map of aging in the cardiovascular system of elderly patients, accompanied by the emergence of AH, cannot be described only on the basis of standard methods of clinical research.

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

Modern methods and technologies of molecular analysis of large interactomes (blood, urine) and human tissues (myocardium, vascular wall)—including methods of genomics, transcriptomics, proteomics, and metabolomics—allow us to explore pathways of aging of target organs in elderly AH patients.

According to multicenter studies, modern hypotensive drugs indirectly eliminate remodeling processes in the myocardium and vascular walls, mainly by reducing systolic and diastolic blood pressure (BP). Today we need progress in the development and clinical application of the hypotensive drugs which impact key genomic-epigenomic interactions underlying the aging processes of the cardiovascular system, taking into account the results of population studies.

Materials and Methods

To address this need, we conducted a comparative prospective cohort study with parallel design. The study included 306 persons divided into two groups: Group 1

(Control) included 150 elderly persons without AH, and Group 2 included 256 elderly patients (early old-age pension, between 65 and 74 years) with ISH, according to the inclusion/exclusion criteria. Patients with ISH corresponded to the criteria for the classification of BP levels (SBP>140 mmHg and DBP<90 mmHg) and risk stratification – middle (n=87) and high (n=69) additional risk proposed by the ESH/ESC (2013) Guidelines for the management of arterial hypertension [4]. ISH duration was 13.5 years. All patients of Group 2 were divided into three subgroups depending on the combination of drugs [(amlodipine (calcium antagonist), indapamide-retard (diuretic), and valsartan (angiotensin receptor blocker)] at the beginning of the study.

Group 2a (n=53) received amlodipine (5mg/day) and indapamide-retard (1.5mg/day), Group 2b (n=53) received valsartan (80mg/day) and indapamide-retard (1.5mg/day), and Group 2c (n=50) received amlodipine (5mg/day) and valsartan (80mg/day). The duration of therapy was 5.2 years. The subgroups were matched for age, sex, SIH Grades, middle/high additional risk stratification, and disease duration. The antihypertensive efficacy of different treatment regimes was evaluated by the BP decrease (more than 15%) and achievement of target BP.

At the stage of data collection and screening, we applied standard methods for identification of ISH and secondary hypertension: the assessment of the patient`s complaints, medical history, physical examination, 24-hour ABPM, ECG (ATES MEDICA, Italy-Russia), echocardiography (Samsung-Medison, South Korea), blood and urine tests, biochemical analysis of blood and urine, blood level of aldosterone and corticosteroids, plasma renin activity, urinary catecholamines and metabolites (ELISA, Siemens 2000, Germany), coagulogram («Instrumentation Laboratory», USA), and an MRI of adrenal glands, kidney and brain (Philips Intera 1.5T, Japan).

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

Based on the data of standard methods of identification of ISAH and molecular phenotyping of blood serum, we conducted a personalized selection of hypotensive drug therapy for each patient. After 3 years of personalized hypotensive drug therapy, parameters of standard methods and molecular phenotyping of blood serum were measured.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the Rostov-on-Don State Medical University Ethics Committee. Written informed consent was obtained from each patient.

Statistical analysis was performed using the statistical software «Statistica 12.0». A probability value of $P<0.05$ was considered statistically significant.

Results

Among Group 2 patients, Grade 1(n=48), Grade 2(n=82), and Grade 3(n=26) of SIH were identified (Table 1). MRI signs of leukoaraiosis were detected in all patients with ISAH.

Table 1.

Clinical and anamnestic characteristics of ISH patients

Parameter	Group 2a (n=53)	Group 2b (n=53)	Group 2c (n=50)
Sex (male/female), n	34/19	33/20	27/23
Age, years	69.2±2.9	67.3±2.5	68.2±2.8
Weight, kg	66.7±1.3	69.4±1.6	68.5±1.5
Height, cm	170.3±1.9	167.5±1.3	169.5±1.5
BMI, kg/m^2	19.6±1.2	20.8±1.3	22.0±1.5
Duration of disease, years	12.3±1.5	15.7±1.9	14.2±1.8
Hypertensive crises, n	16	11	7
Risk factors:			
Heredity			
AH	34	34	35
CHD	34	34	35
Dyslipoproteinemia	34	34	35
Anamnesis			
CVD	25	27	25
Dyslipoproteinemia	34	34	35
Smoking	11	8	10
Poor nutrition	22	13	17
Obesity	-	-	-
Low physical activity	25	23	25
Target organs and associated clinical conditions:			
Brain and eyes (headache, dizziness, impairment of view, speech, TIA, sensory and motor disorders)	34	34	35
Heart (heartbeat, pain in the chest, shortness of breath, swelling)	34	34	35
Kidney (thirst, polyuria, nocturia, hematuria, swelling)	4	5	5
Peripheral arteries (cold extremities, intermittent claudication)	5	8	7
Physical examination:			
Vascular changes in the fundus	34	34	35
Heart (offset heart borders, arrhythmia, CHF)	34	34	35
Peripheral arteries (pulse weakening or disappearance, asymmetrical radial pulse, cold extremities, symptoms of skin ischemia)	8	13	9
Carotid arteries (systolic murmur)	9	12	10
ECG data:			
Sokolov – Lyon index (SV1+RV5-6)>3.5mV, n	34	34	35
Cornell voltage QRS duration product (>244 mV*ms), n	34	34	35

BMI–body mass index, CHD – coronary heart disease; TIA – transient ischemic attack; CHF – chronic heart failure.

At the stage of data collection and screening, we detected intergroup differences between indicators of 24-h daytime and nighttime SBP and DBP and heart rate in ISH patients taking 3 modes of hypotensive therapy. We revealed a significant reduction of these parameters in Group 2c compared to similar indicators in Groups 2a and 2b.

Daytime SBP: 141.2±3.4mmHg (2a), 138.3±3.6mmHg (2b), and 117.6±3.5mmHg (2c), *P2a/2c<0.001, P2b/2c<0.001*;

Daytime DBP: 78.4±1.6mmHg (2a), 73.5±1.3mmHg (2b), and 69.4±2.4mmHg (2c), *P2a/2c<0.001, P2b/2c<0.01*;

Nighttime SBP: 130.9±3.6mmHg (2a), 127.4±3.2mmHg (2b), and 117.6±2.9mmHg (2c), *P2a/2c<0.001, P2b/2c<0.001*;

Nighttime DBP: 81.8±1.4mmHg (2a), 75.4±1.2mmHg (2b), and 67.6±1.8mmHg (2c), *P2a/2c<0.001, P2b/2c<0.01*;

Heart rate: 84.5±1.5bpm (2a), 77.2±1.8bpm (2b), and 72.3±1.2bpm (2c), *P2a/2c<0.001, P2b/2c<0.01*.

The downward trend of LVMI [139.5±4.7g/m^2 (2a), 137.3±4.3g/m^2 (2b), and 129,4±3,9g/m^2 (2c), P2a/2c<0.001, P2b/2c<0.05] was observed in patients taking the combination of amlodipine and valsartan.

Proteomic analysis helped in the detection of differences in the component composition of the serum proteins in ISH patients with varying grades who were taking different modes of hypotensive therapy, compared to Group 1 (Table 2).

Proteomic analysis contributes to the development of a personalized mode treatment in ISH patients, which is the safest and most efficient: 135 ISH patients switched to the administration of the amlodipine+valsartan combination. After 3 years of personalized hypotensive drug therapy, we identified a significant decrease in parameters of daytime/nighttime SBP and DBP, daily indexes, heart rate and of the myocardial performance index, as well as signs of the progression of ischemic, distrophic, metabolic, and morphogenetic disorders in the cardiovascular system in accordance with changes of peptide indicators in patients of Group 2c.

Bioinformatics analysis revealed the presence of molecules that are the participants in the universal pathways of cardivascular aging and the molecular interactions involved.

Discussion

Proteomic analysis revealed an increase in the absolute number of ISH patients with an abnormal profile of serum proteins performing certain biological functions and having various localizations in the intra- and extracellular spaces (Table 2). Molecules interact among themselves and with other molecules as participants in universal pathways in cardivascular aging in ISH patients: RAAS (renin-

Table 2

Qualitative profile of serum proteins in elderly ISH patients and control group

№	Protein name	Number of patients with the expression of the serum protein (n)							MW* (Da)	Functional process (sources: InterPro, Entrez, SWISS-PROT, NRDB, , PDB, KEGG)
		Control (n=150)	Gr. 2a (n=53)	Gr. 2a[1] (n=11)	Gr. 2b (n=53)	Gr. 2b[2] (n=10)	Gr. 2c (n=50)	Gr. 2c[3] (n=135)		
1	Disheveled-associated activator of morphogenesis 1	23	53	8	53	7	50	28	123396	Epidermal cell proliferation, and glucose and lipid metabolism
2	Apolipoprotein D	138	12	3	15	2	35	114	21262	Fatty acid and steroid metabolism
3	Brain spectrin	144	22	6	19	7	34	108	41404	Cytoskeletal protein, neurotransmission, neuromuscular junction
4	Myocardial ischemic preconditioning upregulated protein 1	137	34	4	37	4	44	103	56904	Nuclear factor, cell apoptosis, the gene expression of downstream inflammatory mediators
5	Neprilysin	10	22	10	23	8	27	14	85460	Amyloid beta regulation, the regulation of signaling peptides
6	Gamma butyrobetaine hydroxylase	145	22	3	33	6	45	130	44687	L-carnitine biosynthesis pathway, mitochondrial beta oxidation
7	Endothelial growth factor A	142	32	4	35	7	44	132	27042	Vasculogenesis, neovascular age-related macular degeneration
8	Angiotensin-converting enzyme	4	14	3	10	3	10	2	149715	The convertion of vasoactive peptides
9	Angiotensinogen	3	12	3	9	3	10	1	53154	Blood pressure, body fluid and electrolyte homeostasis
10	Hypoxia inducible factor 1	129	23	7	34	8	42	132	92670	Transcription factor, regulator the hypoxia in cells
11	Peroxisome proliferator-activated receptors D	125	28	6	34	9	40	127	49903	Nuclear hormone receptor, integrator of transcription repression and nuclear receptor signaling
12	Voltage-dependent calcium channels 1D and 1C	26	29	5	32	7	22	47	245141	The regulator of hormone and neurotransmitter release, muscle contraction, cellular functions
13	Nitric oxide synthase, endothelial	147	39	5	41	9	45	132	133289	Vascular tone, cellular proliferation, leukocyte adhesion, platelet aggregation
14	Endothelin I	4	42	7	38	8	31	35	24425	Vasoconstrictor, vascular homeostasis

MW* – molecular weight (Da); Groop 2a[1], Group 2b[2], Group 2c[3] - after 3 years of personalized hypotensive drug therapy

angiotensin-aldosterone system), PPARs, WNT (Wg/Int), NOTCH signaling pathways, mitochondria and ROS signaling pathways (electron-transport chain signaling, stress-induced protein kinases: JNK and MST-1), and genome surveillance pathways (tumor suppressors and antagonistic pleiotropy).

Each protein molecule in the functional group interacts with other protein molecules. For example, the molecular interactions of hypoxia inducible factor 1, alpha subunit (HIF-1alpha) are presented in Fig.1. Serum HIF-1alpha concentration rose in ISH patients, and was most pronounced in Group 2c.

Conclusion

We identified potentially new biomarkers of cardiovascular aging that could help in developing a noninvasive, serum-based diagnostic test. This study is the first step in the development of a new system for creation of personalized hypotensive therapy. The dynamics in the proteome-map of blood serum in ISH patients revealed the molecular mechanism of neuro-, cadio- and vascular protective effects of amlodipine and valsartan, as a geroprotective mode of drug action.

Competing interests

The authors declare that they have no competing interests.

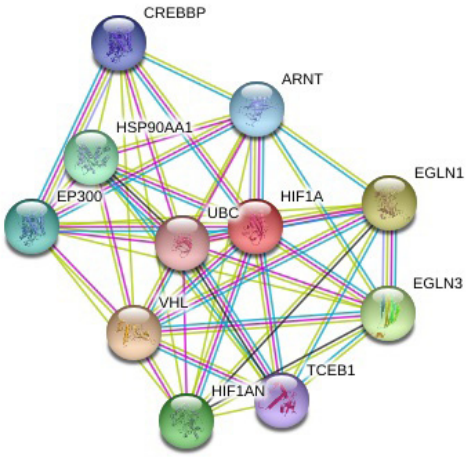

Fig. 1. Molecular interactions of HIF-1alpha (STRING 10.0 database)

VHL-von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase; EGLN1-egl nine homolog 1; EGLN3 - egl nine homolog 3 (C. elegans); HIF1AN-hypoxia inducible factor 1, alpha subunit inhibitor; HSP90AA1-heat shock protein 90kDa alpha (cytosolic), class A member 1; EP300-E1A binding protein p300; ARNT-aryl hydrocarbon receptor nuclear translocator; CREBBP-CREB binding protein; TCEB1-transcription elongation factor B (SIII), polypeptide 1; UBC- ubiquitin C.

References

1. Nielsen WB, Vestbo J, Jensen GB. Isolated systolic hypertension as a major risk factor for stroke and myocardial infarction and an unexploited source of cardiovascular prevention: a prospective population-based study. J Hum Hypertens. 1995; 9(3):175-80.
2. Boutouyrie P, Tropeano A, Asmar R, Gautier I, Benetos A, Lacolley P, at al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. 2002. 39(1):10-5.
3. McEniery CM1, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). J Am Coll Cardiol. 2005; 46(9):1753-1760.
4. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34(28):2159-219.

International Journal of Biomedicine 5(4) (2015) 207-213

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Clinical Profile and Prognosis of Patients with Right Ventricular Dilated Cardiomyopathy: Results of a Prospective Study

Ya.R. Akhmatov[1]*; T.A. Abdullayev[1], PhD, ScD; B.U. Mardanov[2].

[1]*The Republican Specialized Center of Cardiology, Tashkent, Uzbekistan*
[2]*National Research Centre for Preventive Medicine, Moscow, Russia*

Abstract

The aim of our study was to investigate the clinical prevalence of dilated cardiomyopathy (DCM) with predominantly failure of the right-side heart (right ventricular DCM, RV-DCM), and features of the clinical course and prognosis of the disease compared to DCM with biventricular heart failure (BV-HF).

The study design suggests a prospective observation of 300 patients with idiopathic DCM between 2000 and 2012. Herewith, we followed the criteria of the WHO/ISFC task force (1995) on the definition and classification of cardiomyopathies. All patients underwent a comprehensive examination. Two groups were formed for further comparative analysis. Group 1 included 22 patients (mean age 42.9±14.3 years, male/female 5/17) with RV-DCM. Group 2 included 38 patients (mean age 43.6±13.8, male/female 29/9) with DCM and BV-HF. The groups were matched for age, NYHA class II-III, and disease duration. According to our aim, we studied 5-year survival prognosis and analyzed the incidence and causes of deaths, as well as the occurrence of nonfatal complications of the disease. Medical therapy for DCM patients was performed according to the CHF therapy guidelines (ACC/AHA 2001, 2005).The results of our investigations during many years of research have shown that the clinical incidence of RV-DCM was 7.3% among all forms of DCM. The study of life prognosis in patients with 2 forms of DCM showed that 5-year mortality of patients was about 50%. Fatal pulmonary embolism was a leading cause (50%) in RV-DCM patients. **(Int J Biomed. 2015;5(4):207-213.)**

Keywords: *right ventricular dilated cardiomyopathy, biventricular heart failure, prognosis; pulmonary embolism.*

Abbreviations

RV-DCM, right ventricular dilated cardiomyopathy; **CHF**, chronic heart failure; **BV-HF**, biventricular heart failure; **LVM**, left ventricular mass; **LVEDD**, LV end-diastolic dimension; **LVESD**, LV end-systolic dimension; **LVEDV**, LV end-diastolic volume; **LVESV**, LV end-systolic volume; **LVSV**, LV stroke volume; **LVFS,** LV fractional shortening; **LA** left atrium; **RA**, right atrium**; IVST**, interventricular septal thickness; **LVPWT**, LV posterior wall thickness; **EF**, ejection fraction; **ARVD**, arrhythmogenic RV dysplasia; **AF**, atrial fibrillation; **PEmb**, pulmonary embolism; **PE**, peak early filling velocity*;* **PA**, peak atria1 filling velocity*;* **LBBB**, left bundle branch block; **RBBB**, right bundle branch block.

Introduction

The idiopathic nature of right ventricle failure was first described by A. Bahler in 1976 [1]. Describing two cases of RV dilatation and paradoxical movement of the interventricular septum in the absence of any disease or heart disease, the authors suggested that there are certain initial symptoms of cardiomyopathy. Afterwards, the report of WHO/ISFC task force defined cardiomyopathies as diseases of the myocardium associated with cardiac dysfunction and classified the forms of diseases, including DCM with its varieties [2]. The international recommendations to date have provided clear criteria for the diagnosis of alcoholic, peripartum cardiomyopathy and family forms of cardiomyopathy [3-5]. There are a lot of controversial issues related to both the diagnosis and treatment of DCM with predominantly failure of the right ventricle (not to be confused with ARVD). Isolated idiopathic RV-DCM is an equally rare condition like the

***Corresponding author**: Yashin R. Akhmatov. The Republican Specialized Center of Cardiology, Tashkent, Uzbekistan. E-mail: dr.yashin@mail.ru*

familial RV-DCM. Besides the few reports which had been mentioned casually, there are only a few documented cases in the literature. [6-9].The severity of the problem is heightened by a significant limitation of publications related to the clinical course and prognosis precisely of RV-DCM.

Based on the above, the aim of our study was to investigate the clinical prevalence of DCM with predominantly failure of the right-side heart (RV-DCM), and features of the clinical course and prognosis of the disease compared to DCM with BV-HF.

Materials and Methods

The study design suggests a prospective observation of 300 patients with idiopathic DCM between 2000 and 2012 in the Department of Heart Failure and Noncoronary Myocardial Pathology at RSCC. Herewith, we followed the criteria of the WHO/ISFC Task Force (1995) on the Definationa and Classification of Cardiomyopathies [2]. RV-DCM was diagnosed according to the above criteria and the additional exclusion of other conditions characterized by dilatation and right-sided heart failure (primarily ARVD, idiopathic and secondary pulmonary hypertension), as well as clinical, electrocardiographic, and echocardiographic features that were systematized later and proposed as criteria for diagnosis [3]. In addition, to assess the clinical status, the patients underwent 6-minute walk test (6MWT), a standard electrocardiogram («Cardio Lab», Ukraine), transthoracal echocardiography (Siemens Sonoline Verso Pro) at rest by common methods to determine the volumetric and linear heart parameters with calculation of LV myocardial contractility by Teicholz method. We used R. Levine's, T. Gibson's at al. formula: RVEF%=(RVEDV-RVESV)/RWEDV)·100% for the calculation of RVEF [10,11]. Biochemical and immune – enzyme analyses were also carried out.

