

# Neural Tube Defects, Its Etiology: Environmental Exposures and Genes, Possible Risk Factors

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## Abstract

**Aim** The etiology behind abnormal development of the neural tube during embryonic life is not well understood. The evidence for associations of environmental cause and genes remains controversial. However recent analysis suggests excessive corresponding risks at environmental exposure levels and involvement of genes.

**Methods** We analyzed the research on developmental defects of neural tube and developed priorities and strategies to understand its etiology and risks associated with environmental factors and genes involved. We conducted searches for peer-reviewed papers published since 1990, using the term “NTD’s”, AND “Pesticide exposure” AND “Congenital malformations”.

**Results** Research on the investigation of neural tube defects may offer an excellent opportunity to understand and characterize high-risk population and to understand association between environmental factors and gene involved in the etiology of NTDs. Such study may lead us to evaluate etiological hypothesis i.e. the possible environmental factors exposure level and genetic factors that may initiate NTD pathogenesis. The environmental exposures include organic solvents, agricultural pesticides, water nitrates, and heavy metals; ionizing radiation; and water disinfection by products. Our review supports an association between environmental exposures, genetic involvement and NTDs.

**Conclusions** Future research is needed to understand neural tube defects prevalence and its etiological association with the exposure of environmental factors and genetics. Epidemiological and experimental studies will provide us the information regarding the physiology of NTD and analyzing the novel etiological hypothesis. Thus, it is necessary for us to consider maternal exposure and effect on the developing embryo, also the possible interactions among them.

**Key Words:** - Environmental exposures, Congenital malformations, Neurulation, Anencephaly, NTD.

## INTRODUCTION

Defects of the neural tube are the debilitating structural birth defect, among newborn affecting 1 per 1000 births in all over the world population. The neural tube formation occurs between 17 and 30 day after conception i.e. 4 to 6 weeks after the first day of a woman’s last day of menstrual cycle, before a woman knows she is pregnant. During this period development of defects can occur. Congenital malformations (i.e. non-syndrome) isolated NTDs results when the neural tube closure fails during embryogenesis.

**Embryology:**-To understand the embryonic study of neural tube defects, it is important to know the morphogenetic processes and the underlying molecular mechanism involved in neural tube closure. The neural tube development process is called neurulation and it is the fundamental embryonic activity. During foetal development process, neural tube is the leading precursor for brain and spinal cord. The phenomena behind the building of neural tube is an extremely complex procedure where cell changes shape migrates, differentiates and form a hollow tube from a flat sheet of epithelial cells called neural plate. When the neurulation process fails at any stage, neural tube defects occur. The etiology behind NTDs is very complex and involves both genes and environmental factors. Environmental factors that increases the possibility of NTDs includes geography, epidemic trends, socioeconomic factors, maternal age and maternal food habits, maternal disease conditions like diabetes, thyroid disorder and obesity and drug exposure mainly antiepileptic drugs[1].

The brain and spinal cord formation begins with the neurulation process of neural tube development. The dorsal

surface gets thickened and ectoderm folds up and fuses in the midline to create the neural tube and results in origination of the neural plate. Figure. 1 shows the closure of neural tube, the initiation and completion events. Closure is initiated at the hind brain/cervical boundary (closure 1), after that the spreading of fusion occurs in the hindbrain bidirectionally and along the region of the spine. The closure initiation sites gets separated at the midbrain-forebrain boundary (closure 2) and forebrain rostral extremity (closure 3) [2].

Progression Neurulation occurs in two part process and begins when neural plate starts forming, the ectoderm of the post-gastrulation embryo at the dorsal surface gets thickened. In humans, neural plate development into the neural tube occurs via a two-step process

i) Primary neurulation process (21 to 28 days) helps in the neural tube formation which develops into brain and most part of the spinal cord.

