Hyperhomocysteinemia is a Prior Diator of Congenital Anomalies

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ABSTRACT

Currently, the number of congenital diseases is increasing significantly. to prevent and treat the next generation of these congenital diseases, congenital disease markers play an important role as predictors. One of the predictors is maternal serum hyperhomocysteinemia. therefore, we decided to explain in detail the etiology of hyperhomocysteinemia, as well as the toxic effects on the maternal and especially the fetal body, and the mechanism of how hyperhomocysteinemia can cause congenital diseases.

Keywords: hyperhomocysteinemia; folate cycle; hypercoagulopathy; DNA methylation; endothelial dysfunction.

Introduction. Homocysteine is an amino acid that is involved in several key metabolic processes, including the methylation and sulfur pathways. The concentration of homocysteine in the blood is determined by various dietary factors, including folic acid and vitamin B12, physiological changes, and changes in enzyme activity in various pathways as a result of genetic polymorphisms. Impaired maternal and fetal homocysteine metabolism is associated with fetal neural tube defects, with various conditions characterized by placental vasculopathy, such as preeclampsia, fetal abruption, as well as recurrent miscarriage, and other congenital anomalies.

Folic acid is known to play a role in both placental development and fetal growth as it promotes the synthesis of proteins, lipids, and DNA. Folic acids are vital in many metabolic pathways, including the conversion of serine to glycine, histidine catabolism, thymidylate, methionine, and purine synthesis, and DNA methylation, all of which are important for proper gene silencing or expression [31]. Folate contains a methyl group and can affect DNA methylation by altering 1-carbon metabolism. Epigenetic regulation of growth processes through folic acid supplementation may contribute to embryonic development and fetal growth (Figure 1). In contrast, homocysteine has been reported to be inversely related to placental and fetal growth [11].

