Original Article

Hyperhomocysteinemia and Pregnancy Complications: Elucidating the Role of L-Methylfolate

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ABSTRACT

Women have higher requirements of nutrients like folate during pregnancy. An optimal level of this vitamin must be achieved before conception and maintained throughout pregnancy. Low maternal folate level is causally related to various maternal and fetal complications. Therefore, increasing maternal folate intake before conception and during pregnancy can prevent many pregnancy complications.

Folate deficiency can occur because dietary folate intake is low or because of the genetic defect, hampering folate metabolism, viz. methylenetetrahydrofolate reductase (MTHFR) polymorphism. The MTHFR is a critical enzyme in folate metabolism and is responsible for conversion of inactive folic acid into active L-5-methyltetrahydrofolate (L-5-MTHF), also called as 6(S)-5-methyltetrahydrofolate or L-Methylfolate.

The MTHFR directs folate species to homocysteine (Hcy) remethylation. The MTHFR polymorphism affects this activity of the MTHFR enzyme leading to hyperhomocysteinemia and is associated with various pregnancy complications. Global prevalence of MTHFR polymorphism ranges from 20 to 50%, whereas in India, it is between 28 and 35%. Such a high prevalence poses a great burden of functional folate deficiency and related complications. In such a condition, supplementation with the active form of folate, i.e., L-5-MTHF, is a better alternative to folic acid.

Keywords: Hyperhomocysteinemia, L-Methylfolate, Methylenetetrahydrofolate reductase polymorphism, Neural tube defects, Preterm labor.


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INTRODUCTION

In recent years, the clinical significance of raised levels of amino acid homocysteine is being recognized as a risk factor for pregnancy-related complications like neural tube defects (NTDs), congenital heart defects, still birth, preterm birth, low birth weight (LBW), intrauterine growth restriction (IUGR), pre eclampsia, preterm labor, placental abruption, gestational hypertension, recurrent pregnancy loss, and intrauterine fetal death (Fig. 1).1-8

Homocysteine is a sulfur-containing amino acid that is not used for the synthesis of proteins. Foods only contain traces of homocysteine. Homocysteine is formed when cells metabolize an essential amino acid, viz. methionine. The intracellular homocysteine concentration is precisely regulated and any excess of homocysteine is transported to plasma. In plasma, approximately 99% is oxidized to disulfides. The vast majority (70%) of homocysteine is bound to proteins. Nonprotein-bound homocysteine consists of homocysteine (the disulfide of homocysteine) and mixed disulfides of homocysteine with, e.g., cysteine.

Only about 1% of all homocysteine moieties is reduced “free” homocysteine (free thiol group). The term total plasma homocysteine (conventionally abbreviated with tHcy) refers to all these forms of homocysteine in plasma (Fig. 1).9

Typically, a level <15 μM/L is considered normal. A level between 15 and 30 μM/L is considered mildly elevated, 30 to 60 μM/L is considered moderately elevated, and >60 μM/L is considered severely elevated.10

Intracellular homocysteine can be irreversibly degraded to cysteine through the transsulfuration pathway, which is mainly limited to cells of the liver and kidneys. The enzymes in this pathway, cystathionine-β-synthase and cystathionase, are both dependent on pyridoxal-5-phosphate, a biologically active form of vitamin B6, as a cofactor.

Homocysteine can also be remethylated to methionine by the enzyme methionine synthase. This enzyme uses methylcobalamin (a biologically active form of vitamin B12) as cofactor. The methyl group for the latter reaction is donated by L-5-MTHF. This form of folate is produced by the enzyme 5,10-MTHFR (Fig. 2).9

Disturbances in intracellular homocysteine metabolism lead to elevated tHcy concentrations.
Deficiency of these vitamins (folic acid, vitamin B12, and vitamin B6) and MTHFR polymorphisms leads to elevated homocysteine plasma concentrations. Multiple mutations have been identified within the MTHFR gene. The MTHFR gene polymorphism leads to reduced enzyme activity and this is associated with hyperhomocysteinemia, which further leads to pregnancy complications.

