# *Review of literature*

# II REVIEW OF LITERATURE

The literature available pertaining to the research work entitled "**Neural Tube Defects (NTDs) and the Effect of Counseling and Folic Acid Supplementation**" is reviewed under the following headings.

A. Definition, types and prevalence of NTDs
B. Risk factors of NTDs
C. Complications of NTDs
D. Diagnostic tests for NTDs and
E. Folic acid in the prevention of NTDs and related studies

## A. DEFINITION, TYPES AND PREVALENCE OF NTDs

### 1) Definition and types of NTDs

NTDs are a group of serious birth defects that arise when the neural tube fails to develop into the brain and spinal cord during the first month of pregnancy (Unlu, 2002). The main types of NTDs are spina bifida, anencephaly and encephalocele (Heljic *et al* 2002).

According to Scott and Weir (1995), NTDs are birth defects occurring in pregnancies of genetically predisposed women where the foetal neural tube fails to form properly during early embryonic development. These defects occur during early pregnancy, often before a woman knows that she is pregnant (CDC, 2004).

Muller (2003) suggested that NTDs could be considered as a folate deficiency disorder. These defects comprise an important category contributing to infant mortality. While some NTDs may be due to identifiable inherited or specific environmental factors, most are multifactorial, with genetic and environmental factors contributing to their occurrence.

Geisel (2003) reported that NTDs are common foetal malformations. They are multifactorial in origin with folic acid as the primary known environmental factor. According to Unlu (2002), NTDs caused by abnormal neurulation are the major congenital anomalies which result in foetal or embryonic death and medical, financial and social problems.

NTDs are a group of congenital anomalies, which include anencephaly, encephalocele, iniencephaly, meningocele, myelomeningocele, myeloschisis, lipomeningocele, and rachischisis (Wani, 2000). Figure 1 and Plate 1 illustrate the various types of NTDs.

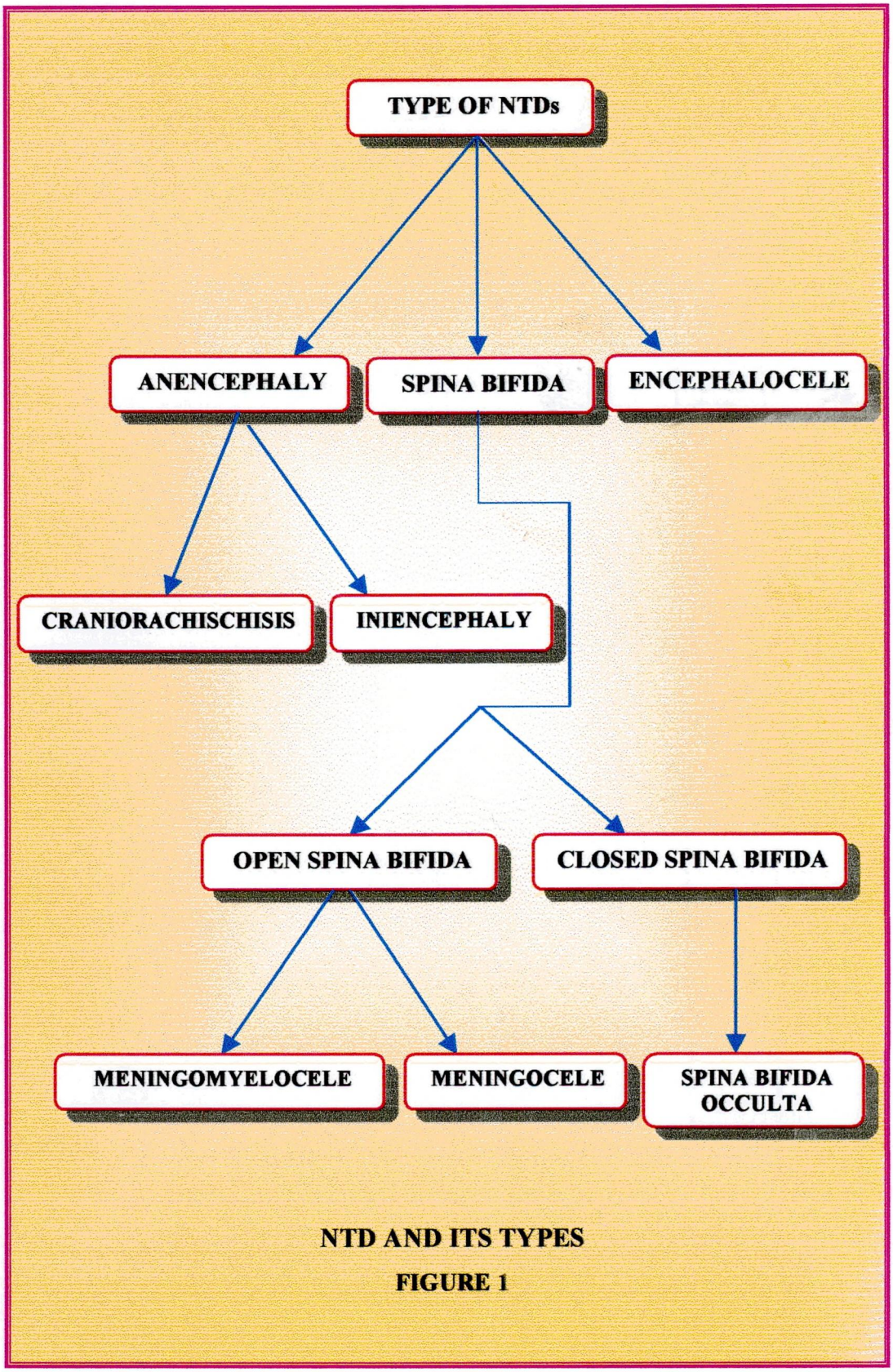
**a) Anencephaly**

According to Esenkaya (2004), anencephaly occurs when the cephalic end of the neural tube fails to close, resulting in the absence of a major portion of the brain, skull and scalp. Many infants with anencephaly are delivered still born, while those born alive are usually blind, deaf, unconscious and unable to feel the pain. If the infant is not still born, then he or she will normally die within a few hours or days after birth (Fishman, 1997).

