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Review Article

FOLIC ACID, VITAMIN B12, AND DNA METHYLATION: AN UPDATE

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ABSTRACT

Epigenetics is one of the exciting and fastest expanding fields of biology; this is above genetics. Methylation is the process involved in the transfer of methyl group to amino acids, proteins, enzymes and DNA of all the cells, and tissues of the body. During cell-division low folate availability may result in decreased production of thymidine wherein uracil may be substituted in the place of thymidine in the DNA sequence. It was reported that folate and Vitamin B12 restricted diet resulted in aberrant methylation patterns. The current review was undertaken to explore the role of folic acid and Vitamin B12 in DNA methylation.

Keywords: DNA methylation, Folic acid, Vitamin B12.

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INTRODUCTION

Epigenetics is one of the exciting and fastest expanding fields of biology; this is above genetics. The modifications of epigenetics play a very important role in the regulation of many cellular processes including DNA replication, gene expression, and recombination. This is achieved through regulatory mechanisms such as DNA methylation, hydroxymethylation, histone modifications, chromatin remodeling, and RNA modifications like methylation. Misregulation of epigenetic mechanism may have adverse effects on health and may lead to neurological disorders, developmental abnormalities and also cancer. Therefore, epigenetic modifications are evolving as very potent diagnostic and prognostic biomarkers in the world of medicine.

Methylation is the process involved in the transfer of methyl group to amino acids, proteins, enzymes and DNA of all the cells, and tissues of the body. Donation of the methyl group is very important in the regulation of cell energy, gene expression, neurological function, detoxification (in liver), immunity, etc. Methylation is one of the important biochemical processes that occur in the body and is catalyzed by different enzymes. This is a process influenced by environmental conditions, decreases with age. Methylation depicts the quality of life in terms of diseased and health conditions.

The methylation processes require two cycles - Cycle A: S- adenosylmethionine (SAM) and Cycle B- folate cycle. The most stable synthetic form of Vitamin B9 is folic acid (FA), and the natural form is termed as folate. The natural dietary folates are polyglutamated consisting of six glutamate molecules linked together by peptide bonds. Mainly there are two dietary folates, 5-methyltetrahydrofolate (5-MeTHF) and 10- formyltetrahydrofolate (10-formyl THF). In the gut, before to the absorption polyglutamates are hydrolyzed to monoglutamates by the enzymes γ-glutamyl hydrolases and is absorbed [1,2]. FA with monoglutamyl residues gets converted to the biologically active form known as THF by the reducing reaction (first it is converted to dihydrofolate (DHF) and then to THF). These reduction reactions are catalyzed by the single NADPH-dependent enzyme DHF reductase (DHFR). THF receives the one-carbon (C_1) units from various donors such as serine, glycine, and histidine during catabolic reactions and can transfer them to specific acceptors for the synthesis of various compounds such as purines, methionine, choline, formyl-methylated tRNA, thymidylate, and serine. The addition of one carbon unit with a simultaneous reduction will produce 5-MeTHF, the main circulating form in the blood. Protein carriers and the tissue-specific folate receptors carry 5-MeTHF into the cells, where they get accumulated and are transformed to polyglutamates. Polyglutamates cannot traverse biological membranes by passive diffusion. Thus, polyglutamylation serves to sequester folate in the cells in which it is required. The enzymes involved in folate metabolism have a higher affinity for polyglutamates than to monoglutamates. FA is involved in remethylation process wherein, 5-MeTHF donates a methyl group for homocysteine in the presence of the enzyme methionine synthase, converting homocysteine to methionine liberating THF. This THF directly gets converted into 5,10-methylene THF by the action of the enzyme serine hydroxymethyltransferase which is present both in mitochondria and cytosol.

5-MeTHF is used in remethylation of homocysteine to methionine in the presence of methionine synthase in all the tissues except red blood cells. This process requires a cofactor the Vitamin B12 (cobalamin). Methylcobalamin is the donor of a methyl group to homocysteine and the transfer mediated through THF getting converted to 5-MeTHF. After donating the methyl group, methylcobalamin is converted back to cobalamin. Methylcobalamin is reproduced from cobalamin by receiving the methyl group from 5-MeTHF. Methionine is an essential amino acid converted to active methionine, i.e., SAM also known as the universal donor. SAM is the active donor of a methyl group for various methylation reactions that occur in the body such as methylation of nucleic acids, proteins, lipids, neurotransmitters, and creatine synthesis [3]. SAM gets converted to S-adenosyl homocysteine (SAH) after donating the methyl group to the acceptors further, gets hydrolyzed to adenosine and homocysteine by the action of the enzyme SAH hydrolase (SAHH). The decrease in 5-MeTHF or cobalamin made lead to the accumulation of homocysteine the potent inhibitor of various methyltransferases. Thus, cobalamin and folate are together involved in methylation process. An absence of cobalamin leads to the cessation of the reaction and build-up of methyltetrahydrofolate (MeTHF) known as "folate trap." Therefore, MeTHF tends to accumulate in Vitamin B12 (cobalamin) deficiency, leads to depletion of other coenzyme forms that are needed for nucleotide synthesis. This is how folate trap hypothesis

explains the anemia of cobalamin deficiency, but it cannot account for the neurological manifestations of pernicious anemia.

