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NEURAL TUBE DEFECTS AND ROLE OF FOLIC ACID

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ABSTRACT

Neural tube defects (NTDs) are a group of congenital malformations with worldwide distribution and complex etiopathogenesis. These are one of the commonest birth defects with high incidence in India. Animal studies indicate that there may be four sites of initiation of neural tube closure (NTC). Selective involvement of these sites may lead to defects varying from anencephaly to spina bifida. Genetic, nutritional and teratogenic mechanisms have been implicated in the pathogenesis of NTDs. Based on animal studies, epidemiologic studies and intervention trials, maternal folic acid is known to be protective for NTDs, primarily spina bifida and anencephaly. To reduce the risk of NTDs, the US Food and Drug Administration mandated that all enriched cereal grain products be fortified with folic acid as of January 1998. Recent data demonstrated that this public health action is associated with increased blood folate levels among women of child bearing age and that the occurrence of spina bifida has decreased by 20%. Birth defect prevention includes a recommended daily dose of 400 µg synthetic folic acid.

KEY WORDS

Neural tube defects, spina bifida, anencephaly, folic acid, fortified.

INTRODUCTION

Neural tube defects (NTDs) are among the most common birth defects contributing to infant mortality and serious disability [1]. Neural tube defects are congenital malformations of the brain and spinal cord caused by failure of the neural tube to close between 21 and 28 days following conception. Defects range from anencephaly through encephalocele to spina bifida which is more variable in severity and effect. Anencephaly is invariably associated with death as a still birth, a neonatal death or occasionally a post neonatal death. Encephalocele and spina bifida may be associated with neonatal death, infant death or with impairments which are frequently severe in absence of surgery e.g. lower limb paralysis, incontinence, convulsions & frequent CNS infections. Even with surgery to close the spinal defect and to insert ventriculo-peritoneal shunts, spina bifida is associated with mortality and a high degree of disability [2].

Birth defects are the leading cause of infant mortality and have been so, for the past 25 years, causing 22% of all infant deaths. Approximately 3-4% of all live births are affected by a birth defect; the etiologies of most of them are unknown [3, 4]. Neural tube defects are an important cause of mortality and morbidity, globally with a conservative estimated incidence of more than 3, 00,000 new cases a year resulting in an estimated 41,000 deaths and 2.3 million disability adjusted life years (DALYs). These defects thus comprise about one tenth of all congenital malformations and are the [5] third largest defects after congenital heart disease and Down's syndrome. Approximately 4, 00,000 infants with spina bifida are born worldwide each year. There is wide variability in its prevalence with higher rates in Northern China [6], certain parts in England and Wales and the state of Punjab in India [7].

A follow up study on 117 operated cases of spina bifida showed that only 46% survived to the age of 35 years and half of the survival had severe disabilities [8]. Based on 1998 cross sectional data, the estimated life time cost of spina bifida is $2, 58,000 per case [9].

NEURAL TUBE DEFECTS: DEFINITION AND SPECTRUM

The formation and closure of neural tube which takes place at about 4 weeks of gestation is an important milestone in the development of the central nervous system. Any defect in the closure of a portion of neural tube could disrupt the differentiation of CNS and the induction of vertebral arches. This result in a number of developmental malformations along the nueraxis from developing brain to sacrum and these are collectively named as neural tube defects.

In spina bifida, the arches of one or more adjacent vertebrae fail to develop. The most common site is the lumbosacral region. The severity of this malformation may range from a failure of fusion of arches in the midline (spina bifida occulta) to defects involving meninges (meningocele) and spinal cord (meningomyelocele). The location of defect is frequently indicated by a tuft of hair, angioma, pigmented naevi or a dimple.

Anencephaly, also known as enencephaly or craniorachischisis is a condition characterized by the absence of the vault of the skull and a greater part of brain. This malformation is caused by failure of the cranial part of the neural tube to close. Here, the normal forebrain is replaced by mass of undifferentiated neural tissue. There are other malformations in which neural folds not only fail to fuse, but also fail to differentiate,

invaginate and finally separate from the ectoderm. These include craniorachischisis foetalis, rachischisis, myeloschisis and retinoschisis.

Encephalocele is a condition in which the brain and meninges herniate through a defect in the calvaria. This defect mostly occurs in the occipital region.