When the anencephaly extends down to the neck, exposing a thin and flattened spinal cord, the condition is known as craniorachischisis (Boyd *et al* 2000).

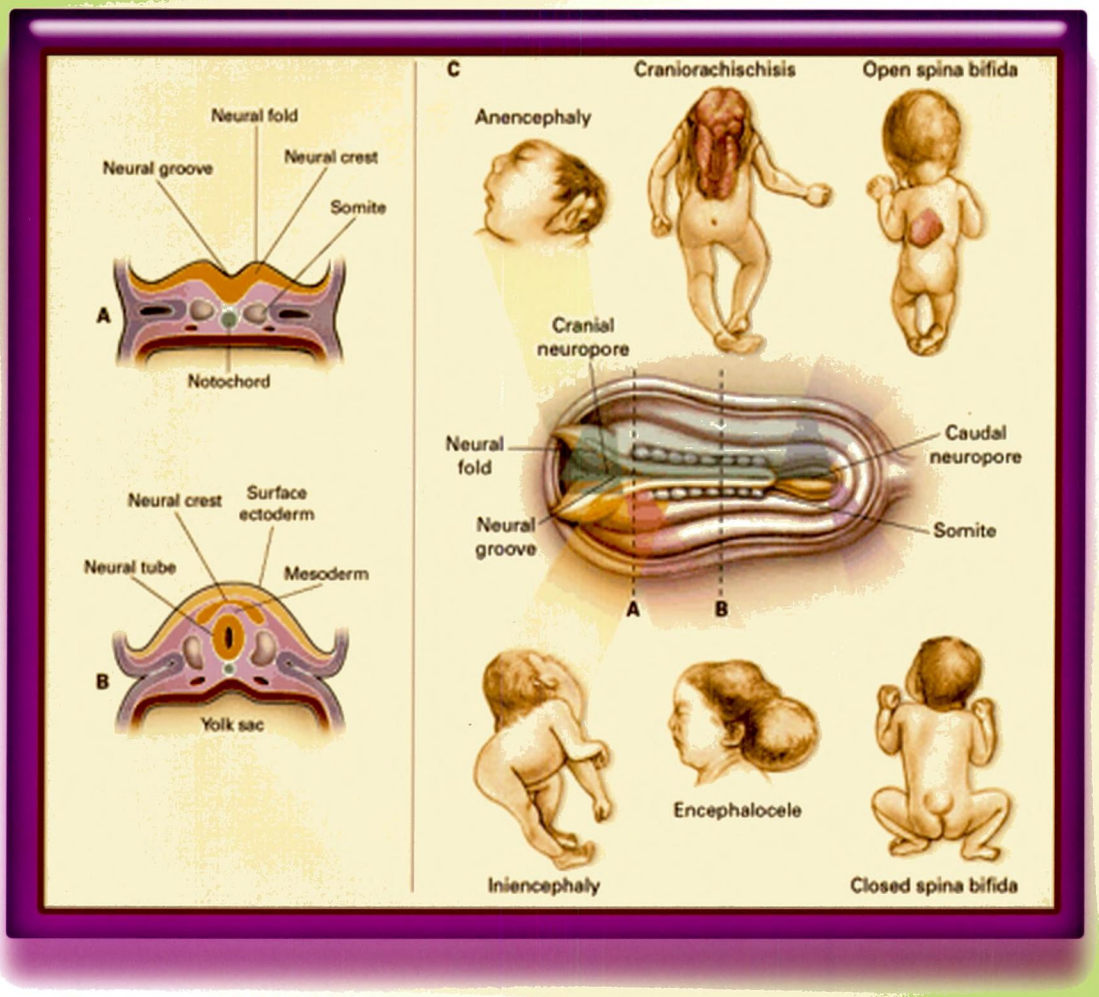
**b) Iniencephaly**

In some infants, in addition to anencephaly and the spinal defects, the baby's head is bent backwards, so that the face looks upwards and generally the neck is absent. This condition is called iniencephaly. The affected infant tends to be short, with disproportionately a large head (Subzposh *et al* 2003).




**NTD AND ITS TYPES**

**FIGURE 1**




***Botto et al (1999)***

## *FEATURES OF NEURAL TUBE DEVELOPMENT AND NTDs*

## *PLATE - 1*

**c) Spina bifida**

Spina bifida is a condition where one or more of the spinal vertebrae do not develop properly. The impact on the baby depends on the degree to which the spinal cord is involved (Cheschier, 2003). According to Greer (2000) spina bifida can be classified into two categories - open spina bifida and closed spina bifida.

**i) Open spina bifida**

Open spina bifida is otherwise called spina bifida manifesta. It is severe but a rare form of birth defect. It is often associated with nerve damages which can result in problems with walking, bladder control and co-ordination (Tunnessen, 1997). Open spina bifida is divided into two classes, myelomeningocele and meningocele.

**Myelomeningocele**

In myelomeningocele, the spinal cord and its protective covering protrude from an opening in the spine. The nerves are often damaged.

**Meningocele**

In meningocele, the spinal cord develops normally but the meninges protrude from a spinal opening. In this case, the deformity may remain covered by the meninges (cystica) or it may be open to the environment (Zambramski *et al* 1986).

**ii) Closed Spina bifida**

Closed spina bifida is otherwise called spina bifida occulta which is the mildest and most common form. One or more vertebrae are malformed and covered by a layer of skin. In some cases, a dimple, depression, birth mark or hairy patch forms over the skin where more than one vertebrae is affected. This is referred to as Occulta Spinal Dysraphism (OSD) (Liptak, 2002).

**d) Encephalocele**

Encephaloceles are sac like protrusions of the brain and the membranes that cover it through openings in the skull, they are often accompanied by craniofacial abnormalities or other brain malformations (McComb, 1997). The prognosis for a baby with an encephalocele varies depending on the type of brain tissue involved, the location of the sacs and the accompanying brain malformations.