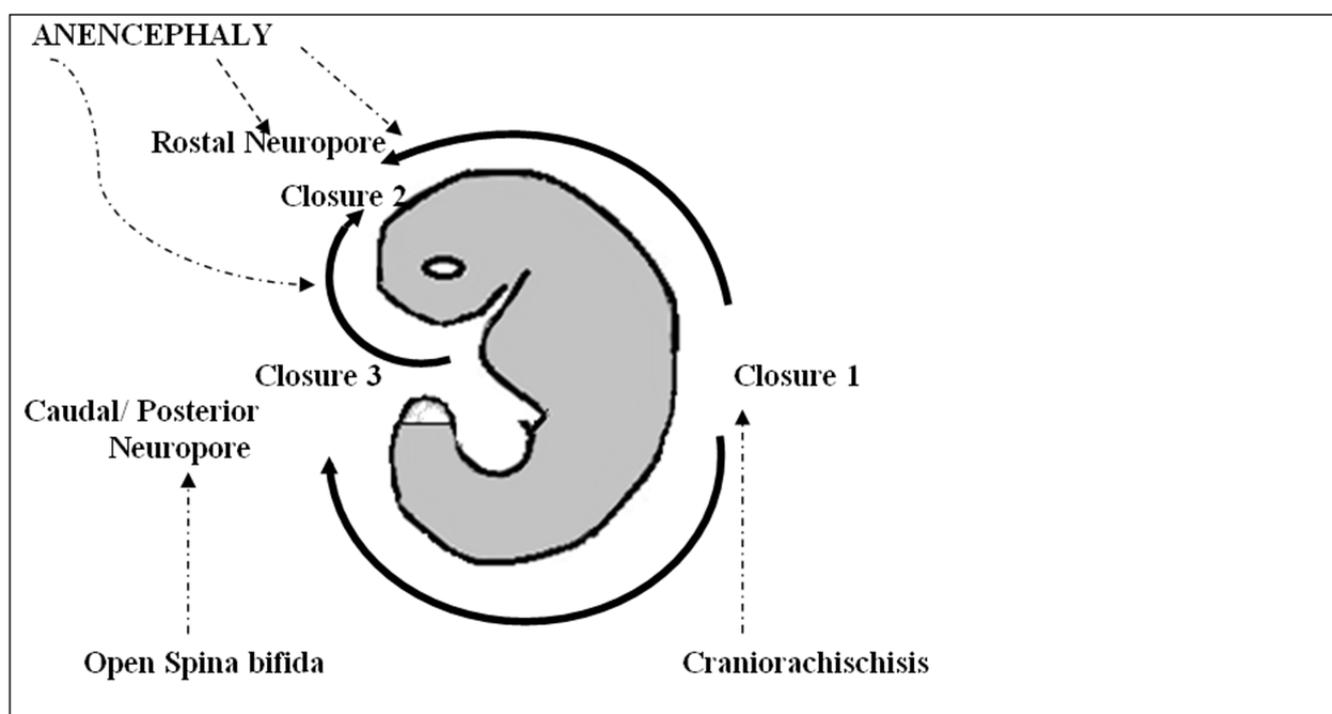
ii) Secondary neurulation (35 to 42 days) forms the neural tube caudal to the mid-sacral region[3].

During the primary neurulation, neural plate folding and shaping occurs with fusion along the midline which forms the tube. The secondary neural tube undergoes proliferation after that condensation followed by cavitation and final fusion occurs with the primary neural tube. This tube is formed from mesenchymal cells, the tail bud[3].

The neurulation mechanism is redundant mechanism driven at both tissue as well as cellular level[4]. Failure of primary neurulation process development results in **open neural tube defects** generally seen in anencephaly (Figure 1), myelomeningocele also known as open spina bifida and craniorachischisis. Any deformity in spinal cord structure

