Epidemiology, prenatal management, and prevention of neural tube defects

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ABSTRACT

تتناول هذه المراجعة الوبائيات، وعوامل الخطر، وفحص ما قبل الولادة، والتشخيص، والوقاية من إمكانات وتأثير الوبائية على عيوب الأنبوب العصبي (NTDs). يبلغ متوسط معدل عيوب الأنبوب العصبي 1/1000 ولادة مع اختلاف جغرافي ملحوظ. في البلدان المتقدمة انخفض معدل حدوث NTDs خلال العقود الأخيرة. ومع ذلك، فأن المعدل لا يزال مرتفعاً في البلدان الأقل نمواً في امريكا اللاتينية، وأفريقيا، والشرق الأوسط، وآسيا، والشرق الأقصى (أكثر من 11-1 لكل 1000 ولادة). تشمل مخاطر NTDs المعترف بها كلا من سكر الحمل، والسمنة، وانخفاض المستوى الاجتماعي والاقتصادي، وارتفاع الحرارة، والتعرض لماسخات معينة خلال فترة الحمل. تقلل مكملات حمض الفوليك أثناء الحمل من معدل انتشار NTDs بنسبة معينة بلغت 50-70% واعتمد إضافة حمض الفوليك للغذاء في العديد من البلدان ليصل للنساء مع حالات الحمل غير المخطط لها والذين يواجهون الحرمان الإجتماعي. وبذلك يمكن الوقاية بشكل أسرع من أمراض NTDs خصوصاً لدى البلدان ذات الدخل المنخفض وتحصين الغذاء الرئيسي في مجتمعاتهم.

This review article discusses the epidemiology, risk factors, prenatal screening, diagnosis, prevention potentials, and epidemiologic impact of neural tube defects (NTDs). The average incidence of NTDs is 1/1000 births, with a marked geographic variation. In the developed countries, the incidence of NTDs has fallen over recent decades. However, it still remains high in the less-developed countries in Latin America, Africa, the Middle East, Asia, and the Far East (>1 to 11/1000 births). Recognized NTDs risks include maternal diabetes, obesity, lower socioeconomic status, hyperthermia, and exposure to certain teratogens during the periconceptional period. Periconceptional folic acid supplementation decreased the prevalence of NTDs by 50-70%, and an obligatory folic acid fortification of food was adopted in several countries to reach women with unplanned pregnancies and those facing social deprivation. Prevention of NTDs can be accelerated if more, especially low income countries, adopted fortification of the staple food in their communities.

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Of human birth defects, those affecting the neural tube account for 0.5-2 per 1000 pregnancies worldwide, with variations in prevalence ranging from 0.2 to 10 per 1000 in specific geographical locations.[1-3] More than 300,000 cases of neural tube defects (NTDs) are estimated to occur worldwide each year, many of these in low income countries (LIC).[4] The magnitude of the resulting economic, social, and psychological impact of NTDs, which arises from a complex combination of genetic and environmental interaction, can easily be visualized given that these inflicted children have many disabled years ahead of them.[5-7] It has been estimated that each year 2,500 infants are born with NTDs in the United States.[8] The annual medical and surgical costs for all persons with spina bifida exceeded $200 million. For each person with typical severe spina bifida, the

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estimated lifetime direct and indirect costs are more than $250,000.[9] This clearly indicates a large burden on health care even in high income countries. For LIC, and lower middle-income countries the required postnatal care of NTDs is beyond the capability of their health infrastructures and other support services. The present review article discusses the epidemiology, risk factors, prenatal screening, diagnosis, prevention potentials, and epidemiologic impact of NTDs.