Hyperhomocysteinemia and Pregnancy Complications—Exploring the Links

Neural Tube Defects

Some authors have presented the theory, which has been gaining increasing acceptance of late, that the teratogenic agent causing NTD in situations of shortage of folates is homocysteine.\textsuperscript{11-20} Hyperhomocysteinemia could cause embryonal toxicity by acting at the level of deoxyribonucleic acid (DNA), either by causing hypomethylation of DNA, or causing structural alterations in the genes implicated in DNA synthesis.\textsuperscript{17,19,21-23} López-Quesada et al\textsuperscript{24} affirmed that hyperhomocysteinemia induces congenital defects by altering the function of the N-methyl-D-aspartic acid receptor.\textsuperscript{3}

Increased total homocysteine has been found in plasma and in amniotic fluid of women with NTD-affected fetuses.\textsuperscript{25} High levels of homocysteine may directly or through indirect metabolic pathways interfere with neurulation and lead to NTDs.\textsuperscript{26}

Vascular-related Pregnancy Complications

There is a strong association between homocysteine and vascular-related pregnancy complications like pregnancy-induced hypertension, recurrent pregnancy loss, preeclampsia, placental abruption, IUGR, miscarriage, and intrauterine fetal death. \textit{In vitro} studies suggest that the pathogenesis of vascular disease associated with homocysteine is related to endothelial dysfunction, smooth muscle cell proliferation, and abnormalities of coagulation.\textsuperscript{27,28} In addition, homocysteine generates reactive oxygen species such as H$_2$O$_2$ that may induce oxidative stress and thereby endothelial dysfunction.\textsuperscript{27} This is in line with research of the last three decades pointing toward the association between hyperhomocysteinemia and both cardiovascular diseases\textsuperscript{28} and vascular-related pregnancy complications.\textsuperscript{26-30}

Steegers-Theunissen et al\textsuperscript{2} demonstrated that hyperhomocysteinemia is associated with an approximately two- to threefold increased risk for pregnancy-induced hypertension, placental abruption, and IUGR.\textsuperscript{2} Pregnant women with hyperhomocysteinemia have a 7.7-fold risk for preeclampsia.\textsuperscript{24} Its etiopathogenesis has been shown to include the existence of vascular damage, with endothelial toxicity being the factor most directly related with development of the disorder.\textsuperscript{31} It has been demonstrated that one of the factors implicated in the vascular dysfunction of pregnant women with preeclampsia is hyperhomocysteinemia, which brings about an endothelial dysfunction as the result of an increase in the
oxidative stress caused by an increase in the concentration of fibronectin, lipid peroxides, and plasma triglycerides. These changes convert the anticoagulant phenotype of the endothelium into procoagulant and this favors the genesis of thrombosis.31

Increased levels of homocysteine have been associated with repeated miscarriages. Hyperhomocysteinemia is a factor of vascular damage that acts by favoring thrombogenesis at the level of the placenta vessels (both arteries and veins), which would reduce fetal blood supply and alter the normal course of pregnancy.3 Early miscarriages may be explained by the damage that excess homocysteine may cause on chorionic and decidual vessels leading to defective implantation of the embryo. Meegdes et al32 conducted histological studies that showed a high number of avascular villosities and a reduction of the vascular density of the placenta vessels in women with spontaneous early miscarriages when compared with controls. On the contrary, Nelen et al33 studied women with repeated miscarriages and found a direct relationship between high levels of homocysteine and alterations in the vascularization of chorionic villosities, which presented reduced vascular areas, perimeters, and diameters. It, therefore, seems evident that the mechanisms in which hyperhomocysteinemia produces vascular alterations or toxicity could be the same as those which also cause thrombosis and infarcts in adults.

On the contrary, it has also been suggested that maternal hyperhomocysteinemia could produce direct toxic effects on the fetus as in vitro experiments have shown that homocysteine has specific embryo toxicity and when it is produced too early, it leads to miscarriage.22

Leeda et al34 described that the incidence of hyperhomocysteinemia in patients with IUGR is seven times greater than in the normal population.