Infants born with any form of spina bifida may have damage in the nerves and spinal cord. The degree of damage will depend on the type and location of the defect. Although a spinal opening can be surgically repaired shortly after birth, any nerve damage is permanent, resulting in varying degrees of paralysis of the lower limbs (Brent, 2000). In addition to physical and mobility difficulties, most individuals have some form of learning disability. McAllister and Chavan (1998) stated that spina bifida also may cause bowel and bladder complications. Many children with spina bifida have excessive accumulation of cerebrospinal fluid in the brain and the condition is called hydrocephalus (Milhorat, 1995). With proper care, most children with spina bifida live well into adulthood.

**2) Prevalence of NTDs**

NTDs are the most common congenital malformation of central nervous system. However, its prevalence varies greatly from place to place and population to population. The incidence of NTDs ranges from 0.6 to 3.7 cases per 1000 live births and varies between socio-economic and ethnic groups. Mother with one or more NTD pregnancies has a 10-fold increase of a subsequent recurrence (MRC,1991). According to Birnbacher *et al* (2002) NTDs have an incidence of 1-5 per 1000 births with marked geographic, ethnic and temporal variations. Population based active surveillance programs that

include prenatal diagnosis have reported NTD rates of 7.2-15.6 per 10,000 live born and still born infants (Cragan *et al* 1995).

Every year 3-4 lakh infants worldwide are born with spina bifida and anencephaly (Shibya and Murray, 1998). The prevalence is approximately 1-5 per 1000 live births and the risk of recurrence is 2-3 per cent (Hall and Sollehhdin, 1999). The highest incidence of NTDs has been reported from Ireland and Wales (6.38-10.92 per 1000 births).

In United States, the prevalence of NTDs is 1 per 1000 live births affecting 4000 pregnancies annually (Cragan, 1995). The rates of NTDs are found to be higher among white population than blacks in United States(Larry and Edmond, 1996). There are racial/ethnic differences in the risk for NTDs. In United States, NTDs prevalence have been reported to be the highest among Hispanics, followed in a descending order by non – Hispanic whites, Native Americans, African-Americans, and Asians ( Hendricks *et al* 1999).

One study reported no significant difference in the risk of anencephaly and spina bifida in infants born to Vietnamese women compared with infants born to non- Hispanic white women in California (Shaw *et al* 2002). Another study reported no significant difference in NTD rates among whites, Far East Asians, Pacific islanders and Fillipinos (Forrester and Merz, 2000). Difference in racial/ ethnic rates may be due to differences in genetic susceptibility to NTDs, cultural behaviors, diet or other factors.

Secular trends have been observed in NTDs prevalence since 1970s.Unambiguous evidence of the effectiveness of periconceptional folic acid in preventing NTDs has been available since 1991 (MRC,1991) and improving folate status sufficiently could result in the prevention of more than two thirds of all NTDs (Busby,2005). Overall rates for anencephaly and spina

bifida have declined in North America, Europe, Australia and New Zealand (McDonnel *et al* 1999), although one study reported an increase in NTD rates in the 1980's-1990's (Forrester and Merz, 2000).Australian state birth register of South Australia, Victoria and Western Australia report a steady rate of NTDs (1.6-2.0 per 1000) until 1996, with a 35-45 per cent fall in prevalence from 1996 onwards(Bower *et al* 2000 and Owen *et al* 2000).

The proportion of NTDs that were electively terminated has increased with time in some regions studied (Whiteman *et al* 2000). Thus, some of this decline in NTDs prevalence may be attributed to increased prenatal diagnosis and elective termination of NTDs affected pregnancies. However, this trend was observed prior to widespread use of prenatal diagnosis and has occurred in areas where elective termination is not allowed. NTD rates in the United States and Canada have shown a decrease from east to west. Anencephaly prevalence is generally lower in continental Europe than in the British Isles and shows a decrease from west to east.

According to Ahramsky *et al* (1999) NTDs, which comprise of open spina bifida, anencephaly and encephalocele, complicate 1.5 per 1000 pregnancies in U.K. and represent the first congenital malformations to be preventable through public health measures.

In China, one lakh infants are born annually with spina bifida and anencephaly. Northern China is identified as high risk area for NTDs with a prevalence of 5-6 per 1000 live births. The occurrence of the rare category of NTDs (i.e.) iniencephaly and craniorachischisis is considerably high when compared to other parts of the world (Berry *et al* 1999).

The prevalence of NTDs from different parts of India has been reported to vary from 0.5 to 11 per 1000 live births (Sharma and Gulati, 1992).

Cherian *et al* (2005) reported elevated prevalence of NTDs (8.2 per 1000 live births) in the Ballrampur District, Uttar Pradesh, India. They also stress the lack of planning for NTD prevention by periconceptional folate supplementation in India and urge Indian ministry of health to develop a comprehensive strategy to reduce the incidence of NTD without delay.

The prevalence of NTDs in consanguineous couples was found to be 16.3-20.6 per 1000 compared to 5.9-8.41 per 1000 in couples without consanguinity rates. However, with similarly high consanguinity rates in other Southern States, the prevalence of NTDs from Mysore and Chennai has not been high (Lakshminarayana, 1998). Open NTDs in babies occurring over a 10 year period (1982-91) in four major maternity hospitals of Lucknow showed an overall incidence of 3-9 per 1000 live births (Sharma *et al* 1994).

According to Ellenbogen (2003) anencephaly is most common in the Western World and is seen 37 times more frequently in females than in males. Recurrence in families is possible and can occur at a rate as high as 35 per cent. The highest incidence of anencephaly can be found in Ireland, Scotland, Wales, Egypt and New Zealand while the lowest is in Japan.

Studies have shown that the incidence of NTD varies between socio economic and ethnic groups. The differences in racial / ethnic prevalence rates may be due to differences in genetic susceptibility to NTDs, cultural behaviours, diet or other factors. Consanguinity, low socio economic status, illiteracy and lake of nutritional knowledge are the possible factors for the incidence of NTDs. Policies and plans of raising periconceptional folate status has reduced the prevalence of NTDs in several countries.

