RISK FACTORS OF NEURAL TUBE DEFECTS: A STUDY

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Abstract - Neural tube defects (NTDs) are birth defects of brain and spinal cord. The incidence of neural tube defects dates back to Ancient Egyptian times, (Obladen, 2011) when fetuses and infants with anencephaly, myelomeningocele and craniorachischisis were reported. Thomas Mc Keown and RG Record, in a series of case control studies conducted on commonly occurring major defects in 1940s, observed that factors like season and year of birth, maternal age, birth rank and socioeconomic status play important role in causing these defects. They mentioned the role of folic acid in reducing the incidence of neural tube defects (Leek, 1996). Many research findings have confirmed their work. So far, nutritional deficiency in mother, genetic factors and some environmental factors have been found to play important role in causing these defects. The present paper attempts to review the contribution of some principal risk factors in the etiology of NTDs, on the basis of studies and findings of various researchers.

Keywords- NTDs, Birth Defects, Nutritional Deficiency, Folic Acid.

1. INTRODUCTION

In embryos of chordates, including vertebrates, neural tube is an embryonic structure which is precursor of brain and spinal cord and forms its central nervous system. The development and closure of the neural tube is completed in about 28 days after conception. The abnormal neural tube closure, results in the development of a neural tube defect in the embryo (Imbard et al. 2013). As reported by Mitchell (2005), an average of 1 in every 1000 established pregnancies worldwide is affected with NTDs. Olney and Mulinares (1998) has recorded a geographic and temporal variation in the incidence of some major NTDs. Furthermore, Fuchtheim et al. (1999) in their study, observed that within a specified geographical area and time period, a significant variation in the prevalence of NTDs occurs depending upon the race and ethnicity and type of NTD.

Neurulation: The process by which neural tube is formed in an embryo is called neurulation. It initiates with the development of the neural plate as a thickening of the dorsal ectoderm, followed by molding of neural plate, with the processes including convergent extension. The neural plate then curves, elevates and starts moving towards the midline. The extremities come into contact and merge together to create the neural tube, which, subsequently, gets covered by epidermal ectoderm. Closure of the cranial neural tube is considered to be vital for the appropriate development of brain, as well as initial formation of major part of the skull (Imbard et al. 2013).

In mammalian embryos, neurulation occurs in two phases: primary neurulation and secondary neurulation. These two phases occur in separate areas along the rostro-caudal axis of the embryo. The entire neural tube rostral to the caudal neuropore, is produced by primary neurulation. This process occurs in the third and fourth weeks of development. During this process, the flat layer of ectodermal cells overlying the notochord gets transformed into a hollow tube. Secondary neurulation is restricted to the tail bud region, which lies ahead the caudal neuropore. Contrary to primary neurulation, the secondary neurulation takes place by proliferation of stem cells, which leads to the formation of a rod-like condensation that subsequently undergoes cavitation. As a result of cavitation, the rod gets transformed into a tube and the lumen of this tube becomes continuous with the lumen of the tube formed during primary neurulation (Detrait et al. 2005).

2. CLASSIFICATION OF NEURAL TUBE DEFECTS

On the basis of embryological considerations and the presence or absence of exposed neural tissue, the NTDs can be classified as: Open NTDs and Closed NTDs. Open neural tube defects occur due to failure of primary neurulation and often include the entire central nervous system. They are characterized by presence of uncovered neural tissue which is associated with cerebrospinal fluid (CSF) leakage. Closed neural tube defects, on the other hand, arise due to defective secondary neurulation. They generally remain confined to the spine, rarely affecting the brain. They are rarer type of NTDs in which the neural tissue is not exposed and the defect is covered by the skin. The NTDs present in the cranial region include Anencephaly, Encephalocele (meningocele or meningo(myelo)cele), Craniorachischisisotalis and Congenital dermal sinus, whereas those present in the spinal region include Spina bifida aperta (cystica), Myelomeningocele, Meningocele, Myeloschisis, Congenital dermal sinus, Lipomatous malformations (lipomyelomeningoceles), Split-cord malformations, Diastematomyelia, Diplomyelia and Caudal agenesis (Jallo,2015).