Dekker et al35 found a 17.7% prevalence of hyperhomocysteinemia in pregnancies with IUGR, whereas de Vries et al36 mentioned figures as high as 24% (Graph 1).

Fetal death may be brought about by a multitude of causes, although in the majority of cases, there is an alteration of the placenta and its vascularization. Some cases of fetal death are the result of extreme IUGR, pre-eclampsia, or detachment of the placenta. In all these cases, there is some form of vascular placental damage and increased level of homocysteine.3 Atherothrombotic mechanisms would, therefore, be a determining factor in the physiopathology of these intrauterine fetal deaths, with hyperhomocysteinemia possibly being one of the factors bringing about the vascular damage.3

**MTHFR Polymorphism—A Genetic Mutation that hampers Pregnancy Outcomes**

Folic acid is a synthetic compound that has no biological function unless it is reduced by a multistep reaction into L-5-MTHF.52

The MTHFR is an enzyme involved in the metabolism of folate. The MTHFR catalyzes the conversion of 5,10-MTHF to L-5-MTHF, the major circulating and active form of folate. In turn, L-5-MTHF is involved in the conversion of homocysteine to methionine. The MTHFR has an important role in maintaining folate and methionine levels, as well as helping to keep circulating homocysteine levels low. The MTHFR is also involved in the methylation pathway, which has multiple, wide-ranging roles in the body, including regulation of gene expression and enzymatic activities.37

Multiple mutations have been identified within the MTHFR gene. One of the most common and best characterized mutations is the substitution of a thiamine (T) for a cytosine (C) at position 677 (677C→T polymorphism), resulting in the amino acid alanine being replaced by valine in the MTHFR enzyme. The 677C→T mutation results in the expression of MTHFR enzyme with reduced activity.37,38

There are three possible MTHFR genotypes at this position: the wild-type CC, CT, or TT. The frequency of the three alleles differs between various populations, and the 677TT genotype is more common among Caucasians and Hispanics in the United States than in African-Americans.38

The 677C→T mutation results in a reduced specific MTHFR activity in isolated lymphocytes (~34% residual activity in 677TT and ~71% residual activity in 677CT relative to 677CC) which leads to higher tHcy concentrations.9

The MTHFR polymorphism is associated with an elevated plasma level of homocysteine as a result of a decreased production of 5-MTHF; this is especially noticeable when the folate levels are low.37

Other mutations are also found in the MTHFR gene. Another important mutation is at position 1298, where there is substitution of a C for an A. There are three possible genotypes at this position: the wild-type AA, AC,
or CC. Approximately 30% of the population has at least one C allele at position 1298.38

Various Clinical Studies have demonstrated the Association between MTHFR Polymorphism and Pregnancy Complications

The 677C→T variant of the MTHFR gene has been identified as a genetic risk factor for NTD42,43 and may account for up to 19% of NTD cases.44-46 Study conducted by Hobbs et al47 demonstrated that obese women carrying the MTHFR TT genotype were 4.6 times more likely to have an affected pregnancy compared with normal weight women carrying a CC genotype. Results indicate that functional polymorphisms in folate-related genes increase the risk of having a fetus with CHD when maternal lifestyle factors that alter folate metabolism are present. In a similar study conducted, it was observed that maternal MTHFR polymorphism in combination with no use of periconceptional folate supplements was associated with a three- to sixfold increased risk for conotruncal heart defects in offspring.48 Similarly, MTHFR polymorphism is associated with placental abruption, IUGR, LBW infants, and gestational hypertension.49-51

L-5-MTHF—A Genetic Nutrient to Counteract MTHFR Polymorphism and associated Pregnancy Complications

The L-5-MTHF is the predominant form of dietary folate which is normally found in the circulation, and hence, it is this folate that is normally transported into peripheral tissues to be used for cellular metabolism. The L-5-MTHF is also available commercially in crystalline form of the calcium salt, which has the stability required for use as a supplement.53

Folic acid is a synthetic form of the vitamin, which is only found in fortified foods, supplements, and pharmaceuticals. It has no biological function, lacks coenzyme activity, and must be reduced to the metabolically active tetrahydrofolate form within the cell.52,53

