POSITIVE EFFECT OF BETAINE-ARGININE SUPPLEMENT ON IMPROVED HYPERHOMOCYSTEINEMIA TREATMENT IN MARRIED COUPLES WITH REPRODUCTIVE DISORDERS

[1,2]Rossokha Z., [1,2]Fishchuk L., [2,3]Sheyko L., [1] Medvedieva N., [2,3]Gorovenko N.

[1]State Institution Reference-centre for Molecular Diagnostic of Public Health Ministry of Ukraine, Kyiv;
[2]State Institute of Genetic and Regenerative Medicine National Academy of Medical Sciences of Ukraine, Kyiv;
[3]Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine

Polymorphic variants of folate exchange and thus conditioned hyperhomocysteinemia (HHcy), recognized as a hereditary metabolic disorder, is a relevant factor of reproductive disorders e

For instance, the United States has shown that the fortification has given the possibility to remove genetic testing of the *MTHFR* gene variants from the clinical guidelines for the diagnostic of inherited thrombophilia and not to be carried out for patients with spontaneous abortions due to the folate status change on the population level. However, identified gene variants and moderate HHcy, as noted by clinical guidelines, in any cases, require medical genetic counseling to properly assess their impact on existing clinical symptoms. Genetic counseling should take into consideration the reason for the referral to a genetic test [12]. Some authors believe that after fortification the risk of folate-mediated diseases on the population level decreases considerably and it is impossible to determine the genetic constituent. But, genetic testing with gene-factor associations analysis would help find persons, which are at risk of developing clinical symptoms related to *MTHFR* gene variants, in order to conduct them personalized prevention measures with the consideration of the whole group B vitamins spectrum and metabolically related compounds [22].

Current precaution against folic acid (FA) "*Folic acid fortification and supplementation – good for some but not so good for others*" is the name of one of the first publications of Kim Y.I. about negative clinical effects of folic acid consumption [15], currently described [10]. Thus, the detection and personalized treatment of HHcy patients, personalized prescription optimal dose of FA in combination with other vitamins and nutrients to improve folate status and achieve stable decrease of homocysteine (Hcy) level is a conceptually new – precision medicine [3]. Timely and efficient HHcy treatment or prevention are especially necessary for patients of reproductive age to prevent undesired clinical consequences of folate-deficient conditions [4, 13].

Aim of the study was to investigate the main risk factors in the developing of HHcy in married couples with reproductive disorders and to evaluate the efficiency of short-term application of betaine-arginine supplement in treatment of identified patients.

Material and methods. The studies were conducted in a total of 206 couples with reproductive disorders. 33.49% of these had natural idiopathic sterility and 66.51% had previous reproductive losses in their family history. The inclusion criteria were determined during the study planning. A couple was not included into the study if any spouse had karyotype anomalies, obesity, somatic and oncologic pathology, acute and chronic infectious diseases. Additional exclusion criteria for men were azoospermia and presence of Y-chromosome deletions. All the patients gave their informed consent to the participation in the study, provided the results of clinical, laboratory and instrumental tests, conducted prior to their being referred to medical-genetic consultation, and filled in the questionnaire, which covered their medical data and information about their lifestyle. The approval of the bioethics committee was obtained for the study. The tests for all the folate exchange indices were conducted for all the study participants: Hcy in blood plasma, FA and vitamin B12 in blood serum and polymorphism of folate exchange genes *MTHFR* (C677T, rs1801133; A1298C, rs1801131), *MTRR* (A66G, rs1801394), *MTR1* (A2756G, rs1805087), *RFC1* (G80A, rs1051266) using previously described methods [23, 24]. 116 (out of 118 detected) patients with HHcy were divided into Group 1 and Group 2. The patients in both groups were prescribed vitamins, containing 800 μg FA (in combination with 4 μg vitamin B12 average). Group 1 included 58 patients, prescribed a sachet of betaine-arginine dietary supplement - Betargin® (contains 1 g betaine, 1 g arginine and citrate ions) twice a day in addition to vitamins preparations. Group 2, also comprised of 58 patients, was prescribed vitamins preparations and not prescribed betaine-arginine containing supplement - Betargin®. Folate exchange indices (Hcy in blood plasma, FA and vitamin B12 in blood serum levels) were defined twice: before treatment and two weeks after vitamins administration.

The methods of single-factor statistical analysis were used to assess the impact of gene variants and other factors on the development of HHcy in the couples. The analysis of basic clinical characteristics and the assessment of quantitative laboratory indices involved the estimation of the mean value (M)±standard deviation (SD). The investigated indices were checked for the normality of distribution using Kolmogorov-Smirnov test. In case of normal distribution, the probability of differences in quantitative results for different groups of investigated patients was determined using Student's t-test; in case of distribution, which differed from the normal one, Mann-Whitney U-test was applied or the normalization of indices was done. Quality criteria, incidence of cases and genotype frequencies were analyzed using criteria $\chi 2$ and odds ratio (OR) within 95% of the confidence interval (CI). The differences were deemed reliable for all the types of analysis at the level of significance (p) under 0.05. The risk factors were also analyzed using the multivariate method – binary logistic regression. The calculations were done using Microsoft Excel Pro Plus 2016 and SPSS v.27 programs.

