INTRODUCTION

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 folate acid and Vitamin B12 in DNA methylation.

Keywords: DNA methylation, Folic acid, Vitamin B12.

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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 DHFR 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-methylenetetrahydrofolate (MTHF) by the enzyme serine hydroxymethyltransferase THF monomethyltransferase 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 methytransferase 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 [15]. 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. (Shelnut 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 Shelnut both 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 methylations 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 hypermethylated DNA in subjects with homozygous MTHFR C677TT genotype. The biochemical mechanism behind this is the MTHFR C677T 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 methylation. The studies showed that 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 contradictory results in different 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.
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 is that a high degree of LINE-1 methylation in peripheral blood mononuclear cells, a one-carbon nutrient related epigenetic alteration, is associated with a lower risk of developing cervical intraepithelial neoplasia. Nutrition 2011;27:513-9.

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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 (T3 and T4) 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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