## B) RISK FACTORS OF NTDs

Genetic and epidemiological studies have suggested high risk group (Iqbal,2000) which include those who have past history of NTDs (Elwood *et al* 1992), maternal age of less than 20 or more than 35 years (Hendricks, 1999), low or high parity (primi para and grand multipara ) (Elwood *et al* 1992), low socio economic status and gross nutritional deficiency and inadequate antenatal care (Wasserman *et al* 1998). Also, the etiology of NTDs is very heterogenic with the impact of a given risk factor varying by the types of NTDs and the presence or absence of other defects (Sever, 1995).

### 1) Genetic disorders

A number of specific chromosomal or single-gene disorders, presumably not affected by environmental influences are associated with the development of NTDs but such syndrome cases account for a small proportion of NTDs in live-born infants (Frey and Hauser, 2003). The researchers learned that having only one copy of a variant gene is enough to increase the chances of being born with a NTD. Researchers believed that two copies of the gene are needed to increase one's risk of being born with a NTD (NICHD, 2004).

The common sporadic type of isolated NTD is considered to be a polygenic multi-factorial disorder (Tolmie, 1996). It implies that multiple genes are involved in predisposition of NTD. But besides genes, environmental factors also play an important role in causation of the disorder. The role of genes in causation of NTD is supported by higher risk of recurrence in first degree relatives compared to general population. However, the genes predisposing to NTD in humans have not yet been identified. The observation that periconceptional folic acid supplementation can prevent recurrence and even the first occurrence of NTD has led to investigation of the role of genes involved in folic acid metabolism (Fleming,1998).

It has been reported that C to T mutation at nucleotide 677 in the MTHFR gene is 1.7- 1.9 (95% CI 1.1 to 3.1) times more prevalent in mothers, fathers and foetuses affected with NTD compared to general population (Schneider *et al*, 1998). For identification of human genes, familial cases of NTD are an important resource. Once the predisposing genes are identified they can serve as tool to screen for high- risk individuals who can be targeted for primary prevention (Smithells, 1997).

**2) Environmental factors causing NTDs**

One investigation found risk of anencephaly and spina bifida, but not encephalocele, to be higher with lower altitude (Castilla *et al* 1999). Seasonal variations in NTD rates have been reported by some studies (Castilla *et al* 1990).

The pharmacology of NTD is a complex issue. Several theories regarding the etiology of NTD emphasize the importance of interactions between genetic, environmental and biochemical factors. One such factor is chronic drug therapy, a potential consequence of which is the formation of toxin drug metabolism, including the radicals which have been implicated in the etiology of NTDs (Pippenger, 2003). One of the high-risk groups for having children with NTDs is epileptic women receiving antiepileptic medications during pregnancy (Finnell, 2003).

Various studies have suggested that NTD risk is higher among families of lower socio economic status, although other studies have failed to support this view (Vrijheid *et al* 2000). One investigation reported higher rates of anencephaly among individuals with proximity to industries like soap and detergents, iron and steel machinery, photographic and rubber industries and encephalocele with proximity to fur dressing and dyeing industry (Castilla *et al* 2000). Another study found increased risk of spina bifida with

maternal occupational exposure to electro magnetic fields (Blaasaas *et al* 2002).

According to the reports of Matte *et al* (1993) maternal nursing occupation was identified to have higher risk of anencephaly and spina bifida. One study revealed no significant association between NTDs and a variety of paternal occupations (Irgens *et al* 1998). A study by Blatter *et al* (1996) reported increased risk for women working in agricultural occupations or in cleaning work. Close relatives in families who have a child with a NTD are at greatly increased risk of having an affected child (Pedreira, 2003). A relative folate deficiency was said to arise from a metabolic defect in the utilization of dietary folate that was manifested early in gestation under the stress of pregnancy (Scholl and William, 2000).

Since NTDs are rare birth defects, it is hard to definitely associate an environmental exposure of specific NTD. However a number of studies have identified exposures that may cause NTDs. Table I presents the range of exposures that may give rise to NTDs.

**TABLE I**

**ENVIRONMENTAL EXPOSURES ASSOCIATED WITH INCREASED RISK OF NTDs**

| EXPOSURE | TYPE OF NTDs | EXPOSURE TYPE | REFERENCE |
|---|---|---|---|
| Glycol ethers | All Combined | Occupational | Cordier *et al* (1997) |
| Nitrates | Anencephaly | Drinking water nitrates >45 mg/L | Croen *et al* (2001) |
| Lead | Isolated NTDs | Drinking water lead 10mcg / L | Bound *et al* (1989) |
| Tri halomethanes | Spina bifida | Every other day of consuming water | Klotz and Pyrch (1999) |
| Valproic Acid | Spina bifida | Anti-seizure medication | Arpino *et al* (2000) |
| Tea | Spina bifida | Maternal Consumption | Correa *et al* (2000) |
| Oxytetracycline (Antibiotic) | All Combined | Treatment during second month of pregnancy | Czeizel and Rockenbauer (2000) |
| Pesticides (Paternal) | Spina bifida | Spraying in orchards and green houses | Kristensen *et al* (1997) |
| Pesticides (Maternal) | All Combined | Professionally applied to home living within ¼ mile of an agricultural plot | Shaw *et al* (2003) |
| Land fill sites | All Combined | Living within 3 km of landfill site | Dolk *et al* (1998) |
| Heat | All Combined | Hot tub use | Milunsky *et al* (1992) |

**3) Maternal conditions contributing to risk of NTDs**

There are a number of maternal conditions that may contribute to an increased risk of NTD affected pregnancy. Shaw *et al* (1998) found an odds ratio of 1.91 for NTDs among infants of women who had fever in the first trimester of their pregnancy. It is not clear whether the exposure to high temperatures or to the bacteria or virus is the cause for the improper development of the neural tube. Maternal common cold in the first trimester of pregnancy has been reported to increase the risk of anencephaly and spina bifida (Zhang and Cai, 1993)

Janssen *et al* (1996) found that women with diabetes are four times more likely to have pregnancies involving NTDs. Women who are obese prior to pregnancy and not diabetic also are more likely to have children with spina bifida. Watkins *et al* (2003) found that obese women had an odds ratio of 3.5. One study showed two fold or greater risk of NTD affected pregnancy among women who were obese (Shaw *et al* 2000).