Results and discussion. The excessive Hcy level, as was proven, has unfavorable effect on the pregnancy course and associated with the birth of children with certain pathologies, impaired development, smaller weight for the gestational age. Detected differences in some results shown the presence of gene-factor interactions, when additional consumption of FA eliminated the incidence of *MTHFR* gene related pathologies on the population level. [4, 5, 12]. Regardless of this fact, the attention of researchers to HHcy has not decreased [22, 24]. 10th revision of ICD-10 (October, 2017) defined hereditary HHcy related to folate gene variants as an autosomal recessive inherited metabolic disorder (code E72.1), but it still does not have clear reference values for the Hcy level, while WHO published reference values for blood serum FA and erythrocytes FA clearly [30]. Based on existing recommendations and some studies, we considered the Hcy level in blood plasma, equal and over 12 μmol/l to be excessive in our study [19,26].

The level of Hcy in plasma, exceeding 12 μmol/l, is deemed

to be cytotoxic. The increased level of Hcy is detected among healthy population but prevails among patients with cardiovascular diseases and reproductive disorders, especially in the countries without any fortification of food products. Additional risk factors (excessive consumption of coffee, meat, unbalanced diet, smoking, alcohol consumption, etc.) increase the total HHcy risk additively and synergistically, especially in case of existing variants in folate exchange genes, thus, must be recommended plasma Hcy level under 10 μmol/l. The studies found that the levels of Hcy correlated with the indices of FA and vitamin B12 in blood serum. The sufficient level of FA in the organism of a woman prior to the pregnancy and on early stages was proven to promote the prevention of thrombophilic disorders in women and normal development of the embryo [4,26,28]. WHO defined the threshold levels of FA in blood serum: under 3 ng/ml – deficiency; 3-5.9 ng/ml – probable deficiency; 6-20 ng/ml – normal level. These threshold indices, indicated by WHO, highlight the need to control the level of folic acid in blood serum, which is first and foremost necessary for women of reproductive age, starting with the stage of planning the conception. It is believed that the optimal level of FA in blood serum prevents HHcy [30].

Our study detected HHcy in 118 (28.64%) cases (in 42 women and 76 men) among the couples with reproductive disorders, examined by us, when FA and B12 blood serum level among most was optimal (Table 1). The number of couples with the detected HHcy was 28.64% from the total number. In 18.93% of the couples, either the husband or the wife had HHcy, and in 9.71% – both husband and wife had HHcy which was conditioned by their specific diet (predominant excessive consumption of meat). Table 1 presents the results of comparison between the basic clinical and genetic charac-

Table 1. The comparison of the basic clinical and genetic characteristics in investigated groups

Characteristics		**Patients with HHcy** (≥12 μmol/l), n=118	**Patients without HHcy** (less 12 μmol/l), n=294	**Statistical differences**
Age, years		33.97±5.01	33.25±5.13	p>0.05
BMI		24.59±5.00	23.57±3.35	p>0.05
male/female, n (%)		76 (64.41%)/ 42 (35.59%)	130 (44.22%)/ 164 (55.78%)	OR=2.28 (1.47-3.55), χ^2=13.73, p=0.0002
Homocysteine, μmol/l		16.95±11.96	8.88±1.81	p<0.05
Folic acid, ng/ml		7.98±5.20	11.60±5.74	p<0.05
Vitamin B12, pg/ml		294.11±107.40	476.58±139.16	p<0.05
MTRR A66G, gene variants n (%)	66AA	29 (24.6%)	51 (17.3%)	OR=1.55 (0.93-2.60), χ^2=2.81, p=0.09
	66AG	49 (41.5%)	148 (50.3%)	OR=0.70 (0.45-1.08), χ^2=2.62, p=0.11
	66GG	40 (33.9%)	95 (32.3%)	OR=1.07 (0.68-1.69), χ^2=0.1, p=0.76
	66A	0.45	0.43	OR=0.89 (0.66-1.21), χ^2=0.55 p=0.46
	66G	0.55	0.57	
MTHFR C677T, gene variants n (%)	677CC	19 (16.1%)	161 (54.8%)	OR=0.16 (0.09-0.27), χ^2=51.16, p<0.0001
	677CT	63 (53.4%)	121 (41.2%)	OR=1.64 (1.07-2.52), χ^2=5.10, p=0.0239
	677TT	36 (30.5%)	12 (4.1%)	OR=10.32 (5.13-20.74), χ^2=57.13, p<0.0001
	677C	0.43	0.75	OR=4.08 (2.97-5.62), χ^2=79.5, p<0.0001
	677T	0.57	0.25	
MTHFR A1298C, gene variants n (%)	1298AA	76 (64.4%)	122 (41.5%)	OR=2.55 (1.64-3.97), χ^2=17.71, p<0.0001
	1298AC	38 (32.2%)	136(46.3%)	OR=0.55 (0.35-0.86), χ^2=6.82, p=0.009
	1298CC	4 (3.4%)	36 (12.2%)	OR=0.25 (0.09-0.72), χ^2=7.53, p=0.006
	1298A	0.81	0.65	OR=0.44 (0.31-0.64), χ^2=19.92, p<0.0001
	1298C	0.19	0.35	
MTR1 A2756G, gene variants n (%)	2756AA	43 (36.4%)	194 (66.0%)	OR=0.3 (0.19-0.46), χ^2=30.08, p<0.0001
	2756AG	64 (54.2%)	83 (28.2%)	OR=3.01 (1.94-4.69), χ^2=24.81, p<0,0001
	2756GG	11 (9.3%)	17 (5.8%)	OR=1.68 (0.76-3.69), χ^2=1.67, p=0.20
	2756A	0.64	0.80	OR=2.31 (1.65-3.22), χ^2=24.82, p<0.0001
	2756G	0.36	0.20	
RFC1 G80A, gene variants n (%)	80GG	20 (16.9%)	95 (32.3%)	OR=0.43 (0.25-0.73), χ^2=9.88, p=0.0017
	80GA	62 (52.5%)	132 (44.9%)	OR=1.36 (0.89-2.09), χ^2=1.97, p=0.09
	80AA	36 (30.5%)	67 (22.8%)	OR=1.49 (0.92-2.40), χ^2=2.68, p=0.10
	80G	0.43	0.55	OR=1.59 (1.17-2.15), χ^2=8.98., p=0.0027
	80A	0.57	0.45	

