Neural Tube Defects: Pathogenesis and Folate Metabolism

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Abstract

Neural tube defects (NTDs) are a group of congenital malformations with worldwide distribution and complex aetio-pathogenesis. 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. The NTC involves formation of medial and dorsolateral hinge points, convergent extension and a zipper release process. Proliferation and migration of neuroectodermal cells and its morphological changes brought about by microfilaments and other cytoskeletal proteins mediate NTC. Genetic, nutritional and teratogenic mechanisms have been implicated in the pathogenesis of NTDs. Folate is an important component in one carbon metabolism that provides active moieties for synthesis of nucleic acids and proteins. Several gene defects affecting enzymes and proteins involved in transport and metabolism of folate have been associated with NTDs. It may be possible in future, to identify individuals at higher risk of NTDs by genetic studies. Epidemiological and clinical studies have shown that dietary supplementation or food fortification with folic acid would reduce the incidence of NTDs. The protective effect of folic acid may be by overcoming these metabolic blocks through unidentified mechanisms. Genetic and biochemical studies on foetal cells may supplement currently available prenatal tests to diagnose NTDs. Antiepileptic drugs (AEDs), particularly valproate and carbamazepine have been shown to increase the risk of NTDs by possibly increasing the oxidative stress and deranging the folate metabolism. Accordingly, it is recommended that all women taking AEDs may use 1-5 mg folic acid daily in the pre conception period and through pregnancy.

INTRODUCTION

Neural tube defects (NTDs) are a group of congenital malformations that cause foetal loss and considerable disabilities in surviving infants. Approximately 400,000 infants with spina bifida are born worldwide each year. There is wide variability in its prevalence with higher rates in northern China,† certain parts of England and Wales and Punjab State in India.‡ The prevalence at birth of both anencephaly and spina bifida has decreased considerably. This secular trend can be seen even after adjusting for prenatal diagnosis and elective termination. In England, Scotland and Ireland, the rates have been declining since the 1970s. In North America, the peak was seen in 1930s and since then the rate has been declining. The affected infants with NTDs who survive beyond infancy suffer from various levels of disability. 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 survivors had severe disabilities.§ Based on 1988 cross-sectional data, the estimated lifetime cost of spina bifida in the USA is $258,000 per case.¶

Neural tube defects: Definition and spectrum

The formation and closure of the neural tube that happens around 4 weeks of gestation is an important landmark in the development of nervous system. The failure of closure of a portion of the neural tube could disrupt the differentiation of central nervous system and the induction of vertebral arches. This can result in a number of developmental malformations along the neuroaxis from the developing brain to the sacrum and are collectively termed neural tube defects (Table 1).

In spina bifida, the arches of one or more adjacent

<table>
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<th>Table 1: Spectrum of neural tube defects</th>
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<tr>
<td>1. Spina bifida</td>
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<td>(i) Spina bifida occulta</td>
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<td>(ii) Meningocele</td>
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<td>(iii) Meningomyelocele</td>
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<tr>
<td>2. Anencephaly</td>
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<td>3. Encephalocoele*</td>
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* There is ambiguity in medical literature whether encephalocoele should be included in the spectrum of neural tube defects.
vertebrae fail to develop. The most common site is the lumbosacral region. This occurs due to a failure of the mesenchyme to grow in between the neural tube and the surface ectoderm to form the vertebral arches. The severity of this malformation may range from a failure of fusion of the arches in the midline (spina bifida occulta) to defects involving the meninges (meningocele) and spinal cord (meningomyelocele). The location of the defect is frequently indicated by a tuft of hair induced by the underlying abnormality or by an angioma, pigmented nevus orimple.

Anencephaly, also known as exencephaly or craniorachischisis is a condition characterised by the absence of vault of the skull and a greater part of the brain. This malformation is caused by a failure of the cranial part of neural tube to close. The normal forebrain is replaced by an exposed dorsal mass of undifferentiated neural tissue. Most anencephalic infants are still born or die shortly after birth. There are other malformations in which the neural folds not only fail to fuse but also fail to differentiate, invaginate and finally separate from the ectoderm. These include craniorachischisis totalis, rachischisis or myeloschisis and inionschisis.

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

**Development of neural tube**

By about 16 days after fertilisation, the embryo is in the form of a trilaminar disc. The process by which the intraembryonic mesoderm is formed and the embryo becomes a trilaminar disc is called gastrulation. One or two days after gastrulation, the notochord can be seen along the midline of the embryo. The notochord induces a wide strip of ectoderm overlying it to get thickened to form the neural plate. Some cells at the junction between the neural plate and the rest of the ectoderm become specialised to form the primordia of the neural crest.