that are covered by skin are called **closed neural tube defects**. It ranges from asymptomatic spina bifida occulta to severe spinal cord tethering and is traceable when secondary neurulation are disrupted. Skin covered spinal defects are called closed NTDs and examples are lipomyelomeningocele, lipomeningocele, and tetheredcord. Defects when meninges (with or without tissues of brain and spinal cord) become exteriorized through pathological opening present in the skull or vertebral column are called **Herniation neural tube defects**[5]. Some active processes are essential for neural tube closure and that includes cranial mesenchyme expansion, convergent extension movement of cell, neural plate bending, actin filaments contraction, and neural fold adhesion. There are many mouse models that provide the mechanisms of NTDs occurrence as a result of gene mutations [6]. Neural tube closure, a discontinuous process proceeds bi-directionally. Severe forms of NTDs including open defects where neural tube closure failure occurs, in which the interior of the brain or spinal cord communicates directly with outside. **Table 1** indicates the various forms of open NTDs. **Encephaloceles** and **meningoceles** are the moderate form of NTD. In these defects herniation occurs through an opening in the skull or vertebral column of brain and meninges. The third group of dysraphic defects where spinal cord having closed abnormalities occurs, especially in the lower part of the lumbar vertebrae and sacral regions[7]. Table 2 represents the dysraphic defects. Among the defects of the neural tube, prevalence of anencephaly reported was highest i.e 2.1 per 1000 births after that spina bifida as the second highest i.e. 1.9/1000

births [9]. Epidemiological, genetics and surgical studies are the major advances which help us in understanding NTD, its prevention and treatment [10]. NTDs can be classified into two categories, according to their identification; they are biological and positional. On the basis of biological categorization genes that are identified from research, done in animal models and also from biological pathways are important for studying NTDs. Identification of some genes can also be done through positional methods i.e. rearrangements of chromosomes in genomic screen in linkage analysis or NTD patients [11]. Clinical research studies demonstrated that supplementation of folic acid decreases the chances of NTDs occurrence as well as their recurrence risk [12, 13]. According to March of Dimes (MOD) and Global Report on Birth Defects [14], worldwide annually 7.9 million births occur with birth defects and from which 94% of defects occur in the middle and low income countries. A joint meeting of WHO and MOD reports shows that defect at birth accounts for 7% mortality rate in infants and 3.3 million under five deaths. Prevalence of birth defects in India varies from 61 to 69.9 per 1000 live births [15]. Major birth defects include Congenital Heart defects, Down syndrome, defects in neural tube (NTDs), hemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency, which causes infant mortality 20% and responsible for increase in the number of childhood hospitalizations [16]. During early pregnancy maternal hyperthermia is related with increased risk of birth defect such as NTD [17]. Some study reports 70% of birth are preventable [15].



**Figure 1.** Diagrammatic representation showing neural tube closure, the initiation and completion events are joined by unidirectional or bidirectional neural tube zippering (dark arrows). Dotted arrows indicates the affected events causing NTDs. Secondary neurulation process starts from the level of the closed posterior neuropore, with the help of canalization inside the tail bud (white in colour).

**Table 1:** Neural tube Defects (Open): Types

Open NTDs	Occurrence
<b>Anencephaly</b>	Part (nearest to the head), neural tube fails to get close; absence of large part of the brain, skull, and scalp. Infants having this disease are born without forebrain and cerebrum. Infants are usually blind, deaf, unable to feel pain and unconscious. There is an unknown reason behind anencephaly and it is most likely a “multifactorial” birth defect, including genetic, nutritional, and/or environmental factors[8].
<b>Encephalocele</b>	Cranial contents get protruded beyond the normal confines of the skull through a calvarium defect. It is identified by herniation of meninges and brain, a defect occur in the cranial vault midline or at the base of the skull.
<b>Myelomeningocele</b>	Failure of spinal neural tube closure, especially in the lumbosacral region. It involves underlying layers that includes the spinal cord, nerve roots, vertebral bodies, meninges and skin. Meningeal sac present in the open spinal cord, herniates because of vertebral defect; this is called spina bifida cystica. In case of myelocele, flat lesion occurs at the open spinal cord.
<b>Craniorachischisis</b>	Neural tube closure is completely absent in this disease, which affects both brain and spine. Initiating event of neurulation in the early embryo fails, results in craniorachischisis[7].