teristics of the examined couples depending on the detected HHcy using the methods of single-factor analysis.

Significant genetic determinant of HHcy was variants of *MTHFR*, *MTR1*, *RFC1* gene. The protective impact on HHcy development was determined for *RFC1* gene variant (80GG), but other *MTHFR*, *MTR1* gene variants increased the risk. HHcy was found significant frequently among men from the couples with reproductive disorders compared to women. Other significant factors of HHcy risk were the level of FA and B12 in serum blood at the beginning of the study, but their mean value in both groups (Table 1) located within known described threshold levels [2,6,30]. FA serum blood level was reliably lower among male patients (not shown in the Table 1) with HHcy (6.94±3.67 ng/ml) compared to females with HHcy (9.87±6.86 ng/ml).

Historically, the deficiency of vitamin B12 was determined and studied in clinical conditions, when the symptoms, caused by malignant anemia, malabsorption or severe vegan diet, were studied [1]. However, it has been determined that vitamin B12 deficiency is widely common among many groups of population and is considered to be the healthcare system problem. According to the data of different studies, the incidence of vitamin B12 deficiency is about 40 % [11]. The total concentration of vitamin B12 in blood serum or plasma is used as a biomarker of the first line of deficiency. According to WHO recommendations, the threshold value is 203 pg/ml [6]. But, there are other threshold values in the scientific literature – from 100 to 350 pg/ml, and the researchers indicate that vitamin B12 deficiency may occur even in case of its normal concentrations [2]. Our analysis found that the mean index of vitamin B12 in blood serum was within the threshold values in both investigated groups but considerably lower in patients with HHcy compared without (Table 1).

Some authors proved that the increase within the threshold values of folate status indices, namely, the levels of FA and vitamin B12 in blood serum, in the couples during IVF planning increased the live birth rate. These studies were conducted by A.J. Gaskins et al. for the population of women, residing in the country with fortification practice [8]. A similar effect was noted for men in the work of J. Hoek et al., who demonstrated the relevance of the folate status of men, and determined that both low and high pre-conception levels of folates in erythrocytes of men were associated with the delayed embryonic growth in spontaneous pregnancy [13]. The paternal status of folic acid is an impact factor for embryo programming and endometrium sensitivity [17]. The study of J. Hoek et al. demonstrated that the increased levels of FA were as harmful as the decreased ones, and considering possible epigenetic events, they may be a relevant unpredictable risk factor for the health of the progeny. In this study, such risk effect was determined only for spontaneous pregnancy, not the ones via IVF [13]. Therefore, it is an urgent task to search for the balanced personalized approach to improving folate status indices and HHcy prevention with the consideration of genetic specificities of patients. It also requires the population-wise specificities of FA consumption and current welfare of people of reproductive age. As seen from the results of our study, HHcy in the patients under our investigation was conditioned by variants of folate exchange genes and was more remarkable for men from the couples with idiopathic sterility or spontaneous abortions. It is also known that the HHcy level is usually higher in men than women, which increases on the level of population with age [4,26].

The method of binary logistic regression was used to assess the impact of variants of the investigated genes of folate exchange on the risk of HHcy development in our patients. A reliable genetic model of risk was built with the consideration of variants C677T of *MTHFR* gene and A2756G of *MTR1* gene and had the highest predictive value – 77.2 % (Table 2).

The next analysis included gene variants, basic clinical indices, diet specificities, personal consumption of folic acid and other vitamins, determined the highest predictive value – 85.7% for the significant model of risk (Table 3), which covered variants of genes, gender, level of vitamin B12 in serum blood and additional consumption of vitamin B12 before beginning this study. Significant determinants of HHcy development were male gender, *MTHFR, MTR1* gene variants, low vitamin B12 level and its consumption. Therefore, it is important for the patients in our investigation to have optimal intake of vitamin B12 and its increasing level in blood serum during and after HHcy treatment.