In the course of examination of patients, two groups were formed for further comparative analysis. Group 1 included 22 patients (mean age 42.9±14.3 years, male/female 5/17) with RV-DCM. Group 2 included 38 patients (mean age 43.6±13.8, male/female 29/9) with DCM and BV-HF. The groups were matched for age, NYHA class II-III, and disease duration. According to our aim, we studied 5-year survival prognosis and analyzed the incidence and causes of deaths, as well as the occurrence of nonfatal complications of the disease.

Medical therapy for DCM patients was performed according to the ACC/AHA 2001, 2005 Guidelines.

Statistical analysis was performed using using the statistical software "Biostatics" (v.4.03"). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Group comparisons with respect to categorical variables are performed using chi-square tests or, alternatively, Yates' $\chi 2$ when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the Republican Specialized Center of Cardiology Ethics Committee. Written informed consent was obtained from each patient.

Results

During the study period, we had the opportunity to assess the clinical and hemodynamic parameters of the patients; readmissions frequency and outcomes were recorded, including an active remote observation. The initial patients' characteristics— clinical and functional, anamnestic, gender and hemodynamic—are shown in Table 1. The vast majority of our patients (77%) identified with RV-DCM were female, while almost all epidemiological studies suggest that DCM as a whole is more common in men. The patients' mean age (more than 40 years), as well as the age when the disease manifested, was comparable in both groups.

Table 1.

The initial patients' characteristics

Variable	Group 1 (n=22)	Group 2 (n=38)	*P*
Average age, years	42.9±14.3	43.6±13.8	*NS*
Male/female	6/16	29/9	*<0.05*
Average age for disease onset, years	38.2±14	38.7±14	*NS*
Diagnosis of DCM during the primary examination	2 (9%)	8 (21%)	*NS*

Among RV-DCM patients during the primary examination, DCM was diagnosed only in 9% of cases vs. 21% of cases among DCM patients with BV-HF ($P<0.05$). The higher percentage among patients with BV-HF was possibly caused by obvious signs of LV failure and the detectable LV dilatation. The majority of Group 1 patients were treated and observed with other diagnoses, such as chronic hepatitis, COPD, coronary heart disease, liver cirrhosis,etc.

Clinical evaluation of the patients revealed some features that depended on the character of myocardial failure. We analyzed the symptom scores to assess the differences (Table 2) between Groups.

Table 2.

Clinical characteristics of the studied patients

Variable	Group 1 (n=22)	Group 2 (n=38)	*P*
Dyspnea	1.35±0.49	1.42±0.51	*0.614*
Patient's position in the bed	0.89±0.16	1.58±0.51	*0.000*
Jugular venous distention	1.05±0.72	0.89±0.66	*0.394*
Congestive moist rales	0.75±0.58	1.26±0.73	*0.009*
Gallop rhythm	0.40±0.50	0.58±0.51	*0.200*
Liver	1.35±0.58	1.37±0.68	*0.911*
Edema	1.40±0.82	1.11±0.66	*0.145*

Thus, RV-DCM patients had fewer pronounced signs of LV failure: lower position of the head end of the bed (0.89±0.16 versus 1.58±0.51, in Group 2, $P=0.000$) and less congestive

moist rales in the lungs (0.75±0.58 versus 1.26±0.73 in Group 2, *P=0.019*). Such indicators as jugular venous distention and edema in Group 1 patients were prevalent but not statistically significant.

ECG analysis revealed most significant differences in the incidence of "dextrogram" and the ratio of RV6/RVmax amplitude. Particularly, right axis deviation was registered in 16(80%) cases among RV-DCM patients and only in 4(10%) cases in DCM patients with BV-HF (*P=0.000*). RV6/RVmax ratio reached 0.87±0.38 and 2.98±1.29 in Group 1 and Group 2, respectively (*P=0.000*). In addition, 8(40%) patients with RV-DCM had T- wave alternans, which was not recorded in Group 2 (*P=0.000*), while a T-wave inversion occurred in 6(30%) and 13(32%) of cases in Group 1 and Group 2, respectively (Table 3). The patient proportion with persistent AF in Group 1 was slightly above that in Group 2; complete LBBB occurred only in DCM patients with BV-HF (*P=0.129*), and incomplete RBBB was significantly more frequent in RV-DCM patients (5 vs. 1, *P=0.04*). The same frequency of first-degree AV block occurred in both groups, but one RV-DCM patient developed a complete AV block that required pacemaker implantation (Table 3).

Table 3.
ECG parameters of DCM patients

Variable	Group 1	Group 2	*P*	χ2
Right axis deviation, high R wave in leads V1-V3 and deep S wave in leads V5-V6, n	16	4	*0.000*	24.258
T wave inversion in leads V1-V3, without RBBB, n	8	-	*0.000*	Yates' χ2 12.952
T wave inversion in leads V5- V6 without LBBB, n	6	13	*0.578*	0.31
Increases of QRS complex duration >110ms in V1-V)	4	5	*0.8791*	Yates' χ2 0.023
Permanent form of AF, n	6	8	*0.583*	0.301
Complete LBBB, n	-	6	*0.129*	Yates' χ2 2.305
Incomplete RBBB, n	5	1	*0.04*	Yates' χ2 4.219
Complete RBBB, n	3	2	*0.518*	Yates' χ2 0.418
Left posterior hemiblock, n	3	-	*0.085*	Yates' χ2 2.961
Left anterior hemiblock, n	1	4	*0.747*	Yates' χ2 0.104
First-degree AV block, n	3	2	*0.518*	Yates' χ2 0.418
Complete AV block, n	1	-	*0.780*	Yates' χ2 0.078

A number of significant differences in the linear and volume indicators were revealed by comparative analysis of Echo-CG parameters (Table 4.) Despite the right-sided heart dilation (RV=50.54±8.43mm, RA=47.98±9.92mm), the left-sided heart size remained in normal range (LVEDD=39.9±9.8 mm; LVESD=29.12±10.15mm; LA=35.75±12.69mm) that is typical for RV-DCM patients. LV myocardial contractility also remained within normal range (LVEF=67.09±10.0%). Right and left heart dilatations with a decrease in inotropic myocardial function were noted in DCM patients with BV-HF.

Table 4.
Echo-CG parameters of DCM patients

Parameter	Group 1	Group 2	*P*
Aortic Root, mm	30.44±3.69	29.11±4.71	*0.274*
LA dimension, mm	35.75±12.69	47.48±5.49	*0.000*
IVST, mm	10.75±1.94	9.62±1.64	*0.021*
LVPWT, mm	10.45±1.74	9.75±1.95	*0.180*
LVESD, mm	29.12±10.15	63,73±7,80	*0,000*
LVEDD, mm	39.9±9.8	74.68±7.34	*0.000*
LVEF, %	67.09±10.01	30.59±7.13	*0.000*
LVFS, %	19.63±5.59	14,80±3,37	*0,000*
RVEF, %	37.86±8.17	47.2±9.31	*0.000*
RV, mm	50.54±8.43	42.78±5.58	*0.000*
RA, mm	47.98±9.92	42.98±5.57	*0.015*
LVEDV, ml	82.30±36.79	296.29±68.61	*0.000*
LVESV, ml	27.90±16.59	200.88±70.65	*0.000*
LVSV, ml	54.40±24.81	95.42±27.64	*0.000*
LVM, g	179.45±67.13	349.46±85.04	*0.000*
Mean PAP, mmHg	37.12±7.77	44.57±10.75	*0.008*
Comparative characteristics of LV/RV diastolic function			
LV PE, cm/sec	0.73±0.23	0.66±0.16	*0.228*
LV PA, cm/sec	0.44±0.21	0.39±0.18	*0,367*
LV PE/PA	1.57±0.13	1.61±0.11	*0.243*
RV PE, cm/sec	0.79±0.17	0.67±0.14	*0.005*
RV PA, cm/sec	0.29±0.31	0.39±0.12	*0.077*
RV PE/PA	2.64±0.23	1.51±0.11	*0.000*

Almost all DCM patients had pulmonary arterial hypertension syndrome. Mean pulmonary pressure, defined as an indexed rate, was significantly higher in patients with classical DCM. All Group 1 patients had pseudonormal LVDD (PE/PA=1.57±0.11), but RVDD was worse in Group 2 patients compared to Group 1. The dysfunction found in Group 2 patients was the restrictive type, against the pseudonormal type in Group 1(2.64±0.23 and 1.57±0.11; *P=0.000*).

Results of biochemical and immune–enzyme analyses in DCM are shown in Table 5.

Table 5.
Biochemical parameters of DCM patients

Variable	Group 1	Group 2	*P*
Total bilirubin, mkmol/l -conjugated -unconjugated	34.83±23.72 14.82±16.21 23.26±14.58	24.44±9.01 6.76±3.51 21.23±5.59	*0.012* *0.02* *NS*
ALT, IU/l	39.55±42.59	44.92±46.78	*NS*
AST, IU/l	41.09±47.91	37.33±27.84	*NS*
Plasma albumin, g/dl	2.9±0.6	3.16±0.48	*NS*
Total cholesterol, mg/dl	113.8±11.7	107.3±8.8	*0.026*

A change in level of at least one of any of the functional markers of liver damage was identified in 81.8% of cases in RV-DCM patients, while this index was only 42.1% in Group 2 (*P=0.01)*. Hyperbilirubinemia was the most typical marker of the liver damage among examined patients, which was recorded in 91% of patients in Group 1 versus 58% of patients in Group 2. The laboratory analysis showed that the total bilirubin level was increased, mainly due to high values of unconjugated bilirubin fraction in both groups. The serum level of conjugated bilirubin was 2 times greater than the normal value only in Group 1 patients. TNF-α and CRP plasma concentrations were determined additionally in 9(41%) and 14(37%) patients of Group 1 and 2, respectively, and also in 10 healthy persons. The serum level of TNF-α was 59.7±1.8 pg/ml and 58.6±1.68 pg/ml in Group 1 and Group 2, respectively, compared to 29.2±1.4 pg/ml in healthy persons (*P<0.005* in both cases).

The increased serum CRP level was without significant differences between groups (9.8±1.1 mg/l and 8.9±1.2 mg/l; *P=0.084*) and more than three times higher than normal values (2.3±0.6 mg/l; *P<0.05* compared to Groups 1 and 2).

In the study of patient prognosis, it was noted that 5-year mortality was almost the same in both groups: 12(54.5%) and 18(47.4%) in Group 1 and Group 2 (χ2=0.287; *P=0.592*). Analysis of the causes of death revealed that fatal pulmonary embolism (PEmb) was a leading cause (50%) in RV-DCM patients (Fig.1).

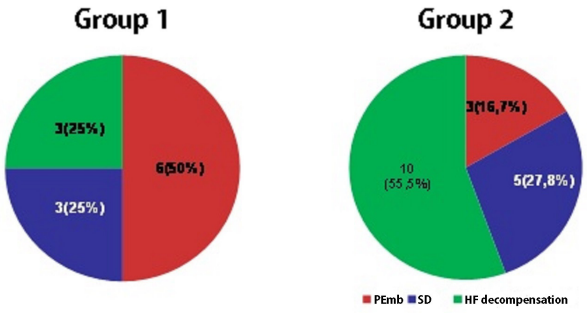

Fig. 1. *Causes of death in DCM patients*

This cause of death was established on the basis of acute symptoms and autopsy results of in-patients, as well as an interview with the relatives when they indicated the suddenly developed dyspnea, cyanosis, hemoptysis which preceded death. The unfavorable outcome of sudden death (SD) in 3(25%) patients of Group 1 occurred as a result of progressive decompensation of CHF. PEmb was the cause of death only in 3(16.7%) cases in Group 2 patients (Yates'χ2=2.388; *P=0.122* compared to Group 1). The majority of DCM patients with BV-HF (55.5%) died due to HF progression (Yates'χ2=1.635; *P=0.201* compared to Group 1). We also analyzed the frequency of patients' readmissions with CHF destabilization and nonfatal thromboembolic complications, such as acute ischemic stroke and PEmb. Thus, the average number of hospitalizations during the observation period was 8.5±3.2 in Group 2 versus 9.3±2.5 in Group 1 (*P>0.05*). The cases of nonfatal stroke and PEmb were registered with different frequency, as expected, on the assumption of morphological and hemodynamic features of heart disease in comparison groups of patients. Acute destabilization of CHF with episodes of hemoptysis was registered in 15(68.2%) of cases in RV-CMP. Thromboembolism was verified by X-ray examination in some patients. The incidence of this outcome was 26,3% (χ^2=10.048; *P=0.0015*) in Group 2. Incidents of ischemic stroke or transient ischemic attack episodes were registered only in patients of Group 2 (7/18.4%; Yates'χ^2 = 2.97; *P=0.08*).

Results and Discussion

It is well known that cardiomyopathy, in particular DCM, is characterized by extremely poor prognosis and a high mortality rate. However, detection of possible differences in the course and outcome of various DCM clinical variants is very important. We focused on gender features of DCM and its clinical variant, RV-DCM, as an initially diagnosed condition. It is a common view that DCM occurs more frequently in men than in women [2,13], which was confirmed by us. However, women significantly prevailed over men (2.7 times) among RV-DCM patients. It is not possible to confirm or deny this gender feature of RV-DCM because of the very limited publications on the subject. Almost all of them are characterized by descriptions of sporadic cases or small clinical studies. For example, a case of idiopathic dilatation and RV dysfunction in a young 16-year-old girl was described by K.Ishikawa et al.[13]. The monitoring results of 14 RV-DCM patients were published by D.Fitchett et al. [14]. At the same time, Indian researchers (Mohan JC et al., 1989) described RV-DCM in 10 patients, and noted the predominance of women [15]. Obviously, this issue can be clarified by a large epidemiological study. History has shown that RV-DCM patients enter cardiology departments very late due to misdiagnosis or a delay in diagnosis because of the lack the LV dysfunction, which is stereotypically associated with DCM. E. Amosova also indicated that most cases of RV-DCM are diagnosed accidentally during patient examination for intercurrent diseases [16]. According to our data, we can assume that the isolated RV failure is clinically more severe than biventricular heart disease. J.Goldstein [17] noted that RV-MI may result in severe hemodynamic compromise associated with a poor clinical outcome. The ischemic, dyskinetic right ventricular free wall exerts mechanically disadvantageous effects on biventricular performance. Depressed RV systolic function leads to a decrease in transpulmonary delivery of left ventricular (LV) preload, resulting in diminished cardiac output. [17]. The first report of concurrent right and left ventricular hypertrophy in response to pressure or volume overload of the right ventricle was made in 1982. In two pressure and volume overload models of canine right ventricular hypertrophy, D.F. Larson et al. have demonstrated significant hypertrophy of both the left and the right ventricles. The extent of hypertrophy was correlated positively to the extent of the increase in plasma epinephrine in both volume and pressure overload models [18].

Several studies of HF of ischemic etiology have shown

that RV function is related to exercise tolerance and determines the severity of the patient's condition more than LV function, and that increasing RVEF is the measure of the effectiveness of revascularization in CHD patients with congestive HF [19-21]. According to G. Karatasakis et al. [22], in patients with advanced heart failure, preserved RV function as indicated by an echocardiographically derived RV shortening >1.25cm was a strong predictor of survival.

In addition, we would like to mention the prevalence of some cases of AF in RV-DCM patients although the differences were not significant. The revealed features seemingly contradict the common pathogenesis mechanisms according to which the AF occurrence is mainly due to the appearance of abnormal impulses in LA remodeling from the mouths of the pulmonary veins and throughout the tissue. Nevertheless, the superior vena cava is one of the sources of ectopies that can initiate AF, which was determined in a study performed by M.Goya et al. [23]. Gaynor SL et al. [24] showed that in dogs with chronic RV pressure overload, RV systolic function was preserved, but diastolic function was impaired. To compensate, RA contractility increased, and the atrium became more distensible to maintain filling of the stiffened ventricle. This compensatory response of the right atrium likely plays an important role in preventing clinical failure in chronic pulmonary hypertension. In DCM, it has been histologically shown that a main cause of AF is fibrosis and reduction of myofibrils precisely, and to a lesser extent, remodeling and genetic contribution [25]. These data apparently explain the prevalence of AF in Group 1 patients.

Despite the right-sided heart dilatation in RV-DCM patients (RV=50.54±8.43mm and RA=47.98±9.92mm), the size of the left-sided heart remained in the normal range (LVEDD=39.9±9.8mm; LVESD=29.1±10,1mm; LA=35.75±12.69mm). The results indicate intact LV myocardial contractility (LVEF=51.7±10.1%). The expected both right and left heart dilatation with marked reduction of inotropic miocardial function was registered in DCM patients with BV-HF. Polak J. F. et al [21] showed that in patients with depressed left ventricular ejection fraction (less than 40%) and clinically evident congestive heart failure secondary to atherosclerotic coronary artery disease the reduction in right ventricular ejection fraction is a useful index not only for assessing biventricular function, but also for predicting patient outcome. These data were confirmed in other studies [5,10].

Studies of the lesser circulation hemodynamics in 150 patients with dilatation cardiomyopathy and in 15 after heart transplantation [26] revealed secondary postcapillary pulmonary hypertension of more than 60mmHg in cases with dilatation cardiomyopathy with systolic pressure in the pulmonary artery (SPPA) in 30% of patients, transpulmonary gradient (TPG) of more than 15 mm Hg and pulmonary vascular resistance (PVR) or more than 4 Wood's U in 15.3% of patients.

One of the anatomical features of the liver is its sophisticated dual blood supply, which makes it quite resistant to hemodynamic overload. It is established that even a slight increase of RA pressure in patients with heart failure may lead to hyperbilirubinemia [15]. Hepatic dysfunction is developed at the overpressure on 10mmHg and more in RA and a cardiac index reducing to lower than 1.5l/min/m². In cases of "Cytolytic" syndrome with elevated aminotransferase levels, jaundice (defined as bilirubin higher than 3 mg/dL) is a consequence of persistent and severe hypotension and hypoperfusion, such as acute LV failure, PE, and cardiogenic shock.

The results of our study also demonstrated that an increase in the level of liver enzymes was not so pronounced and widespread. An increase of at least one aminotransferase concentration higher than the norm occurred in 36.3% and 30% in Group 1 and Group 2, respectively. Earlier studies have also shown that an increase in the level of liver aminotransferase does not usually exceed normal values by 2 to 3 times in patients with cardiovascular diseases [16].