By the 21st day (end of 3rd week), the neural plate gets depressed along the midline and a groove is formed (neural groove) on the neural plate. The neural groove gradually deepens; the two edges of the neural plate come closer and eventually fuse to become the neural tube. With separation of the neural tube from the surface ectoderm, the cells of the neural crest appear as a group of cells lying along the dorsolateral sides of the neural tube.

The neural tube initially closes in the middle. The fusion starts around 23rd day and proceeds both cranially and caudally. The anterior neuropore closes by the 25th day and the posterior neuropore by the 27th day. The closure of neural tube is completed by the end of 4th week.

**MECHANISM OF NEURAL TUBE CLOSURE**

The formation and closure of the neural tube can be conceived in three stages viz: formation of the neural groove, elevation of the neural walls followed by their approximation and lastly fusion of the neural folds. Experimental studies in lower species have shown that notochord induces the transformation of overlying ectodermal cells to form the neural plate. As neurulation progresses, cells in the superficial neuroectoderm undergo an apical constriction thereby shaping the neural groove. The elevation of the neural walls is brought about by several mechanisms.

(i) **Convergent extension**, which has two components: extension in the longitudinal direction and narrowing in the transverse direction at the same time. The cells within the neural plate increase in number, migrate towards the midline and undergo certain morphological changes that lead to the neural groove deepening to form the neural tube. The anteroposterior elongation of the neural plate during convergent extension results in a transverse buckling that promotes the elevation of neural folds.

(ii) **Contraction of the apical microfilament bundles**, which are arranged like a purse string around the cellular apices, is a crucial component of neural fold elevation. As a result of this apical constriction, all the neural plate cells become wedge shaped and as a whole, the neural plate curls up. Apical constriction can result in anteroposterior shortening of the neuroepithelium and convergent extension may compensate for this. The apical constriction of the cells of the neural plate occurs in a radial plane. This produces buckling of the neural plate in all directions and would have resulted in a depression, which is in the form of a cup rather than in the form of a groove. It is the elongation of the neural plate that shapes the depression into a groove.

(iii) **Formation of medial hinge point (MHP)**: The medial neural plate cells decrease in height and become markedly wedge shaped probably under the influence of the underlying notochord. The cell generation time also differs in these regions. The MHP is considered to facilitate the bending of neural plate in its midline and plays a major role in generating the normal cross-sectional morphology of the neural tube.

(iv) **Expansion of underlying mesoderm** is also thought to facilitate the elevation of neural walls.

The approximation of the walls of the neural tube is also mediated by several similar mechanisms. The process of convergence or medial bending of the neural folds, whereby, the distance between the neural folds decreases gradually and finally meet follows the bending and elevation of the neural plate. The dorsal portions of the neural walls and the non-neural ectoderm adjacent to the neural plate make the neural folds. Three
mechanisms are involved in the process of convergence.

(i) **Formation of dorsolateral hinge points**\(^{37,21}\): The cells of dorsolateral hinge point become markedly wedge shaped due to enhanced apical microfilament contraction.\(^{13,14}\) These hinge points are the likely result of interaction between the neural walls and the adjacent ectoderm. With the formation of dorsolateral hinge points, two longitudinal furrows arise along the lateral neural walls allowing the neural folds to bend towards each other.

(ii) **Convergent extension**: The mechanism that advances the elevated neural folds produces only a finite amount of medial movement. For tube closure to be successful, midline convergent extension is required to decrease the width between the folds, reducing the distance the neural folds need to travel in order to meet and fuse.\(^{22}\)

(iii) **The expansion of surface ectoderm**, which occurs due to changes in epidermal cell number, shape and position is a major extrinsic force, that facilitates the bending of the neural plate. Epidermal ectoderm is necessary for full elevation and convergence of neural folds.\(^{23,24}\)

When the neural folds come into contact with each other, the ectoderm adjacent to the neural plate fuses first. This is followed by the fusion of neural ectoderm and the neural crest is released from the neural tube thus formed. This process is mediated by the cell adhesion molecules and by the protrusive activity of neuroectodermal cells.\(^7\)

During neural tube formation, the forces resulting from apical constriction are not distributed uniformly throughout the neuroepithelium (neuroectoderm), but are rather concentrated sequentially at three distinct locations: (i) the floor (during transformation of the neural plate to a V-shaped neuroepithelium),\(^{19}\) (ii) the midlateral walls (during transformation of the V-shaped neuroepithelium into a C-shaped neuroepithelium), (iii) the upper walls (during transformation of the C-shaped neuroepithelium into a closed tube).

