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TITLE: OXIDATIVE STRESS AND GENETIC POLYMORPHISMS IN THE FOLATE METABOLIC PATHWAY AND ITS ASSOCIATION WITH TYPE 2 DIABETES MELLITUS

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Abstract: Type 2 diabetes mellitus is a polygenic and multifactorial disease which is considered as the most common form of diabetes rising alarmingly throughout the world in the recent times. The interesting fact is that the chronic hyperglycemia in diabetes itself leads to increased oxidative stress, which may result in oxidative DNA damage. In addition to that, the genetic polymorphisms in the folate metabolic pathway play a critical role in the etiology of diabetes and its complications. The development of complications is mainly due to excessive generation of free radicals that could ultimately result in DNA damage. Evidence also suggests that aberrations in folate metabolism may induce oxidative DNA damage in diabetes mellitus. In this fascinating background, this chapter was designed to elucidate the role of oxidative stress and polymorphisms involved in the folate metabolic pathway and its association with Type 2 diabetes mellitus.

Keywords: Oxidative stress, Hyperglycemia, Reactive Oxygen Species, Antioxidants, DNA damage, Polymorphism, Folate metabolism, Methylenetetrahydrofolatereductase, Methionine synthase, Reduced folate carrier, Glutamate carboxypeptidase, Type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a multifactorial disease which is characterized by hyperglycemia, insulin resistance, and insulin deficiency or both [1]. Risk factors such as obesity, age, ethnic origin, lifestyle and familial history of diabetes contribute to its development. Genetic component also plays an important role in the pathogenesis of diabetes mellitus. It has been reported that the genotype establishes the conditions for the individual to be more or less prone to environmental effects and lifestyle factors [2]. Evidences suggest that polymorphisms in genes involved in folate metabolism plays a critical role in the etiology of diabetes and its complications. As the complex nature of T2DM reflects the multifaceted genetic background and the varied genetic – environmental interaction, the incidence of T2DM varies substantially from one geographical region to the other [3].

PART I: OXIDATIVE STRESS AND DIABETES MELLITUS

Chronic hyperglycemia in diabetes leads to increased oxidative stress and reduced antioxidant levels, followed by the development of chronic complications [4]. Experimental evidence indicates that these complications are mainly developed because of the production of excessive free radicals, which results in oxidative damage to biomolecules [5]. Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals [6]. This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. Radicals derived from oxygen represent the most important class of radical species generated in living systems [7]. The harmful effect of free radicals causing potential biological damage is termed oxidative stress [8]. Oxidative stress results from an imbalance between radical-generating and radical scavenging systems, that includes increased free radical production or reduced activity of antioxidant defenses or both. Implication of oxidative stress in the pathogenesis of diabetes is suggested, not only by oxygen free-radical generation, but also due to nonenzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes, lipid peroxides formation and decreased ascorbic

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acid levels. Humans have evolved with antioxidant systems to protect against free radicals. These systems include some antioxidants produced in the body (endogenous) and others obtained from the diet (exogenous) [9].

Free radical and oxidative stress induced complications of DM includes both microvascular and macrovascular diseases [10,11]. *In-vivo* studies support the role of hyperglycemia in the generation of oxidative stress leading to endothelial dysfunction in the blood vessels of diabetes patients [12]. Increase in the levels of glucose and insulin along with dyslipidemia in patients suffering from diabetes develop macroangiopathies that cause oxidative stress leading to atherosclerosis [13]. Another finding shows that oxidative stress may play a substantial role in the development and progression of neuropathy in Type 2 diabetes patients [14].

Role of Antioxidant defense system and Diabetes mellitus

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. In a biological system they may protect cells from damage caused by unstable molecules known as free radicals. Antioxidant helps in destroying the free radicals that damage cells, promoting the growth of healthy cells, protecting cells against premature, abnormal ageing, help fight against age-related macular degeneration and provide excellent support for the body's immune system [15].