Table 2. Genetic model of HHcy risk

Genes variants	Regression coefficient	p	Exp (OR) (95% CI for exp (OR))
MTHFR C677T (TT)	3,319	0,0001	27,627 (11,902-64,128)
MTHFR C677T (CT)	1,943	0,0001	6,980 (3,267-14,914)
MTR1 A2756G (AG)	0,926	0,053	2,525 (0,989-6,451)
MTR1 A2756G (GG)	-0,383	0,422	0,682 (0,268-1,735)
Constant	-1,535	0,005	0,215

Table 3. HHcy risk model (including clinical and genetics characteristics)

Characteristics	Regression coefficient	p	Exp (OR) (95% CI for exp (OR))
MTHFR C677T (TT)	3,015	0,0001	20,382 (7,3109-56,8437)
MTHFR C677T (CT)	1,667	0,0001	5,297 (2,075-13,518)
MTR1 A2756G (AG)	0,83	0,145	2,293 (0,751-6,998)
MTR1 A2756G (GG)	-0,425	0,457	0,654 (0,213-2,004)
Gender (male)	1,042	0,001	2,834 (1,498-5,365)
Vitamin B12 level	0,010	0,0001	1,010 (1,007-1,013)
Vitamin B12 ingestion	2,213	0,038	9,144 (1,128-74,092)
Constant	-5,573	0,0001	0,004

Remethylation of Hcy and its disposal occur via methyl groups, formed during metabolic transformation involving betaine or 5-methyltetrahydrofolate [20, 27]. Betaine, a choline derivative, was proved to be extremely useful in the treatment of mild HHcy. It is especially true for patients with variants of *MTHFR* gene with low functionality when the occurring deficiency of remethylation via decreasing the activity of the enzyme produces the increase in Hcy level and the decrease in the level of methionine, whose transformations are required for the synthesis of nucleotides, proteins, neurotransmitters, etc. In some clinical cases of HHcy, related to variants of *MTHFR* gene, betaine was found to be the only supplement, able to improve the Hcy level of patients; as for our patients, the leading factor of HHcy development were variants 677TT and 677CT of *MTHFR* gene [16]. It was determined for healthy people that the level of Hcy decreased reliably better in case of FA administration compared to betaine, but the stabilizing effect on Hcy level was found for betaine [27].

There have long been hypotheses that betaine-dependent remethylation is a leading way to decrease the excessive Hcy level regardless of the reason of its occurrence [14]. Some studies proved that regardless of the genetic basis and the factor of HHcy development, there is an observed decrease in betaine level in blood plasma and tissues (liver, brain, heart, etc., but not in kidneys). It was demonstrated while examining the patients and conducting experimental work with animals. The decrease in betaine was rather considerable, on average 60% below the normal value. The decrease in betaine concentration in patients with remethylation deficiencies (for instance, variants of *MTHFR* gene, deficiency of *MTR1*) is hardly a surprise as for these patients BHMT remains the only enzyme, capable of remethylating homocysteine to methionine. Moreover, for all the reasons of HHcy occurrence there may be observed increased consumption of betaine via accumulation of Hcy – substrate for enzyme BHMT. While monitoring patients with acquired HHcy, treated without betaine, it was found that the concentrations of Hcy and betaine changed towards normalization, starting with the 6th day of treatment with vitamin B12. Therefore, the application of betaine will decrease the load on Hcy remethylation via the reaction with methionine synthase, whose activity is controlled by *MTR1* gene, which in our investigation is a component of genetic and clinical-genetic models of risk and requires vitamin B12 as a reaction cofactor [27].

Considering the described interaction between remethylation pathways and the decrease in betaine concentration in blood and tissues in case of long-term HHcy, it is relevant to consider its efficiency in treating this condition with reproductive disorders. Previous studies, using animal models, proved that deficient MTHFR was accompanied with the decrease in betaine concentration in liver, thus, its application in our patients with HHcy is completely justified as the variant of *MTHFR* gene was a leading genetic risk factor for them, and both folic acid and vitamin B12 are deposited in the liver [25]. The attraction of betaine-arginine supplement for patients with HHcy and reproductive disorders is additionally enhanced due to the determined correlation between the level of betaine and homocysteine, which was observed during pregnancy and reported in the study of Silvia Ferna`ndez-Roig et al. [7]. According to this investigation, a low folate status enhanced the decrease in betaine and its correlative relationship with Hcy level during pregnancy. In addition to a similar correlative effect, another study determined long-term stabilization of Hcy level when betaine was taken, in cases of low folate status and the action of provocative factors [27].

L-arginine, presented in the composition of Betargin®, is a source of nitrogen oxide, regulating NO-synthetase via the feedback mechanism [21]. The physiological function of nitrogen oxide lies in relaxing smooth muscles of blood vessel walls, inhibiting the aggregation of platelets and their adhesion and regulating cardiovascular and genitourinary system as well as the main components of cell immunity. The formation of nitrogen oxide by immunocompetent cells ensures the protection of the organism from bacterial infections and oncologic diseases due to the participation in the regulation of apoptotic processes [18]. Nitrogen oxide impacts the activity of many enzymes and proteins, including the one of antioxidant protection, especially in conditions of disease and stress due to excessive burden, during adaptation processes and correction of metabolic shifts. First of all, the deficiency of nitrogen oxide promotes the development of endothelial dysfunction, which accompanies the development of cardiovascular diseases and reproductive disorders, thus, when L-arginine is used, the state of the vascular wall improves and the vasodilation and fibrinolytic effects develop. Similar clinical effects of L-arginine are required to decrease the toxic effect of increased Hcy on the vascular wall [29].

Group 1 and Group 2 patients with HHcy were treated for 2 weeks. Group 1 patients received betaine-arginine supplement in addition to folic acid and vitamin B12. We found no differences in homocysteine, folic acid, vitamin B12 levels of patients Group 1 and Group 2 before treatment (Fig. 1-3).