METHODS

A detailed review of published literature from Google, PubMed, and MEDLINE was performed and analyzed.

Folate DNA methylation

FA, when consumed in fortified foods or supplements, is primarily metabolized to 5-methyl THF that behaves similar to natural dietary folate. Initially, FA is reduced to DHF in the presence of the enzyme DHF reductase, further converted to THF and enters the folate pool. In some cases, the oxidized form of FA may appear in circulation with an increase in DHF reductase enzyme [4]. The coenzyme THF is converted to 5,10-methyleneTHF by the enzyme serine hydroxymethyltransferase requires Vitamin B6. Further MTHFR irreversibly reduces it to 5-methyl THF. This is a key reaction for the maintenance of the methyl flux essential for the remethylation of homocysteine to methionine in the presence of Vitamin B12-dependent methionine synthase. Methionine is converted to SAM, an active methyl donor wherein numerous SAM-dependent reactions play regulatory roles by affecting gene transcription, genome stability [5] and localization of protein [6], etc.

Along with folate, many other dietary nutrients such as Vitamin B6, Vitamin B12, riboflavin (Vitamin B2), and choline are required for the maintenance of one carbon flux and normal formation of SAM, homocysteine remethylation, and DNA methylation. DNA methylation and one carbon metabolism work under tight regulatory control. Homocysteine remethylation is folate-dependent and requires SAM as an important regulator for this process. Increase in SAM inhibits MTHFR this reduces 5-methylTHF synthesis further hinders homocysteine remethylation. In contrast, remethylation is favored with low concentrations of SAM and SAM-dependent methyltransferase is inhibited by SAH [7,43,44]. Therefore, for the maintenance of normal DNA methylation, there should be a continual conversion of SAH to homocysteine [8] and increased plasma concentration of homocysteine is associated with increased concentration of SAH which in-turn associated with hypomethylation of global DNA [9]. The common genetic variant 677C-T modifies the activity of MTHFR and reduces the formation of 5-methylTHF [10].

DNA methylation and low folate status

The studies on the association of low folate with increased risk of NTDs, cardiovascular disease and multiple cancers are well established but, the mechanism leading to these disorders is yet unclear [11-13]. During cell-division low folate availability may result in decreased production of thymidine wherein uracil may be substituted in the place of thymidine in the DNA sequence. This may increase the frequency of chromosomal breaks to repair the defect made by the mutagenic event. This was studied by a tissue culture where the MTHFR-TT genotype shows the formation of increased micronuclei as a result of multiple chromosomal breakages occurred under low folate conditions [14]. The effects of supplementation of FA may promote or prevent cancers, stated in many studies. In human cancers, the DNA methylation is dysregulated. It shows either hypermethylation or hypomethylation stating that the association of DNA methylation with tumor is cell or tissue or organ-specific. A study showed genome-wide hypomethylation but found 5% hypermethylated patterns defining the characteristics of the specific type of human tumor. These altered DNA methylation cause chromosomal instability and silencing of tumor suppressor genes [16]. However, it is very important to note that decrease in folate status may result either in hyper or hypomethylation leading to the misregulation in the complex system. (Shelnutt *et al*., 2004) On controlled feeding studies observed the association of low folate concentration with reduced DNA methylation in older women but not in younger female adults (Rampersaud *et al*., 2000; Jacob *et al*., 1998). Jacob and Shelnutt both had observed that the folate intake during repletion resulted in increased DNA methylation and the increase was limited to MTHFR TT genotype. Friso *et al*. (2002) and Pufulete *et al*. (2005) also found the same trend of lowered DNA methylation with low serum folate concentration. These studies taken together suggest that the genotype, age, duration, and magnitude of exposure should be considered as the response of global DNA methylation to the folate status is different at different conditions. In the above studies, variations in DNA methylation were found between the sexes and ages (El-Maarri *et al*., 2007); (Fraga *et al*., 2005) justifying the observations of different methylation patterns with low and high folate status.

MTHFR is an essential enzyme involved in the irreversible conversion of 5,10 MethyleneTHF to 5-methylTHF thus, playing important role in DNA synthesis, DNA methylation and maintenance of balanced nucleotide pool (Friso *et al*., 2002; Kim *et al*., 1999). Analyses of MTHFR polymorphisms are included to investigate the folate status and DNA methylation in humans. As many observational studies had established the association of low folate with hypomethylated DNA in subjects with homozygous MTHFR C677TT genotype. The biochemical mechanism behind this is the MTHFR C677TT polymorphism causes thermolability thereby reducing the MTHFR activity by lowering 5-methylTHF levels and leading to the accumulation of 5,10methyleneTHF, increase in plasma homocysteine levels and finally changing the cellular composition of one-carbon folate derivatives stating that there is a greater risk of global DNA hypomethylation in the women carrying MTHFR T allele as it is involved in the impairment of enzyme activity modulating both gene and genome-specific DNA methylation (La Merrill *et al*. 2012).