Antioxidant defense mechanisms include both enzymatic and non-enzymatic pathways. Their functions are to counterbalance toxic, reactive oxygen species (ROS) in the human system. Common antioxidants include the vitamins A, C, and E, glutathione (GSH), and the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx) [16]. SOD, CAT and GPx are enzymes that protect against the damage caused by oxidative stress by scavenging free radicals. The primary ROS produced in the course of oxygen metabolism is superoxide (O^{2-}), which is a highly reactive, cytotoxic ROS. O^{2-} is commonly produced within aerobic biological systems, and superoxide dismutases (SODs) provide an important defense against it. Thus, SOD is the front line of defense against ROS-mediated injury which catalyzes the dispolymorphism of the superoxide anion into hydrogen peroxide and molecular oxygen [i]. Therefore this enzyme is one of the most important antioxidant enzymes [17].

$$SOD + 2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2 \text{ [i]}$$

H_2O_2 that is produced by the action of SODs or the action of oxidases, such as xanthine oxidase, is reduced to water by CAT and the GSH- Px. Catalase (CAT, H_2O_2: H_2O_2 oxidoreductase) is an enzyme that decompose hydrogen peroxide (H_2O_2) to molecular oxygen (O_2) and water (H_2O) [iii]. This activity of CAT is known as catalytic activity. It also exhibits peroxidatic activity and catalyses the oxidation of various hydrogen donors in the presence of relatively lower concentrations of hydrogen peroxide. Although CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells [18].

$$CAT + H_2O_2 \rightarrow (CAT - H_2O_2) \text{ (Complex I) [ii]}$$
$$(CAT + H_2O_2) + H_2O_2 \rightarrow CAT + 2\ H_2O + O_2 \text{ (Catalytic activity) [iii]}$$
$$(CAT + H_2O_2) + AH_2 \rightarrow CAT + 2\ H_2O + A \text{ (Peroxidative activity) [iv]}$$

Glutathione peroxidase belongs to a family of tetrameric enzymes that contain the unique amino acid selenocysteine within the active sites. GPx uses low-molecular-weight thiols, such as GSH, to reduce H_2O_2 and lipid peroxides to their corresponding alcohols. There are four different forms of mammalian GSH-Pxs which includes GSH-Px-1, GSH-Px-2, GSH-Px-3 and GSH-Px-4. GSH-Px-1/cellular GSH-Px is ubiquitous and reduces H_2O_2 and fatty acid peroxides and not esterified peroxyl lipids. GSH-Px-4/phospholipid hydroperoxide GSH-Px reduces the esterified lipids. GSH-Px-2/gastrointestinal GSH-Px is localized in gastrointestinal epithelial cells which reduces dietary peroxides. GSH-Px-3/extracellular GSH-Px is the only member of the GSH-Px family that resides in the extracellular compartment and is believed to be one of the most important extracellular antioxidant enzyme in mammals [19].

Antioxidant enzymes constitute one of the major cellular protective mechanisms against oxidative stress in the human body. Several different mechanisms have been proposed to explain the role of oxidative stress in diabetes mellitus. The major mechanisms involved are increased production of ROS and decreased antioxidant defenses. Hyperglycemia in diabetes may increase ROS production and decrease antioxidant defenses due to reduction in plasma total antioxidant status (TAS) [20]. It has been reported that due to low antioxidant defense in plasma also causes problems like blindness, nerve damage, cardiovascular disease and nephropathy [21]. Such type of complications can be reduced by dietary antioxidants and is one of promising therapeutic approach for the treatment of diabetes mellitus [15].

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Association between DNA damage and Diabetes mellitus

Hyperglycemia causes tissue damage by the following mechanisms: (i) increased flux of glucose and other sugars through the polyol pathway, (ii) increased intracellular formation of advanced glycation end-products (AGEs), (iii) increased expression of the receptor for AGEs and its activating ligands, (iv) activation of protein kinase C (PKC) isoforms and (v) overactivity of the hexosamine pathway. All these mechanisms are activated by a single upstream event: mitochondrial overproduction of ROS. When increased intracellular glucose generates increased ROS in the mitochondria, free radicals induce DNA strand breaks [22].

Oxidative stress in response to hyperglycemia in diabetes patients induces peroxidation of cellular membrane lipids, increased oxidative modification of amino acids and DNA [23]. It has been reported that high blood glucose levels *in vitro* may impair the cellular DNA repair and increase DNA cleavage [24]. Several studies have shown that oxidative DNA damage in lymphocytes and plasma antioxidant potential are used as biomarkers to measure oxidative stress. A study conducted in the Egyptian population indicated that diabetes patients have more oxidative DNA damage than controls [25]. Another study demonstrates that elevated levels of oxidative DNA damage are seen in the blood cells of type 2 diabetes patients in the Bangladeshi population [26]. It has been suggested that poor glycemic control might further aggravate the damage. Several studies have shown that elevated glucose level may result in oxidative stress. This has been reported both experimental diabetes in animals and in diabetes patients [20,27, 28].