**Table 2:** Dysraphic defects

Defects	Occurrence
<b>Diplomyelia</b>	Duplication of side by side or anteroposterior part of spinal cord occurs.
<b>Diastematomyelia</b>	Spinal cord gets longitudinally divided into two usually unequal portions by a midline septum, extending upto 10 thoracolumbar segments.
<b>Hydromyelia</b>	Overdistension of central canal occurs.
<b>Lipomenigocele</b>	Fatty tissue deposition occurs along with the dysraphic spinal cord.
<b>Spina bifida occulta</b>	Posterior bony components defects of the vertebral column occurs without involving cord or meninges.

### Epidemiological Studies on Neural Tube Defects

A study shows the incidences of NTDs in Yaounde, the birth record was exploited during the last ten years period (1997-2006). This study was conducted in three main hospitals of Yaounde and a total of 52,710 births were recorded, out of which 98 cases of NTD was registered showing incidence of 1.99/1000 births. Spina bifida cystica (myelomeningocele and meningocele) (71%), encephalocele (21.1%) and anencephaly (5.4%) were observed [18].

An association between maternal and child characteristics and having an infant with neural tube defect in Colorado was observed between 1989 and 1998. Data was obtained from population based case control study, utilizing birth certificate records and state wise registry of neural tube defects cases. During this ten year period study, 251 confirmed cases of neural tube defects were found in Colorado, 224 of whom were born to women belong to either United States or Mexico. From this study it was concluded that low maternal education is an important predictor of having a child with neural tube defect. For reducing the incidences of neural tube defects, researchers should target women having low educational status [19].

A population-based study of mothers who lives in remote villages, a least-developed area in India was done with the help of door to door survey method. This data showed NTDs incidences, 6.57–8.21 /1000 live births, which is highest among worldwide [20]. The trends in neural tube

defects from July 1998 to June 2004 were identified by Mahadevan and Bhatt, 2005 in the Jawaharlal Institute of Postgraduate Medical Education and Research, India. In this study it was observed that total number of babies born with neural tube defects were 310, with overall frequency of 5.7/ 1000 births and spina bifida was most common i.e. 54.8% then anencephaly 31.6% followed by encephalocele 11.6%. Parents of consanguineous marriage showed a significant higher rate of NTDs among babies born [21]. A pilot study of North Indian population shows that dyslipidemia during pregnancy may result in the increased risk of neural tube defects. This study involves 129 pregnant women having gestation period of 16 to 18 weeks. Out of 129 women, 80 had normal pregnancy and 49 were at clinically high-risk of NTDs [22].

### Experimental Research on Implications for Neural Tube Defects Etiology

An inherent implication of research on genetics and environmental factors is that it may eventually lead to a better understanding of neural tube defects etiology [23]. Incidences of NTDs vary with socioeconomic status of the parents, geographical location and seasonal variation.

### Genetic factors affecting NTD

Animal study shows the effect of folic acid intake on risk of NTDs in splotch (Sp2H) mice, carrying mutation in Pax3 was investigated and it was found that induction of deficiency of folate in splotch (Sp2H) mutant embryos causes significant increase in the frequency of cranial NTD,

which results from interaction between gene – environmental factors between folate status and loss in the functioning of Pax3 [23]. Reduction in the NTD-affected pregnancies due to folic acid supplementation was evidenced by both observational studies and randomized trials; however 30 to 50% cases of NTDs are not folate preventable. For these types of NTD, consideration of environmental agents must be done [24].

Some animal studies shows more than 200 genes are responsible for NTDs which fall into diverse functional classes that include regulators of cell adhesion, actin dynamics, DNA damage repair, electron transport, and other processes [25, 26]. The effect on planar cell polarity function (non-canonical) pathway and sonic hedgehog signaling over activation leads to the causes of NTD, with requirements also for inositol and retinoid signaling, the key signaling pathways for NTDs. Folic acid supplementation in humans and mice are the preventive method for the risk of NTDs, although the mechanism of folate action on embryo remains unclear. Inositol supplementation in mice can pervert folic acid-resistant cases, raising the possibility that this may be another preventive strategy for human NTDs in future [2].