High folate and DNA methylation

Many studies on folate insufficiency and the effect of low folate in humans are well established. Low folate has detrimental effect on the embryo and increases the risk of NTD's, increases the possibility of long-term risk of diabetes and also may lead to many other health outcomes [26-29]. High folate concentrations and increased folate supplementation had shown contradicting results in different in controlled feeding trial studies. Certain studies have shown the association of high folate with high global DNA methylation and reduced risk of cancer [30,31]. A recent study had shown reversal effect that is, the increased folate supplementation resulted in stimulation and progression of existing tumors and altered normal DNA methylation patterns [32-34]. As DNA methylation is a regulatory process depends on tissues, sequence of DNA, genome region, stage of transformation, degree, duration and exposure of folate intervention, timing, and other regulatory proteins and enzymes involved in the process. Hence, we should include all these factors to study the effect of high folate, whether high folate leads to increased risk or benefit.

Folate, Vitamin B12, and placental DNA methylation

Placenta is an important organ. Proper development and function of placenta are crucial for the growth, health, and survival of developing fetus. Many studies had established links between epigenetic changes in the placenta and the risk of disease in gestation and early life (Kim *et al*., 2009). On examination of the epigenetic changes occurred in the placenta had evolved interest in the research of biomarkers of exposure, pathogenesis of the disease and the biology of the development of the disease [36]. Presently, many studies on nutrition during pregnancy and placental outcomes are taken up to understand the basis of disease seen in early or later in life. Nutrition and epigenetic changes are the emerging topic of interest in the present scenario to understand the effects of increased supplementation of micronutrients like FA and learning the importance of balancing the different micronutrients in the diet to avoid unbalanced nutritional disorders and other health complications later in life. One such study is carried on rats to know the effect of FA supplementation in utero on the epigenetic changes in the offspring. It was observed that maternal dietary folate during pregnancy led to placental DNA hypomethylation and showed that there is a significant correlation between folate levels of placenta and placenta genomic DNA methylation. On the other hand, the study also stated the importance of maintaining the ratio of folate and Vitamin B12. As

it has a significant role in determining genomic methylation patterns. Wherein, high folate in the absence of Vitamin B12 resulted in placental DNA hypomethylation [37]. It has also shown the association of maternal folate deficiency with DNA hypomethylation [38]. Yet, another animal study in sheep with maternal folate and Vitamin B12 restricted diet resulted in aberrant methylation patterns, i.e., only 4% of cytosine-guanine dinucleotide islands (CpG islands) out of 1400 CpG islands were methylated. Along with hypomethylation the adult male offspring also showed increased adiposity, altered immune function, high blood pressure, and insulin resistance [39].

A study conducted by Kulkarni *et al*. [37] on the adverse effects caused by excess FA supplementation in the presence or absence of Vitamin B12 deficiency and correlated it with global DNA methylation patterns. The team observed a reduction in the levels of global DNA methylation on excess maternal FA supplementation with low plasma Vitamin B12 concentration. They also observed the effect of Vitamin B12 deficiency with excess or normal FA levels on docosahexaenoic acid (DHA) levels. In this study, the team for the first time had identified the DHA plays an important role in one-carbon metabolism, influencing the placental global DNA methylation. Further learned that on the supplementation of omega three fatty acids, the DHA levels in placenta got increased and the DNA methylation levels were reverted back to that of the control group. This study suggested that the altered ration of FA and Vitamin B12 during pregnancy have effect on DNA methylation thereby influence imprinting in the embryo and could be associated with adverse pregnancy outcomes. These epigenetic changes caused, may alter the gene expression and could be carried throughout the lifespan of an individual [40].

Till date, there are only a few studies performed to learn the association of FA and Vitamin B12 and together their effect on thyroid hormones. The well- established knowledge regarding the two micronutrients (folate and Vitamin B12) is (i) deficiency of Vitamin B12 is very common in hypothyroidism (ii) both the micronutrients are very important for fetal development and the imbalance ratio of Vitamin B12 and FA (high folate with low Vitamin B12) may lead to small for gestational age infants [42-44] and other detrimental effects on the growing fetus, and (iii) both folate and Vitamin B12 are important along with other micronutrients such as Vitamin B6, choline to prevent NTD's, and proper methylation process to occur.

CONCLUSION

Nutrition and epigenetic changes are the emerging topic of interest in the present scenario to understand the effects of increased supplementation of micronutrients like FA. The available information is that the increased folate supplementation had a suppressive effect on thyroid hormones (T_3 and T_4) and may possibly lead to motivational deficits and memory impairments in adolescent rats and also observed that the animals were functionally hypothyroid during the administration of folate. The increased levels of folate supplementation alone may be harmful not only during fetal development and on growing infant but also in the adolescent period. If the same effect implies in humans with increased folate supplementation during pregnancy, i.e., high folate intake may lead to suppression of maternal plasma thyroid hormonal levels, would have alarming implication on the health of the fetus.

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