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NUTRI-GENETIC DETERMINANTS OF NEURAL TUBE DEFECTS IN INDIA

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ABSTRACT

Justification: *Neural tube defects (NTDs) are one of the commonest birth defects with a high incidence in India. However, few studies have systematically looked into the etio-pathogeneis of NTDs, which mainly includes nutritional deficiencies and genetic predisposition. Efforts are afoot for universal food fortification with folic acid in the hope of preventing NTDs, without factual evidence of folate deficiency in the target population.*

Evidence acquisition: *We conducted a review of Indian literature on NTDs focusing on the role of folate and vitamin B_{12} nutrition and common genetic polymorphisms in 1-carbon metabolism. We performed a literature search of Medline and Indian Medlars (www. indmed.nic.in) for articles using following search terms: Neural tube defect and India, published up to November 2008, on human subjects. We did not include individual case reports and case series describing surgical and medical management, genetic syndromes where NTD was only one of the features or unusual associations of NTDs with other clinical findings.*

Results: *Absence of anationally representative large study, lack of interventional studies and methodological differences were conspicuous during this review. Larger studies are, therefore, urgently needed to delineate gene-nutrient interactions in association with NTDs in India. We urge that caution should be exercised before widespread folic acid fortification of food, without addressing the issue of concurrent B_{12} deficiency.*

Keywords: *Etiopathegenesis, Folic acid, India, Neural tube defects.*

Neural tube defects (NTDs) top the list of birth defects in India contributing to both morbidity and mortality. The number of NTDs diagnosed is progressively increasing with the advent of prenatal screening with ultrasound and maternal serum alpha-fetoprotein.

In spite of the mounting incidence of NTDs, few studies have systematically explored the nutritional, genetic or other determinants of NTDs in India. Efforts are afoot towards universal folic acid fortificationofflourinthehopeofpreventing NTDs, despite lack of specific Indian evidence(1). Indian obstetricians are routinely using 5 mg or more folic acid beginning at the first antenatal visit, in the hope to prevent NTDs, often quoting the recommendation from MRC (Medical Research Council, UK) trial(2).

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We reviewed the literature on NTDs in India with special focus on the role of folate and vitamin B_{12} nutrition and common genetic polymorphisms in 1- carbon metabolism in its etiology.

METHODS

We performed a literature search of Medline and Indian Medlars (*www. indmed.nic.in*) for articles using following search terms: Neural tube defect and India, published up to November 2008, on human subjects. We also examined bibliographies of all studies for other potential citations, but did not search studies published in languages other than English. Only one unpublished personal communication with the Birth Defects Registry of India, run by a non-governmental organization, Fetal Care Foundation and Research, Chennai, (*http:// www.mediscansystems.org/fcrf*) was used to include the latest incidences of NTDs in various major Indian cities. Papers related to NTDs in Indian and migrant Indian population (for example, Sikhs in Canada), including original epidemiological studies, case-control studies, studies discussing specific risk factors as well as review articles were included in this study. We did not include individual case reports and case series describing surgical and medical management, genetic syndromes where NTD was only one of the features or unusual associations of NTDs with other clinical findings.

Absence of a nationally representative large study, lack of interventional studies and methodological differences were conspicuous during this review. Although efforts were made to include all published articles related to NTDs in India, paperspublishedinnon-indexedjournalsmay not be covered. ***Table* I** summarizes papers reviewed in this article(3-54).