Parameters including age, duration, poor glycemic control, family history, hypertension and dyslipidemia may aggravate the disease and its complications. It has been reported that hyperglycemia contributes to increased oxidative stress and decreased TAS, which would lead to further increase in DNA damage in diabetes patients with complications. Therefore, it is suggested that the assessment of DNA damage might serve as a diagnostic tool for preventing further development of complications of diabetes mellitus [29].

PART II: FOLATE AND TYPE 2 DIABETES

Folate, water-soluble B vitamin (vitamin B_9) is naturally found in foods such as green leafy vegetables, liver, beans, egg yolks, cereals, some citric fruits, kiwis, and strawberries [30]. Folate is not synthesized *de novo* by humans; therefore the daily requirements are met from the dietary intake of folic acid supplements or food rich in B-vitamin [31]. Folic acid is the form of folate that is used therapeutically [32]. Adequate folate intake is vital for cell division and homeostasis due to the essential role of folate coenzymes in nucleic acid synthesis, methionine regeneration, oxidation and reduction of one-carbon units required for normal metabolism and regulation. Folate requiring reactions, collectively referred to as one-carbon metabolism is involved in phases of amino acid metabolism, purine and pyrimidine synthesis, and the formation of the primary methylating agent, *S*-Adenosyl Methionine (SAM) [33]. A deficiency in cellular folates results in aberrant DNA methylation, point polymorphisms, chromosome breakage, increased frequency of micronuclei, as well as in defective chromosome recombination and aneuploidy [34] and has been linked to several human pathologies including cancer, congenital diseases, cardiovascular diseases, neurological and neuropsychiatric disorders [35]. The results of recent study showed that folic acid deficiency decreased intracellular folate, whereas it increased Hcy concentration, ROS levels and oxidative DNA damage [36].

Genetic polymorphisms in Folate Metabolism Genes and Type 2 diabetes

DNA sequence that codes for a protein is referred as gene. Alternate form of gene is referred as allele. Humans have two alleles at each genetic locus, with one allele inherited from each parent. Each pair of alleles represents the genotype of a specific gene. Genotypes are homozygous if there are two identical alleles at a particular locus and heterozygous if the two alleles differ. Single nucleotide polymorphisms, frequently called SNPs (pronounced "snips"), are the most common type of genetic variation among people. Most commonly, these variations are found in the DNA between genes. The DNA of humans may contain many SNPs, since these variations occur at a rate of one in every 100–300 nucleotides in the human genome. They can act as biological markers, helping scientists to locate genes that are associated with disease. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene's function. Polymorphisms that have been proven to influence gene functions are called functional polymorphisms.

Genes involved in folate metabolism are polymorphic [40]. Polymorphisms in enzymes involved in folate metabolism play a critical role in the etiology of diabetes and its complications [37]. Folate metabolism plays a vital role in nucleic acid synthesis, methionine regeneration, shuttling and redox reactions of one carbon units required for normal metabolism and regulation [38]. Folate metabolism related disorders can be caused by genetic or environmental factors that include an individual's genetic variability and diet [39].