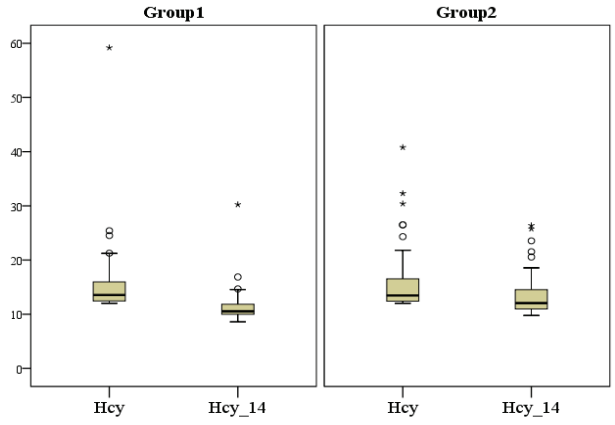

Fig. 1. Hcy (μmol/l) in patients: before (Hcy) and after treatment (Hcy_14)

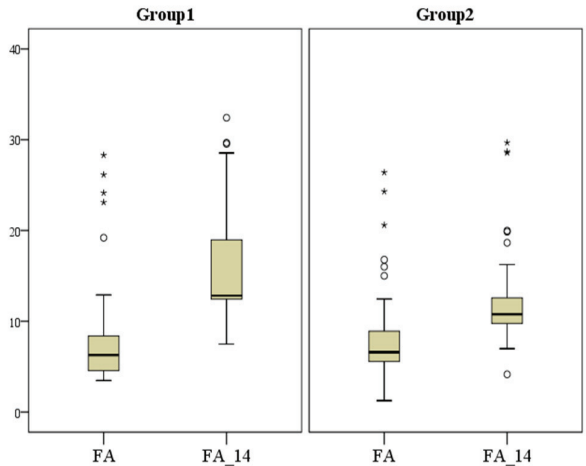

Fig. 2. FA (ng/ml) in patients: before (FA) and after treatment (FA_14)

Hcy mean values among patients Group 1 and Group 2 were 15.34±6.62 μmol/l and 15.93±5.73 μmol/l respectively, before treatment (Fig. 1), significantly decreased after treatment in both groups: 11.28±3.00 μmol/l and 13.52±3.82 μmol/l, respectively. Hcy mean values was significantly lower after treatment among patients of Group 1 compared to patients of Group 2 (Fig. 1).

FA mean values among patients of Group 1 (8.07±5.59 ng/ml) and patients of Group 2 (7.86±4.75 ng/ml) did not differ significantly at the beginning of treatment and increased significantly after the ingestion of vitamins (Fig. 2) in both groups. But patients of Group 1 (15.43±5.35 ng/ml) had significantly higher level then in Group 2 (12.21±4.82 ng/ml) after treatment.

Our analysis of B12 vitamin mean values identified the same significant rise as for FA mean values after 2 weeks of vitamins admission (Fig. 3). B12 vitamin mean values significantly increased after treatment in comparison before treatment. Also no reliable difference was in B12 vitamin mean values before vitamins consumption: Group 1 (300.95±125.69 pg/ml) and Group 2 (288.37±87.34 pg/ml) (Fig. 3), then a significantly increased indices was observed in Group 1 (542.40±137.84 pg/ml) compared to Group 2 (432.31±85.31 pg/ml) after treatment.

The patients of Group 1 with betaine-arginine supplementation had significantly lower Hcy mean values compared to patients of Group 2. The number of patients with Hcy level over 12 μmol/l significantly decreased in 47 patients from 58 (81,03%) in Group 1 against 29 patients from 58 (50%) in Group 2 (χ^2=12.36, p=0.0004).

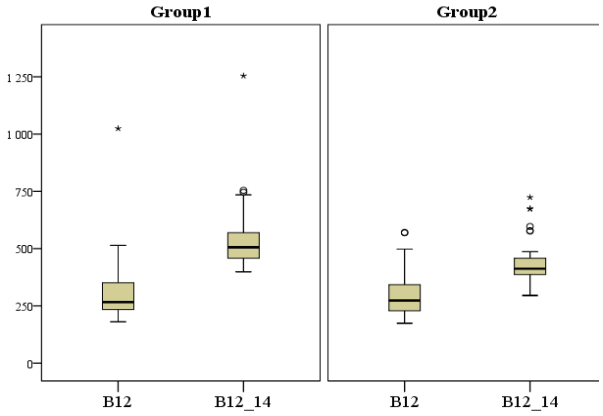

Fig. 3. Vitamin B12 (pg/ml) in patients: before (B12) and after treatment (B12_14)

Thus, the most efficient treatment was in patients group where prescribed betaine-arginine supplement in addition to FA and vitamin B12. The mentioned clinical effect indicated that the application of a betaine-arginine supplement improved the folate status of patients with reproductive disorders considerably, which is relevant in increasing the live birth rate in the family in the future.

Conclusions. The highest predictive value of hyperhomocysteinemia development in patients with reproductive disorder was identified for the risk model included *MTHFR, MTR1* gene variants, male gender, low level of vitamin B12 in blood serum and its low additional consumption. The prescription for hyperhomocysteinemia treatment in patients with reproductive disorder folic acid, vitamin B12 and betargin during two weeks significantly improved all indices (homocysteine in plasma blood, folic acid and vitamin B12 in blood serum) compared to patients without betargin consumption. Betaine-arginine supplementation provided significantly the best reduction of homocysteine level (less than 12 μmol/l in patient). The application of betaine-arginine supplement in the couples with reproductive disorders is a promising way of restoring all folate status indices quickly if variants of *MTHFR, MTR1* genes in patients are present. Further studies are required to determine the duration of betaine-arginine intake to achieve the target homocysteine level and to estimate the durability of the achieved effect.