INCIDENCE

Thereported NTD incidence in India varies from 0.5 to 11/1000 births while the incidence in the USA and Europe is reportedly below 1/1000, with progressive decline with periconceptional folate fortification (40), barring a few countries like Ireland. The incidence tends to vary within various states of India and is reportedly also higher in Indians living abroad. The northern states have been consistently reporting a higher incidence compared to the southern states except for Davangere, Karnataka(4-6). Recent unpublished data from the Birth Defects Registry of India has identified Visnagar, Gujarat, as another high NTD reporting area (Birth Defects Registry of India, unpublished data). The incidence of NTDs in Sikhs living in British Columbia, Canada, was reported to be 2.86/1000 while the overall rate was 1.26/1000 in that area(7). Michie, *et al.*(8) quoteda higher incidence of NTDs in Indians living in the North Thames (West) region of UK. We found only one paper by Henry, *et al.*(9) from South Africa who have quoted a relatively low incidence of 0.37/1000 births in Indians compared to another contemporary British series quoting an incidence of 2.5/1000, during 1963-69.

TABLE I DISTRIBUTION AND TYPE OF PUBLICATIONS REVIEWED

Type of study	Number	Study (Reference)
Incidence/prevalence		
Population based	1	3
Hospital based	22	4,5,6,7,8,9,10,11, 12,13,14,15,16,17,18, 19,20,21,22,23,24, 25
Case-control studies	1	26
Intervention studies	1	27
Mechanistic		
Embryological	5	18,28,29,30,31
Infections	1	32
Trace elements (Fluoride, Zn)	2	30,33
Vitamin deficiency (Folic acid, B12)	4	33,34,35,36
Genetic	3	37,38, 39
Review/Recommendations	6	1,2,40,41,42,43
Other/cross-references	11	4,45,46,47,48, 49, 50, 51, 52, 53, 54

Most epidemiological studies are hospital- based(10-25), except for a study by Cherian, *et al*.(3) that explored the incidence of NTDs in community, in the remote village clusters in one of the poorest regions of India, Balrampur District, Uttar Pradesh between October 2002 to September 2003. This door to door survey revealed a high NTD incidence of 6.57-8.21/1000 live births. ***Table* II** shows the reported incidence of NTDs in India over the last 4 decades.

EPIDEMIOLOGY

Neither maternal age nor parity seems to be significantly different in relation to NTDs in the fetuses. Thehighestincidenceof NTDsisreportedin women in the age group 20-25 years (4,14,17), which is the commonest childbearing age in India. Cherian, *et al*.(3) and Mahadevan, *et al*.(17) report a higher incidence of NTDs in females (M: F: 1:1.5 and 0.6: 1.0 respectively) while Kulkarni, *et al*.(4) as well as Roy-Choudhury, *et al*.(14) did not report any significant gender difference. It may not be possible to precisely report gender as major NTDs may result in early pregnancy loss.

Most of the Indian studies have not found a seasonal variation in the occurrence of NTDs, except for a study in West Bengal in 1989(14) that reported a higher incidence in the rainy season.

TABLE II INCIDENCE OF NEURAL TUBE DEFECTS IN INDIA

NTD	Place Incidence	Year of Reporting	Study Reference
>7/ 1000	Chandigarh	1967,	15
	Davangere	1985-87	6
	Balrampur	2002-03	3
>3–7/ 1000	Lucknow	1982-1991	18
	Agra	1984	23
	Delhi	1991	22
	Pondicherry	1998-2004	17
	Pune	2007	Unpublished*

	Mumbai	2007	Unpublished*
	Chennai	2007	Unpublished*
	Visnagar	2007	Unpublished*
1–3/ 1000	Mysore	1967-69	12
	Kolkata	1976-1987	21
	Wardha	2000	16
	Bangalore	2007	Unpublished*
	Mumbai	1968-1972	19
<1/ 1000	Hyderabad	2007	Unpublished*

** Unpublished data from Birth Defects Registry of India run by Fetal Care Research Foundation, Chennai (http://www. mediscansystems.org/fcrf).*

Historically two main sites of neural tube closure (anterior and posterior neuropores) at 3rd and 4th week of pregnancy have been considered. Defective closure of anterior neuropore may result in upper level defects such as anencephaly, encephalocele while defective closure of the posterior neuropore may cause spina bifida, meningomyelocele etc. Kulkarni, *et al*. in 1989(4) and Verma, *et al*.(5) in 1978 reported the upper NTDs to be more prevalent than the lower NTDs. However recent studies from Mahadevan(17) and Cherian(3) reported an excess of spina bifida. There are isolated case reports of multiple site NTDs such as double or triple meningomyeloceles(28, 29).