A myristoylated, alanine rich substrate for protein kinase C is F52. It was found that F52 disruption in mice identifies a gene, when mutation occurs in that gene; it results in isolated NTDs [28]. Disorders of DNA methylation in NTD affected fetuses are related with the risk of NTDs. Insulin like growth factor 2 (IGF2) genes have its major role in foetus development. Differentially methylated regions (DMRs) 0 and 2 can partly controls IGF2 transcription. It was found that IGF2 DMRO methylation level was significantly raised in the brain tissues of NTD affected fetuses. IGF2 contains two intragenic DMRs, namely DMRO located between exons 8 and 9. The methylation level of H19 DMR1 was significantly high in NTD group than in control group. This result indicates that IGF2 DMRO hypermethylation is a risk factor of NTDs. In addition contribution to DMRO hypermethylation was gender-dependent. Significant hypermethylation of DMRO was existed between female NTDs. These results suggests that normal fetus development is likely to be more sensitive to modification of methylation and female infants are more afflicted with NTDs. Female NTD-affected fetuses show more sensitivity to environmental effects on DMRO methylation [29].

A study demonstrated the role of canonical complement 5a receptor (C5aR) in the mammalian neural tube development in maternal folic acid deficiency. They observed C5aR and C5 expression throughout the neurulation period in wild type mice and localized the expressed C5aR ad C5 to the cephalic regions when neural tube is developing. C5aR was also expressed in the neuroepithelium of early human embryos. High prevalence of neural tube defect associated congenital anomalies was seen after ablation of C5ar1 gene or when specific C5aR peptide antagonist was administered to folic acid-deficient pregnant mice [30].

### Environmental factors affecting NTDs

There has been an increasing concern about the possible role of occupational exposures and exposures to chemical agents in the ambient environment and its role in the etiology of adverse reproductive outcomes including NTDs during the last 10-15 years. Increased incidences of NTDs have been observed in areas with a excessive use of agricultural chemicals [31]. Using data from population based National Birth Defects Prevention Study, the relation between occupational exposure of mothers to aromatic solvents, chlorinated solvents and in case of early pregnancy and NTDs and or facial defects were examined. This study suggested that occupational exposures of mothers to chlorinated solvents during early pregnancy are associated positively with the incidences of NTDs in offspring [32].

Animal studies shows that maternal alcohol and pesticides exposures, led to excess neural cell death, results in the closure of the neural tube. It was also observed that alcohol exposure can result in folic acid deficiency, suggesting that alcohol exposure might play an indirect role in the progression of NTDs through folic acid depletion. More specifically alcohol administration in rats resulted in reduction in plasma folate levels by increasing folic acid excretion through kidney [33, 34]. Pesticides are the chemicals which can cross the placenta and affects the embryonic development. These chemicals alter proliferation and differentiation of neuroepithelial cell during neurulation and lead to excessive neuroepithelial cell death, affecting closure of neural tube. Exposures to toxic chemicals such as amide, benzimidazole, methyl carbamate, or organophosphorus pesticides and with increasing numbers of pesticides are related with the increased risks of NTDs and anencephaly or spina bifida subtypes. For NTD subtypes, it was observed increases in anencephaly are associated with organophosphorus pesticides and spina bifida with amides, benzimidazoles, and methyl carbamates [35]. Persistent organic pollutants (POPs) are lipid soluble and resistance to metabolism, can accumulate in the human body. The common POPs include polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs), and among others. Adverse health effects such as carcinogenicity and teratogenicity are associated on exposure to these POPs [36, 37, 41]. Stockholm Convention organized by UNEP in 2001, ratified a global treaty on persistent organic pollutants was further adopted in the year 2004, enlisted the organic pollutants as the ‘dirty dozen’ dieldrin, aldrin, chlordane, endrin, heptachlor, DDT, PCBs, hexachlorobenzene (HCB), mirex, toxaphene, dibenzodioxins, and dibenzofurans [42]. These pollutants effects depend upon their interaction with the environment and how they remain in the environment for extended period of time. They are highly halogenated and they degrade very slowly in air, water and soil [46]. Despite the fact that many are currently regulated and have not been in production, they do not easily break down and persist in the environment for decades. They get bioaccumulated and

biomagnified when they move up the food chain [44]. In the food chain, the higher consumers such as humans and other carnivores are exposed to higher concentrations than the consumers at the bottom, which only eat vegetation. Exposure of POPs in humans begins prenatally as they can cross the placenta. After birth exposure of these POPs occurs through breast milk of lactating mothers [45, 46] and also through inhalation, ingestion, and skin contact [37].