Epidemiological characteristics. Several epidemiological characteristics of NTDs have been recognized. A preponderance of female fetuses (up to 3:1 for anencephaly and 2:1 for spina bifida) has been observed.[10] In the United Kingdom and Ireland, the yearly prevalence of NTDs declined from 4.5/1000 in 1980 to 1-1.5/1000 in the 1990s.[11] This decline predated the periconceptional folic acid supplementation policy initiative. Nevertheless, despite this 32% fall in prevalence, it remained higher than the overall European levels close to 1/1000 births.[11] A geographic gradient has been observed in the British Isles with higher rates of NTDs at birth in the northwest and lower rates in the southeast.[12] The estimated incidence of spina bifida in Sweden during the period 1999-2004 was 0.61/1000.[13] On the other hand, the incidence of anencephaly and spina bifida was found to be 2.11/1000 in a northern population of Russia compared with 1.08/1000 in Norway.[14] There is also a marked geographic variation in the rates of NTDs occurrence.[15,16] Africans were reported to have the lowest incidence. In Malawi,[17] the incidence of spina bifida (with or without hydrocephalus) was found to be 0.63/1000. In South Africa,[18] the prevalence of NTD was lowest in blacks (0.95/1000), highest in the white population (2.56/1000), and intermediate in those of mixed ancestry (1.05/1000). However, other studies found higher NTDs rates in Africans. A hospital-based study reported 2.6/1000 live births in Tanzania,[19] and 7/1000 deliveries in the middle belt of Nigeria.[20] Mexico and Northern China have high rates of NTDs, and in China the rates are higher in the North than in the South.[21,22] In a rural area in Northern China, the incidence of NTDs was 6/1000 before the supplementation of women in the child bearing age with folic acid when the incidence dropped to 2/1000.[21] Celts (Eastern Irish, Western Scots, and all Welsh) have an incidence recorded as high as 1:80 in the recent past.[23] Historically, the rates of NTDs have been higher among Hispanics, intermediate among non-Hispanic whites, and lower among African-Americans, a trend that follows the relative frequency of the 677C-T homozygosity in the *MTHFR* gene.[24] The reported incidence of NTDs in India ranges between 0.5 and 11/1000 births and varies within its various states, with the northern states generally reporting a higher incidence compared to the southern states.[25]

The Spanish also have a high incidence of myelomeningocele, and this has been attributed to "Arabian influences".[26] In Tunisia, the incidence of spina bifida was reported to be 1.05/1000,[27] whereas in Algeria[28] the reported incidence was high at 7.5/1000 and was suggested to reflect the high prevalence of consanguinity. Another high incidence of NTDs (5.49/1000 births) was recorded among Palestinians.[29] It is noteworthy that there was a marked fall in the frequency of anencephaly among Arabs in Kuwait, particularly Bedouins, following dietetic counseling of mothers of children born with NTDs. This mass educational dietetic program, aimed at Bedouin women, has emphasized the importance of fresh vegetables and fruits rich in folic acid.[30,31] A study from Kuwait[32] estimated the incidence of NTDs to be 1.19/1000 and was pioneering in highlighting the contribution of syndromic causes of NTDs in Arab communities, as has been later reported.[33-36] Similar rates were recorded from The Gulf Cooperation Council (GCC) countries. The incidence was found to be 1.14/1000 in the United Arab Emirates,[37] 1.507/1000 in Bahrain,[38] and 1.25/1000 in Oman.[39]

An unusually high incidence (3.7-6.96/1000) of NTDs was observed among Egyptians and has been attributed to the high coefficient of inbreeding.[40] A prospective hospital-based longitudinal study in Khartoum, Sudan reported an incidence of 2.4/1000 for NTDs.[41,42] Another prospective case-control study from Omdurman (one of the 3 cities forming Khartoum State), found a higher incidence of 3.48/1000.[43] However, most of the mothers of affected babies in the latter study, as well as their controls belonged to tribes from western Sudan who migrated recently to Omdurman. In a retrospective study of 43 patients with NTDs from Sudan, 83.7% were from Arab tribes and 16.3% had African ancestry.[44]

Several studies, addressing the epidemiology of NTDs, have been reported from different parts of Saudi Arabia.[45-58] All of these retrospective studies have been directed mainly toward estimating the incidence of the disease. A study[49] from King Fahad Hospital at Al Khobar in the Eastern province revealed an incidence, over a 10 year period, of 1.83 per 1000 live births. Magbool et al,[50] reported an incidence of 1.04 per 1000 live births, and Khaliji et al,[52] also from the Eastern Province, found a higher incidence of 1.6/1000. The incidence reported by Murshid[54] in Al Madina (1.09/1000) and

Safdar et al[56] in Jeddah (1.3/1000) is much higher than the reports from the Southwest Asir area in 1992 (0.82/1000),[51] and in 2001 (0.78/1000).[55] Both studies from Asir[51,55] were likely to be an underestimation of the true incidence since infants with anencephaly and spina bifida occulta were not referred from peripheral hospitals to the main referral hospital (Asir Central Hospital) as stated by Asindi and Al-Shehri.[55] A study by Hakami and Majeed-Saidan[57] reported an incidence of 0.44/1000 from Riyadh in the Central Region of Saudi Arabia post fortification of flour by folic acid (2001-2010). However, they excluded from analysis 8 cases of dysraphism occurring as part of specific syndromes.