ETIOPATHOGENESIS

Neural tube defects (NTDs) are believed to originate from complex interactions between various environmental and genetic factors (***Table* III**). Maternal nutrition is considered to be one of the

TABLE III ETIOLOGY OF NEURAL TUBE DEFECTS

Etiology	Examples
Nutritional	Folate, B_6 and B_{12} deficiency, Zn deficit
Maternal illness	Diabetes
Teratogenic	Anti-epileptic drugs: phenytoin and valproate, warfarin; Hypervitaminosis A and D; Addictions like cocaine, alcohol; TORCH infections, ? Dengue* and Hyperpyrexia
Chromosomal	Trisomy 13, Trisomy 18
Single gene defects	Meckel-Gruber syndrome
Associations	Currarino triad, including pre-sacral meningomyelocele, sacral dysgenesis and anal atresia/ stenosis
Complex eco-genetic	Predisposing polymorphisms in genes e.g., MTFR C677T polymorphism

* *Prenatal exposure to dengue fever during an epidemic in 1988 in Rohatak has been associated with increased incidence of NTD from basal 6.8 to 18.8/ 1000 births(32).*

most important determinants of fetal growth and development. Micronutrients including B group of vitamins especially folates, vitamin B_{12} and B_6 and minerals including zinc have been of special interest.

Kulkarni, *et al*.(4), made an important observation that fall in the infant mortality rate was not paralleled by the incidence of NTDs which remained high. They postulated a role of micronutrient deficiency. Agarwal(40) in a review article in 1999, discussed the role of hyperhomocysteinemia in the etiology of NTDs and peri-conceptional folate supplementation for prevention of NTDs. However, no supportive Indian interventional study was quoted. A question was also raised about 30% of NTDs that occur despite folate supplementation in the western world.

Maternal Hyperhomocysteinemia, and Folate and Vitamin B_{12} Deficiency

Hyperhomocysteinemia

Plasma homocysteine (Hcy) is considered to be a good integrated marker of folate and vitamin B_{12} status. There is a progressive decrease in plasma Hcy during pregnancy attributed to increased GFR during pregnancy, lower plasma albumin that binds to Hcy and increased cortisol level during pregnancy(44). This necessitates use of different cut-off points for hyperhomocysteinemia during pregnancy and 10 mmol/L has been used as the cut-off(45). Inherited defects in enzymes of 1-Carbon metabolism (*e.g.* methionine synthase) or cofactors such as folates and/or vitamin B_{12} cause abnormal Hcy metabolism resultinginhyperhomocysteinemia(44) Perturbation in this pathway leads to accumulation of inter- mediates like homocysteine and deficiency of methyl donors leading to defects in DNA synthesis, cellular growth as well as methylation reactions.

Folates

Folates is a generic term for compounds with pteroylglutamic acid-like activity and naturally occurring folates are present both in animal and plant foods. In India, major sources of folates are legumes and green leafy vegetables. Folates are required for purine and pyrimidine synthesis and methylation reactions including methylation of Hcy to form methionine. Folate deficiency might result from dietary deficiency, genetic defects in folate metabolism or both.