Hyperbilirubinemia is caused by the violation of the synthesis, metabolism, transport and excretion of bilirubin. This condition is typical for a sufficiently wide variety of pathological conditions, including hepatitis, toxic lesions, biliary obstruction and hepatocellular dysfunction, and hereditary cholestatic syndromes. A secondary liver damage indicator is hyperbilirubinemia, which is characterized mainly by increasing unconjugated bilirubin. However, our results confirm once again the fact that serious damage to the right-sided heart and severe CHF leads to an increase in the level of conjugated bilirubin; at the same time, the jaundice may not manifest clinically before plasma bilirubin levels increase more than 3 mg/dl. Currently, the clinical forms of liver disease in CHF are determined to be congestive hepatopathy, ischemic hepatitis, and cardiac hepatic fibrosis [19]. Changes of liver function test indicators in our study had moderate severity without development of varied clinical symptoms with rare exceptions(refractory ascites, encephalopathy, hemorrhagic complications), which indicated congestive hepatopathy.

The estimation of liver synthetic function is extremely important for the evaluation of the lesion depth. We identified a moderate hypoalbuminemia in both groups of patients (Table 5). It should be noted that the serum albumin does not change in acute viral hepatitis. Drug-induced liver injury, biliary obstruction, and hypoalbuminemia are more common in congestive genesis of liver lesions, enteropathy, nephropathy, and as a complication of heart failure.

In our work, we prospectively analyzed the prognosis of RV-DCM patients on medical therapy according to the CHF therapy guidelines [27,28]. The 5-year mortality of our patients was about 50%, which is consistent with the 5-year survival of 50% to 70% reported in more recent studies on the prognosis of non-ischemic DCM [29-31]. The explicit prevalence of fatal pulmonary embolisms in RV-DCM patients is an important feature of RV-DCM outcomes. Ribeiro and colleagues were the first to suggest that patient with RV failure may have increased mortality. "RV dysfunction when diagnosis of PE is established is associated with mortality rate" [32].

Conclusion

The results of our investigations during many years of research have shown that the clinical incidence of RV-

DCM was 7.3% among all forms of DCM. The study of life prognosis in patients with 2 forms of DCM showed that 5-year mortality of patients was about 50%. Herewith, we detected the differences in causes of death depending on the type of heart damage, primarily development of fatal PE. Obviously, the results we anticipated have limitations, including lack of an analysis of the treatment regimens. Nevertheless, we believe that this pilot study, as well as a number of other small studies, contributes to the clarification of one of the least studied non-coronarogenic myocardial diseases—isolated RV-DCM.

Competing interests

The authors declare that they have no competing interests.

References

1. Bahler AS, Meller J, Brik H, Herman MV, Teichholz LE. Paradoxical motion of the interventricular septum with right ventricular dilatation in the absence of shunting: report of two cases. Am J Cardiol. 1976;38(5):654-7.
2. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, et al. Report of the 1995 World Organization International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. Circulation. 1996; 93(5):841-2.
3. Coignard E, Blanchard B, Jault F, Dorent R, Vaissier E, Nataf P, et al. Alcoholic cardiomyopathy and cardiac transplantation /Coignard E., Blanchard B., Jault F. et al. Arch Mal Coeur.1998;91(1):45-51. [Article in French].
4. Homans DC. Peripartum cardiomyopathy.N Engl J Med.1985;312(22):1432-7.
5. Mestroni L, Maisch B, McKenna WJ, Schwartz K, Charron P, Rocco C,,et al. Guidelines for the study of familial dilated cardiomyopathies. Eur Heart J. 1999;20(2):93-102.
6. Matsui S, Murakami E, Takekoshi N, Tsuji S. Idiopathic dilated right ventricular cardiomyopathy: a report of eight cases. J Cardiogr. 1985;15(4):1027–38.
7. Samanta S, Vijayverghia R, Vaiphei K. Isolated idiopathic right ventricular dilated cardiomyopathy. Indian J Pathol Microbiol. 2011;54(1):164–6.
8. Ibsen HH, Baandrup U, Simonsen EE. Familial right ventricular dilated cardiomyopathy. Br Heart J. 1985;54(2):156–9.
9. Rowland E, McKenna WJ, Sugrue D, Barclay R, Foale RA, Krikler DM. Ventricular tachycardia of left bundle branch block configuration in patients with isolated right ventricular dilatation. Clinical and electrophysiological features. Br Heart J. 1984;51:15-24.
10. Levine RA, Gibson TC, Aretz T, Gillam LD, Guyer DE, King ME,, et al. Echocardiographic measurement of right ventricular volume. Circulation 1984; 69(3):497-505.
11. Gibson TC, Miller SW, Aretz T, Hardin NJ, Weyman AE. Method for estimation right ventricular volume by planes applicable to cross-sectional echocardiography: correlation with angiographic formulas. Am J Cardiol. 1985;55(13Pt 1):1584-8.
12. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopaties: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2008; 29(2):270-6.
13. Ishikawa K, Yokoyama K, Araki T, Miura T, Goto T, Saito H. A case of right ventricular cardiomyopathy, which involved left ventricle. Kokui To Junkan 1990;38(12):1269-72.
14. Fitchett DH, Sugrue DD, McArthur CG, Oakley CM. Right ventricular dilated cardiomyopathy. Br Heart J. 1984;51(1):25-9.
15. Mohan JC, Chutani SK, Sethi KK, Arora R, Khalilullah M. Dominant right ventricular dilated cardiomyopathy: clinical, echocardiographic and haemodynamic profile. Indian Heart J 1989;41(3):177-81.
16. Amosova EN. Cardiomyopathy: A Guide. Kiev: Kniga Plus, 1999. [in Russian].
17. Goldstein JA. Right heart ischemia: pathophysiology, natural history, and clinical management. Prog Cardiovasc Dis. 1998; 40(4):325-41.
18. Larson DF, Womble JR, Copeland JG, Russell DH. Concurrent left and right ventricular hypertrophy in dog models of right ventricular overload. J Thorac Cardiovasc Surg. 1982 Oct;84(4):543-7.
19. Baker BJ, Franciosa JA. Effect of the left ventricle on the right ventricle. Cardiovasc Clin. 1987;17(2):145-55.
20. Ostroumova EN, Kormer AY, Gureev SV Ermolenko AE. Ejection fraction of the right ventricle as an indicator of effectiveness of myocardial revascularization in patients with coronary heart disease and congestive heart failure. Kardiologiia. 1996; 4:57-61.
21. Polak JF, Holman BL, Wynne J, Colucci WS. Right ventricular ejection fraction: an indicator of increased mortality in patients with congestive heart failure associated with coronary artery disease. J Am Coll Cardiol. 1983; 2(2): 217-24.
22. Karatasakis GT, Karagounis LA, Kalyvas PA, Manginas A, Athanassopoulos GD, Aggelakas SA, et al. Prognostic significance of echocardiographically estimated right ventricular shortening in advanced heart failure. Am J Cardiol. 1998; 82(3):329-34.
23. Goya M, Ouyang F, Ernst S, Volkmer M, Antz M, Kuck KH. Electroanatomic mapping and catheter ablation of breakthroughs from the right atrium to the superior vena cava in patients with atrial fibrillation. Circulation. **2002;** 106(11):1317-20.
24. Gaynor SL, Maniar HS, Bloch JB, Steendijk P, Moon MR. Right atrial and ventricular adaptation to chronic right ventricular pressure overload. Circulation. 2005; 112(9 Suppl):I212-8.
25. Mroczek AG, Atroschenko ES, Ostrovsky YP, et al. Diagnosis and treatment of atrial fibrillation. National guidelines. Minsk, 2010. [in Russian].
26. Shumakov VI, Kormer AIa, Kozakov EN, Chestukhin VV, Shemakin SIu. The hemodynamics of the lesser circulation before and after heart transplantation in patients with dilated cardiomyopathy. Grud Serdechnososudistaia Khir. 1993;6:4-7. [Article in Russian].
27. Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS,, et al. ACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult: Executive Summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2001; 104: 2996-3007.
28. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult- Summary Article: a report of the American College of Cardiology/American Heart Association Task Force on

Practice Guidelines. Circulation 2005; 112:154- 235.
29. Martins E, Cardoso JS, Campelo M, Amorim S, Moura B, Maciel MJ, Gonçalves FR. Survival of patients with familial dilated cardiomyopathy on optimal heart failure. Rev Port Cardiol. 2009; 28(3):263-8.
30. Felker GM, Thompson RE, Hare JM, Hruban RH, Clemetson DE, Howard DL, et al. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N Eng J Med 2000; 342(15): 1077-84.
31. Di Lenarda A, Pinamonti B, Mestroni L, Salvi A, Sabbadini G, Gregori D, et al. The natural history of dilated cardiomyopathy: a review of the Heart Muscle Disease Registry of Trieste. Ital Heart J Suppl 2004; 5(4):253-66. [Article in Italian].
32. Ribeiro A1, Lindmarker P, Juhlin-Dannfelt A, Johnsson H, Jorfeldt L. Echocardiography Doppler in pulmonary embolism: right ventricular dysfunction as a predictor of mortality rate. Am Heart J. 1997;134(3):479-87.

International Journal of Biomedicine 5(4) (2015) 214-218

ORIGINAL ARTICLE

Oncology and Hematology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Clinical Use of the Interferon Inducer IIBI in Patients with Refractory Hodgkin's Lymphoma

Daniar R. Aspetov[1]; Maria N. Omarova[1]; Lyazzat Zh. Orakbay*[1]; Raiymkul K. Karakulov[2]; Kairat S. Doskhozhayev[2]; Bayan Kh. Zhumatova[1]; Meruyert D. Chazhayeva[1]

[1]Scientific Centre of Hygiene and Epidemiology
[2]Kazakh Research Institute of Oncology and Radiology
Almaty, Kazakhstan

Abstract

This study evaluated the effects of the immunobiological preparation "Interferon inducer bacterial liquid" (IIBI) combined with chemoradiotherapy (CRT) in patients with refractory Hodgkin's lymphoma (HL) are presented. The clinical application of IIBI increases the efficacy of treatment. IIBI in combination with CRT induces the production of highly active endogenous IFN-α/β, provides a marked regression of the affected lymph nodes in a short period (within 7 to 10 days) of treatment, relieves *B* symptoms, and increases by 2 times the length of remission in patients with refractory HL. (**Int J Biomed. 2015; 5(4):214-218.)**

Keywords: *Hodgkin's lymphoma; chemoradiotherapy; IFN-α/β; IFN inducer.*

Introduction

Hodgkin's lymphoma (HL) is a heterogeneous malignancy with a complex etiology and epidemiology. Therapy for classical HL has considerably improved during the last two decades [1]. With current therapeutic approaches consisting of polychemo- and small-field radiotherapy, up to 80 % of all patients can be cured long term. In refractory or relapsed HL, intensified treatment including high-dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT) is associated with progression-free survival (PFS) of 50 %. [2]. The long-term toxicity of current regimens, however, is still strikingly high, providing a need for alternative strategies. Evaluating novel drugs in multiple relapsed or refractory cases and reducing treatment-related side effects are the focus of modern research.

The blockade of immunological checkpoints has been successfully employed for the treatment of various solid neoplasms [3]. A recent study indicates that the vast majority of patients with advanced, heavily pretreated HL also respond to a monoclonal antibody targeting programmed cell death 1 (PD-1) [4]. Thus, checkpoint blockers may soon become part of our therapeutic armamentarium against hematological tumors. This would be particularly important as it would spare (at least some) patients the deleterious toxic effects of combinatorial chemotherapies and bone marrow transplantation.

Immunotherapy of Hodgkin's lymphoma, however, has to take into account that the malignant $CD30^{+}$Hodgkin/Reed-Sternberg cells (H/RS) persist in small numbers in the lymphoma lesion and are accompanied by massive infiltrations with benign cells [5-9]. H/RS cells secrete a variety of cytokines and chemokines favoring a T helper-2 (Th2) immune response which likely contributes to disease progression through restraining cellular reactivity [6], [10-12].

Type I interferons (endogenously produced IFN-α/β) are important components of the cancer immunoediting process [13]. The fact that IFNs can bring about long-term remissions in certain malignancies is well established [14-19].

Type I IFN is represented by several partially homologous genes of IFN-α and a single gene of IFN-β and those genes can be expressed by almost any type of cell in response to stimulation of an array of receptors by pathogens [20]. Regarding that, type I IFN are more than just anti-viral as they play a major role in linking innate to adaptive immunity [21-23]. IFN-α is a type of interferon that may be effectively used to treat lymphoma [24,25]. Yet experts remain uncertain

*__Corresponding author:__ *Lyazat Orakbay, PhD, ScD. Scientific Centre of Hygiene and Epidemiology named after Hamza Zhumatov. Almaty, Kazakhstan. E-mail: ncgigieny@mail.ru*

about the benefits of interferon, and there are questions about its suitability in combination with chemotherapy. N. Batty et al. [25] performed a phase II trial to determine the safety and effectiveness of IFN-α and standard doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) chemotherapy (IABVD) in patients with newly diagnosed advanced stage classic Hodgkin lymphoma (HL). Treatment consisted of six cycles of ABVD with concurrent IFN-α followed by radiation therapy if indicated. The 3-year event-free survival rate was 71% (95% confidence interval [CI], 56-90%), and the 3-year overall survival rate was 96% (95% CI, 89-100%). Continued research is needed to improve the long-term survival of HL patients and to lessen the toxicities associated with therapy. It should be noted that many preparations of synthetic interferon applied in the clinic are quite expensive and not without side effects. An alternative and more harmless method is interferonization with the induction of endogenous interferon. Classically, exposure of cells to type I IFN induces an antiviral state that prevents productive viral infection. This premise was postulated by Isaacs and Lindenmann about 60 years ago when they demonstrated the cell ability to resist a virus infection. This phenomenon was then attributed to type I IFN cytokine considered the factor responsible to interfere in the viral infection [26].

Besides viruses, an increased number of pathogens, such as bacteria, protozoa, and fungi, have been reported to be inducers of type I IFN [21,27-29]. Type I IFN related to prevent bacterial infection was first demonstrated for pathogens from Chlamydia genus [30]. The limitation of bacterial infection by type I IFN induction was also observed in the case of *C. pneumoniae* and *Legionella pneumophila* [31,32]. The augmentation of pro-apoptotic stimulus in macrophages and lymphocytes was described in infection with *Listeria, Mycobacteria*, and *Chlamydia spp* [33-35].

In 2005, the first observation that *Brucella* induces type I IFN was made by L. Huang et al. [36] when IFN-α was detected in serum of wild type mice injected with HKBa and the level was markedly reduced in the $TLR9^{-/-}$ mice serum, demonstrating that HKBa induces IFN-α in a TLR9-dependent manner. Additionally, it has shown that *B. abortus* is able to induce IFN-β in DCs [37].

In 2012, the new bacterial INF inducer IIBI was developed in the Laboratory of Virology at the Scientific Center for Hygiene and Epidemiology (Kazakhstan) and included in the State Register of medicinal products to treat cancer patients. IIBI contains a unique bacterial substance (*Brucella melitensis*) and induces the production of endogenous INF-α/β [38]. Studies to assess the antitumor properties of IIBI were performed in the Kazakh Research Institute of Oncology and Radiology during 2012-2014.

The purpose of this study was to explore the interferonogenic, anti-inflammatory, and detoxifying properties of IIBI on the background of CRT in patients with refractory HL.

Materials and methods

The study included 138 patients with refractory HL (from 30 to 55 years). The disease duration ranged from 1 to 11 years. The diagnosis of HL was confirmed histologically via an endoscopic biopsy of the mediastinal or abdominal lymph nodes. Patients had Ann Arbor stage II (54.3%), III (41.3%) or IV (4.3%) disease. B symptoms were observed in 77.5% of patients.

Histological sub-classification was performed in line with WHO classification [39]: nodular lymphocyte predominant HL (NLPHL) and classic HL (nodular sclerosis HL, lymphocyte rich classic HL, mixed cellularity HL, and lymphocyte depleted HL).

Immunohistochemistry (IHC) was performed using an antibody panel for Reed-Sternberg cells (RSCs). Sections were analyzed by a semi-quantitative method, which differed depending on the antibody used. Antibodies against CD15, CD30, CD3, CD20, CD45, EMA and EBV LMP-1 were used for paraffin section IHC. Of the panel of antibodies, CD30 was the most useful in identifying RSCs in classical HL. IHC was also used for differential diagnosis between non-HL and NLPHL [40].

According to the therapy regimen, patients were divided into 2 groups. Study group patients (Group 1, n=70), after 6 months of their last CRT course (6 cycles of ABVD/MOPP and radiation therapy), were assigned to intramuscularly IIBI (10MM IU/ml) injections every other day (first 3 injections), and after that one injection per week for 4 weeks. Immediate and 2-year outcomes of treatment were identified. In the control group (Group 2, n=68), IIBI was not administered according to protocol.

After each IIBI injection, we determined serum IFN-α/β activity. Serum IFN-α/β was defined by the micromethod in plastic 96-well microtiter trays with a monolayer of RD cells, according to A. Novokhatskiĭ et al. [41]. Determination of T- and B-lymphocytes was performed by an E-rosette test and EAC-rosette test, respectively. Side effects of IIBI therapy were analyzed.

The study was conducted in line with the requirements of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (2008). It was approved by the Kazakh Research Institute of Oncology and Radiology Ethics Committee. Written informed consent was obtained from all participants.

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

Results

As can be seen from Table 1, both groups were comparable in terms of Ann Arbor stage, histological type of HL, and *B* symptoms (fever, night sweats, and weight loss).

Table 1.

Clinical characteristics of HL patients

HL characteristics	Group 1 (CRT+IIBI) n=70	Group 2 (CRT) n=68
Ann Arbor stage:		
II	39 (55.7)	36 (52.9)
III	28 (40.0)	29(42.6)
IV	3 (4.3)	3(4.4)
Histological classification:		
lymphocyte rich classic HL	13 (18.6)	16 (23.5)
nodular sclerosis HL	18 (25.7)	18 (26.5)
mixed cellularity HL	20 (28.6)	20 (29.4)
lymphocyte depleted HL	19 (27.1)	14 (20.6)
Stage A	16 (22,9)	15 (22,1)
Stage B	54 (77,1)	53 (77,9)

In both groups, most patients (81.9%) received radiotherapy according to the radical program. The total focal dose on the struck zones was 38-42Gr, on the subclinical zones it was 30-36Gr. Ten and more ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) or MOPP (mechlorethamine, vincristine, procarbazine, prednisone) regimens were ineffective. In addition to chemoradiotherapy, patients received antitoxic therapy: the antiemetic preparation ondem (12 mg/day), prednisone (10 mg/day), and a weekly infusion of physiological saline during the CRT course. Tumor regression was observed only in 20%-30% of cases. Relapses of the disease occurred after 4-5 months following CRT.