REFERENCES

1. Allen LH, Miller JW, de Groot L, Rosenberg IH, Smith AD, Refsum H, et al. Biomarkers of nutrition for development (BOND): Vitamin B-12 review. // J Nutr. 2018; 148(4): 1995S-2027S.
2. Aparicio-Ugarriza R, Palacios G, Alder M, González-Gross M. A review of the cut-off points for the diagnosis of vitamin B12 deficiency in the general population. // Clin Chem Lab Med. 2015; 53(8):1149-5944.
3. Aronson S, Rehm H. Building the foundation for genomics in precision medicine. // Nature. 2015; 526: 336-42.
4. Bitsadze VO, Samburova NV, Makatsariya NA, Mishchenko AL. Folate deficiency in obstetrics and the problem of its correction. Obstetrics, Gynecology and Reproduction. 2016; 1: 38-48.
5. CDC: Centers for Disease Control and Prevention [Internet]. Atlanta, GA, 2020 [reviewed 2020 February 21; cited 2020 August 15]. Available from: https://www.cdc.gov/ncbddd/folicacid/index.html
6. de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. // Food Nutr Bull. 2008; 29(2): S238-44.
7. Fernàndez-Roig S, Cavallé-Busquets P, Fernandez-Ballart JD, Ballesteros M, Berrocal-Zaragoza MI, Salat-Batlle J, et al. Low folate status enhances pregnancy changes in plasma betaine and dimethylglycine concentrations and the association between betaine and homocysteine. // Am J Clin Nutr. 2013; 97(6): 1252-9.
8. Gaskins AJ, Chiu YH, Williams PL, Ford JB, Toth TL, Hauser R, et al. Association between serum folate and vitamin B-12 and outcomes of assisted reproductive technologies. // Am J Clin Nutr. 2015;102(4):943-50.
9. Gorovenko NG, Rossokha ZI, Kiryachenko SP, Sheiko LP, Brisevac LI. [Features of gene-gene and gene-factor intractions in men from couples with infertility and reproductive losses]. Ukrainian medical journal. 2018; 5(2): 28-31.Ukrainian
10. Gromova OA, Serov VN, Torshin IYu, Kerimkulova NV, Limanova OA. [A Role of folic acid-containing vitamin-mineral complexes in prophylaxis of congenital heart diseases and neural tube defects]. Akusherstvo i Ginekologiya. 2015; 4: 4-12.
11. Guney T, Yikilmaz AS, Dilek I. Epidemiology of vitamin B12 deficiency. In: Kasenga F. editor. Epidemiology of Communicable and Non-Communicable Diseases - Attributes of Lifestyle and Nature on Humankind. InTech; 2016. p.103-112.
12. Hickey SE, Curry CJ, Toriello HV. ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing. // Genet Med. 2013; 15(2): 153-6.
13. Hoek J, Koster MPH., Schoenmakers S, Willemsen SP, Koning AHJ, Steegers EAP, et al. Does the father matter? The association between the periconceptional paternal folate status and embryonic growth. //Fertil Steril. 2019; 111(2): 270-9.
14. Imbard A. Benoist JF, Esse R, Gupta S, Lebon S, de Vriese AS, et al. High homocysteine induces betaine depletion. //Biosci Rep. 2015 Apr 28;35(4):e00222.