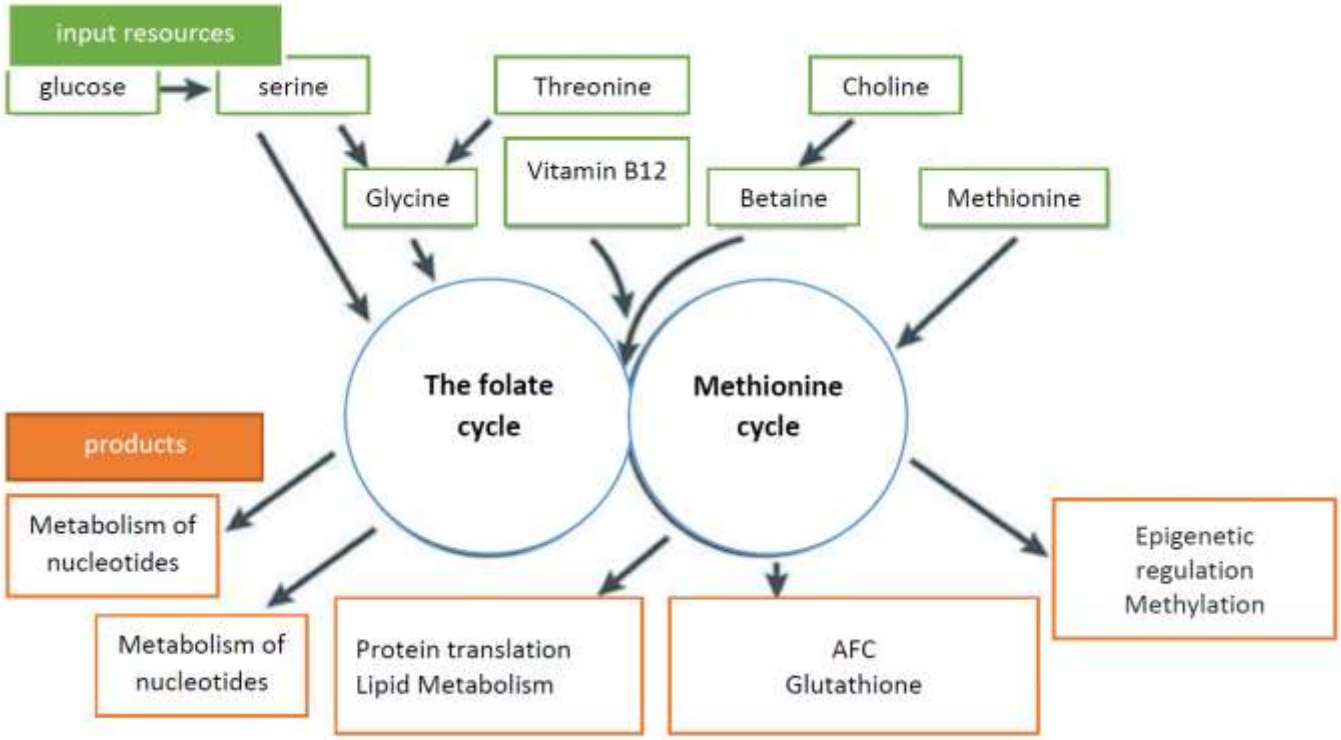

Figure 1. Demonstrates the main roles of the folate and methionine cycle in the body. NADPH - Nicotinamide-β-adenine dinucleotide phosphate; ROS is an active form of oxygen.

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 and is a toxic product. The source of homocysteine in the human body is methionine. It is an essential amino acid used in protein synthesis or the synthesis of S-adenosylmethionine [4]. But due to the reverse conversion to methionine (and to a relatively lesser extent through transsulfuration to cysteine) with the help of folic acid and B12 vitamins, homocysteine does not normally accumulate in the body [2,6]. The metabolism of homocysteine begins with the intake of folic acid from food. Folic acid enters the human body in the form of folate polyglutamate. In the brush border of the small intestine, cleavage of glutamic acid residues occurs, then folate-monoglutamate is absorbed into the bloodstream using the PCFT transporter (Fig.) and is reduced to tetrahydrofolate (THF). Folate is transported into cells by the transporter of reduced folates, RFC1, and by endocytosis upon binding to folate receptors, FRα, FRβ, and FRγ. These proteins are differentially expressed in various tissues; the placenta, yolk sac, and neural epithelium are especially rich in folate receptors before neural tube closure. FR and RFC1 transport 5-methylTHF, but FR also has a high affinity for THF [1,8]. In the cell, folylpolyglutamate synthetase reattaches glutamate residues to folate, which is necessary for its accumulation in the cell. 5-Methyl-tetrahydrofolate is formed from 5,10-methylene-tetrahydrofolate in a reaction catalyzed by flavoprotein methylenetetrahydrofolate reductase (MTHFR - The MTHFR gene is located on the short arm of chromosome 1 (1p36.3) and consists of 11 exons. The length of the entire coding region is about 1980 base pairs . 5-methylTHF transfers a one-carbon group to homocysteine to form methionine while being converted to THF by methionine synthase (MTR). Methionine enters the methylation cycle: under the action of methionine adenosyltransferase, S-adenosylmethionine (SAM) is formed from it, which is used by methyltransferases as a universal methyl donor. There are more than 80

methylation reactions in the human body, including DNA and RNA methylation, the synthesis of choline, phosphatidylcholine, adrenaline, etc. During these reactions, SAM is converted to S-adenosylhomocysteine (SAH), which is then hydrolyzed by adenosylhomocysteinase to adenosine and homocysteine and completes the cycle. In addition to participating in the methylation cycle, homocysteine reacts with serine to form cystathionine under the action of cystathionine β-synthase. Cystathionine further serves as a substrate for the formation of cysteine. The bioavailability of dietary vitamin B12 varies with the amount of vitamin B12 in the diet, but typically averages around 50% [1,9]. Vitamin B12 in food is bound to protein and is released in the stomach under the action of an acidic environment and proteolysis under the action of pepsin. The released vitamin B12 first binds to R-binders, which are food proteins that have an affinity for vitamin B12. The stomach contains specialized parietal cells that contain H+, K+-ATPase, which produces gastric acid. In humans, these cells also secrete a 50 kDa glycoprotein called intrinsic factor (IF), which can bind vitamin B12. When the vitamin B12-R binder complexes pass through the small intestine, the R binders are hydrolyzed by pancreatic proteases and the released vitamin B12 binds to IF [18]. The IF receptor (cubulin) recognizes the IF-vitamin B12 complex, not vitamin B12 or unligated IF, and this complex is internalized by a receptor-mediated endocytic process. Endosomes fuse with lysosomes, IF is destroyed, and vitamin B12 is released into the cytosol [8].

Methylcobalamin is a cofactor of the previously described folate-dependent methionine synthase (MTRR) involved in homocysteine remethylation. Methionine synthases are zinc metalloproteins and contain three domains: a catalytic domain containing binding sites for 5-methyl-H4PteGlun and homocysteine, a vitamin B12 domain in which vitamin B12 binds a cofactor, and an additional protein domain [2].