ETIOLOGY OF NEURAL TUBE DEFECTS (NTDS)

Over 95% of all NTDs are first occurrence with a small proportion being repeat events in women with a previously affected pregnancy [10]. The risk for NTDs is higher among families of lower socio-economic status [11]. Currently identified risk factors for NTDs include a mother who previously had a NTD affected pregnancy, maternal diabetes [12], hyperthermia [13, 14], obesity [15], certain antiseizure medications, genetic variants, race, ethnicity and nutrition (particularly folic acid deficiency).

Genetic and environmental factors are likely to cause NTDs. Genetic disorders associated with NTDs include single gene mutation (e.g. Meckel's syndrome) and chromosomal abnormalities (e.g. trisomy 13, trisomy 18). Maternal age, maternal exposure to excess of vitamin A and lead, fever during pregnancy, alcohol consumption and tea in 1st trimester may be causally associated with the pathogenesis of NTDs. Certain parental occupations are also associated with the occurrence of NTDs [16].

Higher maternal preconceptional intake of zinc is found to decrease the risk of NTDs in the offsprings [17]. Even in higher income countries, lower maternal education status is associated with higher risk of NTDs [18, 19, 20].

As we know that NTDs occur early in fetal development, prevention would be most effective at the earliest phase of pregnancy, often before women know that they are pregnant. Hence the best public health interventions must target all fertile women (millions of women who are of child bearing age).

The relationship between serious birth defects and their prevention by folic acid is well established. Much of the birth defect data focus on the well substantiated relationship between folic acid and prevention of neural tube defects and this emphasis is reflected in this review.

FOLIC ACID: STRUCTURE AND FUNCTION

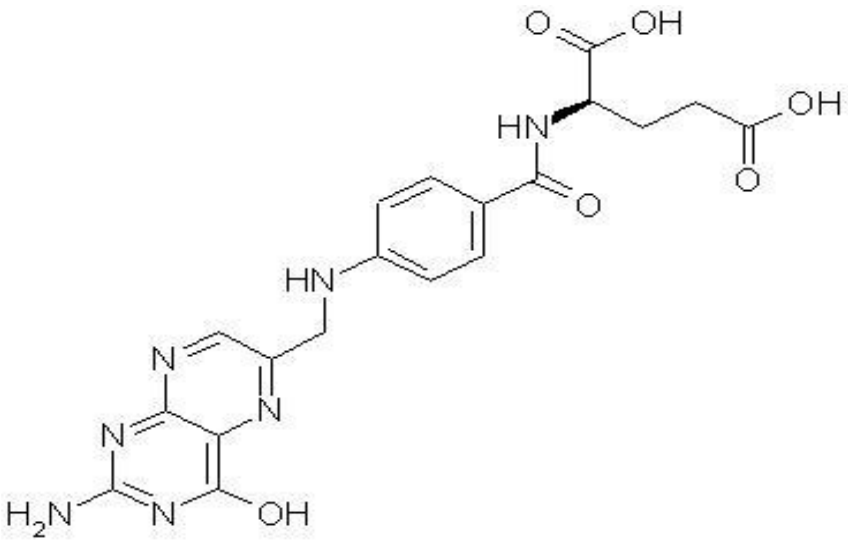

Figure 1.
The Chemical Structure of Folic Acid

Folic acid, also known as pteroyl-L-glutamic acid is a synthetic compound used in dietary supplement and fortified foods. The term folate includes all compounds that have the vitamin properties of folic acid including folic acid and naturally occurring compounds in food[21]. A small comparison study suggests that blood folate concentrations are increased much more by folic acid supplementation than by naturally occurring folate in the diet [22].

Within the intestinal cells, folic acid is reduced first, to 7, 8 dihydrofolic acid and then to tetrahydro folic acid (THFA) by a NADPH dependant folate reductase. It is then methylated to N5 methyl THFA. Folic acid is transported in blood as methyl THFA bound to plasma proteins.

Folic acid is a water soluble vitamin that has no known toxicity. However, higher doses of folic acid can correct the anemia of vitamin B_{12} deficiency (pernicious anemia), which might be an important clue to the presence of vitamin B_{12} deficiency in some instances.