It is now generally accepted that a Body Mass Index >29 doubles the risk of NTDs (Shaw *et al* 1996). Another study reported higher NTD rates among women who gained less weight during pregnancy (Shaw *et al* 2001). Since weight gain during early pregnancy, the time when NTDs occur, is small and is not consistent, it is not likely that low weight gain is causal for NTDs. More likely, carrying an NTD affected foetus caused low weight gain throughout the pregnancy, or both conditions are the consequences of a common factor.

Maternal psychological stress may increase the risk of NTDs. Carmichael and Shaw (2000) found that women who experienced death of someone close, job loss, separation or divorce either themselves or some one

close to them in the very early stages of pregnancy had an odds ratio of 1.5. Suarez *et al* (2003) counted job changes, residential moves and major injuries as stressful events in the year prior to conception in Mexican Americans. Women with at least one such event had almost three times the risk of NTD affected pregnancy. Women with low levels of emotional support also had an increased risk of NTD affected pregnancy.

Maternal malnutrition is an important risk factor for the development of NTDs. Studies till date have shown decreased maternal folate levels in NTD affected pregnancies (Yates, 1987). Behavioural factors such as smoking cigarettes, using alcohol or using oral contraceptives are also associated with poor folate status (Bendich, 1991).

Hypothyroidism does not appear to be associated with NTDs, although risk of encephalocele but not spina bifida or anencephaly may be increased with hyperthyroidism (Khoury *et al* 1989).

A number of studies have reported maternal age risk for NTDs to be U-shaped, i.e. highest among youngest and oldest women, while other studies have found the risk to decrease with increasing age or the reverse (Owen *et al* 2000) or clear maternal age trend (Forrester and Merz, 2000). Paternal age has not been clearly linked to NTD risk (Shaw *et al* 1994), although one investigation identified increased NTD risk with lowest and highest paternal age groups (McLntosh *et al* 1995).

Parity has shown either a U-shaped pattern of NTD risk, i.e., risk being higher for the lowest and highest number of births (Little and Elwood, 1991) or increasing risk with increasing parity (Whiteman *et al* 2000).

NTD risk seems to be greater if women have had a previous foetal or infant death, even if the previous infant or foetus did not have an NTD (Canfield *et al* 1996). However, it is not clear to what extent recall be as or an undiagnosed NTD in the previous foetal death may contribute to this observation (Little and Elwood, 1991).

NTD rates may also be higher among multiple births and with lower birth weight (Mastroiacova *et al* 1999). However, one study reported no relationship between NTD risk and plurality (Kallen, 1986).

The recurrence risk for NTDs is approximately 3-4 per cent, with the risk being slightly higher if the previous infant or foetus had anencephaly. However, 95 per cent of the infants with NTDs are born to parents with no family history of the defect. With respect to consanguinity, NTD rates have been found to be higher when the parents are related (Little and Elwood, 1991), although not all studies identified this association (Stollenberg *et al*1997).

**4) Diet**

Folic acid is a nutrient that is already needed for proper neural tube development (Watkins, 1998). The metabolism of folic acid is very complex, and there are many reasons why an individual may not have sufficient folic acid to reduce the risk of a NTD affected pregnancy. For example, valproic acid and lead interfere with folic acid metabolism and therefore may lead to NTDs by reducing the folic acid available to the foetus in the first few weeks of pregnancy (Fornoff *et al* 2004).

Li *et al* (1996) reported that more consumption of dried and pickled vegetables, consumption of meat, egg and beans contribute to NTDs. According to the studies of Scholl and William (2000) there exists an association of gestational problems with population subgroup of low-income

women who have diets low in folate before pregnancy. A recent study by Velie *et al* (1999) has also suggested that periconceptional intake of zinc can reduce NTD risk, and another study reported the use of methionine to decrease NTD risk (Shoob *et al* 2000).

Genetic disorders, environmental exposures, maternal illness during pregnancy and lack of necessary nutrients at the time of foetal development may influence the occurrence of NTDs.

**C) COMPLICATIONS OF NTDs**

Brent *et al* (2000) stated that spinal cord damage due to spina bifida manifesta may lead to complications like inability to control urine or bowel movements (incontinence), constipation, little or no feeling in the legs and feet, inability to move or feel paralysis of the legs and less often the arms. Spina bifida often results in curvature of the spine-solios kyphosis (hunch back) or both.

Spina bifida may lead to disability. Associated complications depend on the level and severity of the lesion. Downward displacement of cerebellar vermis, fourth ventricle and brain stem (Arnold Chiari malformation) is another serious complications, requiring decompression and the signs include vocal cord paralysis, central apnoea, aspiration, dysphagia, hypotonia, quadriparesis, nystagmus and strabismus (Leask, 2005). Dias and McLone, (2003) reported that an open NTD allows fluid to escape from the cranial vesicles, altering the intracranial environment and leads to brain changes. Decompression of the intracranial vesicles causes over crowding, decrease in the size of the third ventricles and changes in the foetal skull. It also permanently links the intracranial ventricular system to the spinal cord central canal.

Groenen *et al* (2003) reported that in spina bifida group, myo-inositol, glucose and zinc concentrations in amniotic fluid gradually declined throughout pregnancy. Myelomeningocele may lead to neurological problems and hind brain herniation (Walsh *et al* 2001). The serum from women with a pregnancy complicated by NTD contains auto antibodies that bind to folate (Rothenberg *et al* 2004).

Basbug *et al* (2003) found significantly higher leptin levels in both amniotic fluid and maternal serum of patients who had foetuses with a NTD. The main source of leptin in amniotic fluid of pregnant women who had foetuses with a NTD is the leakage into amniotic fluid from cerebrospinal fluid.

