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THE ROLE OF HOMOCYSTEINE IN FETAL ANOMALIES

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Summary: Determination of the level of homocysteine should be used in the prenatal diagnosis of malformations of the central nervous system, heart and chromosomal abnormalities in the fetus. The use of methylfolate three months before conception and intake throughout pregnancy reduces the level of homocysteine and reduces the development of malformations in the fetus.

Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid that is not included in the structure of proteins and takes part in the methylene cycle, is an intermediate product of transmethylation. Homocysteine was isolated in 1932. Conditions accompanied by hyperhomocysteinemia and homocystinuria were first described in 1962 when examining children with mental retardation [17]. With this pathology, a Marfan-like phenotype, visual impairment, and a tendency to thrombosis of any localization are observed, which leads to early death of patients [3]. In 1975, the homocysteine theory of atherosclerosis was formulated [23] based on the revealed pathogenic effect of homocysteine on the endothelium. Currently, hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, pregnancy complications (recurrent miscarriage, hypertensive disorders), the occurrence of certain types of fetal malformations, cognitive impairment, neurodegenerative diseases, psoriasis, and carcinogenesis [24]. Interest in the study of hyperhomocysteinemia in malformations is associated with the difficulties of early diagnosis of these conditions, the need to clarify the etiology of the disorders that have arisen, and the search for effective methods of prevention due to the significant economic costs of treating children with congenital anomalies. Currently, the main method for diagnosing congenital fetal malformations is ultrasound, which makes it possible to visualize up to 70% of gross pathology [5, 10]. The source of homocysteine in the human body is methionine. It is an essential amino acid used in protein synthesis or in the synthesis of S-adenosylmethionine [24]. When methionine interacts with ATP (the process is catalyzed by the enzyme methionine adenosyltransferase), S-adenosylmethionine (SAM) is formed. This reaction is observed in almost all tissues. SAM is a universal donor of methyl groups for methylation reactions carried out by numerous methyltransferases [12]. About 100 reactions are known that are accompanied by the transfer of a methyl group to such substrates as proteins, nucleic acids, and lipids [15]. For example, DNA methylation is an important regulatory mechanism for gene expression, the basis of epigenesis. DNA hypomethylation leads to chromosome instability and promotes mutagenesis [18]. The result of the transfer of the methyl group is the formation of S-adenosylhomocysteine (SAH), which, in turn, is an inhibitor of methyltransferase reactions, and therefore must be rapidly metabolized. This process is carried out with the help of the corresponding hydrolase and leads to the production of homocysteine. SAH can also bind to intracellular proteins or be removed from the cell. Homocysteine is further involved in the synthesis of cysteine (transsulfurization) or remethylated to methionine [15, 24, 26]. The sources of the methyl group in the latter reaction can be methylenetetrahydrofolate or betaine. Excess homocysteine is eliminated from the cell and appears in the blood plasma, where its normal level usually does not exceed 5 μmol/l. The level of homocysteine in blood plasma increases with age [11], has circadian rhythms, and depends on the quality and quantity of food intake [22]. During pregnancy, there is a slight decrease in the level of homocysteine, which can be explained by changes in the volume of circulating plasma. An increase in homocysteine content of more than

15 μmol/l is interpreted as moderate (1630 μmol/l), medium (31-100 μmol/l) and severe or severe (more than 100 μmol/l) hyperhomocysteinemia [21]. In plasma, homocysteine binds to proteins (75%) or forms homocystin, i.e. is in the oxidized state. The reduced homocysteine accounts for only 1%. The sum of all forms of homocysteine in plasma is called total homocysteine (tHcy). The key enzyme of Hcy transsulfurization is cystathionine-P-synthase, the coenzyme of which is vitamin B6. Under physiological conditions, the reaction is irreversible [26]. The resulting cysteine is used for protein synthesis or for the formation of glutathione. Excess cysteine is oxidized to taurine and inorganic sulfates. About 70% of methionine is utilized in this chain, which emphasizes the importance of this metabolic pathway [16].
The remethylation of homocysteine involves cobalamin (vitamin B12) and folic acid, namely 5-methylenetetrahydrofolate as a methyl group donor. The formation of methylenetetrahydrofolate is catalyzed by methylenetetrahydrofolate reductase (MTHFR) in an irreversible reaction. Remethylation of homocysteine is carried out with the help of methionine synthase, the coenzyme of which is vitamin B12. The result of the reaction is the formation of tetrahydrofolate and methionine. Another donor of methyl groups for remethylation of homocysteine is betaine (a derivative of choline). The enzyme of this reaction is betaine homocysteine methyltransferase. This enzyme is mainly present in the liver [26]. It has been shown that in humans the amount of homocysteine undergoing remethylation and transsulfuration is approximately equal [15]. The regulator of homocysteine metabolism is the level of S-adenosylmethionine in the cell. High concentrations of this metabolite inhibit MTHFR, which reduces the flow of homocysteine in the reaction catalyzed by methionine synthase. In turn, this promotes the metabolism of homocysteine via transsulfuration (cystathionine P-synthase is activated), which occurs mainly in the liver and, to a lesser extent, in the kidneys. In other tissues of the body, remethylation reactions mainly predominate. Thus, the liver plays an important role in the metabolism of homocysteine, since it is in it that the main amount of cystathionine-P-synthase is located. Hyperhomocysteinemia can occur with:

1) increased rate of homocysteine formation;

2) violation of transsulfurization processes;

3) a decrease in the rate of remethylation to methionine; here, the provision with vitamins (B2, B6, B9, B12) and microelements (Zn) plays an important role; it has been proven that hyperhomocysteinemia in 75% of cases is due to inadequate intake of these factors into the body [30];

4) the use of anticonvulsants;

5) administration of a folic acid antagonist, metatrexate;

6) MTHFR gene polymorphism; a mutation of the replacement of cytidine in position 677 by thymidine (C677 ^ T) was described, leading to the incorporation of alanine instead of valine in the polypeptide chain and the appearance of thermolabile properties of the enzyme with a decrease in its activity by 50%; the frequency of this mutation varies in different regions and among different ethnic groups, averaging 10–20% in Europeans [24, 26];

7) food load with methionine.

Currently, the relationship between hyperhomocysteinemia and the occurrence of fetal malformations is being intensively studied. Folate has been shown to be protective against neural tube defects [26]. Interestingly, the first literary data on this issue date back to the 18th century, when a Danish midwife in her notes noted an increase in the number of children with defects of the central nervous system after lean years [25] and the predominance of this pathology among the poorest segments of the population. Such manifestations were often noted as a consequence

of natural disasters and wars. An increased incidence of malformations was reported in Holland after World War II, or a 3-fold increase in central nervous system malformations in children in Jamaica after a devastating hurricane [14].

The aim of our work was to determine the level of homocysteine in the plasma of pregnant women with normal fetal development and in the presence of congenital malformations. Introduce methylfolate 1000 mg into the treatment complex in patients with hyperhomocystemia.

Material and research methods. 60 pregnant women with various types of fetal anomalies were examined. The comparison group consisted of 39 patients with normal fetal development. The studied groups did not differ in age, parity, the onset of menstrual function, the presence of genital and extragenital pathology. detector. Statistical processing of the obtained results was carried out using nonparametric statistics.

Results. It was found that in the blood of pregnant women whose fetuses were diagnosed with malformations, there was a significantly higher level of homocysteine than in patients of the comparison group. It was significantly higher in women whose children suffered from congenital heart defects, central nervous system and chromosomal abnormalities. There was no significant difference in the content of homocysteine in the blood plasma of pregnant women whose fetuses had such malformations as skeletal dysplasia, anomalies of the abdominal wall (omphalocele, gastroschisis), polycystic kidneys and lungs, and others (this subgroup includes cystic fibrosis, sacrococcygeal teratoma, atresia of the gastrointestinal tract). Noteworthy is moderate hyperhomocysteinemia detected in pregnant women of the comparison group. Only 28% of pregnant women in this group had a normal plasma homocysteine level, and in 33% this figure exceeded 30 μmol/l. It should be noted that 35.1% of women in the comparison group had hypertensive disorders in late pregnancy. Identified hyperhomocysteinemia in women of the comparison group may indicate inadequate preconception preparation, the main component of which is folate intake. According to the results of a survey conducted among women in both groups, it was found that only 11.1% (!) of patients took folic acid on the eve of the planned pregnancy and in the first 3 months of the onset of pregnancy. 37.1% of women reported taking folic acid during pregnancy, from about 7 to 8 weeks, although the formation of the neural tube and 4-chambered heart occurs up to 28 days from the moment of conception and up to 8 weeks of pregnancy, respectively. Therefore, the appointment of folic acid in this period and later loses its protective effect. Finally, 51.8% of women did not take folic acid even in the first 12 weeks of pregnancy! Moreover, about a third of the patients learned about the need to take folic acid to prevent fetal malformations only during the survey.

Discussion. The data obtained indicate the need for a significant change in the approach to preconception preparation, which is a significant reserve for reducing perinatal losses. No correlations were found between the level of homocysteine and the age of pregnant women, parity, the onset of menstrual function and sexual life in women of the main group. In the comparison group, a direct correlation was found between the homocysteine content and the age of the patients; there was no correlation between the homocysteine level and other studied parameters, which is consistent with the literature data [21].