Studies in chick embryo have shown that additional processes may be involved in the formation of the neural tube. It was found that, in the presumptive mesencephalic region, the two neural walls align parallelly and zip into apposition, starting near the floor plate and proceeding dorsally. The lumen of neural tube nearly disappears after apposition and then reopens to form the lumen. The mechanism is best compared with a zipping-up releasing model. In a transverse view, the shape of the neural groove changes from \(V \rightarrow Y \rightarrow I \rightarrow O\). The walls and folds are sequentially in contact by the ventrodorsal zipping-up mechanism, thereby avoiding the possibility of misalignment of neural folds.\(^{25}\)

**Multi site closure of neural tube**

According to conventional studies, the closure of the neural tube initiates in the cervical region and extends bi-directionally to close the anterior and posterior neuropores. Studies in mouse embryos have shown that there are multiple closure initiation sites and multiple transient neuropores.\(^{26,27}\) Closure I is in the cervical region. Closure II takes place at the forebrain-midbrain transition and like closure I proceeds bi-directionally, dividing the anterior neuropore into a forebrain and a midbrain/hindbrain neuropores. Closure III is unidirectional beginning adjacent to the stomodeum and proceeding caudally to meet closure II, thereby closing the forebrain neuropore. Finally, closure IV takes place where it meets closure II to close the mid/hindbrain neuropore. The posterior neuropore is closed by caudal continuation of closure I and remains the longest. Such a multi site closure was also found to be present in other animal models.\(^ {16,28}\) In humans, such a multi site closure pattern was suggested to occur as well.\(^ {29,30}\)

**Reopening of the neural tube**

Neural tube defects are thought to be due to a failure of the neural tube to close properly. It has been proposed that neural tube defects may also occur due to secondary reopening of the neural tube.\(^ {31}\)

The above discussion has shown that several complex mechanisms that cause hyperplasia, migration, and transformation of the neuroectodermal cells are involved in the formation of the neural tube. These complex mechanisms are governed by genetic factors and are influenced by several environmental factors. The link between these factors is poorly understood.

**Aetiology of NTDs**

Genetic and environmental factors are likely to cause NTDs. Genetic disorders associated with NTDs include single gene mutations (e.g. Meckel’s syndrome,) and chromosomal abnormalities (e.g. Trisomy 13, Trisomy 18.). More commonly NTDs have a multi factorial inheritance in which the genetic predisposition is polygenic and is influenced by gene-gene interactions. Environmental factors are also important as revealed in several epidemiological studies. The risk for NTDs is higher among families of lower socio-economic status.\(^ {32}\)

Other factors identified in previous studies include maternal use of anti epileptic drugs, maternal diabetes,\(^ {33}\) hyperthermia\(^ {34,35}\) and obesity.\(^ {36}\) Maternal age, alcohol consumption, maternal exposure to excess vitamin A and lead, febrile illness, heat exposure and tea usage in the first trimester may be causally associated with the pathogenesis of NTDs. Previous pregnancy wastage, parity and foetal birth weight have also been implicated as factors influencing the occurrence of NTDs. Certain parental occupations are also associated with an increased occurrence of NTDs.\(^ {37}\) A variation in micronutrient status of the mother is another important environmental factor associated with NTDs. Higher maternal preconceptional intake of zinc is found to decrease the risk of NTDs in the offsprings.\(^ {38}\) It is widely
observed that periconceptional folate administration reduces the incidence of NTDs, but the precise mechanism is uncertain. The protective effect of folate varies among different populations thereby reflecting a complex interaction between genetic and environmental factors in the genesis of NTDs.

**Epidemiological correlation between folate and NTDs**

The possible relation between folic acid deficiency and NTDs in humans was first reported by Hibbard in 1964. He observed that there was a higher incidence of aberrant folate metabolism in women who had pregnancies associated with foetal malformations. Smithells and co-workers in 1976 observed that maternal red blood cell folate levels were significantly lower when the foetus had NTDs in comparison to when the foetus did not have NTDs. This observation led to the possibility of preventing neural tube defects by periconceptional vitamin supplementation. A randomised control trial had shown that periconceptional supplementation of folic acid (4 mg) reduced the risk of recurrence of NTDs. Another non-randomised trial had shown that administration of multivitamins containing 360 micrograms of folic acid in the immediate pre-pregnancy period to women who had earlier pregnancies with NTDs resulted in 86% reduction in risk of NTDs.