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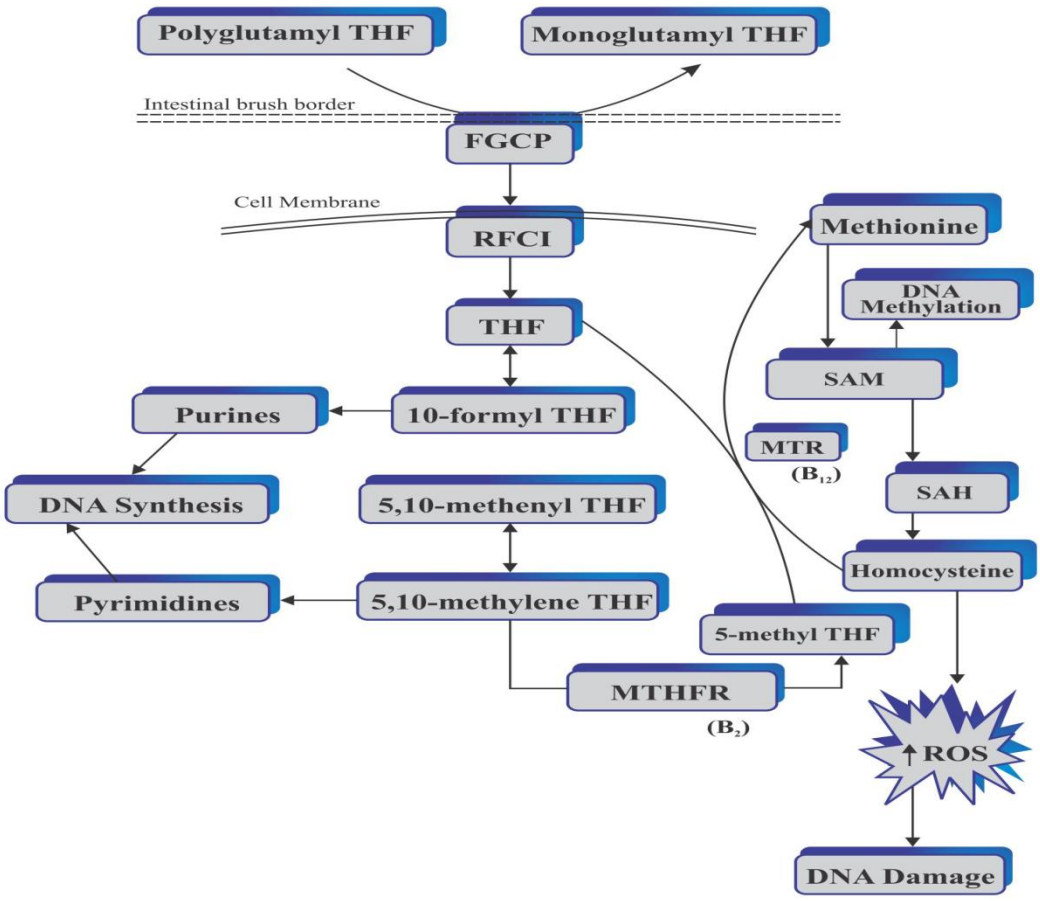

Fig. (1). Schematic pathway showing folate metabolism.
The enzymes encoded by genes involved in the pathway are indicated: [FGCP/GCPII- Folyl poly-γ-glutamate carboxypeptidase, RFCI- Reduced folate carrier, MTHFR- Methylenetetrahydrofolatereductase and MTR- Methionine synthase]

Functional polymorphisms in folate genes are associated with elevated plasma homocysteine (Hcy) levels, decreased functional folate and SAM [41]. Decreased levels of folate are known to be associated with defective synthesis of purines and uracil misincorporation in DNA [42]. Higher rates of occurrence of uracil misincorporation may induce double-strand breakage of DNA [43]. Impairment in folate metabolism may influence oxidative stress as the cycle provides metabolic intermediates that either react with pro-oxidants or promote antioxidant defense [44]. Recently, several studies have investigated SNPs of the genes involved in folate metabolism and their role in various pathology.

The most commonly studied functional genetic polymorphisms in the folate metabolic pathway were as follows,

i. Methylenetetrahydrofolatereductase (*MTHFR*) C677T
ii. Methylenetetrahydrofolatereductase (*MTHFR*) A1298C
iii. Methionine synthase (*MTR*) A2756G
iv. Glutamate carboxypeptidase (*GCPII*) C1561T and
v. Reduced folate carrier (*RFCI*) A80G

Methylenetetrahydrofolatereductase (*MTHFR*) C677T gene polymorphism

MTHFR gene is located on chromosome 1 (1p36.3) and shows single nucleotide polymorphism (SNP) in exon 4 at nucleotide 677 involving a transition of C to T which inturn results in an alanine to valine substitution at codon 222 (*Ala222Val*) [45]. The polymorphism of *MTHFR* C677T (rs1801133) is located in the catalytic domain of the enzyme, which results in a thermolabile enzyme with a decrease in enzyme activity of 50% [46]. The *MTHFR* C677T polymorphism with decreased enzyme activity significantly lowers the serum folate level and consequently increases the blood level of Hcy [47,48]. The resulting hyperhomocysteinemia is

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one of the indicators of impaired methylation capacity and could lead to a number of pathological processes [49]. Earlier studies have shown that the elevated level of Hcy is linked with T2DM complications such as cardiovascular disease, stroke and nephropathy [50-52].