In contrast to folic acid that is not found in foods in significant amounts, L-5-MTHF is the predominant natural form of folates in many foods. It is also the essential form in which folates occur and are stored in the human body.54

In contrast to folic acid that needs to be reduced in two steps (catalyzed by the enzyme dihydrofolate reductase) before it can serve as a cofactor in the activated methyl cycle, L-5-MTHF can be used directly in methylation reactions.54

It is, therefore, hypothesized that L-5-MTHF may have a higher bioefficacy in methylation reactions involving this type of folate, such as the methylation of homocysteine. Supplementation with L-MTHF produced a slightly yet significantly larger reduction of plasma homocysteine levels than the supplementation with an equimolar amount of folic acid, pointing to slightly higher bioefficacy of the natural folate.55

In the United States, L-5-MTHF-Ca, meeting appropriate food grade specifications and produced in accordance with current GMP practices, is “Generally Recognized As Safe” for use as a source of folate in conventional and medical foods and dietary supplements.54

The European Scientific Committee on Food in its report in 2000 stated that there is no evidence for risk associated with high intakes of natural reduced folates.54

Polymorphism in the gene coding for MTHFR reduces efficiency of folic acid metabolism. The MTHFR polymorphism leads to production of MTHFR enzyme with reduced activity. Due to this, the active form of folic acid is not synthesized. Thus, polymorphisms increase the risk of hyperhomocysteinemia-associated NTDs and other pregnancy complications. Since L-5-MTHF is the active form of folic acid, its action is unaffected by genetic polymorphism.

Apart from the above-mentioned advantages of L-5-MTHF over folic acid, the potential for masking the hematological symptoms of vitamin B-12 deficiency may be reduced with L-5-MTHF. Masking of vitamin B-12 deficiency is unique to folic acid. The predominant naturally occurring form of folate, L-5-MTHF, is unlikely to mask vitamin B-12 deficiency. The conversion of L-5-MTHF into tetrahydrofolate, which is the precursor of folate forms involved in DNA synthesis, is vitamin B-12 dependent, whereas folic acid can be converted to tetrahydrofolate, independent of vitamin B-12. Therefore, folic acid may maintain intracellular DNA synthesis and ameliorate megaloblastic anemia.53,56

Also, L-5-MTHF may be associated with an increased interaction with drugs that inhibit dihydrofolate reductase.53

Thus, supplementation of the natural form, 5-MTHF, appears to be a better alternative to supplementation with folic acid. Supplemental 5-MTHF can effectively improve folate biomarkers in young women in early pregnancy in order to prevent NTDs and other pregnancy complications.52

L-5-MTHF/Folic Acid and Prevention of NTDs

It has been very well established that folic acid supplementation before conception and during pregnancy (first trimester) significantly reduces the incidences of NTDs. Documented evidences suggest that the decrease in incidences is directly proportional to the dose of folic acid and the maximum protection from NTDs is achieved at a dose of 5 mg/day (Table 1).
The Motherisk guidelines suggest use of 5 mg/day folic acid several months before conception and until the end of first trimester. According to the guidelines, compliance is less than optimal among women using prenatal vitamins, rendering many women unprotected against NTDs. Taking a higher dose of folic acid will allow achievement of protective folate levels, even with partial compliance.59 Similarly, the Society of Obstetricians and Gynaecologists of Canada guidelines recommend 5 mg folic acid per day additionally with folate-supplemented diet at least 3 months before conception and continued until 10 to 12 weeks after conception.60

The bioavailability of folic acid and L-5-MTHF was assessed in a randomized, double-blind, four-period crossover study on 21 healthy women given a single oral dose of folic acid (400 µg) or equimolar L-5-MTHF with or without prior loading with folic acid (1 mg/day for 10 days). The plasma folate concentration was measured by immune assay in fasted subjects and every hour for 8 hours after intake of test material. The area under the curve (AUC) of concentration–time was calculated for L-MTHF without preloading (AUC ratio, 156%; 90% confidence interval, 137–177%) and for folic acid with preloading (AUC ratio, 143; 90% confidence interval, 124–164%). The bioavailability of L-MTHF was slightly higher at the start of the study but not at the end of the supplementation period. Overall, the bioavailability of L-MTHF was similar to that of folic acid.61