The British Medical Research Council (MRC) had conducted a large multi centre randomised control trial to study the effect of supplementing folic acid to women who had NTDs in earlier pregnancies. This study has conclusively shown that supplementation of 4 mg folic acid significantly reduced the risk of recurrence of NTDs. In another major community based study, the protective effect of periconceptional administration of folic acid (multivitamin containing 800 microgram of folic acid) in the immediate pre-pregnancy period to women who had earlier pregnancies with NTDs resulted in 86% reduction in risk of NTDs.

**Folic acid: Structure, Functions and Pathways**

Folic acid exists as polyglutamates in green leafy vegetables and other natural sources. These polyglutamates are hydrolysed to monoglutamates by the intestinal enzymes before it can be absorbed in the jejunum. Within the intestinal cells folic acid is reduced first to 7,8 dihydrofolic acid, and then to tetrahydrofolic acid (THFA) by NADPH dependent folate reductase. It is then methylated to N methyl THFA. Folic acid is transported in blood as methyl THFA bound to plasma proteins.

**FUNCTIONS OF FOLATE**

THFA has an important role in the metabolism of one carbon (1C) groups (THFA is the carrier of one carbon groups) such as formyl (-CHO), formimino (-CH=NH), methyl (-CH3), methenyl (-CH=), methylene (-CH2-) and hydroxymethyl (-CH2OH) groups (Fig. 1). These compounds are derived from glycine, serine, histidine, tryptophan and choline. They are vital in several important metabolic pathways and are essential for the synthesis of nucleic acid (Table 2). Several genetic defects of the enzymes involved in folate and 1C metabolism have been identified in NTDs in clinical and experimental settings. It possible that supplementation of folic acid may overcome these metabolic blocks and reduce the risk of NTDs.

The enzyme methylene tetrahydrofolate reductase (MTHFR) that irreversibly converts N,N,N-trimethylene THFA to methyl THFA and the enzyme methionine synthase that converts homocysteine to methionine play crucial roles in 1C metabolism (Fig. 2). Genetic defects of these and certain other enzymes have been associated with NTDs. All 1C groups are finally converted to methyl THFA since the conversion of N,N,N,-trimethylene THFA to methyl THFA catalysed by methylene tetrahydrofolate reductase (MTHFR) is irreversible. So a deficiency of folate and thereby 1C groups will result in the channelling of all the remaining available 1C groups into this reaction resulting in non-availability of 1C groups for nucleic acid synthesis. N methyl THFA is needed for the conversion of homocysteine to methionine. S-adenosyl methionine (SAM), which is derived from methionine, is the donor of methyl group in biological

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**Table 2: Role of THFA in various biochemical reactions**

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<thead>
<tr>
<th>Reaction Description</th>
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<tr>
<td>1. N,N,N trimethylene THFA is necessary for the conversion of d-UMP to d-TMP (nucleic acid synthesis)</td>
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<td>2. N,N formyl THFA contributes the second carbon atom of purine ring (nucleic acid synthesis)</td>
</tr>
<tr>
<td>3. N,N,N methenyl THFA contributes the eighth carbon atom of purine ring (nucleic acid synthesis)</td>
</tr>
<tr>
<td>4. Synthesis of N formyl methionine of tRNA requires N,N formyl THFA.</td>
</tr>
<tr>
<td>5. Conversion of glycine to serine requires N,N,N methylene THFA.</td>
</tr>
<tr>
<td>6. N methyl THFA is needed for the conversion of homocysteine to methionine.</td>
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Supplementation of folate reduces the incidence of neural tube defects by possibly 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 $80A \rightarrow G$, which is a common polymorphism in the RFC-1 gene, may contribute to NTD susceptibility. The risk for NTDs is increased when this polymorphism is associated with other gene defects.48

(b) Defects in receptor mediated folate uptake

Receptor mediated folate transport is another mechanism of folate transport across mammalian cell membranes. Folate receptors (FR) are crucial for the assimilation, distribution and retention of food folates. Murine Folate Binding Proteins 1 and 2 (FBP1 and FBP2) are homologues of human FR. Mouse foetuses without FBP-1 gene die during gestation and show defects in neural tube closure. Such mice and also those with only one FBP-1 allele have lower folate concentrations. Thus defects in folate receptor genes or its promoter alleles can be involved in aetiology of NTDs.49 A recent study has also linked maternal auto-antibodies against folate receptors to NTDs.50