A meta analysis of 29 studies conducted among different ethnic groups such as Japanese, Russian, Polish, Chinese and Arab groups showed that 677 TT genotype was associated with the risk of diabetic nephropathy [53]. The findings of a recent meta-analysis of 15 studies have also reported that the development of diabetic nephropathy is associated with *MTHFR* C677T polymorphisms in Asian populations, especially in early Type 2 diabetes [54]. Furthermore, Zhou *et al*., (2015) in their meta analysis recruited 33 studies which included Asians, Caucasians and Africans suggested that *MTHFR* 677 TT genotype might be a significant genetic, molecular marker to determine the risk of diabetic nephropathy in individuals with Type 2 diabetes [55]. However, *MTHFR* C677T gene polymorphism is not considered as a risk factor for the development of T2DM and its vascular complications in the south Indian population [56,57].

Table 1: Prevalence of the MTHFR C677T gene polymorphism in T2DM patients among different populations [57]

Country	Population	No. of Subject	Disease risk	Mutant 'T' allele frequency	Reference
India	South Indian	P=200 C=100	Vascular complications with T2DM	0.07	(Nithya *et al*., 2017)
China	Chinese	P=162 C=302	Type2 Diabetic Nephropathy	0.35	Wang *et al*., 2017
Tunisia	Tunisian	P=160 C=200	Vascular complications	0.33	Fekih-Mrissa *et al*., 2017
UAE	Emirati	P=169 C=209	T2DM	0.14	Chehadeh *et al*., 2016
Turkey	Turkish	P=107	Cardiovascular disease with T2DM	0.89	Bahadir *et al*., 2015
India	East Indian	P=227 C=44	Hyperhomocysteinemia with T2DM	0.13	Chakraborty *et al*., 2015
Croatia	Croatian	P=85	Type2 Diabetic Nephropathy	0.15	GojoTomic *et al*., 2013
Egypt	Egyptian	P=60 C=60	T2DM	0.29	AbdRaboh *et al*., 2013
Brazil	Brazilian	P=50 C=52	T2DM	0.14	Mello *et al*., 2012
Egypt	Egyptian	P=50 C=20	Type2 Diabetic Nephropathy	0.20	Sharaf *et al*., 2012
Egypt	Egyptian	P=80 C=40	Ischemic Stroke with T2DM	0.27	Mohammed Mackawy and Badaway, 2011
India	South Indian	P=120 C=100	Acute myocardial infarction with T2DM	0.14	Angeline *et al*., 2009
Tunisia	Tunisian	P=360 C=400	Type2 Diabetic Nephropathy	0.36	Mtiraoui *et al*., 2007
Turkey	Turkish	P=249 C=214	Left Ventricular Hypertrophy with T2DM	0.31	Yilmaz *et al*., 2004

The highest mutant allele frequency of MTHFR C677T gene polymorphism was observed among Turkish (0.89) and the lowest frequency (0.09) was observed among south Indian population (Table 1). Various studies have been conducted in the MTHFR C677T polymorphism in different populations, but the results are controversial. This might be due to the differences in geographical regions and ethnic groups in which the studies were conducted.

Methylenetetrahydrofolatereductase (*MTHFR*) A1298C gene polymorphism

SNP in *MTHFR* A1298C (rs1801131) leads to a glutamate to alanine substitution within the C terminal regulatory domain of the enzyme due to an A to C transversion that occurs in exon 7, that results in a decrease in *MTHFR* activity [47]. Low MTHFR activity reduces DNA methylation [58], thereby resulting in accumulation

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of Hcy in blood [41]. Several studies have also shown that elevated levels of Hcy may induce DNA damage either by an increased production of ROS or by biological mechanisms directly associated with an excessive misincorporation of uracil in DNA and the process of DNA methylation [59-61]. In addition, few studies have found the association of this polymorphism with coronary heart disease in a Chinese population [62], ischemic stroke in a Tunisian population [63] and retinopathy in an Egyptian population [64]. Also, *MTHFR* A1298C gene polymorphism is considered as a risk factor for the development of T2DM and its complications among south Indians [65]. Another study conducted in the Tunisian population has reported that the MTHFR A1298C gene polymorphism was significantly associated with Type 2 diabetes [66].