In Group 1, IIBI therapy was well tolerated with no patients requiring IIBI dose reduction or discontinuation because of side effects. The serum IFN-α/β activity increased after the first IIBI administration compared to the initial background values (<2IU/ml) (Table 2). With increasing frequency of IIBI administration (from first to third injection), the serum IFN-α/β activity increased up to the highest level (more than double) after the third injection (152±25.8 IU/ml). INF activity induced after 5 to 7 IIBI injections remained on the therapeutic level (within 60 IU/ml).

Table 2.

The serum IFN-α/β activity after the IIBI administrations

Number of IIBI injections	Quantity of patients	Activity of serum IFN-α/β (IU/ml)	
		Before IIBI administration	After six hours of IIBI administration
1	10	<2	52.0±8.6
2	9	48.4±6.4	84.0±14.0
3	10	67.2±12.0	152.0±25.8
4	8	62.0±6.6	68.4±8.2
5	8	50.2±6.8	59.8±7.4
6	8	54.6±6.2	64.8±6.8
7	8	51.2±5.4	62.2±8.4

After treatment, *B* symptoms disappeared, affected nodes decreased in size, and laboratory parameters were normalized in 22.5% of the patients. Clinical improvement was associated with an increase in serum IFN-α/β activity. In the remaining 77.5% of patients with severe symptoms of intoxication, IIBI administration had no significant therapeutic action. In these patients, the serum IFN-α/β activity remained low (39 ± 9.34 IU / mL) during treatment.

Hematologic parameters were improved during the first week of therapy. In particular, we noted an increase in the number of lymphocytes (3.3 ± 0.17 vs. 4.5± 0.26x10^9/l, P<0.05) and a decrease in ESR level (29± 2.3 versus 13±1.9 mm/h, P<0.05).

The levels of T- and B-lymphocytes in peripheral blood were evaluated in Group 1 patients (n=57) after 4 injections and the full course of IIBI therapy, in Group 2 patients (n=37), and in healthy donors (n=10) (Table 3). IIBI application had a positive influence on the quantitative content of T- and B-lymphocytes and reduced the side effects of CRT.

Table 3 .

The levels of T- and B- lymphocytes in peripheral blood of HL patients during treatment

Group of patients	Quantity of patients	TLC %	BLC %
Group 2			
Before treatment	37	21± 3.8	14±2.3
After treatment	35	25±4.2	22±3.7
Group 1			
Before IIBI administration	57	18 ± 3.0	12± 2.0
After the 4th IIBI injection	56	38 ± 6.0*	28 ± 6.0*
After full course of IIBI therapy	54	46±7.7*	37±6.2*
Healthy donors	10	55 ± 3.4	22 ± 2.7

*TLC-- T-lymphocytes; BLC-- B-lymphocytes; *- P<0.05 compared to data before IIBI administration*

When evaluating the antitumor activity of IIBI in combination with CRT, we noted that the efficacy of this treatment was inversely related to the initial tumor size. The majority of patients (62.9%) had lymph nodes (LNs) up to 5cm in diameter. The highest percentage of complete regression of tumors was observed in lymph nodes with a small size (<5cm) (Table 4). The most significant decrease in the size of lymph nodes was observed after the 3 injections of IIBI (within 7-10 days of treatment). The rate of lymph node regression during the subsequent IIBI injections was less pronounced.

Table 4.

Regression of lymph nodes in HL patients during treatment (CRT+IIBI)

Average size of LN	Before treatment (number of LNs)	After treatment (number of LNs)	P, $\chi 2$
<2 cm	19	4	$\chi 2$=11.706; P<0.001
≥5 cm	33	17	$\chi 2$=7.964, P<0.005
≥10 cm	7	4	$\chi 2$=0.888, P>0.05

In conclusion, the clinical application of IIBI increases the efficacy of treatment. IIBI in combination with CRT induces the production of highly active endogenous IFN-α/β,

provides a marked regression of the affected lymph nodes in a short period (within 7 to 10 days) of treatment, relieves *B* symptoms, and increases by 2 times the length of remission in patients with refractory HL.

Competing interests

The authors declare that they have no competing interests.

References

1. Evens AM, Hutchings M, Diehl V. Treatment of Hodgkin lymphoma: the past, present, and future. Nat Clin Pract Oncol 2008;5(9): 543–56.
2. Bröckelmann PJ, Engert A. The GHSG Approach to Treating Hodgkin's Lymphoma. Curr Hematol Malig Rep. 2015 Sep; 10(3):256-65.
3. Kroemer G, Galluzzi L. Immunotherapy of hematological cancers: PD-1 blockade for the treatment of Hodgkin's lymphoma. Oncoimmunology. 2015;4(6):e1008853.
4. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med. 2015;372(4):311-9.
5. Jahn T, Zuther M, Friedrichs B, Heuser C, Guhlke S, Abken H, Hombach AA. An IL12-IL2-antibody fusion protein targeting Hodgkin's lymphoma cells potentiates activation of NK and T cells for an anti-tumor attack. PLoS One. 2012;7(9):e44482.
6. Küppers R, Schwering I, Bräuninger A, Rajewsky K, Hansmann ML. Biology of Hodgkin's lymphoma. Ann Oncol.2002; 13 Suppl 1: 11–18.
7. Maggio E, van den Berg A, Diepstra A, Kluiver J, Visser L, Poppema S. Chemokines, cytokines and their receptors in Hodgkin's lymphoma cell lines and tissues. Ann Oncol. 2002; 13 Suppl 1: 52–56.
8. Kennedy-Nasser AA, Hanley P, Bollard CM. Hodgkin disease and the role of the immune system. Pediatr Hematol Oncol. 2011; 28(3):176–86.
9. Stein H, Bob R. Is Hodgkin lymphoma just another B-cell lymphoma? Curr Hematol Malig Rep.2009; 4(3):125–8.
10. van den Berg A, Visser L, Poppema S. High expression of the CC chemokine TARC in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltratein Hodgkin's lymphoma. Am J Pathol. 154(6):1685–91.
11. Poppema S, van den Berg A. Interaction between host T cells and Reed-Sternberg cells in Hodgkin lymphomas. Semin Cancer Biol. 2000; 10(5):345–50.
12. Skinnider BF, Elia AJ, Gascoyne RD, Patterson B, Trumper L, Kapp U, et al. Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. Blood. 2002; 99(2):618–26.
13. Dunn GP, Bruce AT, Sheehan KC, Shankaran V, Uppaluri R, Bui JD, et al. A critical function for type I interferons in cancer immunoediting. Nat Immunol. 2005; 6(7):722-9.
14. Ferrantini M, Capone I, Belardelli F. Biochimie. Interferon-alpha and cancer: mechanisms of action and new perspectives of clinical use. Biochimie 2007; 89(6-7):884-93.
15. Dunn GP1, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002; 3(11):991-8.
16. Swann JB, Hayakawa Y, Zerafa N, Sheehan KC, Scott B, Schreiber RD, et al. Type I IFN contributes to NK cell homeostasis, activation, and antitumor function. J Immunol 2007;178(12):7540-9.
17. Hasan UA, Caux C, Perrot I, Doffin AC, Menetrier-Caux C, Trinchieri G, et al. Cell proliferation and survival induced by Toll-like receptors is antagonized by type I IFNs. Proc Natl Acad Sci USA. 2007; 104(19):8047-52.
18. Shmulevitz M, Pan LZ, Garant K, Pan D, Lee PW. Oncogenic Ras promotes reovirus spread by suppressing IFN-beta production through negative regulation of RIG-I signaling. Cancer Res. 2010;70(12):4912-21.
19. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. Nat Rev Immunol. 2015; 15(7):405-14.
20. Decker T, Müller M, Stockinger S. The yin and yang of type I interferon activity in bacterial infection. Nat Rev Immunol. 2005 Sep;5(9):675-87.
21. Gomes MT, Campos PC, de Almeida LA, Oliveira FS, Costa MM, Marim FM, et al. The role of innate immune signals in immunity to Brucella abortus. Front Cell Infect Microbiol. 2012;2:130.
22. Nagarajan UM, Sikes J, Prantner D, Andrews CW Jr, Frazer L, Goodwin A, et al. MyD88 deficiency leads to decreased NK cell gamma interferon production and T cell recruitment during Chlamydia muridarum genital tract infection, but a predominant Th1 response and enhanced monocytic inflammation are associated with infection resolution. Infect Immun. 2011;79(1):486-98.
23. Monroe KM, McWhirter SM, Vance RE. Induction of type I interferons by bacteria. Cell Microbiol. 2010;12(7):881-90.
24. Petropoulos D, Worth LL, Mullen CA, Lockhart S, Choroszy M, Chan KW. Interferon-alpha after autologous stem cell transplantation in pediatric patients with advanced Hodgkin's lymphoma. Bone Marrow Transplant. 2006;38(5):345-9.
25. Batty N, Hagemeister FB, Feng L, Romaguera JE, Rodriguez MA, McLaughlin P, et al. Doxorubicin, bleomycin, vinblastine and dacarbazine chemotherapy with interferon for advanced stage classic Hodgkin lymphoma: a 10-year follow-up study. Leuk Lymphoma. 2012;53(5):801-6.
26. Isaacs A, Lindenmann J. Pillars Article: Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci. 1957;147;258–267
27. Meissner NN, Swain S, Tighe M, Harmsen A, Harmsen A. Role of type I IFNs in pulmonary complications of Pneumocystis murina infection. J Immunol. 2005;174(9):5462–71.
28. Monroe KM, McWhirter SM, Vance RE. Induction of type I interferons by bacteria. Cell Microbiol. 2010;12(7): 881–90.
29. Sharma S, Deoliveira RB, Kalantari P, Parroche P, Goutagny N, Jiang Z, et al. Innate immune recognition of an AT-rich stem-loop DNA motif in the Plasmodium falciparum genome. Immunity. 2011;35(2):194–207.
30. de La Maza LM, Peterson EM, Goebel JM, Fennie CW, Czarniecki CW. Interferon-induced inhibition of Chlamydia trachomatis: dissociation from antiviral and antiproliferative effects. Infect Immun. 1985;47(3):719–22.
31. Buss C, Opitz B, Hocke AC, Lippmann J, Van Laak V, Hippenstiel S, et al. Essential role of mitochondrial antiviral signaling, IFN regulatory factor (IRF)3, and IRF7 in Chlamydophila pneumoniae-mediated IFN-beta response and control of bacterial replication in human endothelial cells. J Immunol. 2010;184(6): 3072–8.

32. Plumlee CR, Lee C, Beg AA, Decker T, Shuman HA, Schindler C. Interferons direct an effective innate response to Legionella pneumophila infection. J Biol Chem. 2009;284(44):30058–66.
33. Carrero JA, Calderon B, Unanue ER. Lymphocytes are detrimental during the early innate immune response against Listeria monocytogenes. J Exp Med. 2006;203(4): 933–40.
34. O'Connell RM, Saha SK, Vaidya SA, Bruhn KW, Miranda GA, Zarnegar B, et al. Type I interferon production enhances susceptibility to Listeria monocytogenes infection. J Exp Med. 2004;200(4):437–45.
35. Qiu H, Fan Y, Joyee AG, Wang S, Han X, Bai H, et al. Type I IFNs enhance susceptibility to Chlamydia muridarum lung infection by enhancing apoptosis of local macrophages. J Immunol. 2008;181(3): 2092–102.
36. Huang LY, Ishii KJ, Akira S, Aliberti J, Golding B. Th1-like cytokine induction by heat-killed Brucella abortus is dependent on triggering of TLR9. J Immunol. 2005; 175(6):3964–70.
37. Salcedo SP, Marchesini MI, Lelouard H, Fugier E, Jolly G, Balor S, et al. Brucella control of dendritic cell maturation is dependent on the TIR-containing protein Btp1. PLoS Pathog. 2008;4(2):e21.
38. Interferon inducer bacterial liquid.State Register of medicinal products of the Republic of Kazakhstan. 2012; *IDA* 42-33-68-11.
39. Jaffe ES. The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. Hematology Am Soc Hematol Educ Program. 2009. 523-31.
40. KovriginaAM,ProbatovaNA.Morphoimmunohistological differential diagnosis between Hodgkin's lymphoma with lymphoid prevalence and large B-cell lymphoma. Arkh Patol. 2005; 67(4):10-6 [Article in Russian].
41. Novokhatskiĭ AS, Cherkashina NP, Ershov FI. Accelerated method of determining the antiviral activity of interferon inducers in experimental infection. Vopr Virusol. 1979;3:303-6. [Article in Russian].

International Journal of Biomedicine 5(4) (2015) 219-223

ORIGINAL ARTICLE

Ophthalmology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Dynamics of Morphological Changes in the Anterior Segment of the Rat Eye after Experimental Alkali Burn Depending on the Acetylator Phenotype

Khalidjan M. Kamilov, PhD,ScD; Laylo M. Maksudova*

Tashkent Institute of Postgraduate Medical Education
Tashkent, Uzbekistan

Abstract

The purpose of this study was to identify the nature and dynamics of morphological changes in the anterior segment of the rat eye (ASE) in the aspect of regeneration opportunities under an experimental alkali burn (EAB) in the fast and slow acetylators.

Materials and Methods: The experiment was performed on 50 adult outbred male Wistar rats weighting from 180 to 200g. According to results of testing, all animals were divided into 2 groups: fast acetylators and slow acetylators. The experimental chemical eye burn model was performed according to standard procedures by applying a 10% NaOH solution for 10 seconds. The tissues of ASE were the object of this study. The morphological examination was performed on the 3rd, 5th, 12th, and 30th day after EAB.

Results: The morphological changes of eye tissues after EAB are formed immediately after the injury involving the deep tissues and are characterized by severe manifestations of the colliquative burn of all eye structures. The intensity of the pathological process increases over time with subsequent reduction of pathological changes on day 30 post-injury. The intensity of morphological changes and their dynamics vary depending on the N-acetylation, given equal external conditions. Regarding the features of drug biotransformation in slow acetylators, it is advisable to take acetylation phenotype into account when choosing the treatment method. (**Int J Biomed. 2015;5(4):219-223.)**

Keywords: *rat's eye; alkali burn; morphological changes; acetylation phenotype.*

Introduction

Injuries and burns of the cornea over the past three decades have been the leading cause of eye injuries (68% to 70%) [1-4]. The structural and functional restoration of a chemical burn-injured cornea remains challenging. In severe cases, the eye limbus and central epithelium can be lost, leading to loss of vision. Slow epithelialization, persistent ulceration, corneal perforation and angiogenesis are the main complications and result from the processes of inflammation, neovascularization, and conjunctivalization of cornea [1,2,5]. Strategies to treat corneal chemical burns include antibiotics, tear substitutes, corticosteroids, ascorbic acid, collagenase inhibitors and surgical treatments such as penetrating keratoplasty and amniotic membrane transplantation [6]. However, the structural and functional restoration of alkali burn-injuries to the cornea remains a challenge, despite these therapies and thus prompting the search for novel treatment strategies [7-9]. A detailed study of regenerative processes under the influence of various drugs is a top priority of modern ophthalmology.

Our analysis of the contemporary literature on the study of morphological changes in the organ of vision under the influence of chemical burns has identified some common features in the views of researchers on this problem. In burn injuries, the focus of interest of the majority of contemporary researchers is the final result of the application of a pharmacological agent without analysis of the dynamics of the processes. In most cases, the final result is expressed as: (a) negative result - no effect of the pharmacological treatment; (b) moderate result - an insufficient (incomplete) effect of the pharmacological treatment; (c) positive result - a complete recovery of visual functions during pharmacological treatment. It should be noted that for the patient only complete

***Corresponding author**: Laylo M. Maksudova. Tashkent Institute of Postgraduate Medical Education. Tashkent, Uzbekistan. E-mail: doclaylo@rambler.ru*

recovery of vision might be considered an effective treatment, while moderate or insufficient effect is a doctor's defeat in the fight for the preservation of vision.

The effect of treatment depends on the drug metabolism and rate of elimination of toxic products of chemical burns. Many pharmacological agents, as well as some toxic products of chemical burns, are metabolized primarily by acetylation by liver N-acetyltransferase. The rate of acetylation is genetically determined. The quantitative assessment of the rate and extent of acetylation of many chemical substances yields bimodal distribution, representing fast and slow acetylators [10-12]. Recently, it has been suggested that slow acetylators are at greater risk of developing severe pathological processes [13,14]. But there have been no studies of the association between the dynamics of morphological changes during reparative processes with alkali burns of the eyes, on the one hand, and genetic differences in xenobiotic elimination and metabolic rate on the other.

Based on the above, ***the purpose*** of this study was to identify the nature and dynamics of morphological changes in the anterior segment of the rat eye (ASE) in the aspect of regeneration opportunities under an experimental alkali burn (EAB) in the fast and slow acetylators.

Materials and Methods

The study was conducted between October 2013 and May 2014 in accordance with the principles of ARVO Statement for the use of animals in ophthalmic and visual research. The experiment was performed on 50 adult outbred male Wistar rats weighting from 180 to 200g. Acetylation phenotype (AF) was determined according to the Brodie and Axelrod method (1949) as modified by T.A. Popova and O.B. Leonenko (1977), using Sulfathiazole, by analyzing the drug metabolite ratio in urine after *per os* administration of 10 mg/kg Sulfathiazole. According to results of testing, all animals were divided into 2 groups: fast acetylators (FAs) and slow acetylators (SAs). The study group consisted of 20 slow acetylator rats (SARs) and 20 fast acetylator rats (FARs) (Fig.1- 2).

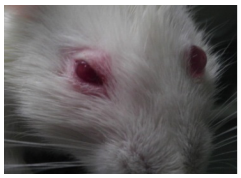

Fig.1. *The eye of SAR. EAB.*

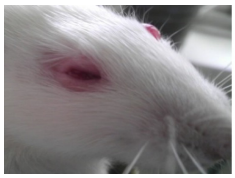

Fig. 2. *The eye of FAR. EAB*

In the study group, the experimental chemical eye burn model was performed according to standard procedures by applying a 10% NaOH solution for 10 seconds. The control group consisted of 5 SARs and 5 FARs who underwent application of a 0.9% NaCl solution in the same exposure. All animals were kept in the same vivarium conditions. The tissues of ASE were the object of this study. The morphological examination was performed on the 3rd, 5th, 12th, and 30th day after EAB. Procedures for euthanasia were performed according to MMRI Policy for the Humane Care and Use of Laboratory Animals in a manner consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia, unless a deviation is justified for scientific reasons in writing by the investigator".

Morphological studies were conducted using standard light microscopy techniques (H&E staining). Light optical micrographs obtained with different magnifications using microscopes Carl Zeiss AxioScope, Biolam-I, and Biolam I2 combined with a digital camera.