Folic acid deficiency disrupts the process of homocysteine remethylation and leads to hyperhomocysteinemia, which, according to some authors, is the cause of neural tube defects and congenital heart anomalies [4, 26]. Moreover, both methionine synthase and cystathionine P-synthase are present in embryonic tissues already at the early stages of development [27]. Interesting data on folate deficiency are given in the work of Japanese authors [31]. Although Japan is considered to be a safe country in terms of vitamin sufficiency, the so-called "Westernization" of lifestyle, including in matters of nutrition, has led to an increase in the incidence of Down syndrome. The authors attribute this to an increased level of homocysteine in the blood plasma of the women examined by them. In the United States, in 1993, a government decree was adopted (in force since 1998) on the saturation of cereal products with folic acid [26]. The results of these actions will be known in the near future. Many authors believe that the cause of fetal malformations may be the direct teratogenic effect of homocysteine [28]. In addition,

homocysteine and its derivative, homocysteine-thiolactone, disrupt the processes of apoptosis, the main mechanism for the formation of cavities and organ configuration in the fetus (the appearance of cleft face and hard palate [32]), affect the process of neuron migration, and regulate ion fluxes. Ca2+ through membranes, inhibit the synthesis of antioxidant enzymes, activate oxidative stress and stress of the endoplasmic reticulum [19, 24, 28]. It should be noted that homocysteine-thiolactone can induce apoptosis in the cytotrophoblast [20] and, in addition, can be incorporated into the structure of the polypeptide chain and change the conformational properties of proteins, ultimately leading to disruption of their normal functioning [19].

Our data confirm the role of homocysteine in the genesis of malformations of the central nervous system and heart (see table). The level of homocysteine is also significantly increased in chromosomal abnormalities (in our study, these were cases of Down's syndrome and Klinefelter's syndrome). A possible cause of chromosomal pathology may be DNA methylation disorders, or the so-called epimutations. For example, DNA hypomethylation in the tissues of a developing embryo leads to an increased frequency of neural tube defects [26].

We found that none of the patients from the main group had well-known genetic risk factors. The average age of pregnant women was 25 years.

Study groups	Number of observations	Homocysteine level (μmol/l) /Me (25% - 75%)
Hydrocephalus	8	48.55 * / 26.32 - 80.44
Neural tube defects	2	60.25 * / 48.55 - 71.89
Chromosomal pathology	6	71.06 * / 20.96 - 109.47
Skeletal dysplasia	5	25.24 / 15.7 - 52.17
UPU	6	38.31 * / 29.54 - 74.62
Abdominal wall pathology	8	25.36 / 16.71 - 39.68
Polycystic kidneys and lungs	7	22.25 / 10.33 - 35.75
Other vices	5	34.62 / 28.46 - 61.85
Comparison group	39	22.52 / 3.285 - 52.44

Congenital anomalies in the fetus were detected by chance during multiple ultrasound examinations and after invasive diagnostic methods (chromosomal abnormalities). This indicates the presence of epigenetic disorders in them. The term "epigenesis" refers to the modification of certain DNA regions (cytidine-guanosine), which regulates the implementation of the cell's genetic program without changing the nucleotide sequence of the genome [29]. The main mechanism of epigenetic regulation is DNA and histone methylation. It has been established that homocysteine directly or as a result of accumulation of SAH suppresses the activity of DNA methyltransferases and thus affects epigenesis [14]. Given that a significant number of congenital anomalies have a weak genetic basis (chromosomal diseases account for only 3% of all congenital malformations [7]), this mechanism may explain the occurrence of anomalies in the fetus. Perhaps hyperhomocysteinemia should be regarded as one of the epigenetic risk factors.

Interestingly, the described metabolic disorders can be identified at the preconception stage, and this will allow us to assess both the risk of obstetric complications (including the likelihood of congenital malformations in the fetus) and the quality of preconception preparation. Unlike ultrasound, which is inherently "stating", the detection of elevated levels of pathogenic metabolites long before the onset of pregnancy and the timely correction of the identified conditions can play a huge role in preventing the occurrence of fetal malformations.

Conclusions

1. Our data confirm the role of homocysteine in the genesis of fetal malformations. Determination of homocysteine levels should be used in the prenatal diagnosis of malformations of the central nervous system, heart and chromosomal abnormalities in the fetus.
2. The use of methylfolate at a dose of 1000 mg three months before conception and intake throughout pregnancy reduces the level of homocysteine and reduces the development of malformations in the fetus.

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