Methionine synthase (*MTR*) A2756G gene polymorphism

MTR is one of the key enzymes that participate in the folate metabolism and it is involved in DNA synthesis, repair and methylation [67]. Methylation governs vital processes such as gene expression, Hcy metabolism and neurotransmitter synthesis and degradation [68]. The enzyme *MTR* requires 5-MTHF as a methyl group donor for the remethylation of Hcy to methionine, and the formation of this radical depends on the action of the enzyme *MTHFR* [69]. Also, *MTR* is the only enzyme that can regenerate tetrahydrofolate (THF) from 5- MTHF [70]. Single nucleotide polymorphism in *MTR* A2756G (rs1805087) leads to change from aspartic acid to glycine at codon 919 (D919G), resulting in a lower enzyme activity followed by Hcy elevation and DNA hypomethylation [71]. It has also been demonstrated that the *MTR* A2756G polymorphism contribute to alterations in the Hcy and folate levels [72]. Some authors have also suggested that MTR A2756G gene polymorphism can alter Hcy levels, which may lead to DNA damage that may further contribute to the pathogenesis of diseases [73,74]. In diabetes, the oxidative stress is augmented leading to DNA damage which is potentially linked to the complications [75]. While various studies were conducted among different populations in the folate metabolism, the *MTR* A2756G polymorphism was found to be a risk factor for deep vein thrombosis, non-syndromic cleft lip, autism and intimal medial thickening in Type 2 diabetes [76-79].

Genetic and nutritional factors play a major role in determining the functionality of the folate and methionine cycle. An analysis of gene-nutrient interactions with vitamin B_{12} revealed no interaction with *MTR* A2756G polymorphism on Hcy, SAM and S-adenosylhomocysteine concentrations in the healthy individuals [80]. It has been noted that polymorphism in the folate pathway may alter enzyme activities, thereby interfering with DNA methylation, DNA synthesis, as well as the genomic stability, which finally results in pathological conditions [72].

Glutamate carboxypeptidase (*GCPII*) C1561T gene polymorphism

Evidences suggest that the *GCPII* gene encodes for three different splice variants namely, N-acetylated-α-linked acidic dipeptidase (NAALADase), folyl poly-γ-glutamate carboxypeptidase (FGCP) and prostate specific membrane antigen (PSMA), which are predominantly expressed in brain, intestinal mucosa and prostate gland respectively [801-83]. Dietary folates primarily exist in polyglutamate forms and are unable to cross the cell membrane when the glutamate tail is longer than three residues [84]. *GCPII*, also referred to as folate hydrolase (FOLH1), cleaves polyglutamates from dietary folate or from folate located within tissues to facilitate transport into or out of cells *via* folate transporters. Therefore, in the small intestine of humans where folate is absorbed, the folates are first hydrolyzed into monoglutamates; this process is catalyzed by an exopeptidase glutamate carboxypeptidase II that is anchored to the intestinal apical brush border membrane [85]. After hydrolysis, this mono glutamyl folate is transported into the cells by facilitative anion exchange [86]. In this mechanism, it involves the use of a facilitative anion exchanger, reduced folate carrier (RFC) which has a higher affinity for reduced folates than folic acid. The *GCPII* C1561T polymorphism (rs202676) is located in exon13 at the putative catalytic domain of the enzyme and is associated with a 53% reduction of enzyme activity [87].