Results

Dynamics of morphological changes in ASE of FARs after EAB

Figure 3 presents the morphological picture of the healthy rat cornea. On the third day after EAB, morphological changes in the cornea of FARs were characterized by the development of edema with the separation and dissociation of collagen fibers in the central area of burn injury (Fig.4). Cellular elements of the anterior corneal epithelium were completely absent. The lamina limitans anterior, having a fibrillar structure, was partially preserved. The corneal stroma was represented by irregularly aligned connective tissue plates, which intersected at different angles and consisted of the separated and dissociated collagen fibers of different thicknesses. The posterior corneal epithelium, consisting of flat polygonal cells, was also edematous and amorphous in most cases and separated from the Descemet's membrane. Revealed morphological changes allow us to conclude that rude and often irreversible structural changes in the affected tissues of the eye are formed by the second day after EAB.

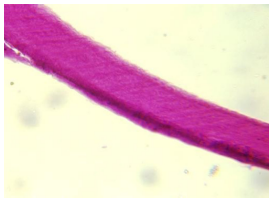

Fig. 3. *The morphological picture of the healthy rat cornea. H&E, 10x10.*

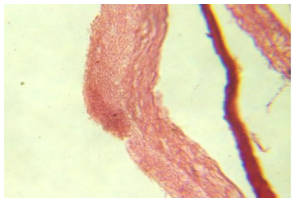

Fig. 4. *Exfoliation of the anterior corneal epithelium. FA. The 3rd day after EAB. H&E.10x40.*

On the fifth day after EAB, the infiltration and ulceration of the burn surface of the cornea were intensified, and signs of edema with the involvement of the large areas of the corneal stroma increased. The anterior corneal epithelium was absent in the central zone of edema, and, toward the periphery, the transition from a single-layer epithelium into the two- or pseudostratified epithelium was observed. Areas of degradation in the corneal stroma were identified (Fig. 5 and 6).

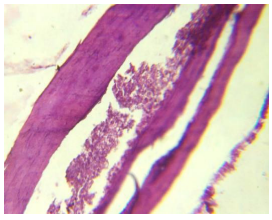

Fig.5. *Edema and areas of degradation in the corneal stroma. The 5th day after EAB. FA. H&E. 10x16.*

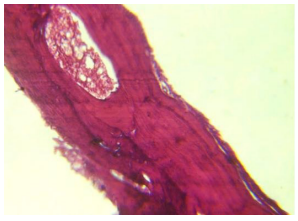

Fig.6. *Elements of the corneal stroma destruction, the surface erosions. The 5th day after EAB. FA. H&E. 10x16.*

It should be noted that hyperemia and overflow of blood capillaries, causing edema severity, are characteristic morphological changes in the eye tissues on the fifth day after EAB.

By the 12th day after EAB, pathological changes in the connective tissue of the sclera had increased and the dissociation with fragmentation of collagen fibers, as well as colliquation of tissues ("colliquatio"), were detected (Fig. 7 and 8).

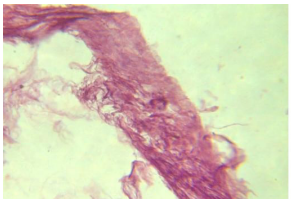

Fig.7. *Dissociation with fragmentation in the connective tissue of the sclera. The 12th day after EAB. FA. H&E.10x16.*

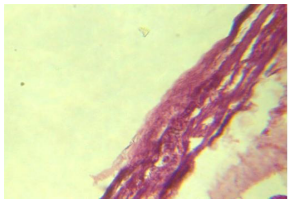

Fig.8. *Signs of scleral cell death. The 12th day after EAB. FA.H&E. 10x16.*

The absence of morphological signs of scab was manifested by hydrolysis of proteins and cell membranes. Signs of cell death caused by the spread and deep alkali penetration were also identified. All structures of the eye, including the conjunctiva (Fig.9), eyelids (Fig.10), cornea, sclera, lens, and even the retina, were involved in the pathological process. Edema and the leukocyte infiltration of the connective tissue of the eyelids were spread.

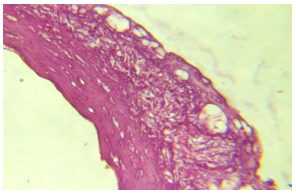

Fig.9. *the conjunctiva: edema and hyperemia. The 12th day after EAB. FA.H&E. 10x16.*

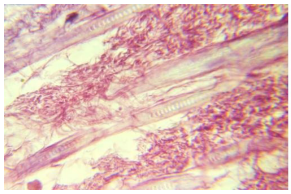

Fig.10. *Edema and leukocyte infiltration of the connective tissue of the eyelid. The 12th day after EAB. FA. H&E.10x16.*

By the 30th day after EAB, tissue swelling persisted, but it was not so pronounced. The phenomena of tissue destruction came to the fore, namely the destruction of the accommodative apparatus with hemorrhagic manifestations of varying severity from an imbibition to the extensive hemorrhages (Fig. 11-12).

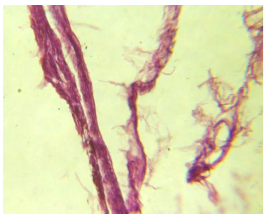

Fig. 11. *Dystrophy of the elastic fibers; the bundle and necrosis of the sclera. The 30th day after EAB. FA. H&E. 10x16*

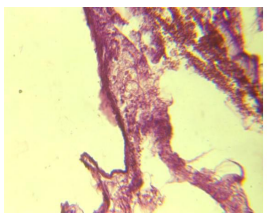

Fig.12. *The anterior chamber angle. Edema and destruction of accommodative apparatus. The 30th day after EAB. FA. 10x16*

Dynamics of morphological changes in ASE of SARs after EAB

On the third day after EAB, the morphological changes in the rat cornea were characterized by the formation of pronounced edema. The multiple areas of small erosions on the outer surface of the cornea with elements of cellular debris were detected (Fig.13). Sclera was characterized by edema; collagen fibers were loose and had relatively parallel positions to the surface of the eyeball (Fig.14). The outer surface of the sclera was eroded in some places, so that the free edges of the fibrous tissue were behind the epithelium anterior which had a tendency to separate from the stroma. The auxiliary apparatus of the eye, eyelids, and lacrimal apparatus were also involved in the pathological process (Fig.15). On the fifth day after EAB, symptoms of edema were intensified; there were tears and a lamination of the corneal fibers; an accumulation of large amounts of blood cells, mainly red blood cells, was identified in the lumen of the tears (Fig.16).

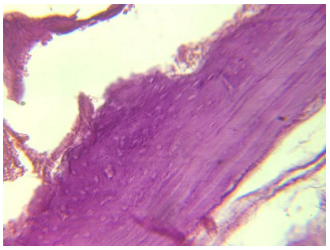

Fig.13. *The cornea. The 3rd day after EAB. SA. H&E. 10x16*

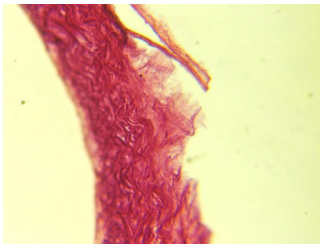

Fig.14. *The sclera. The 3rd day after EAB. SA. H&E. 10x16*

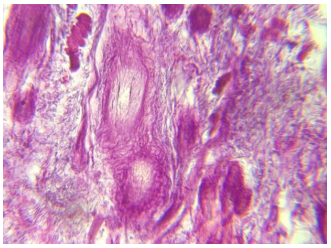

Fig.15. *Eyelid, hair. Stasis and sludge of erythrocytes. The 3rd day after EAB. SA. 10x16*

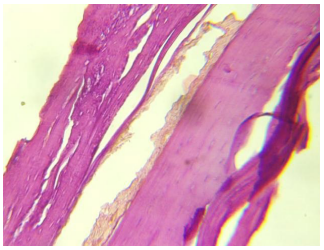

Fig.16. *Edema, tears and a lamination of the corneal fibers. The 5st day after EAB. SA. 10x16.*

Morphological changes in the angle of the eye were characterized by a diffuse increase of edema. The cavities of venous sinuses of sclera and Schlemm's canal were empty; blood cells were absent in the wide vascular lumens (Fig.17). The spread of the alkali burn went deep into the tissues of the eye with involvement in the pathological process of the entire organ, damaging the receptor and neurosensory apparatus. We have identified the characteristic changes in the form of increasing diffuse edema in the tissues that were infiltrated with blood (imbibitions with red blood cells); areas of hemorrhage were also found. Blood cells were found within the lumen of blood vessels, mainly in parietal areas. Figure 18 presents the stagnation and stasis of blood in the back wall of the eye.

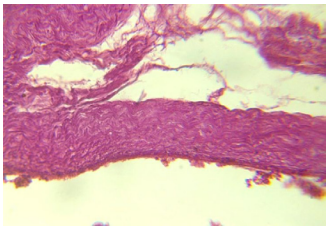

Fig.17. *Schlemm's canal. The 5st day after EAB. SA. 10x16.*

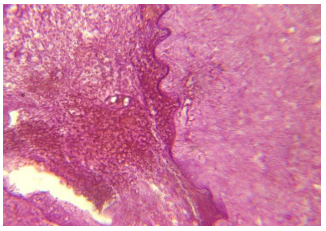

Fig.18. *The back wall of the eye. The 5st day after EAB. SA. 10x16.*

On the 12th day after EAB, we identified a progression of degradation processes, signs of epithelial tissue regeneration with the participation of the cellular elements of the non-keratinized stratified squamous epithelium, the anterior corneal epithelium, that could be traced ubiquitously (Fig.19).

On the 30th day after EAB, we found the negative dynamics characterized by the severe processes of hypotrophy and atrophy, with loss of vision. The morphological picture of trophic tissue disorders was due to processes of the generalized blood circulation disorders with thrombosis on the background of an edema, as well as disturbances of the drainage function of the venous and lymphatic system (Fig.20).

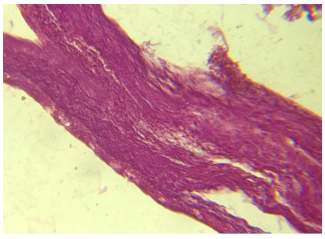

Fig.19. *Signs of epithelial tissue regeneration. The 12th day after EAB. SA. 10x16.*

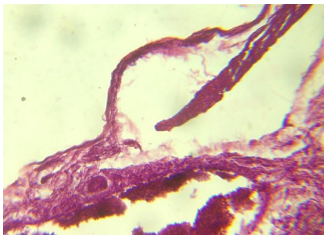

Fig.20. Corner of the eye. *The 30th day after EAB. SA. 10x16.*

Discussion

To analyze of the morphological changes in the tissues of the eye after EAB, it is advisable to remember the fundamentals of the damaging effects of alkalis. Alkalis, leading to saponification of proteins, fats and fat-like substances of cell membranes, penetrate intracellularly and change the plasmalemma cellular pH, which leads to dissolution and colliquation of proteins and determines the degree of degradation. The character of morphological changes in tissues of the eye on the background of EAB is also associated with features of supply and regeneration of the cornea, which occurs due to the diffusion of micronutrients from the front chamber of the eye and blood vessels of the limbus, because of the absence of blood vessels in the cornea. Moreover, against the background of pronounced edema and loss of structure in all histological layers of the cornea, a corneal lymphatic system consisting of narrow gaps usually is isolated from the possibility of adequate communication with the venous plexus.

Our results confirm the previously published data that alkali burns, dissolving tissue proteins and inducing a colliquative necrosis [15-17], quickly penetrate deeply into tissues and the ocular cavity and affect the inner shells of the cavity. According to the authors, some alkalis are found in the anterior chamber of the eye after 5 to 6 minutes following their introduction into the conjunctival cavity. In this regard, we can assume that the possibility of achieving the minimization of dioptric apparatus defeat in the early stages of treatment in FAs does not exclude the penetration of alkali in the deeper layers of the eye. In this case, not only are the cornea and conjunctiva affected, but also the inner shells of the front chamber of the eye. Hemorrhagic manifestations against the backdrop of the ongoing inflammatory response will contribute to the widening of the connective tissue structures and, consequently, a lesion of the functional activity of the accommodative apparatus of the eye, even on the background of the saved morphological organization of the dioptric apparatus.

The differences in the morphological changes in tissues of the eye with alkali burn injuries in animals with different AF, in particular, the severity of the lesions in SARs, may be important to optimize the treatment of this severe injury. Regarding the features of drug biotransformation in SAs, it is advisable to take AF into account when choosing the treatment method. In particular, we recommend including AF definition in the standards of diagnosis and treatment of chemical burns of the eye in order to predict the possible outcome of therapy and its optimization.

In conclusion, it should be noted that the morphological changes of eye tissues after EAB are formed immediately after the injury involving the deep tissues and are characterized by severe manifestations of the colliquative burn of all eye structures. The intensity of the pathological process increases over time with subsequent reduction of pathological changes on day 30 post-injury. The intensity of morphological changes and their dynamics vary depending on the N-acetylation, given equal external conditions. Regarding the features of drug biotransformation in slow acetylators, it is advisable to take acetylation phenotype into account when choosing the treatment method.

Competing interests

The authors declare that they have no competing interests.

References

1. Wagoner MD. Chemical injuries of the eye: current concepts in pathophysiology and therapy. Surv Ophthalmol. 1997; 41(4):275–313.
2. Brodovsky SC, McCarty CA, Snibson G, Loughnan M, Sullivan L, Daniell M, et al. Management of alkali burns: an 11-year retrospective review. Ophthalmology. 2000; 107(10):1829–35.
3. Ramakrishnan KM, Mathivanan T, Jayaraman V, Babu M, Shankar J. Current scenario in chemical burns in a developing country: Chennai, India. Ann Burns Fire Disasters. 2012; 25(1):8-12.
4. Iakymenko S. Forty-five years of keratoprosthesis study and application at the Filatov Institute: a retrospective analysis of 1060 cases. Int J Ophthalmol. 2013; 6(3):375-80.
5. Adamis AP, Aiello LP, D'Amato RA. Angiogenesis and ophthalmic disease. Angiogenesis. 1999; 3(1):9–14.
6. He J, Bazan NG, Bazan HE. Alkali-induced corneal stromal melting prevention by a novel platelet-activating factor receptor antagonist. Arch Ophthalmol. 2006; 124(1):70–8.
7. Han Y, Shao Y, Liu TT, Li SM, Li W, Liu ZG. Therapeutic effects of topical netrin-4 in a corneal acute inflammatory model. Int J Ophthalmol. 2015; 8(2):228-33.
8. Ke Y, Wu Y, Cui X, Liu X, Yu M, Yang C, Li X, et al. Polysaccharide hydrogel combined with mesenchymal stem cells promotes the healing of corneal alkali burn in rats. PLoS One. 2015; 10(3):e0119725. doi: 10.1371/journal.pone.0119725. eCollection 2015.
9. Yao L, Li ZR, Su WR, Li YP, Lin ML, Zhang WX, Liu Y,

Wan Q, Liang D. Role of mesenchymal stem cells on cornea wound healing induced by acute alkali burn. PLoS One. 2012; 7(2):e30842. Epub 2012 Feb 17.
10. Evans DA, White TA. Human acetylation polymorphism. J Lab Clin Med. 1964; 63:394–403.
11. Evans DA. An improved and simplified method of detecting the acetylator phenotype. J Med Genet. 1969; 6(4):405-7.
12. Gelber R, Peters JH, Gordon GR, Glazko AJ, Levy L. The polymorphic acetylation of dapsone in man. Clin Pharmacol Ther. 1971; 12(2):225-38.
13. Thomas L. Lemke, David A. Williams. *Foye's Principles of Medicinal Chemistry*. 7th ed. LWW; 2012
14. NAT2 slow acetylation and bladder cancer risk: a meta-analysis of 22 case-control studies conducted in the general population. Pharmacogenetics. 2000; 10(2):115-22.
15. Sykes RA, Mani MM, Hiebert JM. Chemical burns: retrospective review. J Burn Care Rehabil. 1986; 7(4):343–7.
16. Mozingo DW, Smith AA, McManus WF, Pruitt BA Jr, Mason AD Jr. Chemical burns. J Trauma. 1988; 28(5):642–7.
17. Palao R, Monge I, Ruiz M, Barret JP. Chemical burns: pathophysiology and treatment. Burns. 2010; 36(3):295-304.

International Journal of Biomedicine 5(4) (2015) 224-227

ORIGINAL ARTICLE

Public Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Primary Health Care Challenges in Rural/Remote Areas of Yakutia and Use of Automated Systems for the Medical Screening Examination of the Pediatric Population

Sardana A. Evseeva[1]*, PGS; Vyacheslav G. Chasnik[1], PhD, ScD;
Tatiana E. Burtseva[2]*, PhD, ScD; Maya S. Savvina[2], PhD;
Fecla V. Vinocurova[1], PGS

[1]*Saint Petersburg State Pediatric Medical Academy, Saint Petersburg, Russia*
[2]*Yakutsk Research Center for Complex Medical Problems, Yakutsk, Sakha Republic, Russia*

Abstract

The negative consequences of social and economic changes in recent decades have primarily affected the rural population and violated the main principles of medical care organization for this group. The reduction by one third in the number of district hospitals, uncompensated by adequate development of outpatient care, and a shortage of doctors in rural clinics led to reduced availability of primary care. Specialized medical assistance in regional and national hospitals has also become less accessible to the rural population due to the high cost of travel. The number of doctors and nurses in rural areas is lower by 3.4 and 1.6 times, respectively, than in cities. In this regard, the burden and responsibility for rural health workers is much higher.

Study of the opinions of the medical staff of the Northern and Arctic regions is an important part of the decision-making system in health care, allowing us to carry out modernization programs in the industry and increase their efficiency through feedback mechanisms.This article presents the available data on the problems of organizing medical assistance for residents of the Northern and Arctic regions of Yakutia, because dealing with these problems is still the most socially significant task for the authorities and carries a great load of negative experience, stereotypes, and scientific-methodological errors.