FUNCTION OF FOLATE

Tetrahydrofolic acid has an important role in the metabolism of one carbon (1C) groups like formyl (-CHO), formimino (-CH=NH), methyl (-CH_3), methynyl (-CH=), methylene (-CH_2) and hydroxymethyl (-CH_2OH) groups. These compounds are derived from serine, glycine, histidine, tryptophan and choline. They are vital in several important metabolic pathways and are essential for the synthesis of nucleic acid.

ROLE OF THFA IN VARIOUS BIOCHEMICAL REACTIONS

1) N_5N_{10} methylene THFA is necessary for the conversion of d-UMP to d-TMP (nucleic acid synthesis).
2) N_{10} formyl THFA contributes the second carbon atom of purine ring (nucleic acid synthesis).
3) N_5N_{10} methylene THFA contributes the right carbon atom of purine ring (nucleic acid synthesis).
4) Synthesis of N formyl methionine of t-RNA requires N_{10} formyl THFA.
5) Conversion of glycine to serine requires N_5N_{10} methylene THFA.
6) N_5 methyl THFA is needed for the conversion of homocysteine to methionine.

Several genetic defects of the enzymes involved in folate and 1C metabolism have been identified in the NTDs in clinical and experimental settings. It is proved that supplementation of folic acid may overcome these metabolic blocks and reduce the risk of NTDs.

Plasma homocysteine level is considered to be a good integrated marker of folate and Vitamin B_{12} status. There is progressive decrease in plasma homocysteine levels during pregnancy which is attributed to various factors like increased GFR, increased cortisol level during pregnancy and lower plasma albumin binding.

Increased serum or RBC folate concentration are associated with decreased risk of NTDs. Serum homocysteine levels are inversely proportional to folate levels. Hence, one of the big mysteries is, whether birth defects, including NTDs are due to low folate or high homocysteine levels or both.

Although some cases of NTDs are induced by hyperhomocysteinemia metabolism may be involved in the etiology of NTDs. Although some cases of NTDs are induced by hyperhomocysteinemia resulting from genetic polymorphism of a thermobolic enzyme, the cause of most NTDs are unknown [23, 24].

GENETIC DEFECTS RELATED TO FOLATE METABOLISM THAT MAY LEAD TO NTDs

1) Defects related to folate transport

a) Reduced folate carrier (RFC) polymorphism
b) Defects in receptor mediated folate uptake

2) Defects related to THFA metabolic pathway

i) Methylene tetrahydrofolate reductase mediated
ii) Methionine synthetase gene defects
iii) Methionine synthetase reductase gene defects
iv) Methylene tetrahydrofolate

dehydrogenase gene defects
v) Cystathionine beta synthase (CBS) gene defects

Supplementation of folate reduces the incidence of NTDs possibly by overriding genetic defects in folate metabolism.

a) Reduced folate carrier (RFC) polymorphism

The transport of reduced form of folate in mammalian cells occurs by a carrier mediated mechanism. The reduced folate carrier is an integral membrane protein that is primarily responsible for this transport. The 80A →G which is a common polymorphism in RFC – 1 gene may contribute to NTD susceptibility.

b) *Defects in receptor mediated folate uptake*

Receptor mediated folate transport is another mechanism of folate transport across mammalian cell membrane. Folate receptors (FR) are very important for assimilation, distribution and retention of food folate. So defects in folate receptor genes or its promoter alleles can be involved in etiology of NTDs [25]. A recent study has also linked maternal antibodies against folate receptors to NTDs [26].

i) *Methylene tetrahydro folate reductase (MTHFR*)

The C677T mutation in MTHFR gene results in the thermolabile variant of MTHFR enzyme with reduced activity that leads to elevated plasma homocysteine concentration [27,28,29].

Certain studies have shown an increased prevalence of MTHFR gene in NTD patients and their mothers [30, 31]. Another mutation, the A1298C in the MTHFR gene is also associated with decreased enzymatic activity [32].

