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Research Article

Pathogenetic Justification for the Use of Folates for the Prevention of Congenital Malformations

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Abstract
The frequency and severity of congenital malformations (CMF) do not tend to decline in modern society. CMF for etiologic factors are referred to the pathologies of a multifactorial nature. Among the many causative factors of CMF there is a hereditary predisposition.
The objective of the study was to increase the effectiveness of complex individualized prophylaxis of congenital malformations in women with polymorphic alleles of genes of folate cycle enzymes, the second phase of the detoxification system through the use of diagnostic, preventive, and therapeutic measures.
Materials and methods. 120 women of reproductive age who live in the city of Odessa and the Odessa region were examined. The alleles of the genes of the folate cycle enzymes of methylenetetrahydrofolate reductase (MTHFR), 5-methyltetrahydrofolate homocysteine methyltransferase reductase (MTRR), glutathione-S-transferase M1 (GSTM1), folate acid, cyanocobalamin were determined.
Results. The determination of the polymorphic alleles of the genes of the folate cycle enzymes of methylenetetrahydrofolate reductase (MTHFR), 5-methyltetrahydrofolate homocysteine methyltransferase reductase (MTRR), glutathione-S-transferase M1 (GSTM1), low folate, cyanocobalamin indicates the presence of a hereditary predisposition to the emergence of CMF, before and during pregnancy. Polymorphism of genes that control the synthesis of GSTM1 may alter the activity of detoxification enzymes. Expression of genes of GSTM1 enzymes begins in the embryonic period. Glutathione-dependent detoxification plays a key role in disinfecting of DNA peroxides. Mistakes of metabolism, functions of the corresponding enzymes are realized by chromosomal abnormalities and the risk of the occurrence of CMF, which requires the use of antioxidant therapy before and during pregnancy.
Conclusions. It was found that a high (about 55%) frequency of the polymorphic alleles of the genes of folate cycle enzymes MTHFR (homozygous - 10.0%, heterozygous - 16.7%), 5 MTRR (homozygous 12.5%, heterozygous - 15.5%) , the second phase of the GSTM1 detoxification system (homozygous - 13.3%, heterozygous - 15.8%), the content below the reference values of folic acid in 26.7%, cyanocobalamin - in 63.4% of observations indicates a hereditary predisposition and may contribute the emergence of CMF, which explains the expediency of timely use of preventive measures including folates, antioxidants.

Keywords
MTHFR; MTRR; GSTM1; folic acid; vitamins B12; pregnancy; prevention

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Problem statement and analysis of the recent research

Prevention of congenital malformations (CMF) is one of the most urgent tasks of modern medicine and society as a whole. The urgency of this problem lies in the high frequency of occurrence of CMF (stable morphological changes in the organ, organs that arise as a result of disturbances in morphogenesis in the antenatal period), congenital anomalies of development (structural, functional, metabolic disorders of the organ, organs that arise in utero) and their dissemination [1].

The emergence and formation of CMF is recognized as a polyethiological process. The pathogenetic mechanism of multifactorial pathology is complex and is constantly being studied. At the heart of the formation of embryonic developmental defects, the term gestation, the direct damaging factor, the destruction of tissues, the dysentogenesis, etc. is important. [6]. The formation of an organ in the embryonic period is determined by the interaction of three main factors: 1) the genetic mechanism; 2) internal epigenetic factors (enzyme systems, hormones, etc.); 3) external epigenetic factors (the influence of the external environment) [5]. The complexity of isolating one primary isolated causative factor explains the constant search for scientists in this direction.

It is generally recognized that metabolic processes, including the folate cycle and the tricarboxylic acid cycle, occur with the participation of vitamins, macro- and microelements under the control of the corresponding genes. Folates, vitamins and vitamin-like compounds of group ”B” are part of enzymes, coenzymes [2, 4]. Deficiency of nicotinamide, flavoprotein

dehydrogenases, ribonucleic acids leads to a disruption in the synthesis of proteins, cellular enzymes, compounds such as amino acids, carbohydrates, fatty acids, the energy source of ATP (adenosine triphosphate), and complex metabolic shifts. The folate cycle is a cascade process controlled by enzymes that have folate derivatives as coenzymes. The key point in this process is the synthesis of the amino acid methionine with homocysteine. Violation of the folate cycle is dangerous for embryonic cells, which quickly divide. Hyperhomocysteinemia leads to endothelial dysfunction, hypermethylation of DNA, disruption of chromosome separation, contributes to the emergence of CMF [3, 5]. The foregoing emphasizes the relevance of the problem under study and explains the appropriateness of an individual approach to preventing the occurrence of CMF by correcting the content of folates, trace elements, antioxidant vitamins, amino acids, and carrying out individualized pre-conceptual preparation for future parents from the perspective of predictive medicine.