Myelomeningocele is a common birth defect that is associated with significant lifelong morbidity. Postnatal surgery is aimed at covering the exposed spinal cord, preventing infection and treating hydrocephalus with a ventricular shunt (Adzick and Walsh, 2003). Long-term treatment of children with meningomyelocele requires multidisciplinary approach that includes surgeons, orthopedists, pediatricians and physical therapists, in order to improve the quality of life of surviving child (Heljic *et al* 2002).

Foetuses with myelomeningocele did have a significantly lower biparietal diameter and head circumference ($p<0.001$) (Williams *et al* 2004). Early experience with foetal myelomeningocele repairs suggests a decreased need for ventriculo peritoneal shunting, arrest or slowing of progressive ventriculomegaly and consistent resolution of hindbrain herniation (Johnson *et al* 2003). Club foot and hydrocephalus are the most common associated anomalies of neural tube fusion defects (Nugud *et al* 2003).

Children with spina bifida manifest fluid in the brain (hydrocephalus) and if not treated, the brain may fail to develop properly, which can lead to

difficulty in swallowing and choking, hoarseness, breath-holding and breathing problems during sleep, below average intelligence, seizure disorders which occur in 15 per cent of children with severe spina bifida and strabismus (sometimes called cross eyes, walleye or squint) a vision problem in which the eyes do not look in the same direction (Greer, 2000).

According to Mazzitelli *et al* (2002) anencephaly has been associated frequently with intrauterine growth retardation, consistently with adrenal hypoplasia and occasionally with an enlarged thymus. Babies with NTDs were poor in birth quality, higher perinatal mortality and poor prognosis (Zhou *et al* 2002).

**(D) DIAGNOSTIC TESTS FOR NTDs**

Folate status is most often assessed through measurement of folate levels in the plasma, serum or red blood cells (Brody, 1991). The first stage of folate depletion can be assessed by measuring serum folate levels which drop prior to tissue depletion and which are paralleled by a reduction in red blood cell (RBC) folate. RBC folate levels, more reflective of folate tissue status than serum folate, represent vitamin status at the time the red blood cell was synthesized. RBC folate may indicate liver folate stores. A low red blood folate may however occur with a vitamin $B_{12}$ deficiency (Bailey, 1990).

An abnormal deoxyuridine suppression test (dUST) provides biochemical evidence of folate depletion. The d UST first becomes abnormal in bone marrow cells, followed by peripheral blood lymphocytes. The d UST is based on inadequate suppression by non-radioactive deoxyuridine of the incorporation into DNA of subsequently added radioactive thymidine, with the abnormality corrected by the addition of methyl-folate (Herbert, 1990).

In controlled folate depletion (Sauberlich *et al* 1987) and supplementation studies (Tamura *et al* 1990) , dU, suppression measurements provided a functional indication of folate status. According to Tamura *et al* (1990) the test was reported to be not as discriminating as erythrocyte folate levels and more tedious to perform, which reduces the practicality of using it in population surveys.

Erythropoiesis becomes impaired in the same time sequence as changes in biochemical functions, as evidenced by abnormal erythrocyte morphology and a reduction in heamoglobin concentration. Synthesis of DNA is impaired, which results in a change in the morphology of the peripheral white blood cells, referred to as neutrophil hypersegmentation. The neutrophil tube index has been found to be useful by some researches (Colman, 1981); others consider the index unsatisfactory for the detection of earlier stages of folate depletion (Sauberlich *et al* 1987). During the time of reduced DNA synthesis, the morphology of the erythrocyte changes such that the mean cell volume (MCV) increases and the cells become oval in shape (macro ovalocytes) and the hemoglobin concentration falls below normal. This is the final stage in the progression of negative folate balance and is the stage in which clinical signs of folate depletion are quantifiable (Herbert, 1990).

One of the first clinical manifestations of folate deficiency is hypersegmentation of the neutrophils, followed by the production of megaloblastic marrow cells, macrocytic red cells and ultimately macrocytic anemia (Gibson, 1990).

Serum folate is a sensitive indicator of the folate available to replicating cells with higher turnover rates, whereas red cell folate reflects folate status over preceding weeks. A metabolic effect of folate deficiency is an elevation of homocysteine (Bakker and Brandjes, 1997).

Low maternal red cell folate (RCF) and serum folate concentrations have been strongly associated with the occurrence of NTDs (Wald *et al* 1996) and maternal RCF concentrations in particular have been shown to correlate with the occurrence of the NTDs in a concentration dependent fashion (Daly et al 1995). Kirke *et al* (1993) also stated that through determination of the maternal plasma and red cell folate concentration, NTDs could be identified.

Homocysteine, a sulphur containing amino acid and a byproduct of methionine metabolism, reflects an inadequate folate intake or abnormal folate metabolism. Homocysteine concentrations are significantly higher among women who have given birth to an offspring with NTDs (Vanderput *et al* 1995). Serum homocysteine levels are a sensitive but not a specific indicator of folate status (Manson, 2003).Prenatal screening along with ultrasonography is used as a tool for identification of NTDs (Forrester and Merz, 2000).

Recent improvements in ultrasound diagnosis of NTDs, with sensitivity and specificity approaching 100 per cent when performed by expert sonographers at major screening centers, have caused some experts to recommend the use of ultrasound instead of MSAFP in pregnancies at low risk for NTDs (Watson *et al* 1991).

The diagnosis of NTDs can be confirmed by high resolution foetal ultra sonography and amniotic fluid acetyl choline esterase estimation, both of which are capable of making correct diagnosis of NTDs in great majority of cases (Agarwal, 1999 and Burton *et al* 1985).

Noninvasive prenatal diagnosis by ultrasound and maternal serum screening offered at 16 to 20 weeks and 15 to 20 weeks of gestation respectively would identify 95 to 100 per cent of NTDs (Chodirker *et al* 2001).

According to Pilu and Hobbins (2002) the ultrasound imaging of the cranium and the identification of cranial scalloping (lemon sign) and cerebellar crowding (banana sign) in association with mild ventriculomegaly is diagnostic of an open myelomeningocele.