In a recent study from Riyadh,[58] the incidence of NTDs during a period spanning 14 years (1996-2009) was 1.2/1000 livebirths. The prefortification of flour with folic acid incidence was 1.46/1000 compared with a postfortification incidence of 1.04/1000 (p=0.103). Syndromic, genetic (mainly inherited as autosomal recessive), and chromosomal defects were more prevalent than in other populations, and constituted around 20% of total NTDs.

Risk factors. Recognized risk factors associated with NTDs include maternal diabetes,[59] and maternal obesity (defined as body mass index of ≥30k/m^2). Maternal diabetes causes NTDs, as well as other birth defects, by disrupting expression of genes that control essential developmental processes.[60] Oxidative stress is also involved, and antioxidants including vitamin E, vitamin C, a combination of antioxidants and lipids, or N-acetylcysteine, might have a protective impact on the outcome of pregnancy.[61] On the other hand, obese women have a 1.5-3.5-fold risk for having a child with NTDs than normal-weight women.[62] It is noteworthy that certain data on risk factors for the development of NTDs are from a model system rather than humans.[61]

Maternal exposure to certain teratogens has also been documented to increase the risk for NTDs. Two anticonvulsant medications in current use, valproic acid, and carbamazepine, have been demonstrated to be risk factors in several studies. Valproic acid is associated with NTDs in 1-2% of exposed children, as well as urogenital, craniofacial, and cardiac malformations.[63,64] The risk of malformations increases with doses above 1000 mg/day. It was found to promote folic acid deficiency and downregulate the folate receptor gene (folr1) in a dose responsive manner.[65] Carbamazepine is also associated with a 1% risk of spina bifida.[66] Other teratogens include exposure to lead,[67] arsenic,[68] and tetrachloroethylene-contaminated drinking water. Tetrachloroethylene was found to leak into public water supplies from the inner vinyl lining of asbestos cement water distribution pipes.[69] The use of sulphonamide during early pregnancy was found to be associated with anencephaly,[70] and the use of trimethoprim, which disturbs folate-related metabolism, was also linked to the causation of NTDs.[71]

Hyperthermia is a potent NTD-causing teratogen in rodents, and NTDs following episodes of maternal fever or extreme sauna usage in early pregnancy have been reported.[72,73] Maternal "flu" in the first trimester has also been implicated.[74] A combination of hot-tub use, febrile illness, or sauna use was associated with a 6-fold increase in risk.[12]

Maternal exposure to pesticides, especially use of pesticides within the home and periconceptional residence within 0.25 miles of cultivated fields, is a known risk factor for the development of NTDs.[75] Maternal consumption of fumonisin-contaminated maize during early pregnancy has also been associated with increased risk of NTDs in populations that have maize as their main dietary staple.[76] Fumonisins are mycotoxins that are produced by the fungus *Fusarium verticillioides*, a common contaminant of maize (corn) worldwide.

Studies on the association between NTDs and parental occupation found increased odds ratios associated with both maternal and paternal occupations.[77-79] These included agriculture, cleaning, welding, transport, healthcare occupations (nursing, dentistry), and painting (paternal). In China, living near coal mines is a significant environmental risk factor for the development of NTDs.[79] Also, several studies have reported that NTDs occurred more frequently among children born to women of lower socioeconomic status.[80] This has been observed in North America, Europe, and several other regions.[12]

Consanguinity was a significant risk factor for the development of NTDs in the report of Murshid[54] from Al Madina, since 89% of the spina bifida parents were consanguineous compared with 67% of the controls (p<0.0005). On the other hand, the recurrence risk for siblings of children with NTDs is approximately 2-5%, representing up to a 50-fold increase over that observed in the general population.[81] As will be detailed later, inadequate periconceptional folic acid is the major and universally preventable risk factor.[79]

Diagnostic technique and prenatal management. Prenatal diagnostic techniques to detect NTDs have been growing in availability over the last 3 decades. Screening for NTDs is based on biochemical testing of maternal blood for alpha-fetoprotein (AFP) or the use of traditional 2-dimensional (2D) ultrasound. The 2 techniques are combined in several screening programs.