To assess the quality of medical care, we conducted an anonymous survey of parents and medical staff of the Northern and Arctic regions of Yakutia. A total of 1,415 parents and 322 health specialists were interviewed between 2011 and 2012. The results of the anonymous survey revealed that in the Northern and Arctic regions of Yakutia there is a deficit of qualified specialists of different profiles, an unsatisfactory infrastructure of medical offices and hospitals, and a low level of income for medical personnel and the whole population. All above listed are some of the reasons for developing and implementing health care information technologies to improve the quality of medical services in remote settlements of Yakutia. **(Int J Biomed. 2015;5(4):224-227.)**

Keywords: *healthcare; Yakutia; automated medical examinations, children's health; quality of diagnosis.*

Introduction

Yakutia (the Sakha Republic) is the largest subject of the Russian Federation; Yakutia occupies one-fifth of Russia. It is one of the coldest regions in the world. The average annual amplitude of air temperature is over 100°C. According to the Russian State Statistics Committee (2013), Yakutia has a population of 955,580 people; the population density of the republic is 0.32 persons per 1 km^2, while in a number of the Arctic regions, it ranged from 0.1 to 0.01 persons per 1 km^2. About 40% of the territory lies above the Arctic Circle, where only 7% of the population lives, including the indigenous peoples of the North who lead a traditional nomadic way of life. Russia has 40 small ethnic groups that are officially classified as "indigenous small-numbered peoples of the North, Siberia and the Far East". Demographic information is available on twenty-six ethnicities, of which approximately 91,000 live north of the Arctic circle, and 70,000 inhabit sub-Arctic territories north of the 60th parallel. Nearly three quarters of the indigenous minority people live in rural areas

***Corresponding authors:** *Tatiana E. Burtseva, PhD, ScD. Deputy Director of the Yakutsk Research Center for Complex Medical Problems, Yakutsk, Russia. E-mail: bourtsevat@rambler.ru*
Sardana A. Evseeva, Postgraduate Student of Saint Petersburg State Pediatric Medical Academy, Saint Petersburg, Russia.
E-mail: sarda79@mail.ru

(80% in the Arctic) and are dispersed across vast territories. All these factors have a certain impact on the medical aid organization for the population [1,2,3].

Currently, Yakutia consists of 36 municipalities, including 34 municipal regions, 2 urban districts, 55 urban settlements, and 586 rural settlements. One-third of the population lives in rural areas; 50% of 676 settlements belong to the category of sparsely populated, 44% to the category of hard-to-reach and remote, and 12 districts have a population below ten thousand [4].

More than ninety percent of the Republic's territory is in the area of seasonal transport service, where communication is mainly by air, water and road (seasonal). Thus, 76% of 34 districts do not have reliable transport links with the center of the Republic and surrounding regions. The most remote village is situated at a distance of 3,189km from Yakutsk, and in the interior, the distance from the medical centers to the Central District Hospital averages about 400km and year-around travel is not possible. Almost 90% of local roads do not meet regulatory requirements. In Yakutia, there are 580 health agencies, including 20 republican institutions. Currently, in Yakutia, as in many regions of Russia, an unstable situation has been formed regarding medical personnel. Eight hundred and twenty-five doctors and 3,146 nursing staff work in health care facilities serving the rural population. During 2012, the number of doctors decreased by 38 people (0.8%), staff nurses by 103 people (0.9%). The ratio of the staff units (doctors:nurses) was 1:2.2. The coefficient of secondary occupations was 1.4 for doctors and 1.3 for nursing staff. The total number of salaried professional positions was 5,614 for doctors and 12,014 for nurses. In the healthcare system, vacant positions totaled up to 131.25 for the doctors and 114.5 for the nurses. The low physician staffing is a main problem for the Arctic regions.

In some regions of the Arctic zone, physician staffing does not reach 50%: for example, 49% in Allaikhovskiy, 49.4% in Verhojansk, 48% in Lower Kolyma, 47.8% in Oymyakon, and 46.6% in Ust-Yana. The increased migration of the population and departure of specialists outside the Republic affects very negatively the provision of medical services by medical personnel. In recent years, the number of retired doctors was approximately equal to the number received to work in health institutions. Thus, in 2012, 627 doctors left their posts: 115(13.7%) were due to natural decline and 428 to self-request and translocation. Newly arrived doctors amounted to 589.

The present situation with staff shortages and the low capacity of medical institutions leads to a lower quality of medical care in the remote and inaccessible locations of Yakutia.

Materials and Methods

To assess the quality of healthcare in Yakutia, we conducted an anonymous survey of parents and medical workers living in this region in 2012. In particular, we conducted an anonymous survey of parents living in 16 districts (Upper Kolyma, Lenskiy, Tomponskiy, Srednekolymskiy, Amginskiy, Verhnevilujskiy, Neryungrinskiy, Ust-Yanskiy, Ust-Maisky, Verkhoyanskiy, Aldanskiy, Olekminskiy, Suntarskiy, Ust-Aldaniy, Namskiy, Megino-Kangalasskiy) to determine the factors influencing the quality of medical care in remote settlements; 1,415 questionnaires were filled out. We also conducted an anonymous survey of medical workers living in 17 districts of Yakutia (Mirniy, Khangalasskiy, Zhiganskiy, Abyiskiy, Aldanskiy, Amginskiy, Neryungrinskiy, Namskiy, Suntarskiy, Kobyayskiy, Olekminskiy, Verhnevilujskiy, Olenekskiy, the Upper Kolyma, Ust-Mayskiy, Verkhoyanskiy, and Yakutsk) to evaluate their professional opinion on the state of rural health care; 322 questionnaires were filled out.

The next phase of the study was devoted to the organization of a pilot project on the implementation of automated medical examinations (ACE), namely, AKDO system in the Olenek district of Yakutia. AKDO systems for prophylactic examination of children are designed to provide comprehensive quantitative assessment of the state of health based on the aggregate of initial medical parameters obtained during prophylactic examination [5]. New models of AKDO system implement an extended range of instrumental examination. They provide:

- ECG with the 12- channel Valenta electrocardiograph
- Rhythmography
- Bicycle ergometry
- Phonocardiography
- Rheography
- Spirometry.

Modern AKDO systems consist of individual structural units designed to solve specific diagnostic problem. The number of pathology profiles screened by individual units may reach 300. AKDO systems provide quantitative comparative analysis of diagnostic data obtained at different periods of time or in different places.

The study was approved by the Yakutsk Research Center for Complex Medical Problems Ethics Committee. Written informed consent was obtained from the child's parents.

Results and Discussion

The results of data analysis of questionnaires were unexpected: on average, 88.3% of the respondents (the rural residents) were not satisfied with number of local medical offices: 63.5% of the residents of settlements nearest to the district center, 73.3% of the residents of the farthest settlements, and 92.8% of the residents of the remote and inaccessible localities.

According to the survey of parents, among the main reasons for the low quality of care was the lack of qualified specialists of different profiles (88.2%) and poorly equipped medical offices (71.7%). Lack of necessary medications, low qualification of the physicians, and expensive medications were noted in 60.2%, 39.8%, and 36.6% of cases, respectively.

One of the problems of rural health care in Yakutia is the poor availability of specialized and high-tech medical care. According to the respondents, they are unable to attend an examination or treatment in the district hospital during the spring and autumn thaw due to the lack of roads or, in summer, poor road conditions (36.2%). Other barriers are lack of money

for travel (34%), duration of travel (about 8-10 hours) to the district center (23.7%), and lack of transport (6.1%).

For example, one question asked, «How often do you visit the doctor to get information about your general health?» Sixty percent of respondents replied «rarely,» 36.4% "constantly,» and 3.6% «never.» To the question «To what purpose do you usually visit a doctor?" overall, 71.2% of the respondents replied «for treatment,» 16.7% «for clinical examination,» 4.5% «for dispanserization," and 1.7% "for preventive examinations." Respondents indicated the greatest need (75.4%) was for such specialists as a cardiologist, a neurologist, an ophthalmologist, and an otolaryngologist.

An anonymous survey of health staff working in the pediatric service of Yakutia revealed a number of negative health and social factors. Overall, 80.6% health workers are not fully satisfied with the quality of care in their healthcare setting. According to the respondents, the following factors influence their work: deficit of qualified specialists of different profiles (71.5%), poorly equipped medical offices (59%), lack of necessary medications (41.6%), and low level of physician and nurse staffing 40.1% and 19.7%, respectively). Most medical professionals (85.9%) consider that an outpatient medical record takes longer time than an examination of a child. Overall, 70.4% of respondents note the lack of cooperation between the family doctors, the staff of the school and preschool departments, and the qualified specialists of different profiles.

According to the respondents, 12.5% said that during the last 5 years the healthcare system in the districts has worsened, 48.5% that it has not changed, and only 39% that it has improved. Only 39.8% of the health staff is satisfied with their work. The majority of health professionals are not completely satisfied with their work due to a lack of time for self-education, low wages, and poor equipment in medical offices.

Based on the foregoing, the next phase of the study was devoted to the organization of a pilot project on the implementation of AKDO system to improve the quality of the medical examination of children in the Olenek district of Yakutia. AKDO systems belong to such equipment designed for early-diversified diagnostics of chronic diseases made within comprehensive examinations of children, i.e. a state program of regular medical check-ups of children [5].

The population of the Olenek district is 4,028 residents (2,679 adults and 1,349 children). The Olenek district consists of four villages. The purchased AKDO complex was directed to the different villages on schedule. In general, AKDO examinations were done as follows: A trained physician teaches the nurses or medical assistants how to perform an AKDO examination. The examination of one child takes about 20-25 minutes. AKDO conclusions are transferred from big and small villages to the doctor responsible for AKDO analysis at the Central District Hospital.

Currently in Russia, AKDO examination is voted as the most convenient for healthcare facilities with a small number of specialists to conduct the combined screening-diagnostic functions of the first stage. In the second stage of our project, a pediatric center specialist visited children in their place of residence to provide diagnostic and therapeutic services, taking into account the identified pathology profiles. The third stage was an expedition of the equipped mobile medical surgical team to the children's places of residence to provide specialized medical care (MoH order No.01-8/4-378 from 07.03.2012).

As shown in Table 1, cardiovascular and rheumatologic diseases together occupy first place (92.8%), followed by pathology of the endocrine system (50.3%), organs of vision (40.4%), dental disease (36.7%), and pathology of the respiratory system (21/1%), etc.

Table 1.

Prevalence of somatic diseases in child population of the Olenek district of Yakutia

Variable		Surveyed children		
		Total (n=166)	Boys (n=82)	Girls (n=84)
Health children	(n)	2	0	2
Children with different pathology profiles	(n)	164	82	82
cardiovascular and reumatic diseases	(%)	92.8	92.7	92.9
endocrine system	(%)	51.2	56.1	46.4
organs of vision	(%)	40.4	36.6	44.0
dental disease	(%)	36.7	36.6	36.9
respiratory system	(%)	21.1	24.4	17.9
nervous system	(%)	20.5	26.8	14
allergy	(%)	13.3	14.6	11.9
orthopedic pathology	(%)	12.0	18.3	6.0
otolaryngology disorders	(%)	11.4	13.4	9.5
gastrointestinal pathology	(%)	7.2	7.3	7.1

In the North, the incidence of health disorders among children is considerably higher than the national average and has increased in the past decade, as up to 70% of children suffer from health disorders [3]. Our early research showed that the overall prevalence of disease in children living in indigenous minority settlements in northern Yakutia was 117 cases per 100 children examined. Among Yakut and Russian children, the rate was 131 cases per 100 children; among children from indigenous minority groups of the North, it was 108 cases per 100 children.

The prevalence of disease in a particular region depends on the region's current level of access to health care [6]. Since the description of the child's condition in terms of AKDO includes the complaints and symptoms that can be recorded even on a pre-medical level, a wide involvement of nurses to perform AKDO surveys of children living in small villages seems reasonable and advisable.

Conclusion

Development of the concept of a comprehensive medical examination of a large number of children in a short period

of time, limited by the ability to involve highly qualified specialists only for a short time, determines the need for pre-qualification of the diagnostic problems to plan a rational medical examination [6-8]. Implementation of the structured approaches for the screening and diagnosis of childhood disease using modern automated technologies would make it possible to solve a number of difficult problems in the remote and inaccessible locations of Yakutia:

•improve early detection of diseases among children and adolescents

•organize standardized monitoring of children's health

•organize monitoring of the curative activity in medical institutions

•allocate healthcare resources judiciously and appropriately

•perform the development of healthcare system structure appropriately and efficiently.

Competing interests

The authors declare that they have no competing interests.

References

1. Petrova PG, Borisova NV. Environment Features and Human Health in the North**.** Int J Biomed. 2014;4(2):114-116.
2. Agadzhanian NA. Human adaptational physiology and ecology: culture, morality, and spirituality. Fiziol Zh 2011; 57(5):23-5. [Article in Russian].
3. United Nations in the Russian Federation. Climate Change Impact on Public Health in the Russian Arctic (2008) http://www.unrussia.ru/sites/default/files/doc/Arctic-eng.pdf
4. Gorokhov AC, Fedotov SF. Implementation of modern information systems in healthcare of the Republic of Sakha (Yakutia). Information Technologies in the Health System. Yakutsk, 2012:10-11.
5. Vorontsov IM, Matalygina OA, Shapovalov VV. Evolution of the automated system for preventive examination of children an automated unit for mass screening examinations. Med Tekh. 2005;3:18-21. [Article in Russian].
6. Burtseva TE, Uvarova TE, Tomsky MI, Odland JØ. The health of populations living in the indigenous minority settlements of northern Yakutia. Int J Circumpolar Health. 2014 Oct 30; 73. doi: 10.3402/ijch.v73.25758. eCollection 2014.
7. Buortseva TE, Tomski MI, Verbitskaya GG, Kanaeva GG, Chasnik VG, Nikolaeva LA, et al. Assessment of the child population's health status in places of compact residence of indigenous peoples of the North in Ust-Yanskiy, Srednekolymskiy, Allaikhovskiy and Lower Kolyma regions of the Republic of Sakha (Yakutia). Proceedings of the scientific-practical conference "Children's Health in the North". Yakutsk, 2008:7-9.
8. Vorontsov I M, Shapovalov VV, Sherstyuk YM. Creation and using of automated systems for monitoring and screening diagnosis of health disorders. SPb.: ed. «Costa», 2006.

International Journal of Biomedicine 5(4) (2015) 228-230

ORIGINAL ARTICLE

Biomedical Engineering

INTERNATIONAL JOURNAL OF BIOMEDICINE

Developing the Structure of a Hardware and Software System for Quantitative Diagnosis of Microhemodynamics

P.V. Luzhnov; T.O. Pika; D.M. Shamaev

Biomedical Engineering Department, Bauman Moscow State Technical University
Moscow, the Russian Federation

Abstract

Currently, vascular diseases are the leading cause of disability all over the world. Recent publications have pointed out microcirculation disorders as the main cause of vascular diseases. In this paper, we present an analysis of the existing diagnostic methods and identify the advantages, disadvantages and limitations of each method. The analysis showed that there are no accurate quantitative criteria for assessment and diagnosis of peripheral circulation in any of the methods. Our results can be used for the development of medical and technical requirements for hardware and software systems for quantitative diagnosis of microhemodynamic disorders. **(Int J Biomed. 2015;5(4):228-230.)**

Keywords: *microcirculation; vascular diseases; quantitative criteria of diagnosis.*

Introduction

Currently, vascular diseases (chronic venous insufficiency, chronic arteriosclerosis obliterans of the lower extremities, diabetic angiopathy, and others) are the leading cause of disability all over the world, including among people of productive age. Furthermore, there is a tendency to a growth in the number of patients with these types of diseases and a decrease in their average age. Recent publications have pointed out microcirculation disorders as the main cause of vascular pathology [1]. The main problems occurring in the research of such disorders are as follows [2]:

(1) a lack of quantitative criteria and accepted methods of diagnosis, which leads to the detection of the diseases at later stages and late treatment,

(2) the impossibility of developing new methods of treatment and the efficiency investigation without monitoring microcirculation condition.

For these reasons, the choice of a method for quantitative evaluation of microcirculation parameters is the main issue in the diagnosis and treatment of patients with vascular diseases.

Methods

The main problems in microcirculation research stem from the extremely small size of microvessels and their high branching. Nailfold capillaroscopy, reflecting microvascular function in all parts of the body, has been the main method of microcirculation research so far. There are several main advantages and disadvantages of this method (Table 1) [3,4].

Table 1.

Advantages and disadvantages of capillaroscopy

Advantages	Disadvantages
Simple and non-invasive	Impossible to give a quantitative characteristics
Instant visualization	Results depend on the experience of the medical staff
The possibility of a detailed evaluation of all parts of the microvasculature	
The availability of biological objects	
A lack of significant anatomical features in the region of interest	

Currently, the most routine methods for research of regional blood flow and microcirculation in clinical practice are the following [5-7]: transcutaneous oximetry, laser Doppler flowmetry (LDF), high-frequency Doppler ultrasound, tissue oximetry, radionuclide methods, impedance plethysmography, and photoplethysmography. We made a comparative analysis of these methods. We found that each method has its advantages and limitations, but none of them complies fully with clinicians' requirements. Due to this deficiency, it is recommended to use combined methods for microvasculature assessment [8].

Results

Our analysis of the existing diagnostic methods showed that when used together they give superfluous information about microcirculation parameters (Table 2). Unfortunately, there are no accurate quantitative criteria for assessment and diagnosis of peripheral circulation in any of the methods. Moreover, each technique has its significant disadvantages and limitations. Therefore, the problem of quantitative assessment of peripheral circulation is urgent and promising.

The purposes of the subsequent stages of the study are to:

- develop a structure of a hardware and software system for quantitative diagnosis of microhemodynamics (solution of diagnostic issues), and
- develop software and algorithmic tools for biorelevant therapy based on the received diagnostic data (solution of therapeutic issues).

A simplified scheme of a developed bioengineering system is shown in Fig.1. It reflects therapeutic and diagnostic circuits. Thus, this system corresponds to the purposes of the subsequent stages of study.

Table 2.