15. Kim YI. Folic acid fortification and supplementation – good for some but not so good for others. // Nutr Rev. 2007; 65(11): 504-11.
16. Lawson-Yuen A, Levy HL. The use of betaine in the treatment of elevated homocysteine. Mol Genet Metab. 2006; 88(3): 201-7.
17. Lonergan P, Fair T, Corcoran D, Evans AC. Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. // Theriogenology. 2006; 65(1): 137-52.
18. Malakhov VO, Zavgorodnya GM, Lychko VS, Dzhanelidze TT, Voloh FO. [The problem of nitric oxide in neurology: monograph]. Sumy: Vydavnyctvo SumDPU im AS Makarenka; 2009. 242 p. Russian
19. Miller ER 3rd, Juraschek S, Pastor-Barriuso R, Bazzano LA, Appel LJ, Guallar E. Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. // Am J Cardiol. 2010 Aug 15;106(4):517-27.
20. Naumau AV, Danilchyk IV, Sarana YuV. [Three ways of homocysteine remethylation]. Journal of the Grodno State Medical University. 2016; 2(54): 27-32. Russian
21. Pimentel AM, Pereira NR, Costa CA, Mann GE, Cordeiro VS, de Moura RS, et al. L-arginine-nitric oxide pathway and oxidative stress in plasma and platelets of patients with preeclampsia. // Hypertens Res. 2013 Sep;36(9):783-8.
22. Reilly R, McNulty H, Pentieva K, Strain JJ, Ward M. MTHFR 677TT genotype and disease risk: is there a modulating role for B-vitamins? // Proc Nutr Soc. 2014; 73(1): 47-56.
23. Rossokha ZI, Kiryachenko SP, Gorovenko NG. Comparative evaluation of genetic risk models of reproductive disorders caused by MTHFR, MTRR, MTR1 gene polymorphism. Medicni perspektivi. 2018; 23 (2): 85-91. Ukrainian
24. Rossokha ZI, Kiryachenko SP, Gorovenko NG. The role of MTHFR, MTRR, MTR1 intergenic interaction in the development of folate metabolism disturbance in patients with reproductive disorders. Ukrainian medical journal. 2018; 3(2): 35-9.
25. Schwahn BC, Chen Z, Laryea MD, Wendel U, Lussier-Cacan S, Genest J Jr, et al. Homocysteine-betaine interactions in a murine model of 5,10-methylenetetrahydrofolate reductase deficiency. // FASEB J. 2003;17(3):512-4.
26. Stanger O, Herrmann W, Pietrzik K, Fowler B, Geisel J, Dierkes J, et al. DACH-LIGA Homocystein e.V. DACH-LIGA homocystein (german, austrian and swiss homocysteine society): consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. // Clin Chem Lab Med. 2003 Nov;41(11):1392-403.
27. Steenge GR, Verhoef P, Katan MB. Betaine supplementation lowers plasma homocysteine in healthy men and women. // J Nutr. 2003 May;133(5):1291-5.
28. Tinelli C, Di Pino A, Ficulle E, Marcelli S, Feligioni M. Hyperhomocysteinemia as a risk factor and potential nutraceutical target for certain pathologies. // Front Nutr. 2019; 6: 49.
29. West SG, Likos-Krick A, Brown P, Mariotti F. Oral L-arginine improves hemodynamic responses to stress and reduces plasma homocysteine in hypercholesterolemic men. // J Nutr. 2005; 135(2): 212-7.
30. WHO. Serum and red blood cell folate concentrations for assessing folate status in populations. Vitamin and Mineral Nutrition Information System. Geneva: World Health Organization; 2015.

SUMMARY

POSITIVE EFFECT OF BETAINE-ARGININE SUPPLEMENT ON IMPROVED HYPERHOMOCYSTEINEMIA TREATMENT IN MARRIED COUPLES WITH REPRODUCTIVE DISORDERS

[1,2]Rossokha Z., [1,2]Fishchuk L., [2,3]Sheyko L., [1]Medvedieva N., [2,3]Gorovenko N.

[1]State Institution Reference-centre for Molecular Diagnostic of Public Health Ministry of Ukraine, Kyiv; [2]State Institute of Genetic and Regenerative Medicine National Academy of Medical Sciences of Ukraine, Kyiv; [3]Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine

Treatment of hereditary hyperhomocysteinemia and the achievement of optimal folate status is necessary for persons of reproductive age in order to increase live birth rate. Patients are usually advised to take folic acid, a key nutrient in homocysteine remethylation. The results of study showed risk factors for hyperhomocysteinemia development in investigated married couples: male gender, *MTHFR, MTR1* genes variants, lower vitamin B12 blood serum and no additional intake of vitamin B12. Since *MTHFR, MTR1* genes variants affect to decrease the efficiency of homocysteine metabolic transformations, to contribute also to endothelial dysfunction in one of patients group we used betargine combined with folic acids and vitamin B12 administration. Patients group with combined administration including betargine within 2 weeks, in comparison with the group without its supplement, had significantly decreased level of homocysteine in plasma, less than 12 μmol/l (81.03% and 50% of cases, respectively). Folic acid and vitamin B12 mean values in blood serum was significantly increased in patients after two week vitamins administration including betargin. Further research is needed to establish the duration of betaine-arginine intake until the target homocysteine level will be reached, as well as to estimate the durability of clinical effect achieved after consumption.

Keywords: married couples, hyperhomocysteinemia, reproductive disorder, treatment, betargin.

РЕЗЮМЕ

ЭФФЕКТИВНОСТЬ БЕТАИН-АРГИНИН СОДЕРЖАЩЕЙ ДОБАВКИ В ЛЕЧЕНИИ ГИПЕРГОМОЦИСТЕИНЕМИИ В СУПРУЖЕСКИХ ПАРАХ С РЕПРОДУКТИВНЫМИ НАРУШЕНИЯМИ

[1,2]Россоха З.И., [1,2]Фищук Л.Е., [2,3]Шейко Л.П., [1] Медведева Н.Л., [2,3]Горовенко Н.Г.

[1]Государственное учреждение Референс-центр молекулярной диагностики Министерства здравоохранения Украины; [2]Государственный институт Генетической и Регенеративной медицины Национальной академии медицинских наук Украины; [3]Национальная медицинская академия последипломного образования им. П.Л. Шупика, Киев, Украина

Лечение наследственной гипергомоцистеинемии и достижение оптимального фолатного статуса необходимо лицам репродуктивного возраста с целью увеличения рождаемости. Пациентам обычно рекомендуют принимать фолиевую кислоту - ключевой нутриент для реметилирования гомоци-

стеина. Результаты исследования выявили факторы риска развития гипергомоцистеинемии в обследованных супружеских парах: мужской пол, варианты генов *MTHFR, MTR1*, пониженное содержание витамина B12 в сыворотке крови и отсутствие дополнительного употребления витамина B12. Поскольку варианты генов *MTHFR, MTR1* влияют на снижение эффективности метаболических преобразований гомоцистеина и на эндотелиальную дисфункцию, в одной из групп пациентов использовали бетаргин в сочетании с фолиевой кислотой и витамином B12. В группе пациентов с лечением, включавшим бетаргин в течение 2 недель, был значительно снижен уровень гомоцистеина в плазме крови, менее 12 мкмоль/л (81,03% случаев) в сравнении с группой без него (50% случаев). Уровни фолиевой кислоты и витамина B12 в сыворотке крови были значительно повышены у пациентов после двухнедельного приема витаминов, включая бетаргин. Необходимы дальнейшие исследования для определения продолжительности приема бетаин-аргинин содержащей добавки до достижения целевого уровня гомоцистеина, а также для оценки продолжительности клинического эффекта, достигаемого после приема.