Alpha Feta Protein (AFP) screening can identify pregnancies of high risk for NTDs (Velie and Shaw, 1996). By monitoring MSAFP and maintaining frequent contact with physician offices and perinatal centres, 85 per cent of these terminations of NTD affected pregnancies were identified (Allen *et al* 1996).

A significant number of foetuses with open NTD are chromosomally abnormal. Sepulveda *et al* (2004) suggested although prenatal chromosome analysis should be considered in all cases on or before 24 weeks of gestation, prenatal ultrasound seems effective in identifying those foetuses with an underlying chromosomal abnormality. Prenatal screening programmes have led to early detection of NTDs and the opinion to terminate NTDs affected pregnancy (Mulinare and Erickson, 1997).

Serum folate is found to be the most frequently used method for assessing human folate status in periconceptional period and prenatal diagnosis by ultrasound at 16 to 20 weeks will possibly identify maximum number of NTDs and can prevent pregnancy wastage.

## E) FOLIC ACID IN THE PREVENTION OF NTDs AND RELATED STUDIES

Several studies and clinical trials have shown that 50 per cent or more of NTDs can be prevented if women consume a folic acid containing supplement before and during early weeks of pregnancy (MRC,1991).A multivitamin study of efficacy of periconceptional folic acid containing vitamin supplementation

in prevention of open NTDs from India revealed the recurrence of open NTD in the vitamin group was 2.92 per cent compared to 7.04 per cent in the placebo group, with a reduction by about 60 per cent (ICMR,2000).According to Green (2002) the etiology of NTDs is multi factorial and there have been many studies highlighting the role of increasing maternal folate levels in the prevention of NTDs. Strategies to achieve adequate levels of folic acid include increased intake of folate rich foods, dietary folic acid supplementation and folic acid fortification of food.

Clinical trials have clearly shown that folic acid supplementation before conception and during the first trimester (13 weeks) of pregnancy reduced both the recurrence of NTDs in women with previous NTDs affected pregnancies as well as the first-time occurrence of NTDs (Czeizel and Dudas, 1992).

Researchers have estimated that intake of 200 mcg per day could reduce the incidence of NTDs by 35 to 40 per cent while the addition of 400 mcg per day is estimated to reduce the incidence by 47 to 53 per cent (Wald *et al* 1998).

Mills *et al* (2001) reported that women of childbearing age should be advised to take a vitamin supplement containing 400 mcg per day of folic acid to obtain the maximum protection against the development of NTDs. An International randomized controlled trial showed a 72 per cent risk reduction on NTD recurrence with the use of periconceptional folic acid supplementation (Ellenbogen, 2002).

According to the reports of Lumely *et al* (2001) periconceptional folic acid supplementation reduced the prevalence of open NTDs by at least 60 per cent. He also concluded that this reduction occurred both among mothers with previously affected pregnancies and among those who had no such risk factors.

Although adequate folate consumption from food was shown to have a protective effect against NTD affected pregnancies, it should be coupled with intake of folic acid supplements (Bailey *et al* 2003). Another clinical trial demonstrated that folic acid supplementation was effective in populations with high and low prevalence of both spina bifida and anencephaly (Berry and Li, 2002).

Honein *et al* (2001) reported a 91 per cent reduction in the prevalence of NTDs since folic acid fortification of foods in 1998. The timing of folic acid supplementation for the prevention of NTDs is very critical since neural tube in the human foetus closes between $17^{th}$ - $30^{th}$ day of post - ovulation, which corresponds to day 2 to 15 post last missed periods (LMP). In a parallel study it was shown that 0.8 mg of folic acid may prevent occurrence of even the first NTD (Czeizel and Dudas, 1992).

The mechanism of action of folic acid in preventing the occurrence of NTD has been a subject of speculation. Women who have given birth to NTD child did show marginally lower serum and red cell folate levels but the difference was not statistically significant (Wald *et al* 1996). Many of these women have been found to have higher level of serum homocysteine and methionine indicating a metabolic block in the folic acid pathway (Steegers, 1994).

Maternal folate deficiency results in selective upregulation of folate receptors and heterogenous nuclear ribonucleoprotein-E1 associated with multiple aberrations in fetal tissues that include increased cell loss, architectural anomalies and premature differentiation (Antony,2007). Salvi and Damania (2005) stressed that folic acid deficiency is a major concern for all developing countries and India is no exception. They also point out a sex bias consisting of an allocation of food to women after the rest of the family. They

comment the daily intake of folic acid in rural areas of various Indian states is far lower than the daily 400mcg necessary to prevent birth defects.

Homocysteine, a sulphur amino acid and a by-product of methionine, reflects an inadequate folate intake or abnormal folate metabolism. Homocysteine concentration are significantly higher among women who have given birth to offspring with NTDs (Steeger *et al* 1994). Likewise, about one-half of the pregnancies occurring in women with hereditary homocysteinuria terminate in foetal demise (Mudd *et al* 1985 ). Thus, although circulating concentrations of folate bear little relation to risk of spontaneous abortion (Neela and Raman, 1997), high concentration of homocysteine may be a marker for increased risk to the developing foetus.

Centre for Disease Control (CDC) has opined that the avenue for primary prevention of NTD except for the fact that most human pregnancies are neither preplanned, nor such compliance on mass scale appears to be feasible. As an alternative it has been proposed to fortify breakfast cereals / bread to provide the recommended daily allowance of folic acid namely, 0.4 mg per day to all women in the reproductive age group (CDC, 1993).

Scholl and William (2000) suggested that a more abundant supply of folate to mother and foetus could, in turn support growth and gestation leading to improved infant birth weight and increased gestation duration. The effect of supplementing the diet with folic acid given preconceptionally or in the first half of pregnancy in an affluent northern country showed a slight increase in birth weight and a decrease in the incidence of preterm labour, infants with low birth weight and small for gestational age infants. The greatest effect was seen in the groups receiving folic acid preconceptionally (Rolschau *et al* 1999).