In a comparative study evaluating the efficacy of L-5-MTHF-Ca and folic acid, equimolar supplements of L-5-MTHF-Ca (113 µg/d), folic acid (100 µg/d), or placebo were administered to women of childbearing age for a period of 24 weeks. Folates were determined in plasma and erythrocytes at regular intervals during the study using a microbiological assay. At the end of the supplementation period, the increases above baseline values of folate levels were similar for L-5-MTHF-Ca and folic acid supplementation. At the applied dose level, a plateau (saturation) was not reached.62

The bioavailability of L-5-MTHF and folic acid (400 µg/d) was compared in a randomized, double-blind, crossover study with 21 healthy female volunteers (Prinz-Langenohl et al).63 Plasma samples were collected in fasted state and for 8 hours (in hourly intervals) after intake of the supplements, prior to, and after 10 days of supplementation. Plasma folate concentrations were measured by immunoassay. Evaluation of the AUC and maximum concentration of the plasma folate concentration indicated that L-5-MTHF had a slightly higher bioavailability at the start of the study, but at the end of the supplementation period, this was no longer apparent.54

From all this, it can be concluded that L-5-MTHF-Ca is bioavailable to an extent similar or slightly higher than folic acid.

Also international organizations like the European Food Safety Authority, Food Standards Australia New Zealand, and Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives have concluded that L-5-MTHF has a similar or slightly higher bioavailability and bioefficacy than folic acid.

Thus, if the optimum dose of folic acid for maximum protection from NTDs is 5 mg and the bioavailability of folic acid and L-MTHF is similar, the optimum dose of L-MTHF is 5 mg.

### Augmenting Hyperhomocysteinemia-lowering Action of L-5-MTHF with Methylcobalamin and Pyridoxine

Apart from folic acid, vitamin B12 and vitamin B6 can also lower the homocysteine levels and augment this action of folic acid. Studies conducted by Bibi et al63 demonstrated that the combination of folic acid, vitamin B12, and vitamin B6 significantly lowers homocysteine levels in hyperhomocysteinemic women. Safety and tolerability of B vitamins are well established. Studies conducted by Maladkar et al64 demonstrated that the fixed dose combination of folic acid and methylcobalamin is effective and well tolerated in the treatment of patients with vitamin B deficiency.

Thus, supplementation with 5-L-MTHF, vitamin B12, and vitamin B6 can act synergistically and effectively reduce homocysteine levels as compared with either of the vitamins alone.

### CONCLUSION

Hyperhomocysteinemia is a risk factor associated with various pregnancy complications. Lowering homocysteine levels during pregnancy can ensure favorable pregnancy outcomes, which can be achieved by administration of combination of vitamins like folic acid, vitamin B12, and vitamin B6. The MTHFR polymorphism hampers folic acid metabolism leading to increased tHcy levels, which ultimately increases the risk of pregnancy complications. Folic acid supplementation in such cases

<table>
<thead>
<tr>
<th>Dose</th>
<th>% prevention of NTD</th>
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<tr>
<td>400 µg</td>
<td>36</td>
</tr>
<tr>
<td>1 mg</td>
<td>57</td>
</tr>
<tr>
<td>4 mg</td>
<td>82</td>
</tr>
<tr>
<td>5 mg</td>
<td>85</td>
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Table 1: Dose-dependent reduction of NTDs with folic acid67,58

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can be inefficient in reducing the homocysteine levels and in improving the folate status. The L-5-MTHF is the active form of folic acid whose action is independent of MTHFR enzyme. Supplementation of L-5-MTHF in women can effectively improve folate status irrespective of MTHFR polymorphism and significantly reduce pregnancy complications and can ensure better pregnancy outcomes. Studies have shown that L-5-MTHF has similar bioavailability to folic acid. Thus, 5 mg of 5-L-MTHF appears to be a better alternative to 5 mg folic acid in preventing pregnancy-related complications associated with hyperhomocysteinemia irrespective of MTHFR polymorphism.

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