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Review Article

Folate Metabolism. Polymorphisms of Methylentetrahydrofolate Reductase. Concurrent Factor in Pathogenic Effects

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Abstract

Vitamin B9, folate in its natural form is present in vegetables, fruits and organ meats. The function of folate is to act in processes of transfer one carbon compounds, methylation on nucleotides, amino acids and phospholipids, processes of vital importance for cell division, synthesis of nucleic acids, amino acids and methionine from homocysteine1. Folic acid is the synthetic form most used as a supplement to compensate low intakes of folate but has not normal biological actions if it is not converted to 5-methylenetetrahydrofolate, by the enzyme methylenetetrahydrofolate reductase. This enzyme may presents polymorphism due to mutation with biotypes of varied activity. The most commonly present, C677C and A1298A, has full activity, but C677T and T677T and A1298C and C1298C with decreased activity between 30 and 70 %, can cause metabolic alterations such as hyperhomocysteinemia and DNA hypomethylation with increased risk for chromosomal abnormalities and various pathological conditions, as neural tube development disorders, early recurrent pregnancy losses, Down syndrome, cleft lip and/or palate, limb reduction, cancer, mediterranean familial fever, polycystic ovarian syndrome and neurological or cardiovascular diseases. Faced with a history of these disorders and the presence of the aforementioned enzyme biotypes, the replacement of folic acid by L 5-methylenetetrahydrofolate supplied together with vitamin B12, B2, and B6 may reduce the risk. Beside this, with excessive intakes of folic acid, especially when low activity of the enzyme is preset, high levels in the blood can be produced, with consequently higher risk of various health problems. To avoid this, recommended intakes of folic acid should not be exceeded.

Keywords: Folate, Metabolism disorders, Methylentetrahidrofolate reductrase, Early pregnancy loss. Down syndrome

Introduction

Vitamin B9, folate in its natural form is present in green vegetables, fruits and organ meats. Folic acid is the synthetic form widely used as a supplement to compensate low intakes of folate as happens with processed and ultra processed foods as habitual feeding. However, for normal biological actions folic acid must be converted into 5-methylenetetrahydrofolate (5-MTHF) by the enzyme Methylenetetrahydrofolate Reductase (MTHFR).

The function of folate is to act together with vitamins B12, B2 and B6 in the transfer of one carbon units, methylation processes on nucleotides, amino acids and phospholipids, processes of vital importance for cell division, synthesis of nucleic acids, amino acids and methionine from homocysteine [1]. Folic acid does not have normal biological actions if it is not converted by the enzyme MTHFR into 5-10-methylenetetrahydrofolate (5-10-MTHF) and 5-MTHF, the predominant form in the circulation. The bioavailabi lity of folic acid is greater than that of folate, which depends on its content in food and its absorption, which can be interfered with by antinutrients, such as alcohol [2,3] or tobacco [4]. Blood folate levels are 15% lower in smokers and reduced transport of folate to the fetus has been observed in pregnant women who smoked or abused alcohol during pregnancy [5,6]. Folate absorption is also decreased by inflammatory bowel diseases such as ulcerative colitis, regional enteritis, Crohn's disease, or celiac disease [7].

Methionine Synthase (MS) produces methionine from the remethylation of homocysteine, methionine by action of the enzyme adenosyl transferase gives rise to its methyl donor form, S-Adenosylmethionine (SAM) essential for many methylation reactions, including methylation of DNA-by-DNA Methyl Transferase (DNMT) essential for its stabilization and control of gene expression, critical during cell differentiation [8,9]. The folate content, as well as other nutrients, varies according to age of the plant, time of the year, soil moisture and form of consumption; fresh preparations without prior cooking and fruits with a low degree of maturity have higher contents. All these differences have implications for the bioavailability of natural folate, while that of folic acid as a supplement is 100%, but when added to food it is reduced to approximately 85% [10].

Dietary folate is mostly reduced molecules while folic acid is fully oxidized. Natural sources of folate occur especially in avocado, orange, beetroot, asparagus, spinach, lettuce and broccoli. Folic acid should be considered as a medication to compensate for low folate intake from diets low in vegetables and abundant in processed foods. Although the majority of folic acid is converted into 5-MTHF, with excessive intake, high levels in the blood that have not been converted can be detected and numerous health problems can occur, such as leukemia, asthma, depression, even the progression of pre-existing neoplastic lesions or preneoplastic diseases [11-14].

Folate metabolism is compartmentalized in the cytoplasm, mitochondria, and nucleus with specific interdependent metabolic pathways. The entry of folate into cells is made by membrane transporters or by folate-binding proteins; 40% of cellular folate is in the mitochondria, 10% in the nucleus, and 50% in the cytoplasm. Folate catabolism is extremely slow but is accelerated by Methotrexat (MTX), which acts as an antifolic, with consequent DNA hypomethylation [15]. Inadequate availability of folate, consequence of a deficit in intake, malabsorption or alterations in methylation processes due to mutation polymorphisms with decreased activity of enzymes involved, mainly MTHFR, can cause metabolic alterations, lower concentration of 5-MTHF, decreased trans-sulfurization for glutathione synthesis with increased oxidative stress [3-18] and increased folic acid and homocysteine and DNA hypomethylation with increased risk for chromosomal abnormalities and various pathological processes [19].