Comparative analysis of different methods for diagnosis of peripheral circulation

	High-frequency Doppler ultrasound	LDF	Transcutaneous oximetry	Tissue oximetry	Radionuclide methods	
					Perfusion scintigraphy	Clearance method
Functional principle	Doppler effect		local heating of tissue	the interaction of photons with biological tissue	RFP loading with registration of relevant parameters	
Probing depth	3.5…8 mm	~ 1 mm	surface layer of the skin	~ 2 cm	skin, muscles	
Registered parameters	- linear and volumetric blood flow velocity; - rheographic index	- microcirculation index; - mean perfusion in the microvasculature; - average blood flow modulation; - coefficient of variation; - bypass index; - spectral characteristic	- partial pressure of oxygen in the surface layers of the skin ($TcpO_2$)	-absorption coefficient (μ_a); - transport scattering coefficient (μ_s'); - concentrations of oxy- deoxy- and total hemoglobin ([HbO_2], [HHb] and [THb]); - tissue oxygen saturation (StO_2)	radiation intensity of accumulated radiopharmaceutical	radiopharmaceutical washout period
Using the functional tests	is required for increase the information content of research	is required as one of the stages of research	is required for increase the information content of research	is required for increase the information content of research	not required	is required for increase the information content of research
Availability of quantitative criteria for diagnosis	-	±	±	-	-	-
Features of the method	- integrated of estimated parameters; - a limited number of technical implementations; - a limited number of measurement techniques				- possibility of investigating micro-circulation in the skin and in muscle; - possibility of differentiating nutritional and bypass blood flow.	
	- audible and visual control of sensor installation; - determine the type of vessel in the form of the curve; - evaluation of the direction of blood flow	- understanding of the results is more difficult than the study itself; - data obtained in the foreign literature can't be used in Russia	- some algorithms successfully used in clinical practice	- insufficient information about the application in Russia; - absence of correct algorithms for calculating parameters		
Examples of devices	Minimax-Doppler-K (Minimax, St. Petersburg)	LAKK-02 (LAZMA, Moscow)	TCM400 (Radiometer, Denmark)	OxiplexTS (ISS Inc., USA)	Sigma–410S (USA, Germany)	DIACAM (Siemens, Erlangen, Germany)

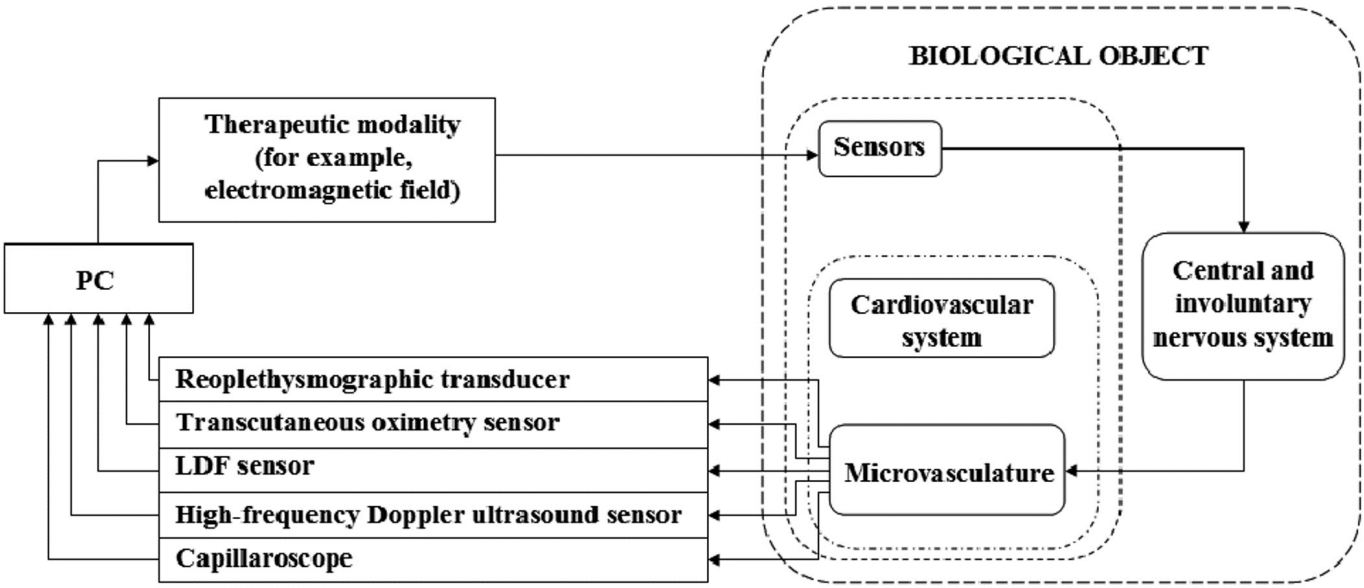

Fig. 1. *A simplified scheme of a developed bioengineering system*

Our results can be used for the development of medical and technical requirements for hardware and software systems for quantitative diagnosis of microhemodynamic disorders.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

This work was supported in part by RFBR, research project No. 15-08-99682.

References

1. Koshkin VM, Karalkin AV, Saitova GD, Nastavsheva OD. Micro- and macrocirculation in the lower extremities in patients with various forms of chronic venous insufficiency. Regional Haemodynam Microcircul. 2004; 10: 47-51.
2. Zolotnitskaya VP, Leontieva NV, Talantov SV, Andrushina NB, Sedletskaya EYu. Radionuclide diagnose in the control of the efficiency of laserotherapy in patients with pathology of lower extremities. Regional Haemodynam Microcircul. 2002; 3:28-33.
3. Berezhanski PV, Melnikova IM, Mizernitski YuL. Modern views on the participation of microcirculatory alterations in the pathogenesis of allergic inflammation. Regional Haemodynam Microcircul. 2012;44: 4-11
4. Kulchitskaya D. Laser Doppler flowmetry in assessing the efficiency of physiotherapy effects. Cosmetics & Medicine. 2009; 2: 22-27
5. Petrishchev NN, Vasin EY. (2009) A method for determining the reactivity and microvascular endothelial vasomotor function using high-frequency Doppler. Retrieved from http://minimax.ru/2011/07/19/sposob-opredeleniya-reaktivnosti-sos/
6. Russian consensus "Diagnosis and treatment of patients with critical limb ischemia." Retrieved from http://diabeticfoot.ru/d/176414/d/rossiyskiy_konsensus.pdf
7. Luzhnov PV, Shamaev DM, Iomdina EN, Tarutta EP, Markosyan GA, Shamkina LA, Sianosyan AA. Transpalpebral tetrapolar reoophtalmography in the assessment of parameters of the eye blood circulatory system. Vestn Ross Akad Med Nauk. 2015;70 (3): 372-7 [Article in Russian].
8. Azizov GA. Estimation of the microcirculation disorders′ rate in vascular diseases of the lower extremities using the functional tests. Regional Haemodynam Microcircul. 2006;17: 37

International Journal of Biomedicine 5(4) (2015) 231-234

ORIGINAL ARTICLE

Psychology and Neuroscience

INTERNATIONAL JOURNAL OF BIOMEDICINE

A Mythologem as a Determinant of Goal-Directed Behavior

Alexander G. Kruglov, PhD, ScD

Central Research Institute of Radiation Diagnosis
Moscow, the Russian Federation

Abstract

The construction of a principled and generalized model of a mythologem (ML) as psychogenic structure having psychological, electric, magnetic, hemodynamic, and time equivalents is the result of this study. Periodic invariant sensory patterns, creating a dominant center, form a stable neural network, the result of which is an ML. Within the framework of the ML model construction, the partial identity of ML and a motive, the image of the goal (IG) is accepted. The location of the IG was attributed to the expected result, which is located outside the attainable. The ML equivalents were defined as properties of a unified psychogenic structure initiating the forms of goal-directed behavior, which are unable to achieve the final goal, and a homeostatic balance, as a final result. We believe that anticipatory, prospective formation of IG, initiating adaptive forms of behavior, is a manifestation of a general principle of "anticipatory response" (the formation of the future in the present moment) inherent for somatic and mental spheres of human ontogenesis. **(Int J Biomed. 2015;5(4):231-234.)**

Keywords: *mythologem; the image of the goal; motive; goal-directed behavior; subjective time.*

Abbreviations

ML, mythologem; **IG,** the image of the goal; **MG**, motivational gradient; **ST**, subjective time; **DC**, dominant center; **GDB**, goal-directed behavior; **EDS**, electrodynamic structure

Introduction

In accordance with modern ideas set out in the research of a number of authors, ML is generally regarded as a figurative, symbolic way to display reality, which is required when the reality does not fit into a formal-logic and abstract display. ML is a stable construct, generally reflecting reality in the form of sensory-specific associations perceived as objective reality [1]. Man lives in the power of illusion, which is so strong that it seems as normal consciousness [2]. ML is capable of directing the activity of the individual and mobilizing this activity to achieve significant goals [3].

The objective of this study was to build a model of ML as a psychogenic, stable construction having psychological, electric, magnetic, hemodynamic, and timing parameters of the neural networks (associations) that provide the formation, the initiation, and the existence of the ML. Understanding the concept of the formation and stability of ML will help in finding the antithesis of the confidence [4] in the invincibility of ML.

Materials and Methods

To create the model of ML as a meaningful unity of the neural networks having the psychological, electrical, magnetic, hemodynamic, and timing parameters, we used our own previously published data (the parameters of hemodynamics and biochemistry of the human brain obtained by invasive methods) [5-8] and data of other authors.

Result and Discussion

ML fixates the sequence, the order of the representation elements about the reality, and serves as the conceptual basis of behavior that allows us to functionally consider ML as a "motive" for specific forms of behavior. The motive is the cause of behavior and consists of a set of dispositions, the most important of which is the need [9]. A need is defined as a state of dissatisfaction of the organism (the personality) and a

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

deficit of what is required to maintain homeostatic balance of the organism (the personality). A special feature of "organized matter" is the selectivity of the reaction that is the subject of urgent needs, i.e. elimination of the gradient between the current status and the target status, the result for which the activity is undertaken, namely, the image of the future results generated by the subject, defined as IG. IG is a leading element of the conscious regulatory process. We think it possible, within ML modeling, to determine the partial identity of the ML and IG (ML~IG).

Association of the interneurons activated to provide GDB, whose ultimate aim is the achievement of parametric equalization and the state of homeostatic balance, we consider as a possible substrate for ML as the long-term circular structure [5]. We believe that the periodic afferent stimulation, which has a constant parametric set during the exposure time (the invariant sensory pattern – training, persuasion, suggestion, etc.) is the initial step in the formation of ML/IG. The sensory afferent stimulation of synapses by the repetitive, monotonous sensory patterns (short- and long-term potentiation) creates the "beaten paths" to facilitate the passage of this particular set of patterns. The formation of the neural "beaten paths" during a quite long afferentation leads to the creation of the sustainable neuronal formation – a dominant center (DC). A DC with the properties of persistence, the concentration of an excitation from other nerve centers, the inhibition of functionally incompatible centers (i.e. filtering the input parameters of the sensory information) determines the nature of the current reactions to the time of its existence. We believe that this consistently structured neural formation creates the IG. It should be noted that the mind has the ability to create the pathological dominants, determinant, and systems [10], and to design images which do not reflect the reality but are accompanied by the sensory, cognitive, and behavioral equivalents with preserved consciousness (phantom pain, some hallucinatory syndromes).

The activity of neurons reflects their involvement in the system, where the organization of all processes is the informative equivalent of the result, i.e. the future [9]. The completion of the IG construction as a result of the successive sensory stimulation, in our opinion, is subjectively experienced as a state known by definition as insight. The IG creation activates the efferent structure described by us [5] as a motivational gradient (MG). The ultimate goal of MG implemented through GDB, is to achieve the status of parametric balance, homeostatic balance. The achievement of the expected utilitarian goals (food, sexual behavior, etc.) leads to the restoration of homeostasis, i.e. elimination of the MG. In contrast to the short-term and "tactical" goals, the forward-looking plans for the achievement of the "strategic" goals covering large time intervals have the discrete structure of MG and the subjective time (ST) (i.e. phenomena, accompanied by the expected changes in the parameters as a result of GDB, may be formed in the interval-separated time periods, at the same time being in the plot(story)-caused sequence of the events) [5].

ML, being a set of universalized symbols, has an ambivalent nature, being both a complete construct and an integral part (element) of the new constructs. Two main interacting elements of a symbol, an image and a meaning, exist only inside of the interpretations [11] where the bijective isomorphic coordinate accordance of any operational IG to the final (ideal) IG is impossible. At the same time, there is convincing evidence of a homomorphism, where full compliance with the ideal IG is impossible, but some degree of similarity is achieved. In the absence of the concretized and utilitarian form (image) during our movement to a symbolic goal, new coordinating and interpretive combinations of IG and a semantic content are formed, which create a fractional and operative image without the possibility of reaching the ideal IG. Consequently, the movement towards a symbolic goal (ML) which has not the specifics of the utilitarian goals and time constraints, creates a new degree of similarity and cannot be completed. In other words, the ML construct has the nature of the expected IG located outside of the actual achievable goal.

Considering the vector of GDB as the direction of a speedy change in the scalar variables, whose values are changed from one point in space to another, we define a "zero" (starting) point of ST in the structure of ML on the expected result of the action, i.e. IG. In psychological time, only the expected outcome (goal), having fixed coordinate specifications, can be taken as an abstract point (which is in the future), while the present is always a certain time interval [12]. Thus, the IG formation is a psycho-physiological stimulus and starting point in the formation of the grading scale of the result achievement, namely, ST. Perhaps a prediction exists as a principle (probably genetic) of the formation of an organism (the personality). In 1930, G.E. Coghill [13] described how during embryogenesis the anatomical structures are formed long before their demand in future behavior (anticipatory structure versus the function). Several authors [14] have described the neurobiological basis of the phenomenon of the "mental anticipatory response." IG formation demonstrates the "anticipatory function" versus the structure.

ML/IG formed by the repetitive patterns of sensory impact on the object has the expected time of existence until the achievement of the ultimate goal, the elimination of the imbalance and the restoration of the parametric equilibrium of the homeostasis. The fundamental properties of the living organisms belonging to the open nonequilibrium systems are their vibrational nature. The organism as a unified formation exists due to the synchronization of the rhythmic changes (the adaptive oscillations) at all hierarchical levels, from the molecular-cellular level to the organism level. Since the ultimate goal (IG) is unattainable, it is impossible to eliminate the imbalances and the restore the homeostatic balance, regardless of the amplitude and duration of the adaptive vibrational changes. The existence of the permanent IG/ML inducing the vector activity initiates the "vibrational" search for forms and vectors of GDB, which is implemented by the cognitive and behavioral activity. The strategies of the IG/ML implementation with (slightly) ineffective negative reverse relations and the conservation (increasing) of the reverse positive relations (filtration of the input sensory information), result in a loss of the correction-concordance of the GDB

stages to the intrasystem and integrative mechanisms of the central nervous system. In addition, the amplitude of the adaptive and search oscillations of homeostasis decreases, a resistance increases, and the system becomes rigid. The natural result is the development of various forms and degrees of change in thinking and consciousness, as well as reduced energy potential and other manifestations of a long homeostatic imbalance and desynchronosis.

The formation of the activity of the neuronal association in the form of the electrodynamic structure (EDS) circulating through the neural network until the achievement of the result is accompanied by the metabolic support of the exchange fields of the activated neurons [8]. The electrical field of the neuron is largely flattened for the external registrars due to nonlinear dissemination of EDS and the electrical insulating properties of the membranes of astrocytes and meninges, which perhaps explains the still existing differences in EEG decoding. The increase in blood flow (i.e. number and speed of blood flows) through the vessels of the brain tissue is an haemodynamic equivalent of this activity. In the tissue in the cortex and white matter of the brain, the regional linear (in the radial vessels) and local (functional hyperemia) streams of the charged particles of blood are intensified. In accordance with V.P. Oleinik [15], the blood is regarded as an electrolyte, while the bloodstream is regarded as a conductor placed in an external magnetic field. In a moving conducting fluid, the ponderomotive forces directed perpendicular to the linear movement of the blood – the magnetohydrodynamic forces (a magnetic dipole) – are formed. Magnetic properties of blood flow (electrolytes), having an essentially linear (radial vessels) character in the regional supply of the increased metabolism of the exchange fields of brain tissue and spreading perpendicularly to the axial potential do not have the insulating barriers in the form of biological tissue, as an interaction with intracerebral structures, as well as for the external recorders. In biological systems, the effect of an induction occurs under impact of the extremely weak (nano- and picotesla) alternating magnetic fields, where the primary receptor is a water-salt component, while the primary target is the nuclear spin of hydrogen atoms [16].

The alternating electric fields, with the parameters imitating the Schumann's resonances (Konig, 1974: cited by Lednev VV et al. [16]) and defining the circadian rhythms (Wever, 1974: cited by Lednev VV et al. [16]), influence the psycho-physical condition of the person. The match between the Schumann resonance frequency and the alpha-rhythm of the human brain (7.83 Hz) suggests that the response to the external action depends on a matching (resonance) with frequency parameters which are identical (close) to the characteristics of the intracerebral electrical and magnetic dipoles (the neural circuits, including an inactive).

The data of Konig, Wever, et al., in our opinion, reflect the external manifestation of the activity of neuronal structures of the first signal system of the brain activated due to external influences: an induction by external magnetic fields. We believe that the main mechanism of this interaction is a response of the brain (neural) structures with electromagnetic properties (dipoles) to the external stimulus with identical (close) frequency characteristics (resonance). In the absence of the primary afferent sensory information from the known receptors, we consider the possibility of the direct resonance effect on the neural structures of the brain during exposure to the external magnetic field with the actualization of the brain dipoles coinciding in frequency characteristics. A demonstrative example of this effect, possibly related to the unconditioned reflexes, in our opinion, is animals sensing that an earthquake is about to happen and fleeing from it, even at a distance of 200 km from the epicenter. Thus, by our assumption, the initiator of mental activity, along with the sensor variables, may be an external vector of magnetic induction with the effect of a direct impact (resonance) on the neuronal circuits (magnetic dipoles) of the brain.

We believe that the registration of the magnetic fields arising during the activation of mental activity largely relates to the parameters of the resulting multipole magnetic field that is a result of vector addition of magnetic dipoles: EDS of the activated neural associations and regional vessels (interdependent distributed systems) having different latency intervals and angular vector differences. We also believe that the differences in the genesis of the magnetic induction should be considered during detailed coding of the results of the magnetographic mapping.

ML, in our opinion, may have objective, measurable parameters: 1) voltage variation of the magnetic fields of the brain of different origin; 2) hemodynamic equivalent with a change in blood flow and shape of the vessel in the exchange field of the activated neurons in the veins and venules of the brain tissue with a diameter of 60–250 microns [5].

In this work, deliberately simplifying the structure of the components, we considered only the most general principles of the structural construction of ML and the gradient behavior under the influence of ML. Our follow-up works will be focused on the following issues: effects of the methods of external influence, which create and initiate ML formation; the filtration of the input information; the transformation of the character traits of the individual under the ML impact; the priority of ML mainstreaming; tunnelization of thinking and narrowing of consciousness; ML interaction and influence on unconditioned reflex behavior; and objectification of ML markers.

Conclusion

Regarding MLs as complexes of the psychological, electrical, magnetic, hemodynamic, and temporary equivalents, we define them as properties of a unified psychogenic construct initiating GDB to achieve the ultimate goal: the homeostatic balance of the subject. Localization of the starting point of ST, the timescale for the rate of the result achievement on ML/IG that is located outside accessibility, determines the coordinates of the actualization of the structures of GDB, which are unable to achieve the ultimate goal.

We believe that anticipatory, prospective formation of IG, initiating adaptive forms of behavior, is a manifestation of a general principle of "anticipatory response" (the formation of the future in the present moment) inherent for somatic and mental spheres of human ontogenesis.