The objective of the study was to increase the effectiveness of complex individualized prophylaxis of congenital malformations in women with a deficiency of B vitamins, polymorphic alleles of folate cycle genes, and a phase II detoxification system using diagnostic, prophylactic, therapeutic measures.

1. Materials and methods of the research

120 native women of Odessa and Odessa region aged from 18 to 35 years before and during pregnancy were examined, who were on dispensary records in women's consultations. In the main group (I) and the comparison group (II), 60 subjects are included, depending on the prescribed preventive, therapeutic measures.

The level of folic acid, cyanocobalamin was determined in the blood by the immunochemical method; alleles of the 677T gene of the enzyme methylenetetrahydrofolate reductase (MTHFR), 5-methyltetrahydrofolate homocysteine methyltransferase reductase (MTRR), glutathione-S-transferase M1 (GSTM1) by polymerase chain reaction (PCR).

For the purpose of correcting the content of vitamins, macro-, microelements, amino acids, stabilization of metabolic processes, all subjects were assigned to use micronutrients in daily doses (food containing natural folates (spinach, broccoli, nuts, peanuts, hazelnuts, almonds, beans, Vitamin complexes Femibion (Natalker) containing folic acid 400 μg and folic acid acid 200 μg, calcium- (Metafolin-equivalent to 200 μg folic acid), vitamins B1 (1.2 mg), B6 (1, 9 mg), B2 (1.6 mg), B12 (3.5 mg), biotin (60 μg), iodine (150 μ), Vitamin C (110 mg), nicotinamide (15 mg), vitamin E (13 mg), pantothenic acid (6 mg), docosahexanoic acid 200 mg) were administered to women in the main group. Women of the comparison group were prescribed drugs, regulated by Order No. 417 of the Ministry of Health of Ukraine on 15.07.2011. The groups of subjects did not include women with hyperthyroidism.

2. Results of the research and their discussion

According to a retrospective analysis of medical records and the results of a prospective examination, the incidence of extragenital pathology, gynecological diseases, and the obstetric history data did not have a significant difference between the groups.

Thus, out of 120 examined women in 63.3% had cardiovascular system diseases, 46.7% had thyroid pathology, 36.7% had hepatopancreatobiliary system diseases, gastrointestinal tract, including chronic constipation, 26.7% - diseases of the urinary system.

13.3% of women suffered from iron deficiency anemia before pregnancy. Birth in the history had 53.3%, medical abortion - 30.0%, spontaneous abortion - 30.0% of the surveyed. Violations of menstrual function noted 20.0%, primary infertility - 6.7% of women.

Analysis of the results of laboratory studies showed that of the 120 women in 26.7% of women, content of the folate acid was lower than the reference values (<4.6 ng / ml), 17.0% at the lower limit of the norm (5.0-7, 0 ng/ml). In 56.3% of the subjects, the level of folic acid was determined at the upper limit of the norm (17.0-19.0 ng/ml). In 36.6% of women the level of cyanocobalamin was lower and at the lower limit of the norm (180.0 - 200.0 pg/ml).

The result of the determination of the alleles of the genes of the folate cycle enzymes showed that the MTHFR polymorphism over the T allele was detected in 32 (26.7%) cases: homozygous - in 12 (10.0%), heterozygous - in 20 (16.7%); MTRR - in 33 (27.5%): homozygous - in 15 (12.5%, heterozygous - in 18 (15.0%, GST M1 - in 35 (29.2%): homozygous in 16 (13, 3%, heterozygotes - in 19 (15.8%).

The combined deficiency of vitamins (folic acid, cyanocobalamin) was determined in 92 (76.7%) of the subjects. The combined polymorphism of the genes of folate cycle enzymes (MTHFR, MTRR) was in 65 (54.2%) pregnant women. The combined polymorphism of the folate cycle enzyme genes (MTHFR MTRR) and the II phase of the detoxification system (GST M1) were in 00 (83.3%) women.