(c) Methylene tetrahydrofolate reductase (MTHFR) gene defects

The C677T mutation in MTHFR gene results in a thermolabile variant of MTHFR enzyme with reduced activity that leads to elevated plasma homocysteine concentration.51-53 MTHFR is the enzyme, which irreversibly converts N5N10methylene THFA to N5methyl THFA, which in turn is the methyl group donor in conversion of homocysteine to methionine. So a defective MTHFR will result in decreased conversion of homocysteine to methionine and thereby elevated homocysteine levels and also decreased levels of methionine (and thereby SAM). Certain studies have shown an increased prevalence of C677T mutation in MTHFR gene in NTD patients and their mothers.54,55 The frequency of C677T allelic variant roughly correlates with the incidence of NTDs.56 It had been demonstrated that the effect of thermolabile MTHFR on homocysteine levels could be reversed by additional folic acid intake.57

Another mutation, the A1298C in the MTHFR gene is also associated with decreased enzymatic activity.58 But this does not lead to elevated levels of homocysteine. However, combined heterozygosity for both the C677T and A1298C mutation might be another genetic risk factor for NTDs.58 A study on the prevalence of this combined heterozygous genotype C677T / A1298C showed an increased prevalence among NTD patients than in controls.59
(d) Methionine synthase gene defects

The most common polymorphism in the methionine synthase gene is substitution A2756G, which leads to a change of aspartic acid to glycine (D919G). The D919G polymorphism probably leads to an improper cofactor oxidation level, which can decrease methionine synthase activity and increase cellular homocysteine level. One study on healthy males reported a moderate but significant increase in homocysteine levels due to A2756G mutation. Methionine synthase along with cofactor Vit B12 catalyses the transfer of methyl group from N\textsuperscript{5}methyl THFA to homocysteine resulting in formation of methionine. Decreased levels of methionine result in decreased levels of SAM. The low enzymatic activity also leads to increased levels of homocysteine. The deficiency of SAM could affect the cells’ ability to methylate important compounds such as DNA, lipids, proteins and myelin, thereby impairing cell function, which could result in defective neurulation. The deficiency of SAM also leads to increased activity of MTHFR by abolition of its inhibition by SAM. This results in a channelling of all the available forms of THFA into the MTHFR reaction. The depletion of other forms of THFA leads to impaired thymidine and purine synthesis. A defective methionine synthase along with deficient Vit B12 could lead to hyperhomocysteinemia, low levels of SAM and thereby defective methylation.

(e) Methionine synthase reductase (MTRR) gene defects

MTRR regulates methionine synthase activity by reductive methylation. Defective MTRR could reduce the functional activity of methionine synthase and thereby decrease methylation of homocysteine to methionine. The most common polymorphism in methionine synthase reductase gene is A66G substitution leading to a change of isoleucine to methionine. This polymorphism does not change the catalytic activity of the enzyme. In one study, the prevalence of 66GG genotype was found to be higher in people with NTDs and their mothers than in the control group. This polymorphism may be of importance in association with low Vit B12 levels.

(f) Methylene tetrahydrofolate dehydrogenase (MTHFD) gene defects

MTHFD enzyme has three activities: NADP dependent methylene tetrahydrofolate dehydrogenase, ATP dependent formyl tetrahydrofolate synthase and methylene tetrahydrofolate cyclohydrolase. Mutation analysis of MTHFD gene in patients with NTDs has led to the discovery of G878A substitution in a patient with familial NTDs. In several patients with isolated NTDs, a substitution G1958A was also observed. The frequency of this mutation in control and patient groups was similar. So its influence on aetiology of NTDs could not be estimated.

(g) Cystathionine beta synthase (CBS) gene defects

CBS catalyses the irreversible synthesis of cystathionine from homocysteine and serine. Disturbances in this process can lead to an increased cellular homocysteine level. Vit B12 is a cofactor in this reaction. Most of the mutations identified so far are correlated with homocysteinuria. Many of these mutations lead to decreased enzymatic activity.