A study on the prevalence of this combined heterozygous genotype C677T/A1298C showed an increased prevalence among NTD patients than in control [33].

ii) Methionine synthetase gene defects

The most common polymorphism in the methionine synthatase gene is a substitution A2756G which leads to a change of aspartic acid to glycine (D919G) [34]. The D919G polymorphism probably leads to an improper cofactor oxidation level which can decrease methionine synthatase activity and cellular homocysteine levels [35].

iii) Methionine synthetase reductase (MTRR)gene defects

MTRR regulates methionine reductase activity by reductive methylation. Defective MTRR could reduce the functional activity of methionine synthatase and thereby decrease methylation of homocysteine to methionine. The most common polymorphism in methionine synthetase reductase gene is A66G substitution leading to a change of isoleucine to methionine [36].

iv) *Methylene tetrhydrofolate dehydrogenase gene defects*

Mutation analysis of MTHFD gene in patients with NTDs has led to the discovery of G878A substitution in patients with familial NTDs.

v) *Cystathionine betasynthase (CBS) gene defects*

CBS catalyzes the irreversible synthesis of uptathionine from homocysteine and serine. Disturbance in this process can lead to increase cellular homocysteine level.

ANTIEPILEPTICS AND THEIR ASSOCIATION WITH NTDS

Most antiepileptic drugs are associated with increased risk for NTDs. Valproate has been directly implicated as a potent neural tube teratogen [37]. Valproate has also been found to elicit its dependant effects on the expression of several genes important in normal embryonic development of the cell cycle and apoptosis genes (bcl-2,p53), growth factor genomes (bgnf, ngf,ngf-R) and folate pathway genes (folbp-1, MTHFR gene)[38,39]. Another proposed mechanism by

which antiepileptics produce fetal malformation is by increasing the total free radical load and thereby free radical mediated cellular damage [40].

FOLATE AND NON NTD BIRTH DEFECTS

While the relationship between folate and NTDs is well established, folate deficiency may also be related to other serious birth defects. A randomized control trial of periconceptional folic acid containing multivitamin supplementation demonstrated a reduced occurrence of urinary tract and cardiovascular congenital anomalies and congenital limb deficiencies(Czeizel et al). The occurrence of orofacial cleftings, cleft lip and cleft palate may also be reduced by high dose of folic acid.

A large population study demonstrating protection against NTDs by folic acid also analyzes other birth defects [41]. The preliminary data suggests that periconceptional folic acid protects against all major defects as a group, ASD & VSD, limb deformities, encephalocele, cleft lip and/or cleft palate [41].

To further explore the relationship between folate and non NTD birth defects, a large epidemiologic database of women taking medication during pregnancy was examined for the results of non-NTD birth defects such as oral clefts, CVS and urinary tract defects in babies born to mothers taking two general classes of folate antagonists [42, 43]. The analysis revealed two important findings.

1) A 2-3.5 fold increased risk of these birth defects following 1st trimester treatment with folate antagonists with the timing of exposure during pregnancy strengthening the argument for causality.
2) In women taking prenatal multivitamins containing folic acid, minimization of risk from drugs that act by inhibiting dihydrofolate reductase, but not from anticonvulsant drugs.

ETHNIC AND GENETIC VARIATION IN NTD RISKS

Population based research has revealed the heterogenous frequency of defects in folic acid metabolism. The most stunning report is 40% increase in incidence of NTDs in offsprings of Hispanic descent compared to Caucasians and African Americans [44].

TABLE. 1.
Relative Risk for Spina Bifida by Race/Ethnicity [44]

RACE/ETHNICITY	ADJUSTED RELATIVE RISK (95% Confidence Limits)
White	1.00
Black	0.80(0.72-0.88)
Hispanic	1.41(1.26-1.58)
Asian/Pacific islander	0.51(0.38-0.70)
Native American	1.13(0.74-1.74)

Adapted from Feuchtbaum et al [44]

RECOMMENDATION OF FOLATE INTAKE

1) Prevention for women with no history of a previous NTD affected pregnancy

American academy of pediatrics (AAP) endorses the U.S. Public Health Services (USPHS) recommendation that all women of child bearing age who are capable of becoming pregnant should consume 400 mcg of folic acid daily. As there is a high percentage of unplanned pregnancy in the United States, the AAP encourages food fortification so as to provide all women with the optimal dose of folic acid. In the absence of optimal fortification, AAP encourages women to consume 400 mcg of

folic acid daily in addition to eating a healthy diet. Many countries like USA and Canada have adopted universal folate fortification of flour following this report. No rational program for primary prevention of NTDs by nutritional supplementation currently exists in India. National health programs such as National Anaemia Prevention Program provide 0.5 mg of folic acid along with 100 mg of elemental iron from the 3rd month of pregnancy. Because the risk for NTDs is not totally eliminated by folic acid use, routine prenatal screening for NTDs is still advisable.