The frequencies with which the MTHFR biotypes 677C>T and 1298A>C occur in the general population have been studied in many countries [20,21]. The dispersion of frequencies in population groups of seven of these countries [22-28] of biotypes CC, CT and TT for 677 are, respectively: 36.5-71.2%, 26-51.2% and 3.9-20.9%; and in 5 of these countries [22-26] of biotypes AA, AC and CC for 1298 are: 39.8-70.1%, 8.8-47.2% and 3.6-13-5%. The determination of these biotypes is carried out by PCR [29] techniques and we have required it in certain clinical cases, but no studies have been carried out in Argentina in order to show frequency in our population.

Methylenetetrahydrofolate Reductase Polymorphism and Related Disorders

Heterozygous biotypes C677T and A1298C show significant pathological associations and even more so, although less frequent, homozygous T677T and C1298C. The reason why these biotypes increase the risk for various pathologies is because they are more thermolabile and have less activity, between 30 and 70% of normal.

Taking into account the very high frequency in the general population of the MTHFR gene mutation that gives rise to the referred biotypes, and the very low prevalence of the pathologies with which associations have been observed, it is necessary to accept the presence of concurrent factors. Genetic factors, homozygous, heterozygous, other enzymes, as MS, SAM, DNMT. Epigenetic factors, mainly habits as alcohol or tobacco [2-6] and nutrition, including for some of these associations, the nutrition of the maternal grandmother in whose womb the oogenesis of the future mother occurs.

Neural Tube Developmental Disorders

The best known and widespread benefit of folic acid administration to prevent pathological alterations is in relation to Neural Tube Developmental (NTD) disorders, pathology in which, before the use of folic acid, prevalence of up to 18.6 per 10,000 births were recorded [30].

With the folic acid supplement, a significant reduction of approximately 75% of this pathology was observed, was prevalence as low as 5 per 10,000 births. To explain cases that are not prevented with folic acid supplementation, the studies carried out show that the most common associated alteration is the presence of the C677T biotype with 2 to 4 times greater risk and secondly, that of A1298C [31-35].

Recurrent Early Pregnancy Loss

In recurrent pregnancy loss, chromosomal abnormalities, uterine abnormalities or acquired thrombophilia may be the cause, but in a large number of cases the etiology is unknown [36].

Meta-analysis of 5,888 cases with 8,400 controls from 39 studies, from Caucasian population groups, showed a significant association with increased risk, with the C677T and A1298C MTHFR [37]. Another study with Chinese women also showed this association and also with a higher risk of male infertility [38].

Down Syndrome

As early as 1999 it was observed that MTHFR mutations with the consequent metabolic alterations could be risk factors for Down Syndrome (DS) [19].

Polymorphisms C677T and A1298C of MTHFR, and elevated plasmatic homocysteine were seen in 72 DS mothers with 194 controls, being a risk factor for DS. Chromosomal non-disjunction was also observed in lymphocytes from DS mothers who showed this association [22,39,40].

Chromosomal aneuploidy, trisomy 13, 18 and mainly the most frequent, trisomy 21, constitute the main cause of fetal death in our species. In early pregnancy losses, during the first 15 weeks of gestation, 50% of the cases show chromosomal aneuploidy and trisomy 21, the most common due to non-disjunction during meiosis in oogenesis, is the most common chromosomal abnormality in newborns. Review studies and meta-analysis carried out on publications up to 2021 show the C677T biotype as the first polymorphism associated with DS [41-44]. Maternal MTHFR polymorphisms in interaction with habits [2-4] (alcohol, tobacco) and food increase the risk of errors in meiosis II in oocytes regardless of maternal age [45]. These factors are even important during the first meiotic division in the maternal grandmother.

Cleft Lip and/or Palate

A significant association was observed between cleft lip and/or palate with maternal biotypes and T677T but not with A1298C [46,47].

Limb Reduction

Defects due to transverse or longitudinal reduction of the extremities are of multifactorial etiology. Although more than 50 genes involved in the development of limbs have been identified, little is known about the genetic etiology of deficiencies in this development [48].

Epidemiological studies show a decreased risk for defects in limb development with periconceptional multivitamin supplementation with folic acid, and risk assessment with MTHFR biotypes show an increased risk with C677T only when periconceptional vitamin supplementation was not used [49].

A prevalence of limb reductions of 2.7 per 10,000 births with the use of folic acid during pregnancy has been reported in northern China, compared to 9.7/10,000 when folic acid was not used, with a statistically significant association for upper limb reductions. In southern China, no differences were observed [50].