Perfluorinated compounds (PFCs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) emerged as a new class of global environmental pollutants and comprises a class of environmentally persistent chemicals that have a wide range of industrial applications. The teratological study evaluated the presence of various skeletal abnormalities in PFOS treated groups which were few in PFOA groups. Neonates were born with low body weight and showed the presence of the bilateral swelling which accompanied by neonatal death, while in PFOA treated group there was only body weight reduction and survival rate [46].

An elevated concentrations of polycyclic aromatic hydrocarbons (PAH) phenanthrene, p,p'-isomers of dichlorodiphenyltrichloroethane (DDT) and metabolites,  $\alpha$ - and  $\gamma$ -hexachlorocyclohexane (HCH), and  $\alpha$ -endosulfan in placenta were associated with high risk of NTD's and these risks increased with the concentrations of these pollutants and found phenanthrene present with maximum concentration]. It was found that in cases of anencephaly and spina bifida the median concentration of PAH, p,p'-DDT,  $\alpha$ -HCH,  $\gamma$ -HCH and  $\alpha$ -endosulfan were all higher in the placental samples. Environmental PAHs have multiple sources, the exposure comes primarily from coal combustion [37, 45, 46]. Highly sensitive 32P-postlabelling assay measured the relationship between PAH-DNA adducts levels in the placental tissue, and the risk of fetal neural tube defects (NTDs). Levels of PAH-DNA adducts were found lower in the NTD group as compared to. The concentration of PAH-DNA adducts below the median was associated with a 3-fold high NTD risk. Women with a lower PAH-DNA adduct level in concert with a higher placental PAH level resulted in a 10-fold increased risk of having an NTD-complicated pregnancy. When the placental PAH-DNA adducts level was low then it was associated with a high risk of NTDs; this risk is increased when a low adduct level was coupled with a high PAH concentration in placenta [37].

One report shows significant correlation between organochlorine pesticides and the level of non-enzymatic oxidative stress markers in preterm delivery cases of humans [38]. The preterm delivery cases of India accounts 31% of neonatal death [38, 39]. The pathophysiology of preterm delivery cases may involved the role of reproductive hormones such as estrogen and progesterone. Uterine quiescence promoting hormone is progesterone whereas activating promoter hormone of myometrial membrane is estrogen which upregulates membrane receptors and gap junctions [38, 40]. Higher levels of Hexachlorohexane (HCH), isomers of endosulfan, p,p'-Dichlorodiphenyldichloroethylene (DDE) and p,p'

Dichlorodiphenyltrichloroethane (DDT) were found in the Preterm Delivery cases than Full Term Delivery. A possible relation of  $\beta$ -HCH with Preterm delivery cases due to estrogenicity was observed [38].

An association of higher water nitrate intake with several birth defects in offspring was reported in a study, however it did not strengthen relation between nitrosatable drugs such as antiemetics, decongestant, antihistaminic etc. and risk of birth defects [50].

A fungal product i.e fumonism is another environmental teratogens with proven effects in humans, causes a doubling of NTD incidence along the Texas-Mexico border in the early 1990s. Fumonism is a potent NTD-causing teratogen in mice, with marked effects on sphingolipid metabolism and it downstream the embryonic gene expression [51].