The content of total homocysteine in the plasma of a healthy person is in the range of 5.0-10.0 μmol. A concentration of 10-30 μmol/l corresponds to moderate, 30-100 μmol/l - medium, above 100 μmol/l - severe hyperhomocysteinemia [5].

The main causes of hyperhomocysteinemia are: Folic acid deficiency; Vitamin B12 deficiency; Polymorphism of the MTR, MTRR, MTHFR genes and other genes involved in the folate cycle, which can cause insufficient expression of these enzymes; Epimutation of these genes leads to underexpression of proteins [6]. Studies of the last 15 years have confirmed and deepened the homocysteine theory of the development of vascular disorders, according to which an increase in the concentration of homocysteine in blood plasma leads to damage and activation of endothelial cells lining the walls of blood vessels, which significantly increases the risk of thrombosis and triggers the atherogenic process. Homocysteine is able to freely cross the placenta and have a toxic effect on the fetus [2,6]. Currently, hyperhomocysteinemia is associated with pregnancy complications (recurrent miscarriage, hypertensive disorders, intrauterine growth retardation), the occurrence of certain types of fetal malformations (neural tube defects, heart defects and facial clefts), neurodegenerative diseases, hydrocephalus (ventriculomegaly), anomalies of the anterior abdominal walls, esophageal atresia, diaphragmatic hernias, cognitive impairment, psoriasis, carcinogenesis, and lower limb defects [4]. The level of homocysteine is significantly increased in case of chromosomal abnormalities (the diagnosis of Down syndrome and Klinefelter syndrome was verified during amniocentesis) and in the detection of cervical hygroma, the presence of which in 50% of cases is also associated with pathology of the chromosomal apparatus [10]. According to Plotsky, a higher level of homocysteine was detected in the plasma of pregnant women in the presence of skeletal dysplasia in the fetus, pathology of the lungs, and tumors of the perinatal period [2,4].

The toxic effect of high levels of maternal blood homocysteine can be divided into a direct toxic effect on endotheliocytes, which disrupts blood circulation in the uterus, which leads to fetal hypoxia, and a genetic effect, which

disrupts normal epigenesis and protein expression.

Since the endothelium is not just a mechanical barrier between the vascular wall and circulating blood, it is a tissue that produces vasoactive substances, mediators, and their inhibitors. With the help of these biologically active substances, the endothelium plays a leading role in the control of vascular tone. One such substance is nitric oxide. It is produced continuously by the endothelium and has several protective properties, including vasodilation, inhibition of smooth muscle cell proliferation, and reduced aggregation of platelets and other blood cells. In addition, under normal conditions, nitric oxide can react with homocysteine and thus "neutralize" it. The result of this interaction is the formation of S-nitrohomocysteine - an additional powerful vasodilator and a weakening of platelet aggregation. However, these protective properties of nitric oxide cannot be realized under conditions of hyperhomocysteinemia, since at elevated concentrations homocysteine harms its activity and synthesis. Homocysteine-induced release of oxygen radicals, intrinsic oxidation, and lipid peroxidation subsequently lead to a decrease in the activity of endothelial nitric oxide synthetase. Thus, under conditions of hyperhomocysteinemia, there is a decrease in the synthesis of the most important factor of vasodilation and protection of the endothelium [8]. And also homocysteine in large quantities significantly reduces the synthesis of prostacyclin (PGI2 - responds to relaxation of smooth muscle fibers of blood vessels and causes platelet disaggregation, contributing to fibrinolysis) and increases the formation of thromboxane A2 (TkA2 - causes platelet aggregation, thereby contributing to thrombosis, has the most powerful vasoconstrictor effect) endothelial cells. Thus, in hyperhomocysteinemia, the TcA2/PGI2 ratio increases, which affects vascular tone and enhances thrombogenesis [7]. Also, high concentrations of homocysteine inhibit the function of natural anticoagulants, such as antithrombin III and protein. In addition, it is able to change the normal antithrombotic properties of the endothelium, which leads to an increase in the activity of coagulation factors - V, X and XII [1,7]

Unfortunately, pregnancy itself is a condition in which the risk of venous thrombosis increases by 5-6 times. Several pathogenetic mechanisms have been proposed to explain this relationship: compression of the inferior vena cava and iliac veins by the pregnant uterus, an increase in blood volume during pregnancy, insufficiency of venous valves, as well as such predisposing factors as: a tendency to stasis as a result of hormonal changes, a state of physiological hypercoagulability, due to changes in the rheological and coagulation properties of blood and inhibition of fibrinolysis [9].