The presence of polymorphic alleles of the genes of folate cycle enzymes (MTHFR, MTRR), the II phase of the detoxification system (GST M1) was assessed as indications for the administration of a complex of vitamins containing directly folate, a microelement of iodine, antioxidant vitamins C, E, docosahexaenoic acid. The presence of polymorphic alleles of the folate cycle genes indicates that synthetic folic acid cannot be absorbed in the body, since corresponding enzymes are necessary for its metabolism, including 5 methyltetrahydrofolate. The only form of vitamin B9 - L-methylfolate has biological activity and is able to participate in the synthesis of DNA, cell replication, methylation.

In the dynamics of monitoring the course of pregnancy, a comparative analysis between the observation groups showed that the decrease in the content of the studied vitamins in the 18-20 and 36-37 weeks of pregnancy was significantly

lower in 28 (46.6%) women who received traditional treatment against 5 (8.3 %) of the group of women who received the drug containing vitamin complex. Individualized approach to the correction of the content of vitamins, macro-, microelements, amino acids by the laboratory (PCR) assessment of the hereditary disruption of enzyme activity to determine the presence of polymorphism (homozygous, heterozygous) and the combined alleles of the folate cycle genes, the II phase of the detoxification system had a positive value of 57 95.0%) of the subjects, which confirms its effectiveness. The offered nutrient and drug therapy helped stabilize the indices that were determined: the content of folic acid, cyanocobalamin, homocysteine, coagulogram, liver tests, general blood and urine analysis, total protein.

The obtained results of laboratory studies were coordinated with the clinical evaluation of the condition of women, the feto-placental complex by trimester of gestation, which significantly confirms the positive value of the individualized pathogenetically grounded approach to the prevention of CMF and gestational complications.

Proposed individualized prophylaxis of CMF includes four stages: I stage-examination; II stage - preparatory; III stage - planning of pregnancy; IV stage - prenatal diagnosis. Important is the laboratory evaluation of the metabolism of connective tissue, the content of vitamins and vitamin-like compounds of group ”B”, homocysteine, expression of genes of folate cycle enzymes (MTHFR, MTRR), and the second phase of the detoxification system (GSTm1).

CMF were not diagnosed in fetuses and newborns in the women surveyed. The proposed therapy contributed to a significant decrease the frequency of the threat of termination of pregnancy in 3.1 times, the threat of premature births - in 3.2 times, placental dysfunction - in 3.5 times, fetal growth retardation syndrome - in 3.0 times, gestational anemia - in 4.1 time, gestational pyelonephritis - in 3.2 times, pre-eclampsia - in 2.5 times ($p < 0.001$) with respect to the comparison group.

3. Conclusions

Prevention of CMF is one of the priorities of physicians of all specialties. It is the prevention of the emergence and formation of embryonic developmental defects that can contribute to the prevention of this pathology. There are no causeless malformations; there is unqualified or late diagnosis of these conditions and untimely preventive measures.

It is advisable to begin prophylaxis of CMF not earlier than 3 - 4 months before fertilization. This makes it possible to evaluate the pre-morbid background of the organisms of future parents, to determine and conduct a pathogenetically substantiated differentiated, individualized, complex, stage, full-fledged correction of metabolic disorders, taking into account the hereditary disruption of the activity of the corresponding enzymes.

About 55% of Russian women are carriers of polymorphic alleles of genes of folate cycle enzymes (MTHFR: homozygous - 10.0%, heterozygous - 16.7%), MTRR: homozygous - 12.5%, heterozygous - 15.5%), II phase Detoxification systems (GST M1: homozygous - 13.3%, heterozygous - 15.8%). The combined polymorphism was 83.3%.

Given the high frequency of the genome polymorphism of the folate cycle enzymes (MTHFR, MTRR), the II phase of the detoxification system (GST M1), the combined polymorphism, a pathogenetically substantiated indication is the direct administration of folates. Preparations of choice for the correction of the content of vitamins, macro-, microelements, aminoacids is the vitamin complex Femibion.

4. Prospects for research

Prospects for research in this area will be further development of the use of folates for the prevention of congenital malformations.

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