Antiepileptics and NTDs

Most anti epileptic drugs (AEDs) are associated with increased risk for NTDs. Valproate has been directly implicated as a potent neural tube teratogen and has been documented to be involved in the inhibition of folate metabolism. Interference in the folate pathway could potentially result in decreased methylation of a number of regulatory genes involved in the normal development. Valproate exposure at critical periods of neurulation may alter the neuroepithelial mitotic rates that drive the elevation, fusion and closure of the neural tube. Valproate has also been found to elicit strain dependent effects on the expression of several genes important in normal embryonic development like cell cycle and apoptosis genes (bcl-2, p53), growth factor genes (bdfn, ngf, ngf-R) and folate pathway genes (folbp-1, MTHFR gene). A strain dependent variability in susceptibility to NTDs for a constant degree of exposure to valproate has been observed. It is possible that valproate could induce NTDs in a genetically predisposed foetus. Most AED therapies induce cytochrome p450 drug metabolising enzymes leading to reduced folate blood levels that may be sufficient to establish a folate deficiency.

Another proposed mechanism by which the AEDs produce foetal malformations is by increasing the total body free radical load and thereby free radical mediated cellular damage. For extensively metabolised drugs like valproate, the free radical burden is greater than for those with only one or two metabolites (Phenytoin, Phenobarbitone, Carbamazepine). Valproate therapy depletes selenium and selenium-deficient persons cannot synthesise glutathione peroxidase at the rates necessary to meet the metabolic demands. Thus in patients on valproate, the free radical burden is increased and also their FRSC is reduced resulting in a higher risk for NTDs.

Recommendations On Folate Intake

Observations from epidemiological and experimental studies have paved way to universal recommendation of folate supplementation in order to reduce the risk of NTDs. However, there is wide variability in the precise dosage, duration and mode of administration. After the release of the British and Hungarian trials, the U.S. Public Health Service (U.S. PHS) recommended in 1992 that all women of child bearing age who are capable of becoming pregnant consume 400 mcg of folic acid per...
day.

The American Academy of Pedatricians (AAP) and the Canadian Task Force have also put forth a similar recommendation. Public health advisories in Australia and Netherlands, have recommended that women plan and prepare for their pregnancies and that they consume 400 mcg of folic acid daily in the preconception period and in early pregnancy. As naturally occurring folate is less readily absorbed than synthetic folic acid in supplements or cereals, the Institute of Medicine in 1998 recommended that women of childbearing age obtain 400 mcg of folic acid daily from dietary supplements or fortified foods. The AAP, Canadian Task Force, American College of Obstetricians and Gynecologists (ACOG) and the U.S. PHS have recommended that higher risk patients (previous NTD affected pregnancy) take a higher dose of 4 mg folic acid daily starting one to three months prior to planned conception and continuing through the first three months of pregnancy. Anti epileptic drugs are known to reduce folate levels in blood.

Higher dosage of folic acid is probably necessary in patients who are taking anti epileptic drugs. In the Indian context, where there is likelihood of more profound deficiency states, concurrent infections, anemia and other conditions, it may be preferable to recommend the higher dose on a regular basis. In India, folic acid is available as 0.4 mg or 5 mg tablets.

**Folic acid fortification**

The increased scientific knowledge on the preventive effects of periconceptional folic acid on NTDs did not translate into any substantial decrease in its incidence. This may be because majority of women were poorly informed on the beneficial effects of folate and the pregnancies were unplanned. This situation has led to an alternate approach of universal food fortification with folic acid. The US policy involves fortification with 140 mcg of folic acid per 100 g of grain. USA and Canada have implemented food fortification programs successfully with resultant improvement in serum folate levels and reduction in incidence of NTDs. In Ontario (Canada), the incidence of NTDs decreased by 47% and in Nova Scotia (Canada), the incidence of NTDs decreased by 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.

**CONCLUSION**

Neural tube defects are an enigmatic problem that occurs as a result of the interplay between a number of genetic and environmental factors. Teratogenic drugs are the proven causative agents in a subgroup of cases. Community studies have shown the effectiveness of folate supplementation in preventing the occurrence of NTDs. More studies are needed to unravel the mystery surrounding the complex interaction between teratogens like AEDs, genetic factors like gene defects and environmental factors like folate deficiency in the pathogenesis of NTDs. The general consensus regarding the dose of periconceptional folate supplementation is that women with no previous history of NTD affected pregnancy take 0.4 mg and high-risk women like women with previous history of NTD affected foetus or women on anti epileptics take a higher dose of 4 to 5 mg. Newer strategies like food fortification with folic acid, need to be considered in order to reduce the incidence of NTDs.

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