As a result of the influence of hyperhomocysteinemia during pregnancy, blood clots are formed and microcirculation is disturbed in tissues, including in the uterine wall and placenta, which leads to a number of obstetric complications both in the early stages of pregnancy (embryo implantation defects, habitual miscarriage) and in the later stages. terms (chronic placental insufficiency, fetal growth retardation, fetal death). Hyperhomocysteinemia is a risk factor for the development of autoimmune processes and antiphospholipid syndrome, which also disrupt the normal development of pregnancy. In addition, homocysteine freely crosses the placenta and can have a direct embryotoxic effect [7].

Many authors believe that the cause of fetal malformations may be the direct teratogenic effect of homocysteine [6]. In addition, homocysteine and its derivative, homocysteine-thiolactone, disrupt apoptosis processes, affect neuronal migration, regulate the flow of Ca2+ ions through membranes, and suppress the synthesis of antioxidant enzymes. It should be noted that homocysteine thiolactone can induce apoptosis in the cytotrophoblast [8], 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 [9].

Since hyperhomocysteinemia is known to affect the extent of DNA methylation, it is likely that abnormal DNA methylation during embryogenesis may be a pathogenic factor for these congenital disorders [3]. DNA methylation is an important regulatory mechanism of gene expression, the basis of epigenesis. DNA hypomethylation leads to chromosome instability and promotes mutagenesis [5]. Also, DNA hypomethylation leads to changes in the centromeric regions of chromosomes, impaired chromosome segregation in oogenesis, and increases the risk of having a child with Down syndrome (trisomy on chromosome 21) . Therefore, one of the main causes of habitual miscarriage of the first trimester is the presence of genomic mutations in the fetus, the occurrence of which in most cases is due to nondisjunction of chromosomes in gametogenesis in parents, which leads to poly- and aneuploidy in the fetus or genetic mosaicism [5].

And also in rapidly dividing fetal cells, a deficiency of methyl groups leads to an increased inclusion of dUMP instead of dTMP in the synthesized DNA chain, which entails excision of nucleotide pairs, breaks in DNA chains, and triggering apoptosis mechanisms. As a result, abnormally easily fragmented DNA is formed, and its synthesis is sharply slowed down [7].

Mammalian sperm and egg genomes are highly methylated compared to somatic cells. However, a few hours after fertilization, rapid demethylation occurs. Until the morula stage, DNA methylation remains reduced and the cells are pluripotent, with all genes potentially active. Remethylation at this stage depends on the part of the affected embryo, and while the ectoderm and mesoderm become hypermethylated, the primary endoderm and trophoblast remain hypomethylated [30]. There appears to be a remethylation sequence that determines the structure and function of each formatting somatic tissue. The methyl groups used for DNA methylation are supplied by S-adenosylmethionine (SAM) and catalyzed by various types of DNA methyltransferases (DNMTs). In such critical cases as DNA remethylation, a sufficient amount of SAM plays an important role for the initial differentiation of cells. If the mother's body is deficient in folate, B12 and other factors that play a significant role in providing the embryo with a methyl group, this disrupts the normal differentiation, migration and apoptosis of embryonic cells. Since due to hypomethylation by a specific DNA locus leads to abnormal overexpression of proteins.

Nutrition is a strong environmental factor that, directly or through the modification of genes and proteins, affects the molecular, biological processes of both the mother and the child during pregnancy. Malnutrition early in pregnancy contributes to congenital malformations, miscarriage, intrauterine growth restriction, and preeclampsia. In the second and third trimesters of pregnancy, maternal nutrition plays a role in the programming of fetal organs.Therefore, an adequate intake of folic acid also contributes to the prevention of other types of birth defects, including heart defects and underdevelopment of the limbs. In addition, low folate intake during pregnancy is associated with an increased rate of preterm birth and low birth weight of the infant . Elevated levels of homocysteine have been measured in women with emotional stress, and elevated levels of homocysteine have been found in the plasma and amniotic fluid of women pregnant with NTD compared with women pregnant without NTD [13].The results of these studies have been introduced into clinical practice. According to WHO recommendations, the daily dose of folic acid for a pregnant woman should be from 400 to 800 micrograms. Reception should begin 2-3 months before the planned pregnancy and continue until at least 3 months of pregnancy [1,6].

Conclusions: Compelling evidence supports the use of folic acid supplements during pregnancy to prevent neural tube defects [11]. According to some reports, women who did not take folic acid during pregnancy were 3.25 times more likely to give birth to a child with congenital anomalies compared to their counterparts [2].

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