Fetal serum concentrations of AFP, a glycoprotein of the fetal yolk sac and liver, are 150 to 200 times that of amniotic fluid.[82] The AFP represents 90% of total serum globulins in the fetus, and leaks into the amniotic fluid and hence into maternal blood with open defective neural tube. In the early 1970's, the detection of elevated levels of maternal serum and amniotic AFP was shown to be associated with open NTDs.[83-85] In the late 1970's, Wald et al[86] found that maternal serum AFP >2.5 multiple of the median (MoM) occurred, when tested at 16-18 gestational weeks, in 88% of cases of anencephaly, 79% of cases of open spina bifida and 3% of unaffected singleton pregnancies. In singleton pregnancies, the detection rate for anencephaly is expected to be >95% and for open NTDs between 65-70% when using 2.5 MoM as the cut off level.[87] Nevertheless, closed NTDs (approximately 10% of lesions) do not increase AFP. Conversely, raised serum AFP can be associated with other abnormalities including fetal demise, conjoint twins, Turner syndrome, omphalocele, gastroschisis, exstrophy of the cloaca, and congenital nephrosis. Moreover, false positive results are encountered in multiple pregnancy and wrong dates. Also, the levels of AFP are age specific and determining precise gestational age is essential for accurate interpretation of the results. The American College of Obstetricians and Gynaecologists (ACOG)[88] suggested that screening for AFP should be offered to all pregnant women unless they plan to have amniotic fluid AFP measurement as part of the prenatal diagnosis of chromosomal abnormalities or other genetic diseases. In a meta-analysis, Wang et al[89] evaluated the studies published in English and Chinese on maternal serum alpha-fetoprotein (MSAFP) screening for NTDs and found that prenatal MSAFP screening can effectively detect 75% of NTDs during the second trimester of gestation. Following data checking by ultrasound and in case of suggestive results,[90] amniotic AFP can detect approximately 98% of all open NTDs. The concomitant assay of amniotic fluid acetylcholinesterase level will add more to the diagnostic accuracy. However, there is a 0.5% risk of post-procedure pregnancy loss in second trimester amniocentesis. In a high risk population, and in the hands of a skilled operator, ultrasonography is around 97% (95% confidence interval [CI] 0.898, 0.996) sensitive and 100% (CI 0.998, 1.0) specific in diagnosing open NTDs.[91] Hence, it has been suggested[92] that high-quality ultrasound will potentially replace routine amniotic AFP testing, although detection of NTDs may be difficult in fetal persistent posterior position and maternal obesity when using standard ultrasound. The traditional 2D ultrasound has largely superseded maternal AFP as a screening tool for NTDs.[93] Sonographic findings include the "lemon sign" due to overlapping of the frontal bones creating a lemon-shaped fetal head (**Figure 1**) and the "banana sign" (**Figure 2**) manifesting as effacement of the cisterna magna due to downward displacement of the cerebellum. The national screening policies current in 2004 for 18 European counties were reviewed by Boyd et al.[94] Fourteen of the 18 European countries had a formal national ultrasound screening policy, and 3 had no official policy, but were regularly performing an 18-to 22-week anomaly scan.

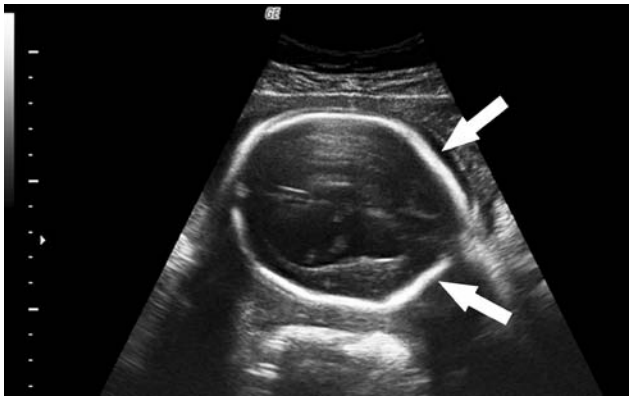

Figure 1 - The "lemon sign" shown on antenatal ultrasound scan. Posterior and inferior displacement of the intracranial contents causes the frontal bones to deflect inferiorly resulting in a lemon-shaped cranium with the frontal bones becoming flattened and inwardly scalloped (arrows).

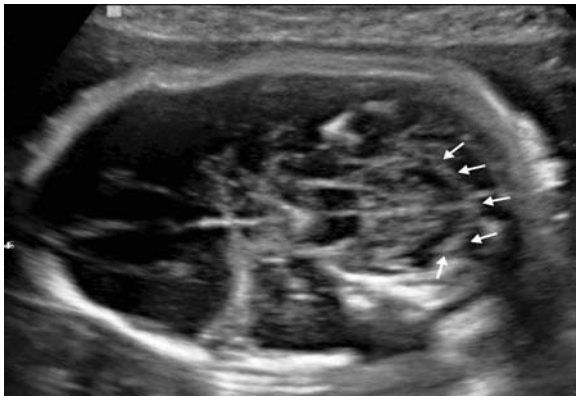

Figure 2 - Antenatal ultrasound scan showing the typical abnormality of the cerebellum found in open neural defects, which appears on sonography as a crescent with the convexity pointing posteriorly, the "banana sign" (arrows).