The most common sources of environmental exposures are air and drinking water. Drinking water includes consideration of water hardness and mineral constituents, water nitrates, organic solvents and water disinfection byproducts. The airborne pollutants in NTDs can be illustrated by studies of vinyl chloride. Several studies have been conducted showing potential exposure to vinyl chloride monomer from industrial sources and NTDs [52]. These studies revealed that central nervous system malformation rates were high in communities with polyvinyl chloride polymerization plants [52, 53]. In animal study, *in utero* exposure to chlorpyrifos, one of the most frequently used organophosphate insecticides, led to large number of cell death in neuroepithelium during neurulation in rat embryos [32, 54]. Teratological study of a pyrethroid insecticide,  $\beta$ -cyfluthrin in developing foetuses of mice showed morphological abnormalities including anophthalmia, microcephaly, micromelia, dysplasia, dysmorphogenesis, and short tail. Morphometric studies including body weight, brain size, crown rump length, length and width of eye, length of both fore limbs and hind limbs and length of tail of foetuses showed significant ( $P < 0.001$ ) differences against controls. This study shows that the pesticides exposure to developing murine fetuses is potentially dangerous and suggests that it may be harmful to foetal development in humans [55].

Lead is a neurotoxin and could cause NTDs by acting directly on developing nervous tissue. Study shows that mothers residing in wards where the proportion of houses with more than 10 $\mu$ g/l lead in their water is higher, and has a higher risk of having baby with a neural tube defect. Also it could act indirectly by causing zinc deficiency with secondary folate deficiency supported by a study of elements in the bones of some stillborn malformed babies. Calcium is a toxicological antagonist of lead. Impaired absorption of folic acid is caused by zinc deficiency which may be caused by lead [56].

Hazardous air pollutants (HAP's) are the toxic substances found in the air environment causes serious health effects [57]. These are large group of pollutants that includes organic solvents such as benzene, toluene, ethyl benzene, and xylene [BTEX] emitted from several sources. Exposure of HAP's to humans can result from inhalation, ingestion and dermal absorption. Benzene crosses the placenta and its

concentration found in cord blood is at levels equal to higher than maternal blood [58]. Benzene could lead to genetic toxicity, it covalently binds to DNA and forms DNA adduct, which if not repaired leads to inhibition of important enzymes, cell death and alteration of other cells by disrupting the microenvironment of the cell. If this occurs during the critical window of development, the complex cellular processes involved in neurulation (e.g folate metabolism, cell proliferation, cellular adhesion, and vascular development) may be disturbed. Oxidative stress also plays a role in teratogenic effects of benzene. After benzene exposure, reactive oxygen species get formed and leads to breakage of DNA strand and fragmentation causing cell mutation. One study conducted in rats demonstrated that increased embryonic oxidation results in defects in neural tube closure. Exposure of mothers during early pregnancy to ambient levels of benzene is associated with the incidences of spina bifida among offsprings [59].

A recent study examined 26 pesticide metabolite present (from pesticides such as chlorpyrifos, carbaryl, naphthalene, lindane, e.t.c.) in urine samples of pregnant women (taken at 26 weeks) who lived near agricultural land and detected 11 pesticides metabolite, 8 occurred in more than 50% of samples. Methyl carbamate, chlorpyrifos, and other organophosphate insecticides are cholinesterase inhibitors, and in animal studies, cholinesterase inhibition shows alteration in differentiation and proliferation of cell during neurulation [60]. The exposure to chlorpyrifos prenatally is related with neurobehavioral deficits in humans and animal models. A study on environmental neurotoxicants reported that prenatal exposure to a widely used neurotoxicants at standard level shows its association with some structural changes in the human brain development [61].

Some studies supports the hypothesis that maternal exposure to agricultural work results in anencephaly and also suggests that pesticide exposure to father during periconceptional period or prior to this can also increases the risk of having an anencephalic child [62]. The toxicological profile for pyrethrins and pyrethroids was reported at the U.S Department of Health and Human Services in 2003. Some young animal study showed the signs of damage to the body's defense system of babies against infection after their mothers were exposed to pyrethroids while their babies were developing in the womb, also it was observed that the developing brain of some very young animals could be affected by pyrethroids [63]. Some researchers have reported that male rats when administered deltamethrin in oral doses as low as 1 mg/kg/day for 65 days was found to exhibit lower weights of testicles, seminal vesicles, and prostate gland. Also, analysis of sperm of treated rats revealed reduction in sperm cell concentration, percentage of live cell and higher percentage of total sperm abnormalities [64].