References

1. Jung KG. *Archetype and Symbol.* M.: Canon; 1991.
2. Berdyaev NA. *Slavery and Freedom*. M.: ACT; 2006. [in Russian].
3. Toporkov AL. *Myth: tradition and psychology of perception. The myth and mythology in modern Russia*. Moscow; 2000. [in Russian].
4. Cassirer E. Technique of modern political myths. Vestn Moscow State University. 1990; (Ser. 7, Philosophy) 2:58-65. [in Russian].
5. Kruglov AG, Gebel GY, Vqsilyev AY. Intracranial venous pressure as a possible determinant of mental regulation. Int J Biomed. 2011; 1(4):245-8.
6. Kruglov AG, Gebel GY, Vqsilyev AY. Impact of intra-extracranial hemodynamics on cerebral ischemia by arterial hypertension (Part 1 and Part 2) IJBM 2012; 2(2):89-101.
7. Kruglov A, Vasiliev AYu. Mathematical approaches in studying the ideal image of the goal. Int J Biomed. 2014; 4(1):46-48.
8. Kruglov A, Vasilyev AYu. Psychological image as an active distributed system. J Int Scien Publ Mat Met Tech. 2014; 8:808-11.
9. Shvyrkov VB. *Introduction to objective psychology. Neuronal basics of psychics*. Moscow: Psychology Institute of RAS (Russian Academy of Sciences); 1995. [in Russian].
10. Kryzhanovsky GN. *Discirculatory pathology of the nervous system*. M.: Medinformagenstvo; 2009. [in Russian].
11. Losev AF. *Essays on ancient symbolism and mythology*. M.:Nauka; 1993.
12. Rubinshtein SL. *Fundamentals of general psychology*. St. Petersburg; 2003. [in Russian].
13. Coghill GE. Correlated anatomical and physiological studies of the growth of the nervous system of amphibia. The mechanism of association of Amblystoma punctatum. J Comp Neurol. 1930; 51:311-375.
14. Rajewski KS, Georgiev VP. Mediator amino acids: neurochemical and neuropharmacological aspects. Moscow; 1986. [in Russian].
15. Oleinik VP. *Fundamentals of the interaction of physical fields with biological substances*. Kharkiv: National Aerospace University; 2006. [in Russian].
16. Lednev VV, Belova NA, Rozhdestvenskaya ZE, Tyros KhP. Biological effects of weak variable magnetic fields and biological precursors of earthquakes. Geophysical Processes and the Biosphere. 2003; 2(1):3-11. [in Russian].

International Journal of Biomedicine 5(4) (2015) 235-237

INTERNATIONAL JOURNAL OF BIOMEDICINE

MINI-REVIEW ARTICLE

Psychology and Human Behavior

Innovation: Love, Passion, or Both?

Nik Tehrani[1]*, PhD; Jahan Ghofraniha[2], PhD; Cynthia Meckl-Sloan[3]

[1]DynoSense Academy
[2]Northwestern Polytechnic University, [3]Argosy University, USA

Abstract

Innovations are heavily influenced by both love and passion. First, innovation happens for the love of what one does, but passion keeps it going. Hence, it is through love and passion by which innovations are both born and nurtured. Human passions fuel innovations, and without these emotions, innovation would be impossible. Passionate innovators share their passion with others. **(Int J Biomed. 2015;5(4):235-237.)**

Keywords: *Love; passion; innovation.*

Introduction

There is an old saying that "necessity is the mother of invention." However, the question arises is how that necessity is formed and prioritized to inspire a solution. While an invention is the application of a new idea, it takes innovation to make it marketable. There are many tools for innovation, such as creating a culture that nurtures innovation, reducing the risk of sharing ideas, having constraints (price, time frame), and risk-aversion [1]. Innovation pioneers, like all innovators, have access to the same tools for innovation that all visionaries have access to, but some visionaries go beyond them to make a better world or by disrupting the current paradigm. Many innovative pioneers changed the future, first through invention followed by innovation.

Many innovators have gone beyond universal basic tools with imagination in their thinking. They have seen potential in the world that did not previously exist and harnessed technology to make it a reality, sometimes being successful and sometimes not. So, then, is it possible that the innovation of visionaries goes beyond basic tools and still have a strong motivation that keeps them going in the face of failures? Might there be another factor or factors that keep them on track until they reach their goals? Is it possible that love and passion are intangible factors that drive success?

Thomas Edison once remarked about his lengthy trial and error toward a working lightbulb that he had not failed, but rather "just found 10,000 ways that didn't work" [1]. Even before reaching 10,000 frustrating failing attempts, many potential innovators might have thrown up their hands and given up, but Edison's passion for finding the right electric lightbulb was stronger than his unsuccessful attempts. Nikola Tesla, who devoted all of his energy to science, is best known as the father of alternating current. He had many ideas that simply did not work the way he wanted them to, yet he kept on going. Tesla was also often ridiculed for suggesting inventions that seemed impossible, but, out of passionate belief that they would work, he invented them anyway [2].

Passion is the driving source on the road to success of startup companies and gives the ability to resist naysayers and stay focused in the face of adversity and failures. If one is not passionate about one's idea, how would he/she be able to successfully communicate the vision and mission of the company to others [3]? Passion is the fuel of the innovative fire and the burning desire to share that passion, but it's inadequate to just love the process; it is essential to have a burning desire to share that passion [4]. Tesla's Elon Musk shared his passion for electric cars by opening the company's technology patents to the automotive industry. According to Musk, Tesla could not conceivably manufacture electric vehicles quickly enough to alleviate the carbon crisis, so he allowed his company to embrace an open-source policy to provide entire industry with proprietary technology to fight the problem together [4].

Musk showed his entrepreneurial spirit early in his life. While a student attending the University of Pennsylvania, he paid his own tuition and expenses by converting his house into a party club and throwing huge parties. Musk went on later to invent Pay Pal, which he sold to eBay in 2002 for $1.5 billion. Since then, Musk has used creating and innovating as his life's

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

purpose by working an average of 100 hours a week. But is hard work what drives Musk toward innovation? [5]. It has been said that Musk is driven by ideas that promise to profoundly alter life today as we know it: space, clean energy and the internet. As a result, his passion has been to create businesses that focus on the commercialization of innovations that have impacted, or will impact all three [6].

Modern-day innovator, Steve Jobs changed the face of modern technology. In 1997, Jobs said, "Apple is not about making boxes for people to get their jobs done, although we do that well. Apple is about something more. Its core value is that we believe that people with passion can change the world for the better" [7]. The one quality of all successful entrepreneurs is that they don't do it for the money. They're passionate about their mission. [8]

Other innovators also have had a relationship with love and passion. Dan Bricklin, inventor of VisiCalc, known as the father of electronic spreadsheet, once said that unless one finds a true calling and love for the craft, the "risks may outweigh the rewards" [4]. It takes more than training or talent, because unless the timing is good, it isn't enough. An innovator needs to have a true passion for the challenges ahead and to be prepared for when the time is right for new ideas. Virgin Group's Richard Branson has said that "innovation begins with either a passion or a problem" [9]. Passion motivates innovation that, in turn, transforms that "passionate idea into a tangible business" [9]. Like Musk, Branson shares innovation with others. "Passions that lead to world-changing insights are rarely self-centered, navel-gazing activities" [9]. Instead, says Branson, sometimes the passions focus on making a positive effect for others.

Undeniably, the power of passion and love is immeasurable and unpredictable. There are no other forces that can be compared to their powerful hold on humans, but they are ignored in most scientific discussions. The language of science has evolved to make personal and subjective themes such as love and passion inconsequential [10]. But, is it through love or passion that human beings achieve the inspiration to innovate? Innovation has been described as doing what you love to do. But, can innovation also spring, not only from what one loves to do, but also from love felt for another human being?

Many innovations are centered on passion on love of humanity by creating medical breakthroughs that alleviate certain kinds of human suffering. The article "Monitor Diabetes From Your Smart Watch" [11] is the story of a father, John Costik, who, out of love for his young son, Evan who was diagnosed with type-I diabetes, altered a smart watch to help him monitor Evan's glucose. Costik and his wife had to keep tabs on Evan at 5 minute intervals because it was difficult to determine the correct dose of insulin for a meal's carbohydrate content, as Evan's blood sugar could shoot up or decrease drastically without warning. Costik and his wife were paranoid and afraid for Evan's life, which made the experience of the disease a lot more restricting for the boy. Not only was Evan missing out on many childhood pleasures, like the freedom to go outside and play, he was also under constant observation and was felt different from his friends. Moreover, Costik and his wife could not keep watch on him every minute of every day, even though they tried. Even when Costik dropped Evan off at the daycare, he wanted school nurse check Evan's glucose level at all times and be alerted when it was progressing out of the acceptable range.

Costik, an engineer, came up with the innovative idea of connecting a Dexicom G4 CGM to a Motorola phone which uploaded all of his son's glucose information to a spreadsheet that viewable in Google with the help of a simple C# program. This allowed the family to receive and monitor all of their son's health data, such as glucose levels, without having to be with him personally all the time. When Costik announced his innovation on Twitter, Lane Desborough, another dad whose child had diabetes, responded. Out of love for his son also, Desborough asked Costik for the C# program and eventually developed Nightscout, a predictive system that could alert glucose levels by accessing essential information compiled in a database. Desborough made the software open-sourced out of love, so others could benefit from it. Desborough, Costik and others continued to collaborate, and in early 2014, they provided an Android app, Pebble watch, and Nightscout code open source, improving the code and making it easier to use.

Love is a strong emotion that motivates humans and has the power to influence physical change. Medical solutions are most often inspired by the innovator's experience with the disease or illness, just as in Costik's case. It was Costik's love for his son to give him as much of a normal life as possible, despite his condition that inspired him to invent a system which could provide his son with the freedom to live a normal life like other children without constant surveillance.

Long [9] correlates the commitment and dedication to get the job done to passion and love for the mission. "You only get out what you put in. It's easy to dedicate everything you have to something you love". He states that most failed ventures do not necessarily fail because of a bad idea but due to lack of passion in their mission. In 2005, Steve Jobs said in a Stanford commencement speech: "You've got to find what you love. The only way to do great work is to love what you do. If you haven't found it yet, keep looking. Don't settle. As with all matters of the heart, you'll know when you find it" [12]. Costik is a father who followed his heart through love to help improve his child's health through innovation, shared his idea, and had the passion to follow it through.

Conclusion

It is not enough to just have a lot of good innovative ideas for success. It also takes love for the journey of discovery and passion to make it happen. Love is an unstoppable force that can overcome pessimists and bring innovation to the brink of obsession. Finding love and passion to face and overcome the challenges of new ideas can result in the impossible becoming possible, just as Nikola Tesla, Thomas Edison, Steve Jobs, Elon Musk, Richard Branson, and John Costik were able to accomplish. In the end, the innovation must be shared for it to actually become a disruptor, like the electric light bulb, or become the foundation for further innovation, much like Costik's glucose monitor.

References

1. Conway Management News. (2015). The innovator's dilemma. Retrieved from http://www.conwaymgmt.com/pdfs/NL21-5-Innovation-NecessityIsTheMotherOfInvention.pdf
2. Earthlink. (2015). Some interesting facts about Nikola Tesla. Retrieved from http://home.earthlink.net/~drestinblack/didyouknow.htm
3. Morris, L. "The Innovation Master Plan", from The CEO Guide to Innovation, Chapter 1, Special Edition for InnovationManagemen.com
4. Newquist, E. (2015). 7 characteristics of highly successful innovators. Retrieved from http://www.innovationexcellence.com/blog/2015/03/13/7-characteristics-of-highly-successful-innovators/?Itemid=92#sthash.jQYLcnO0.dpuf
5. Vance, A. (2015). Elon Musk. Inventing the future. Retrieved from http://www.mahindra.indiareads.com/book/9780753555620/elon-musk-inventing-the-future/biography-autobiography-general/
6. Innovation. (2015). Ordinary people change the world. Retrieved from http://oninnovation.com/videos/detail.aspx?video=1258&title=Ordinary People Change The World
7. Brainy Quotes. (2015). Steve Jobs. Retrieved from http://www.brainyquote.com/quotes/authors/s/steve_jobs.html
8. Gregerson, H. (2015). How Richard Branson turned his passion into a tangible business. Retrieved from http://fortune.com/2015/05/12/how-richard-branson-turned-his-passion-into-a-tangible-business/
9. Long, J. "7 Reasons rock star entrepreneurs hit home run after home run", Entrepreneur, June 15-2015
10. Gaines, S. (2001). Sex, love and science. Nature, 413(6853), 255. doi:http://dx.doi.org/10.1038/35095130
11. Costik, J. (2015). Monitor Diabetes From Your Smart Watch. Spectrum.ieee.org. Retrieved 27 June 2015, from http://spectrum.ieee.org/geek-life/hands-on/monitor-diabetes-from-your-smart-watch/?utm_source=techalert&utm_medium=email&utm_campaign=052115
12. Jobs, S. (2005). 'You've got to find what you love,' Jobs says. Commencement address. Stanford News.Retrieved from http://news.stanford.edu/news/2005/june15/jobs-061505.html

International Journal of Biomedicine 5(4) (2015) 238-239

SHORT COMMUNICATION

Plant Physiology and Biochemistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Non-specific Adaptive Reactions of Athletes: Evaluation and Correction

K. N. Naumova[1]; V.V. Anshakova[1], PhD; B. M. Kershengolts[2]

[1]North-Eastern Federal University
[2]Institute of Biological Problems of Cryolithozone SB of the RAS
Yakutsk, the Republic of Sakha, Russia

Abstract

This work studies changes in non-specific adaptive reactions (NSAR) of athletes who practice Wushu and Qigong and take Kladorod, a biological product made from plant material. The results of our study demonstrate the effectiveness of Kladorod as a remedy to enhance adaptive capacity with the possibility of application for training of athletes without any restrictions within the criteria of doping control. **(Int J Biomed. 2015;5(4):238-239.)**

Keywords: *non-specific adaptive reactions; sport; biocomplexes; mechanochemistry; lichens; salidroside.*

Introduction

The physical state of an athlete can be reliably determined by blood test. According to results, we can reveal different types of NSAR: positive - stable and robust activation of training; transitional - unstable practice and unsustainable activation; negative - stress and reactivation.

The reactions of "training" and "activation" of high levels of reactivity are the most common physiological reactions, constituting the basis of non-specific health standards.

At the present stage of development of the sports, pharmacological support is one of the most popular tools for enhancing adaptive capacity and retention of an athlete in positive NSAR. For this purpose a biological product, Kladorod, which is based on a mechanically activated, ultrafine powder mixture of the lichen thalli of Cladonia and the roots and rhizomes of Rhodiola rosea, was designed [1]. In our earlier studies, it was found that taking a biological product it was found that taking a biological product according to a specific regimen increases its efficiency [2].

The aim of this study was to determine the criteria of disadaptation changes in athletes before and after taking Kladorod.

***Corresponding authors:** Vera V. Anshakova, PhD. North-Eastern Federal University, the Republic of Sakha, Russia. E-mail: anshakova_v@mail.ru*

Material and Methods

Twenty six sportsmen from the Wushu and Qigong Center "Heavenly River" of the Republic of Sakha (Yakutia), with various sports qualifications and aged between 20 and 43, were given 4 Kladorod capsules a day for one month. A general blood test was performed on the hematology analyzer Abacus. The definition of the NSAR phase was estimated by the method of L.H. Garkavi [3].

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

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the North-Eastern Federal University Ethics Committee. Written informed consent was obtained from all participants.

Resilts

The features of phase changes the NSAR of athletes engaged in Wushu and Qigong while taking Kladorod (Group 1, n=10) and those taking mechanically activated roots and rhizomes of Rhodiola rosea (Group 2, n=8), compared to those taking a placebo (Group 3, n=8), are shown in Table 1.

Table 1.

The features of the NSAR phase changes

Group*	NSAR type						Yates' χ2 , P	
	Negative n (%)		Transitional n(%)		Positive n (%)		BAR	ARA
	BRA	ARA	BRA	ARA	BRA	ARA		
Group 1	3(30)	1(10)	2(20)	2(20)	5(50)	7(70)	χ2=0.938 *P=0.625*	χ2=6.338 *P=0.04*
Group 2	2(25)	0	3(37.5)	5(62.5)	3(37.5)	3(37.5)	χ2=0.047 *P=0.977*	χ2=4.547 *P=0.103*
Group 3	1(12.5)	2(25)	3(37.5)	4(50)	4(50)	2(25)	χ2=1.172 *P=0.556*	χ2=0.422 *P=0.809*

**The groups were matched for age and sex. BRA -before remedy application, ARA -after remedy application*

The data obtained can be explained by the fact that the lichen β-oligosaccharides in the composition of Kladorod, binding the Rhodiola rosea salidroside, form a unique complex that provides a higher digestibility of an active ingredient of Rhodiola rosea, increasing 5 to 10 times its bioavailability. The results of our study allow us to conclude that Kladorod leads to increasing the proportion of athletes with the positive types of NARO, compared to pure Rhodiola, which demonstrates the higher effectiveness of Kladorod as a remedy to enhance adaptive capacity with the possibility of application for training of athletes without any restrictions within the criteria of doping control.

References

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

2. Anshakova VV. Increase activity of pharmaceutical substance lichens β-oligosaccharides. Russ J Biopharmaceut. 2012; 4(4):42-46.

3. Garkavi LH, Kvakina EB, Kuzmenko TS. Antistress reactions and activation therapy. The reaction of activation as a way to health through self-organization processes. M.: IMEDIS, 1998.

Save the date

Plant Biology Europe
EPSO/FESPB 2016 Congress

Prague, Czech Republic | June 26–30, 2016

www.europlantbiology2016.org

epso

FESPB

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

XXII.

CONGRESS OF THE
EUROPEAN SOCIETY FOR STEREOTACTIC AND FUNCTIONAL NEUROSURGERY

28 September-01 October 2016
Conrad Hotel, Istanbul

www.essfn2016.org

IJBM

INTERNATIONAL JOURNAL OF BIOMEDICINE

Instructions for Authors

Editorial Policies

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

Manuscript Submission

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

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

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

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

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

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

Pre-submissions

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

Cover Letter

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

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

Manuscript Preparation

Title Page

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

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

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

Abstract

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

Key Words

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

Text

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

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

Authorship

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

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

Acknowledgments

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

References

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

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

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

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

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

Figures and Legends

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

Tables

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

Units of Measurement

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

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

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

Abbreviations and Symbols

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

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

Permissions

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

Processing Fees

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

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

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

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

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

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

°Total Charge for article = $600

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

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

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

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

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

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

AUTHOR'S CHECKLIST

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

• Cover Letter

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

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

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

• Abstract

• Key words

• Text

• Acknowledgments

• References

• Table and Figure Legends

• Figures (individual tiff or eps file format)

• Tables (individual word documents)

• Permissions for the use of any previously published materials

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

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

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

Join us for the

2016 7th Annual ABPP Conference and Workshops

May 11-14, 2016

CHICAGO

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

ABPP
SPECIALTY CERTIFICATION IN PSYCHOLOGY SINCE 1947

THE GWEN
A LUXURY COLLECTION HOTEL
Chicago

Mark your calendars! 2016