Several researchers have analyzed the influence of *GCPII* C1561T polymorphism on folate/Hcy concentrations and their association with the incidence of various disorders such as cardiovascular disease, neural tube defects and gastric cancer [88-90]. Another finding suggests that *GCPII* gene polymorphism may affect folate absorption resulting in lower serum folate levels and increasing incidence of neural tube defects in the eastern region of the Indian population [91]. Also, *GCPII* C1561T polymorphism showed positive association with plasma Hcy in patients with type 2 diabetes. In that study, it has been suggested that increased oxidative stress in subjects with *GCPII* 1561T variant might be mediated through elevated Hcy levels [79]. However, few studies did not find such an association which may be attributed to higher folate intakes in their study population. Food fortification with folic acid may also increase the level of folate intake [92, 93]. Folic acid derived from fortified foods is essentially in monoglutamate form and does not require *GCPII* action for intestinal absorption, therefore nullify the functional role of *GCPII* in the conversion of polyglutamate folates to monoglutamates [94].

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Reduced folate carrier (*RFCI*) A80G gene polymorphism

RFCI is a cell surface transmembrane protein, which is involved in the bidirectional movement of folate across the membrane [95]. RFC has been reported to have a much greater affinity for reduced folates such as 5- MTHF which is the main form of circulating folate in the plasma [96]. The human *RFC1* is shown to be located on chromosome 21q22 and the protein consists of 591 amino acid residues with a molecular mass of 65KD. A SNP of the *RFC1* (rs1051266) gene is an A- to- G transition at nucleotide position 80, replacing histidine (CAC) with arginine (CGC) at codon 27 [97]. It has been noted that expression of RFC is sensitive to dietary folate and mice fed on a low folate diet show decrease in RFC mRNA in the small intestine [98]. Loss of *RFCI* expression or function results in profound physiological or developmental consequences. It has also been reported that synthesis of mutant RFC protein with impaired function results in antifolate resistance due to incomplete inhibition of cellular enzyme targets and low levels of substrate for polyglutamate synthesis [99]. In addition, the *RFC1* A80G gene polymorphism has been linked to several human pathologies including T2DM, parkinson's disease, stroke, down syndrome and cancer [79,100-103].

The findings of Saxena et al. (2010), has reported that the *RFC1* A80G gene polymorphism might consider as an independent risk factor for the development of neural tube defects in the eastern region of Indian population [91]. Another study has also reported that the *RFC1* A80G gene polymorphism is a genetic determinant of ischemic stroke in the Korean population. In that study, they found an association between *RFC1* A80G gene polymorphism and increased level of Hcy and decreased level of folate [101]. Also, a study conducted among south Indians showed that the *RFC1* A80G gene polymorphism confers increased risk for diabetes and its complications [104]. Conversely, a study among Indians revealed no association between *RFC1* A80G gene polymorphism and coronary artery risk [105]. However, such variations are observed due to the difference in the geographical regions and genetic background of the populations. However, contradictory results were also observed in certain studies performed in different populations. This discrepancy in results may be due to differences in the ethnicity, the sample size, the characteristics of the study subjects (e.g. undefined chronic illnesses), presence of other nucleotide polymorphisms, epigenetic alterations, linkage disequilibrium to other sequence variants in the vicinity of the studied locus, and prevailing environmental conditions [106].

Interventions with antioxidants supplementation in patients with Type 2 diabetes

Folic acid supplementation in patients with Type 2 diabetes causes reduction in Hcy levels and, therefore, contributed to better glycemic control [107]. It has been reported that low intake of folate and B_{12} in Type 2 diabetes patients was shown to be associated with hyperhomocysteinemia [108]. Raised serum Hcy concentration levels due to reduction of folic acid and vitamin B_{12} levels increased the risk of diabetic retinopathy [109]. Also, it was reported that the long term depletion of folate from the diet decreases reduced / oxidized glutathione ratio, alter activity of Mn-SOD, CAT and GPx and induce irrepairable oxidative DNA damage [110]. DNA damage as measured by the presence of micronuclei can be reverted by folic acid supplementation, thus reducing the effect of oxidative stress in diabetes patients [111]. Another study showed that administration of folic acid to diabetic rabbits resulted in a decrease in intra-aortic oxidative stress [112].

CONCLUSION

Disorders in Folate metabolism could be caused by genetic or environmental factors that include an individual's genetic variability and diet. Therefore, the interindividual variability with respect to geographical background and lifestyle factors could play a role in the pathogenesis of Type 2 diabetes and its complications. Further, DNA damage and onset of complications in Type 2 diabetes could be prevented by counteracting the oxidative stress by therapeutic interventions using appropriate antioxidants.

CONFLICT OF INTEREST

The authors declare no conflict of interest for this publication.

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