The teratogenicity of a commercial formulation of the insecticide deltamethrin (Decis®) in chick embryos was evaluated and it was found that administration of deltamethrin increased the embryonic mortality. Body weight significantly decreases and percentage of abnormal survivors is higher in a dose dependent manner, suggesting

that deltamethrin exhibits teratogenic and embryotoxic effects in the developing chick embryos [65].

Other factors which are associated with NTDs are the risk of a woman with epilepsy on Anti-Epileptic Drugs of having a child affected with NTDs is 1-2% [66]. Maternal hyperglycemia leads to NTD by activating apoptosis signal-regulating kinase 1 (ASK1) and deletion of this gene was related with reduced neuroepithelial cell apoptosis and neural tube defects development [67].

#### **Role of folic acid during pregnancy**

It is postulated that folic acid supplementation may overcome underlying defect involved in folate metabolism caused by genetic mutation in mother or in the foetus. Folic acid, a water soluble vitamin participates in the transfer of single carbon units in several pathways which includes synthesis of nucleotides and amino acids. The conversion of folate dependent homocysteine to methionine begins early in the development in all tissues and provides a link between folate and homocysteine metabolism. Mild hyperhomocysteinemia and an elevated level of plasma homocysteine have become recognized as a risk factor for NTDs [1, 68]. After entering into the cell, folate acts as a methyl donor for methionine synthesis via homocysteine remethylation. Methionine is the single most important methyl donor for methylation of DNA and tRNA. Folate also acts a donor of one-carbon groups for synthesis of thymidine and purines, the building blocks of DNA. A total no. of 25 proteins are involved in folate and homocysteine metabolism, which have been investigated for association with an increased NTD risk [70]. Few key enzymes are involved in folate and homocysteine metabolism which are 5,10-methylene-tetrahydrofolate reductase (MTHFR), trifunctional enzyme methylene THF dehydrogenase/formyl THF synthase/methylene THF cyclohydrolase (MTHFD), methionine synthase (MTR) and methionine synthase reductase (MTRR). The MTHFR gene is important as it regulates the available folate for homocysteine remethylation, extensively studied as a risk factor for NTDs [1, 70].

A folic acid supplements when its concentration is high in circulation provoke health complaints such as arthritis, leukemia, bowel cancer and ectopic pregnancies and the suppressing of the early hematological symptoms of vitamin B12 deficiency [71,72,73]. It is important for us to observe all the potential benefits and risks and optimal intake of folic acid, the physiological and safety ramification of lifetime exposure to circulating folic acid need to be elucidated [32]. Mitochondrial monofunctional 10-formyl-tetrahydrofolate synthetase encoded by gene MTHFD1L, this gene expressed throughout all the stages of embryogenesis with regions localized along the developing brain, neural tube, craniofacial structures, limb buds and tail bud. Embryo that lacks MTHFD1L exhibits closure of neural tube and includes craniorachischis, exencephaly and/or a wavy neural tube [33]. NTDs have a multifactorial etiology, (i.e genetic and environmentally mediated). Thus, it is essential for us to consider embryonic effects, maternal effects as well as interactions between the two.

**CONCLUSION: -**

Neural tube defects represents a group of congenital malformations occurs due to impairment in the neural tube closure during embryogenesis. Reducing infant mortality and improving the health of children are the main objectives and it is necessary for us, to expand our study of the environment role in etiology of birth defects and better understanding of risk factors for NTD will improve interventions, aimed at reducing NTD prevalence. We summarize the types of NTDs, its etiology and causes in this review. Additional research work is needed and directed towards the etiology of NTDs especially environmental exposures and genetic factors. The main mechanism which represents the role of environmental factors affecting the process of closure of neural tube and their interaction with genes remain a mystery. The factors affecting genes, causing NTDs in animal models is poised to inform high-throughput whole-genome studies of human patients. Identification of folic acid as a primary prevention strategy for NTDs generally achieved through epidemiological studies. The effective method for primary prevention of a proportion of NTDs in humans is the folic acid supplementation.

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