There is substantial evidence from Western countries that maternal peri-conceptional folate supplementation to "prevent" the first occurrence (400 micrograms) and recurrence of NTDs (4 mg) (2, 48,49). Over 50 countries have implemented folic acid fortification of flour and many physicians routinely prescribe pre and peri-conceptional folic acid tablets to eligible women. Kulkarni and Jose(26) followed 55 pregnancies in women with previous history of NTDs; 35 had been prescribed 5 mg folic acid pre-conceptionally while the other 20 women who did receive the supplement. None of the 35 women with folate supplementation had NTDs while 3/20 in the non-supplemented group had NTDs. However, in this observational study, maternal folate status was not documented. The only multi-center Indian study supported by ICMR(27) to explore the role of folic acid supplementation to prevent recurrent NTDs did not measure folate concentrations before or during pregnancy. It wasarandomized control trial using multivitamin preparation: vitamins A, B_1, B_2, B_6, C, D, nicotinamide, zinc, iron, calcium with 4 mg folic acid (placebo with calcium and iron only), to study the role of folic acid to prevent recurrence of NTDs in their future pregnancies. The trial was prematurely terminated after the results of MRC trial were published and showed a non-significant reduction of recurrence of NTDs in 2.97/1000 in folate supplemented *vs* 7.04 in placebo group. It is difficult to individually assess the role of each nutrient including folate, B_6, Zn in

prevention of NTDs in this trial. Folate intake lower than the RDA during pregnancy has been documented, however, no Indian study has measured folate status in women carrying fetuses with NTDs(34). In spite of absence of convincing evidence for maternal folate deficiency as a causative factor for fetal NTDs in India, periconceptional folate supplementation for the Indian women has been advocated(3,35,41,42).

Folate deficiency may be relatively uncommon in Indians compared to the western world mainly dueto vegetarian habits of Indians. Studies looking at maternal folate levels during pregnancy from Pune and Haryana have shown widely varying prevalence of folate deficiency, 0.2 and 26.3%, respectively (45, 46).

Vitamin B_{12}

Vitamin B_{12} is another micronutrient of interest in relation to NTDs due to its role as a cofactor for many enzymatic reactions involved in the folate/1-C metabolism. Refsum, *et al*.(44) have suggested that although there is a worldwide strategy to substan- tially increase folate intake for women in reproductive group, low vitamin B_{12} and elevated homocysteine (Hcy) need to be considered for reducing the NTD incidence.

Most of the Indian literature has heavily relied on the MRC, UK trial recommendations and emphasized periconceptional folic acid supplemen- tation. Vitamin B_{12} deficiency has not been investigated for its role in the etiology of birth defects including NTDs. However, studies in non-pregnant population in India and in Indians living abroad have documented a high prevalence of vitamin B_{12} deficiency and have considered vegetarian diet as a important risk factor(36).

Maternal vitamin B_{12} deficiency during preg- nancy has been documented in relatively recent studies. Pathak, *et al*.(46) reported 74.1% of preg- nant mothers to be vitamin B_{12} deficient using a cut- off of 200 pg/L, in Haryana. Muthayya, *et al*.(47) have reported an association of maternal vitamin B_{12} deficit with low birth weight in the offspring. Pune Maternal Nutrition Study reported vitamin B_{12} deficiency in over 60% (cut-off of 150 pmol/L) of pregnant women(45). A recent study by Ratan, *et al*.(33) reported nutritional status of parents of neonates diagnosed with NTDs. Low RBC folate as well as vitamin B_{12} and high plasma homocysteine were found in both parents of NTD neonates compared to controlparents.

Zinc

Srinivas, *et al*.(30) reported significantly lower hair zinc content in mothers who had delivered

newborns with NTDs compared to controls; however, the difference was not significant for serum zinc concentrations. The multivitamin supplement in the ICMR trial contained both vitamin B_6 and zinc; however, assessing their individual contribution in preventing the recurrence of NTDs was not the aim of that study.

Genetic contribution

Clinical observations suggest a higher frequency of NTDs in offspring of consanguineous couples as well as in twin pregnancies(31), pointing towards a strong genetic contribution to the etiology of NTDs. Empiric recurrence risk of NTDs rises from approximately 3% to 10% of the baseline population risk, if two offspring are affected(51).