At a population level, folic acid food fortification is associated with a pronounced reduction in open NTDs (Ray, 2002). Additionally women planning a pregnancy should take 5 mg folic acid tablets daily, instead of 0.4 mg dose presently recommended (Wald *et al* 2002). Addressing periconceptional folic acid use at a preconceptional consultation improves folate status among women planning to conceive (DeWeerd *et al* 2002).

USA has experienced a decline in spina bifida and anencephaly cases after the initiation of folic acid fortification of cereal grain products nationally (Mathews *et al* 2002). Consumption of 400 mcg of folic acid per day and fortified wheat flour (2.2 mg folic acid per kg) improved folate status in a population of women of reproductive age (Hertrampf *et al* 2003).

Many observational studies of folate during pregnancy suggest a potential benefit of good folate status with an improvement in birth weight of the newborn (Tamura *et al* 1997).

In a prospective, observational study of folate from diet and supplements and serum folate, women with low folate intake, of < 240mcg/d had three times greater preterm delivery and low birth weight infants than women with folate intake, of >240mcg/d ($p<0.05$). Risk of preterm delivery without premature rupture of membranes increased three times ($p<0.05$). Odds of preterm delivery increased 1.5 per cent per unit decrease in serum folate ($p<0.05$) (Scholl *et al* 1997).

According to Pietrzik *et al* (1992) in a case control study of serum folate in women with first trimester spontaneous abortion (n=37) or habitual spontaneous abortions (n=460), compared with parous controls (116) lower concentrations of serum folate were found in habitually aborting gravidas than

in controls (-3.1ng/mL; $p<0.001$) and in the first trimester aborters than in controls (-2.3ng/mL; $p<0.05$).

A randomized controlled trial of folic acid containing multivitamin (0.8 mg/d) (n=2787) or trace minerals (n=2653) in periconceptional period revealed that the folic acid- supplemented group had more ($p<0.05$) diagnosed pregnancies (70.5 per cent folic acid compared with 67.1 per cent trace mineral) (Hook and Czeizel, 1997).

In a prospective observational study of dietary and serum folate in women with risk factors for intra uterine growth restriction (n=1200), folate from diet and supplements correlated with serum folate (r=0.25) at $18^{th}$ and $30^{th}$ weeks. Higher serum folate at $30^{th}$ week predicted higher infant birth weight (2.1g birth weight per unit serum folate; $p<0.05$) and decreased intra uterine growth restriction ($p<0.05$). The birth weight of infants born to mothers whose dietary folate intake was above the $90^{th}$ percentile was significantly higher ($p<0.05$) than that for mothers whose dietary folate intake was below the $10^{th}$ percentile (Tamura *et al* 1997, Goldenberg *et al* 1992 and Neggers *et al* 1997).

Frelut *et al* (1995) studied the red cell folate in gravidas with (n=8) and without (n=13) foetal growth retardation at 27 ± 3 weeks of gestation and found a positive bi-variate correlation between maternal RBC folate and infant birth weight ($r=0.48$, $p<0.02$).

In an observational study of plasma homocysteine and serum folate in healthy nulliparas (without issues) at delivery (37-42 week) (n=35), high maternal homocysteine correlated negatively with low infant birth weight ($r = -0.36$), ($p<0.05$). High maternal serum folate correlated positively with increased birth weight ($r=0.47$, $p<0.01$). Homocysteine and serum folate were negatively correlated ($r = -0.54$, $p<0.001$) (Malinow *et al* 1998).

In women using iron or iron and vitamin C supplements, the serum folate was lowered by 1.1mcg/L in women delivering at less than equal to 39 weeks ($p<0.01$). In another observational study of RBC folate in high- risk women (n=100), the gestational duration was reduced by 0.8 weeks in women with RBC folate less than or equal to 200mcg/L; ($p<0.025$) (Tchernia *et al* 1982).

According to Blot *et al* (1981) in a non-randomized, double blind study of iron and ascorbic acid, compared with iron, ascorbic acid and folic acid (350mcg/d) at $6^{th}$ month of gestation (n=200), it was observed that with folic acid supplementation, the duration of gestation was longer by 0.8 week, birth weight was greater by 150g, birth length was greater by 1.7cm, and placental weight by 56g ($p<0.05$ for each).

In a randomized controlled trial of periconceptional supplementation with folic acid-containing multivitamins, the folic acid group had more multiple pregnancies (3.8 per cent compared with 2.7 per cent, $p<0.05$) and girls (50.1 per cent compared with 48.1 per cent) than did the trace mineral group but the difference was not significant ($p=0.18$) Among all the births, there was a significant excess of low birth weight infants among folic acid supplemented subjects (5.8 per cent) compared with the trace mineral group (4.2 per cent ($p<0.05$). Among singleton pregnancies, rate of low birth weight was 4.3 per cent (folic acid group) and 3.5 per cent (trace mineral group) ($p=0.17$) (Czeizel *et al* 1994).

Supplementation of a pregnant mother's diet with folate was found to protect the developing embryo from birth defects in humans as well as rodent animal models. Folate supplementation not only reversed a potential nutritional deficiency but folate effectively prevented defects even when the mother's

nutritional status was normal. These findings indicate that folate is able to interact with the molecular pathways that control normal embryonic development. Supplementation studies in animals provide the experimental starting point for the identification of such folate responsive pathways. This review summarizes the progress to date in understanding the folate response in genetic models of birth defects in the mouse (Kappen, 2005).

Studies have shown that regular use of supplementary folic acid less than 300mcg increased plasma folate but supplemental folic acid over 300 mcg was required to lower total homocysteine values significantly (Alfman *et al* 2003).

Venn *et al* (2002), suggested that a regular intake of as little as 100mcg folic acid per day was sufficient to lower total homocysteine in persons at upper end of the normal range for total homocysteine. Low level fortification may be appropriate for lowering the risk of NTDs given that, when aggregated from all sources, the total intake of folic acid may be sufficiently high to adequately improve the folate status of young women.

Various studies have shown a potential benefit with supplemental folic acid of 400 mcg to prevent recurrence of NTDs and for women of reproductive age to prevent occurrence of NTDs. A good folate status lowers total homocysteine values and improve the outcome of pregnancy.