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3. CLINICAL FEATURES OF NEURAL TUBE DEFECTS

Clinical severity of NTDs varies greatly depending upon whether the lesion is opened or closed. The experiments of Wood and Smith (1984) on rats have shown that open neural folds undergo growth and differentiation and typically appear to bulge from the developing brain, termed encephalocele. The skull vault is not formed over the open region, which causes degeneration of exposed neural tissue, leading to manifestation of anencephaly. Open lesions like anencephaly and craniorachischisis are found to be unvaryingly lethal before or shortly after birth. Another open NTD termed as encephalocele is characterized by sac-like protrusions of the brain and covering membranes, through the openings of the skull. Encephalocele is generally associated with neurological problems if it is located in the back of the skull. Also, there are various craniofacial or other brain malformations. Hydrocephalus (excessive accumulation of cerebrospinal fluid in the brain), spastic quadriplegia (paralysis of the arms and legs), microcephaly (abnormally small head), ataxia (uncoordinated movement of the voluntary muscles, such as those involved in walking and reaching), delay in development, vision problems, retardation of mental and physical growth, and convulsions, are other abnormalities which are found associated with encephaloceles (National Institute of Neurological Disorders and Stroke, 2007). Presence of open neural folds in the spinal region causes spina bifida, in which the sclerotome-derived vertebral arches fail to cover the neuroepithelium leading to significant damage to the nerves and spinal cord (Copp et al. 2015). The children born with open spina bifida usually survive after birth, but suffer from paralysis of the lower body and learning disabilities (Chobe et al. 2014). Because bladder and bowel movements are controlled by the lowest spinal nerves, bowel and urinary disorders are very common in case of spina bifida (Centers for Disease Control and Prevention, 2011).

Closed neural tube defects are the abnormalities of fat, bone, or membranes. They may be asymptomatic in some people, but others might experience partial paralysis or other symptoms. In some cases, only a dimple or tuft of hair on the spine can be seen externally (Mc Comb & Chen, 1996). The closed NTD termed as spina bifida occulta, is considered to be the mildest type of spina bifida. Also known as “hidden” spina bifida, it is characterized by the presence of a small gap in the spine, with no opening or sac on the back and normal nerves and spinal cord. This type of spina bifida often does not cause any disabilities and may remain undiscovered until late childhood or adulthood (National Institute of Neurological Disorders and Stroke, 2012).

4. RISK FACTORS FOR NTDs

The March of Dimes Global Report on Birth Defects, which details the birth prevalence rates and number of affected births in 193 countries, estimates that, 7.9 million children (6 percent of total births worldwide) are born with a serious birth defect of genetic or partially genetic origin, every year. At least 3.3 million children under five years of age die from birth defects each year and an estimated 3.2 million of those who survive may get disabled for life. According to this report, congenital heart defects (1,040,835 births), neural tube defects (323,904 births), the hemoglobin disorders, thalassemia and sickle cell disease (307,897 birth), Down’s syndrome (217,293 births) and glucose-6-phosphate dehydrogenase (G6PD) deficiency (177,032 births) were the most common five serious birth defects of genetic or partially genetic origin in 2001. The report also mentioned that the impact of birth defects is more severe in middle- and low income countries with 94 percent of the births with serious birth defects and 95 percent of the deaths of children with thses defects (Christianson et al. 2006).

Though the exact pathogenesis of these conditions is unknown, there are numerous and complex risk factors that have constantly been recognized in various studies. In case of neural tube defects, the main risk factors which have been identified and studied can be categorized into nutritional factors, genetic factors, maternal diabetes and obesity, and use of antiepileptic drugs by pregnant women.

5. NUTRITIONAL FACTORS

5.1 Maternal Folate Deficiency

The best known risk factor for fetal NTD is maternal folate deficiency, arising from low levels of vitamin B9 (folic acid). The serum folate, red cell folate, white blood cell vitamin C, and riboflavin values in first trimester were found to be lower than normal in mothers who gave birth to infants with neural tube defects (Smithells et al. 1976). Stover in 2009 found that folate undergoes one-carbon metabolism, involving a complex network of interlinked reactions that facilitates the transfer of one-carbon groups for numerous biosynthetic pathways. Two such pathways are: formation of pyrimidine and purine bases for DNA replication during cell proliferation, and contributing the methyl groups to macromolecules like DNA, proteins and lipids (Copp et al. 2013). Research findings of Smith and Schoenwolf (1987) have shown that alteration of cell cycle causes changes in the neuroepithelial cell shape during bending of chick neural plate. This encourages the hypothesis that folate has an important role in increased cell proliferation during neural tube formation. Thus, all women who have had a pregnancy affected with NTD, can be recommended to have folic acid supplementation starting before pregnancy and all women of child bearing age should be given adequate amount of folic acid in their diet (MRC Vitamin Study Research Group, 1991).