რეზიუმე

ბეტაინ-არგინინის შემცველი დანამატის ეფექტურობა ჰიპერჰომოცისტეინემიის მკურნალობაზე რეპროდუქციული დარღვევების მქონე ცოლქმრულ წყვილებში

[1,2]ზ.როსოხა, [1,2]ლ.ფიშჩუკი, [2,3]ლ.შეიკო, [1]ნ.მედვედევა, [2,3]ნ.გოროვენკო

[1]მოლეკულური დიაგნოსტიკის რეფერენს-ცენტრი; [2]გენეტიკური და რეგენერაციული მედიცინის სახელმწიფო ინსტიტუტი, მეცნიერებათა აცადემია; [3]პ.შუპიკის სახ. დიპლომისშემდგომი განათლების ეროვნული სამედიცინო აკადემია, კიევი, უკრაინა

მემკვიდრეობითი ჰიპერჰომოცისტეინემიის მკურნალობა და ოპტიმალური სტატუსის მიღწევა აუცილებელია შობადობის ამაღლებისათვის რეპროდუქციული ასაკის პირებში. პაციენტებს, როგორც წესი, ურჩევენ ფოლიუმის მჟავას - საკვანძო ნუტრიენტს ჰომოცისტეინის რემეთილირებისათვის. კვლევის შედეგებით გამოვლინდა ჰიპერჰომოცისტეინემიის განვითარების რისკის ფაქტორები: მამრობითი სქესი, გენების ვარიანტები *MTHFR, MTR1*, ვიტამინ B_{12}-ის დაბალი შემცველობა სისხლის შრატში და ვიტამინ B_{12}-ის დამატებითი მიღების არარსებობა. რადგანაც გენების ვარიანტები *MTHFR, MTR1* მოქმედებს ჰომოცისტეინემიის მეტაბოლური გარდაქმნების ეფექტურობასა და ენდოთელურ დისფუნქციაზე, პაციენტების ერთერთ ჯგუფში გამოყენებოდა ბეტარგინი ფოლიუმის მჟავასთან და ვიტამინ B_{12}-თან ერთად. პაციენტების ჯგუფში, ვისთანაც მკურნალობა მოიცავდა ბეტარგინს ორი კვირის განმავლობაში, სისხლის პლაზმაში ჰომოცისტეინის დონე მნიშვნელოვნად ნაკლები იყო – 12 მკმოლ/ლ-ზე ნაკლები (შემთხვევათა 81,03% და 50%, შესაბამისად), ბეტარგინის გარეშე ჯგუფთან შედარებით. ფოლიუმის მჟავას და ვიტამინ B_{12}-ის დონე სისხლის შრატში მნიშვნელოვნად მომატებული იყო პაციენტებში ვიტამინების ორკვირიანი მიღების შემდეგ, ბეტარგინის ჩათვლით. აუცილებელია შემდგომი კვლევების ჩატარება ბეტაინ-არგინინის შემცველი დანამატის მიღების ხანგრძლივობის განსაზღვრისათვის ჰომოცისტეინის სამიზნე დონის მიღწევის მიზნით, ასევე, მიღების შემდეგ მიღწეული კლინიკური ეფექტის ხანგრძლივობის შეფასებისათვის.

MODERN METHODS IN OTORHINOLARYNGOLOGY: POWERED-SHAVER ADENOIDECTOMY

Beridze B., Gogniashvili G.

New Hospitals Ltd, Department of Otorhinolaryngology Tbilisi, Georgia

Adenoids are nasopharynx lymphoid tissues, which participate in the formation of Waldeyer's ring and they were described by Meier for the first time in 1868 [8]. Nowadays, adenoidectomy represents the most common procedure in childhood years, which is performed by resection of adenoids only, or in line with tonsillectomy and/or insertion of ventilation tubes in tympanic membranes [2]. Widespread conventional adenoidectomy was described the first time in 1885 [7]. While precise resection of adenoids along with maximum protection of the adjacent tissues of nasopharynx, complications are avoided, such as: bleeding, incomplete resection of adenoids, stenosis of Eustachian tube and in very rare cases, stenosis of nasopharynx. Incompleteness of conventional method with regard to complete and safe resection of adenoids leads to development of alternative methods, among them, adenoidectomy with shaver [3,6]. This become possible via development of endoscopic surgical instruments [1]. In our study, description of adenoidectomy with shaver is provided in line with its advantages and disadvantages.

Material and methods. 50 patients were enrolled in the prospective study, all of them underwent adenoidectomy with shaver within the period from January 2019 to June 2020 inclusive. Age range was 2-26 years old (average age-14 years old),