Cancer

Polymorphisms due to MTHFR mutations with decreased activity and consequent alteration in blood folate level and increase in homocysteine are associated with various types of cancer, in some cases with chromosomal breaks [51]. Deviations in the complex regulatory mechanism of DNA methylation lead to hypomethylation or hypermethylation with loss of DNA stability or decreased expression of tumor suppressor genes with cancer implications [17,18].

In particular, increased risk has been reported for: breast cancer [52], cervical, ovarian or endometrial cancer [53], hepatocellular cancer [54], acute lymphoblastic leukemia and non-Hodgkin lymphoma in adults [55] and acute lymphoblastic leukemia in children [56].

Familial Mediterranean Fever

Familial Mediterranean Fever, recessive hereditary auto inflammatory disease, showed association with T677T and A1298C [57].

Polycystic Ovary Syndrome

Polycystic ovarian syndrome is an endocrine and metabolic disease that affects women of childbearing age for which no treatment has been reported. Its etiology is unknown, but its pathogenesis is considered to be multifactorial.

Elevated levels of homocysteine are observed, known factors also involved in pregnancy loss and reduced ovulation. Homocysteine levels are strongly determined by enzymatic activity of MTHFR with biotypes C677T, A1298C and MS with biotypes A66G associated with increased risk. Adequate treatment for the metabolic disorder of folate can reduce the risk for this disease [58,59].

Neurological Disorders

According to a review of publications carried out up to 2020 between neurodegenerative diseases with enzyme mutations involved in folate metabolism with high homocysteine levels, folic acid, vitamin B6 and B12 supplementation aimed at reducing homocysteine levels have potential therapeutic applications [60].

Also, mutations in folate 1 receptor have been reported, associated with progressive motor disorders with psychomotor decline and epilepsy with a significant decrease in the concentration of folate in cerebrospinal fluid, profound hypomethylation, decreased choline and inositol in glial cells. Restoration was referred to with folinic acid, with clinical improvement, postnatal myelination and brain development [61,62].

Cardiovascular Disease

Whether due to folate or vitamin B12 deficiency or MTHFR polymorphism, increases in homocysteine can be observed, a known factor that increases the risk of cardiovascular disease. On the risk of cardiovascular disease associated with MTHFR polymorphisms, with biotypes C677T, T677T and A1298C, observations are still insufficient, but they seem to be associated with increases in homocysteine [63-65].

Prevention of Recurrences

Faced with a history of disorders such as those referred, the presence of MTHFR biotypes, mainly C677T and/or T677T, and/or A1298C and/or C1298C, can prevent recurrences by reducing the risk, with the substitution of folic acid supply, by L 5-MTHF supplied together with vitamin B12, B6, B2, C and D with controls to maintain normal levels of the same and homocysteine. 5-MTHF must necessarily be specified to be the left-handed form, L 5-MTHF. The D forms should not be used due to low bioavailability. B12 levels greater than 500pg/ml, folate greater than 10mg% and homocysteine less than 10mg% should be maintained.

In recurrent pregnancy loss with a diagnosis of thrombophilia that did not respond to heparin treatment (generally successful for acquired thrombophilia) and presence of the referred MTHFR biotypes (possible hereditary thrombophilia) in addition to heparin treatment, daily supply of 5mg of L-MTHF during 3months prior gestation and 1mg the entire course of the pregnancy together with B12, B6, B2, C and D, risk of recurrence may decrease.

Side Effects of Folic Acid

A systematic investigation of publications up to 2018 with 108 articles referring to 133 meta-analyses studies with 154 controls, shows beneficial effects of folic acid supplementation in the prevention, evolution and mortality rates in various types of cancer, neurological diseases and in pregnancy and its final product, but in addition to these benefits, adverse effects are recorded, such as increased risk of prostate cancer, allergies, asthma, and depression [11-13].

In interpreting this increased risk for adverse effects, food fortification programs with folic acid should first be taken into account, such as the one implemented in 1998 in the United States, extended to a total of 80 countries, with an estimated contribution of between 100 and 200ug per person per day. In Argentina, the addition of folic acid to wheat flour is regulated along with iron, thiamine, riboflavin and niacin, in containers labeled in this regard, excluding flour intended for the production of dietary products and for export. It should be considered that this addition should be extended to all flours, such as corn and rice to cover differences in eating habits by ethnic groups or sensitivity to gluten gliadin, mainly wheat.

For women of childbearing age, the contribution should be 400ug per day. In peri-conceptional supplementation, 1mg per day should be adequate and only when there is a history of NTD disorder, this supplementation should be 5mg per day.

Higher intakes can cause high levels of circulating unmetabolized folic acid, a reason for adverse effects, especially when there are MTHFR biotypes with decreased activity.

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None.

Conflict of Interest

None.

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