Dinakar from Andhra Pradesh in 1972(11) followed by Kulkarni, *et al*. in 1989(4) have commented on consanguineous marriages in relation to the NTDs. The latter study reported NTD incidence of 20.6/1000 in consanguineous couples compared to 8.4/1000 in non-consanguineous couples. Similar observation was reported by Mahadevan, *et al*.(17) in 2005 where incidence of NTDs was 10.3 and 4.2/1000 in consanguineous and non-consanguineous couples, respectively. Consan- guinity is thought to contribute to Mendelian autosomal recessive conditions by inheritance of pathological mutations and also to polygenic multifactorial disorders such as NTDs by increasing the load of risk alleles.

The genetics of NTDs is complex and is likely to involve interactions amongst multiple genes and between genes and environmental factorsincluding maternal nutrition, infections and diseases. Animal models have provided some evidence for multi-site closure of the neural tube and also evidence for various genes, perturbation in which results in isolated NTDs, as often seen in humans.

POLYMORPHISMS IN GENES INVOLVED IN ONE-CARBON METABOLISM

Polymorphisms in a number of genes involved in the one-carbon metabolism such as MTHFR, MTR, MTRR, TC2 have been associated with increased or reduced susceptibility to NTDs. Kumar, *et al*.(37) reported homocysteine levels to be significantly elevated in individuals adhering to a vegetarian diet (P=0.019) or having MTHFR A1298C polymor- phism (P=0.006). The minor allele frequency of MTHFR for C677T and A1298C was 0.15 and 0.44, respectively. The frequency of A1298C polymor- phism in Indians was found to be higher than Caucasian, Chinese and Japanese populations. A low prevalence of the C677T polymorphism was also reported by Mukherjee, *et al*.(38). Michie, *et al*.(8) had reported a relatively low frequency of MTHFR 677C>T polymorphism in Gujarati women in UK compared to Pakistani women in the UK and speculated

that this polymorphism is unlikely to contribute to folate levels in these women.

Few Indian studies have explored the role of genes in 1-carbon metabolism in relation to NTDs. Ratan, *et al*.(33) have speculated on gene-nutrient interactions because of their finding that paternal hyperhomocysteinemia was the only independent risk factor for NTD in the fetus. Dalal, *et al*.(39) concluded that although the frequency of 677C >T homozygotes was higher in mothers with a previous child with NTD than the controls (*n*=87), the difference was statistically insignificant. There was a significant difference in frequency of T alleles among mothers with a previous child with a 'lower' type of defect compared to controls (OR = 2.15, 95% CI (1.13-4.1), *P*=0.02). No significant association of 1298A–>C polymorphism with the level of NTDs was noted.

FUTURE DIRECTIONS

NTDs are considered to be polygenic, multifactorial condition wherein many genes, nutrients, environment including infections, drugs, and maternal diseases such as diabetes individually or in combination might lead to NTDs. Apart from controlling known diseases like diabetes or avoiding teratogenic medications, the major thrust of primary prevention of NTDs has been on nutritional supplementation with folate. Role of 0.4 mg of periconceptional folic acid leading to 60% reduction in NTDs was demonstrated by MRC, UK(2). Many countries including the USA and Canada have adopted universal folate fortification of flour following this report. No national program for the primary prevention of NTDs by nutritional supplementation currently exists in India. National health programs such as National Anemia Prevention Program provides 0.5 mg of folic acid along with 100 mg of elemental iron from the third month of pregnancy. Private practitioners, especially Obstetricians in India often prescribe 5 mg of folic acid for a variety of conditions falling under the broad category of "BOH" (bad obstetric history) while the prescribed tolerable upper limit level (UL) for folic acid is 1 mg/day. The recent National Family Health Survey of India (NFHS-3) revealed that about 56% of pregnant women sought antenatal care after 16 weeks of gestation(50). Thus, only a small percentage of women receive 'periconceptio- nal' folic acid that might help prevent NTDs in the fetuses of folate deficient mothers. It is important to note that the MRC recommendations were based on population that is predominantly non-vegetarian. ICMR had launched a similar multi-center trial of multivitamin supplementation in 1988, which was prematurely terminated after publication of MRC trial report. This led to an acceptance of MRC recommendations of periconceptional folic acid supplementation even for Indian women who are predominantly vegetarian; without assessing the incidence of the first occurrence and recurrence of NTDs and its nutrigenetic determinants in India.