2) Prevention for women who have had a previous NTD affected pregnancy

Women with a history of previous pregnancy resulting in fetus with an NTD should be advised for a MRI study.

During times in which pregnancy is unplanned, these high risk women should consume 4000mcg of folic acid per day. However they should be offered treatment with 4000 mcg of folic acid per day starting 1 month before the time they plan to become pregnant and throughout the first three month of pregnancy unless contraindicated.

Women should be advised not to attempt to achieve the 4000 mcg daily dosage of folic acid by taking over the counter or prescription multivitamins containing folic acid because the possibility of ingesting harmful levels of other vitamins e.g. Vitamin A [45].

3) Prevention for other high risk persons

No intervention or observational studies address prevention for other high risk persons. Women with a close relative (e.g. sibling, niece or nephew) who has an NTD (risk is approx. 0.3% to 1%), women with type 1 diabetes mellitus (risk is 1%), women with seizure disorders being treated with valproic acid or carbamazepine (risk is approximately 1%) and women or their partners who have an NTD (risk may be 2% to 3%) [46] and are planning a pregnancy should discuss with their physician the risk for an affected child and the advantages and the disadvantages of increasing their daily periconceptional folic acid intake to 4000 mcg[46].

FOLIC ACID FORTIFICATION

The increased scientific knowledge on the preventive effects of periconceptional folic acid on NTDs did not help much as the majority of women were poorly informed on the beneficial effects of folic acid and the pregnancies are unplanned.

The situation has led to an alternate approach of universal food fortification with 40 mcg of folic acid per 100 mg of grain.

USA and Canada have implemented food fortification program successfully with resultant improvement serum folate levels [47, 48, 49, 50] and reduction in the incidence of NTDs [51, 52].In Ontario, Canada, the incidence of NTD decreased by 47% [53] and in Nova Scotia (Canada) the incidence of NTD decreased by 54% [54] after folic acid fortification.The reduction in NTD occurrence associated with improved folate status post fortification indicates the effectiveness of food fortification as an intervention strategy.

POTENTIAL HARM OF EXCESSIVE FOLIC ACID INTAKE

Folic acid in the recommended dosage of 0.4-1.0 mg [55, 56] is not known to cause any demonstrable harm to the developing fetus or pregnant women. Folic acid is water soluble and excess is excreted through urinary tract. The effects of higher intake of folic acid (>1 mg) are not well known, but they include masking the diagnosis of Vitamin B_{12} deficiency. Concerns have been raised that intake of folic acid might cause harmful effects including progression of nerve damage in Vitamin B_{12} deficient persons; excess intake in children; accumulation of unmetabolized folic acid; blunting of anti folate therapy (methotrexate and phenytoin); accelerated cognitive decline in the epigenetic hypermethylation and cancer promotion[57]. However, continued monitoring and research are needed to ensure that folic acid public health recommendation do not have unintended consequences.

PUBLIC HEALTH MESSAGE

NTDs are life threatening and cause lifelong disabilities. Folic acid has been proven to decrease or minimize specific birth defects including neural tube defect, congenital heart diseases, urinary tract anomalies, orofacial clefts, limb defects and pyloric stenosis [58, 59, 60]. Preconceptional folic acid supplementation should be recommended to women who may become pregnant. The dose of folic acid should be adjusted according to the patient history and needs. Fortification of flour and other high consumption, high penetration staple with folic acid is a feasible, economical, safe and effective public health policy to prevent NTDs worldwide.

Current research and increasing fortification efforts have demonstrated the ability to eliminate those NTDs that are sensitive to folic acid.If 50% -70% of NTDs fall in this category and assuming an annual prevalence of 3,00,000 NTDs worldwide folic acid fortification could lead to the prevention of 1,50,000 – 2,10,000 NTDs per year.

ISSUES TO BE DEALT WITH

After 25 years of work on birth defect prevention and folate, preventing all of the folate dependant birth defects and other requires more research on a number of issues. It is our hope that these questions will continue to be made to find answers through appropriate research programs involving animal models, epidemiologic studies and ethically sound clinical intervention trials.

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