5.2 Maternal Vitamin B12 Deficiency

The reduction in the incidence of NTDs after implementation of folic acid fortification by various countries suggests that sufficient intake of folate is vital for prevention of NTDs. However, some researchers have suggested, that adequate vitamin...
B12 levels might also be necessary. The investigations of Ray and Blom (2003) suggested the moderate association between low maternal B12 status and the risk of fetal NTDs. Serum vitamin B12 levels have been found low in women with neural tube defect pregnancy (Wald et al. 1996). Vitamin B12 seems to play some vital role in proper functioning of the enzyme methionine synthase that converts homocysteine to methionine (Refsum, 2001). In countries like India, where much of the population is vegetarian and known to be deficient in vitamin B12, folate alone might not have adequate benefits in preventing fetal NTDs.

5.3 Genetic Factors

Maximum number of NTDs is found to occur sporadically with relatively limited cases of multigenerational families (Greene and Copp, 2014). However, numerous candidate genes have been proposed for understanding the cause of human NTDs based on biochemical and developmental studies, mouse models and positional evidence. In case-control or family-based association studies, on 38 candidate genes, few have shown notable results in human populations (Boyles et al. 2005). Various researchers are of the view that, genetic factors responsible for causing NTDs could involve either folate-related or folate-independent genes. The evaluation of folate-related genes as NTD candidates has been emphasized so far (Molloy et al. 2009). Presence of genetically-determined abnormality of folate metabolism in fetuses having NTD, observed in the analysis of their primary cell lines, also supports the role of folate-related genes (Van der Put et al. 1995). The study of a number of genetic variants have shown variable results in different cohorts and populations and very few of them have been found to have a key causative effect (Human molecular genetics, 2009). The folate-related genes candidate can be further divided into three categories, namely, methylation related genes, folate cycle enzymes required for nucleotide biosynthesis and genes encoding proteins required for folate transport (Greene et al. 2009).

The enzyme 5, 10-Methylenetetrahydrofolate reductase (MTHFR) is known to catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the principal circulatory form of folate which donates carbon for the re-methylation of homocysteine to methionine (Scott and Weir, 1994). The MTHFR 677C→T polymorphism converts alanine to valine, which results in the formation of thermolabile variant of the enzyme (Kang et al. 1988). In a study by Fross et al. (1995), patients with coronary and peripheral artery disease are stated to have reduced MTHFR activity due to presence of its thermolabile variant and plasma homocysteine levels have been found to be considerably high in individuals, homozygous for thermolabile variant of MTHFR (TT). In addition, the NTD affected people are reported to have abnormal thermolabile enzyme in homozygous condition, in DNA sample analysis conducted on people with NTDS, their parents and normal controls (Whitehead et al. 1995). This was the first definite genetic abnormality which explained the association between some NTDs and elevated homocysteine levels, in Irish population.

The second important folate-dependent enzyme is methylenetetrahydrofolate dehydrogenase1 (MTHFD1). MTHFD1, commonly known as “C1-THF synthase”, is a trifunctional, nicotinamide adenine dinucleotide phosphate (NADP+) dependent cytoplasmic enzyme, which catalyzes biochemical reactions involving the conversion of tetrahydrofolate to the corresponding 10-formyl, 5, 10-methenyl, and 5, 10-methylene derivatives. The derivatives 10-Formyltetrahydrofolate and 5, 10-methylenetetrahydrofolate play vital role in biosynthesis of purine and pyrimidine and thus, help in biosynthesis of DNA (Brody et al. 2002).

In a study conducted on Irish populations (Brody et al. 2002) and Italian populations (De Marco et al. 2006), it has been reported that MTHFD1 1958G > A polymorphism in heterozygous and homozygous conditions is the genetic determinant of both maternal and NTD case risk. The MTHFD1 R653Q polymorphism, which lies in the 10-formyltetrahydrofolate synthetase area of MTHFD1, causes reduced C1THF synthase activity in cell lines, which further results in reduced purine biosynthesis (Christensen et al. 2009). In addition to this, a promoter polymorphism (rs1076991C>T) in MTHFD1, that results in reduced transcriptional activity in vitro, was also found linked with NTD case and maternal risk (Carroll et al. 2009).

The third important group of candidate genes comprises those, which encode the proteins required for the transport, uptake and cellular retention of folates. These proteins include folate receptors FRα (Folr1 in mice), FRβ and FRγ, RFC1 (reduced folate carrier), GCPII (folyl-γ-glutamate carboxypeptidase) and FPGS (folylpolyglutamatesynthetase) (Beaudin and Stover, 2009). The studies available till date do not provide any definite evidence to show that common polymorphisms in these genes affect the folate status. No variants have been identified within the coding regions of the folate receptor genes, and the polymorphisms found in noncoding regions of FRs and FRβ have not brought forth any association with NTD risk (O’Leary et al. 2003). In the RFC1 gene, a common single nucleotide polymorphism (SNP) has shown an adequate relationship with NTD risk under conditions of folate deficiency, though with low penetrance level (Shaw et al. 2002). Likewise, the single polymorphism H475Y recognized in GCPII has not found to affect NTD risk in humans (Afman et al. 2003).