The prevalence of NTDs varies amongst the countries as well as within a country. Given the ethnic and dietary diversity in India, it would be interesting to study the genetic and nutritional

aspects of NTDs as well as gene-nutrient interactions underlying NTDs. Apart from the common polymorphisms in the above genes; there are epigenetic changes that affect gene function without altering the DNA sequence. DNA methylation is one such important epigenetic mechanism. An abnormal methylation pattern results in either under or over- expression of involved genes, thereby decreasing or increasing the production of encoded proteins and enzymes. Also, tissue-specific RNA methylation plays an important role in mRNA function and integrity(43). Disturbed methylation activities may interfere with normal fetal growth and development, NTDs being one such obvious effect.

Selhub, *et al*.(52) suggest that high folate could be detrimental in those with a low vitamin B_{12} status, partly because it is associated with a paradoxical rise in homocysteine. Ray, *et al*.(53) have noted B_{12} deficiency associated with NTDs in folate-fortified population. Thus, it would appear that deficiency as well as an imbalance between these two vitamins may be responsible for structural and functional disturbances.

Efforts for universal folic acid fortification of flour in a hope to prevent NTDs in India are afoot without factual evidence of folate deficiency in the target population. Universal folic acid fortification resulting in excess circulating synthetic folic acid is thought to be associated with increased incidence of colorectal cancer and neuro-cognitive decline in the elderly(54). A higher adiposity and insulin resistance has been documented in the children of Indian mothers who were vitamin B_{12} deficient but folate-replete during pregnancy(45). Larger studies are therefore urgently needed to delineate gene-nutrient interactions in association with NTDs in India. ***Table* IV** provides recommendations on information that may be collected while planning such large studies.

We are currently studying the nutritional and center study funded by the Department of Biotechnology, Government of India. Results of this study might throw some light on the underlying nutrient deficits and the genetic predisposition. We urge that utmost caution should be exercised before widespread folic acid fortification of food without addressing the issue of concurrent B_{12} deficiency in India.

TABLE IV RECOMMENDATIONS ON INFORMATION TO BE COLLECTED FOR NTD RELATED STUDIES

I. Parents with NTD offspring
   - Maternal and paternal biochemical studies before/ during pregnancy including plasma B12, plasma/ RBC folate, plasma Hcy, holo-TC, MMA using appropriate standard cut-offs during pregnancy.
   - Detailed dietary history including use of fortified food
   - History of nutritional supplementation of folate/B_{12}/ B_6/ Zn etc specifically during peri-conceptional period
   - Information regarding teratogenic exposure , maternal diabetes, TORCH infection, drugs etc
   - Consanguinity and family history of NTDs and other midline malformations such as cleft lip/palate, congenital heart defects
   - Gestational age (that may help in calculating month of conception)
   - Mode of diagnosis of NTDs (prenatal ultrasound, postnatal finding etc)

II. Fetus/Offspring
   - Sex of affected fetus/ still born or live born
   - Exact type of NTD, all types to be noted in case of multiple neural tube defects (e.g. cervical meningomyelocele with lumbar spina bifida)
   - Associated malformations/ dysmorphism to delineate genetic syndromes

III. DNA samples from both parents and fetus/newborn (fetal tissue/ cord blood) may be stored with appropriate consent for genetic studies at a later date.

genetic associations of NTDs in case and control trios (parents and offspring) as a part of a multi-

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