5.4 Maternal Diabetes And Obesity

Since very long, maternal insulin-dependent diabetes has been connected with congenital malformations. A great majority of researchers have observed that infants of insulin-dependent diabetic mothers have two to three fold chances of getting affected with birth defects, though there is not a collective agreement (Mills, 1982). Single nucleotide polymorphism (SNPs) in maternal genes such as FTO, LEP, TCF7L2, LEPR, GLUT1 and HK1, concerned with glucose metabolism and obesity,
may be responsible for increased vulnerability to NTDs like spina bifida (Davidson et al. 2008). Further, the pathogenesis of obesity has been reportedly found associated with variations in the fat mass and an obesity-associated gene (FTO), though, FTO may have a low-penetrance susceptibility for obesity risk and the association between FTO polymorphisms and obesity risk may vary in different ethnic groups (Peng et al. 2011). In diabetic pregnancies, the fetuses receiving excessive amount of glucose at the time of neural tube formation, may not be capable of controlling these elevated levels and consequently develop congenital malformations (Li et al. 2007).

Maternal obesity, with body mass index (BMI) of $\geq 30$, is another factor that is known to increase the risk of development of NTD in the embryo, twice or more (Shaw et al. 2000). On the basis of a geographically based case-control study conducted on women living in California and Illinois, Waller et al. in 1994 suggested that offspring of obese women have higher risk of neural tube defects and several other congenital malformations. Another population-based case-control study by Watkins et al. (2003), on some particular major birth defects, confirmed the formerly ascertained relationship between spina bifida and prepregnancy maternal obesity and found that maternal obesity is linked with omphalocele, heart defects, and multiple abnormalities among the embryos. Ray et al. (2005), also emphasized that there is increased risk of NTDs in infants of obese women even if they are using folic acid periconceptionally.

5.5 Maternal Use Of Antiepileptic Drugs (Aeds)
The use of antiepileptic drugs (AEDs) by pregnant women has been associated with chronic teratogenic effects on fetus, the most common of which are congenital heart diseases, cleft lip, urinogenital defects, and neural tube defects (Meador, 2008). Valproic acid and Carbamazepine, the two main anticonvulsant medications which are presently in use, have been found to cause these defects (Rose and Mennuti, 2009). In a review of published cohort studies, 14 major congenital malformations have been identified, whose risk increases when the pregnant women undergoes valproic acid monotherapy during the first trimester as compared with no exposure to antiepileptic drugs during the first trimester. Out of these, the risk for spina bifida, was found to be 12 or 16 times higher, depending on the control group used (Jentink et al. 2010). Also, valproic acid is the only AED for which the increase in risk of birth defect depends upon dosage taken, as compared to other AEDs. This has been clearly observed in cases where the dose of valproic acid is above 800-1000 mg/day (Perucca, 2005).

Carbamazepine (CBZ) is another antiepileptic drug that is used mainly for the treatment of epileptic convulsions. In an investigation based on the data pooled from prospective studies which involved 1255 cases of prenatal exposure to CBZ therapy, the higher incidence of congenital abnormalities, mainly neural tube defects, cardiovascular and urinary tract defects, and cleft palate has been reported (Matalon et al. 2002). In other study on NTDs, performed by Rosa (1991), it has been observed that 1% of the infants who had CBZ exposure before birth, got affected with spina bifida. Furthermore, the teratogenic effect gets increased when a combination of CBZ with other antiepileptic drugs is used, in comparison to CBZ monotherapy (Matalon et al. 2002).

6. CONCLUSION
NTDs are congenital birth defects which are one of the major causes of neonatal deaths and lifelong disabilities among children worldwide. The prevalence of NTDs is considered to be more in low income countries and developing countries like India. Though maternal folic acid deficiency has been identified as key causative factor behind NTDs and mandatory folic acid fortification programs in various countries worldwide has resulted in reduced incidence of NTDs, the role of genetic, epigenetic and environmental factors has been found instrumental. Thus, the NTDs comprise a varied group of malformations which occur due to cumulative impact of multiple causative factors, rather than simple vitamin deficiency disorders. Certainly, there are excellent studies that have been carried out on important risk factors involved in etiology of NTDs, the scope for identifying the correlation between various risk factors still exists. Further exploration in this area can help in better understanding of the cause of specific NTDs and thus framing the preventive strategies.

7. REFERENCES


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