Associated malformations are detected in around 20% of NTDs cases, and evaluation of the whole fetus is part of the diagnostic work-up.[95] The type of NTD and extent of the lesion should be accurately ascertained. Closed lesions have a more favorable prognosis, and the majority of children who are ambulatory have lesions at or below L4 level.[93] In this respect, the American Institute of Ultrasound in Medicine has endorsed 3-dimentional (3D) ultrasound for defining the upper level of the lesion and identifying vertebral anomalies.[96]

Further prenatal management of NTDs requires parental decisions regarding fetal karyotyping and whether to continue or terminate the pregnancy. Chromosomal anomaly rates are between 0.66 and 5.56% in cases of anencephaly.[97] However, since the condition is uniformly lethal, karyotyping is unlikely to influence prenatal counseling. Structural anomalies are more common (23-37.5%) in encephalocele,[95,98] and underlying syndromic cases like the autosomal recessively inherited Meckel-Gruber syndrome[34-36] and Walker-Warburg syndrome[33] should be considered. On the other hand, chromosomal abnormalities were reported in 9.7% of cases of spina bifida, the most common being trisomy 18, trisomy 13, and triploidy.[97] When there is a somatic anomaly, the chromosomal abnormality rate approaches 25%.[99,100] The availability of termination of pregnancy for fetal anomaly varies between countries and societies, and operates within differing legal, ethical, and religious frameworks. The overall NTDs termination rates of the 12 countries of European Surveillance of Congenital Anomalies registries were found to be 88%, but these rates vary widely.[94]

Mothers who have NTDs identified in ongoing pregnancy require thorough counseling, including accurate information on recurrence risk and preparation of the parents for the loss of their child at or shortly after birth, or for his disability. Only 5% of 181 liveborn infants with anencephaly lived to one week of age in one series,[101] whereas none survived beyond 28 days in another cohort of 211 cases.[102] On the other hand, encephalocele carries a mortality rate of 60-75% during the first year.[103] Issues that need to be explored and explained in case of spinal dysraphism include mobility or ambulation, mental capacity, and continence (urinary and fecal). Generally, the disabilities in survivors with spina bifida are dictated by the location and extent of the NTD lesion, and the presence, or absence of hydrocephalus. Hydrocephalus requiring shunt placement complicates 80-90% of myelomeningocele cases,[104-108] and shunt related complications adversely affect cognitive outcome,[109] have significant morbidities, and are strongly related to long-term survival.[110]

There is no conclusive evidence regarding the most appropriate mode of delivery in cases of spina bifida. Whereas a prospective non-randomized study suggested that motor deficit increased with vaginal delivery compared to cesarean section,[111] others found that neurological deficit level was not influenced by mode of delivery.[104,112-114] Eventual outcome of NTDs varies with perinatal management and availability of support services, which vary considerably between health care systems around the world.[115]

Fetal surgery for myelomeningocele. Until recently, the neurologic deficits seen in NTDs were believed to have resulted from the aberrant neurulation that occurs during 26-28 days of gestation. However, data from other species showed that in addition to neurulative embryonic deficit, secondary damage occurs because the amniotic fluid is toxic to the exposed neural tissue.[116] Thus, prenatal repair of the skin in utero and covering the neural placode could theoretically decrease the damage to the exposed neural structures by amniotic fluid. Also in utero repair halts loss of CSF through the central canal, which results in progressive hind brain herniation through the foramen magnum (Chiari II malformation) leading to the development of hydrocephalus in 70-90% of cases of spina bifida.[117]

In 1997, researches from Vanderbilt University Medical Center in Nashville, lead by pediatric neurosurgeon Noel Tulipan and obstetrician Joseph P. Bruner,[118] reported the first endoscopic repair of myelomeningocele by intrauterine approach. Later in 1999,[119] they summarized their experience with in utero surgery for the NTDs over the previous decade. Patients who underwent intrauterine repair of NTDs between 24-30 weeks of gestation had a lower incidence of both hydrocephalus (59% versus 91%) and hindbrain herniation (38% versus 95%) compared with the control group. Further studies[120] showed that intrauterine myelomeningocele repair (IUMR) substantially reduces the incidence of shunt-dependent hydrocephalus when compared to conventional NTDs postnatal therapy even when lesion level is taken into account. Patients with lesions above L3 or who were >25 weeks of gestation do not share in this benefit. Also the motor function of patients who underwent IUMR has been reported, at least in the short term, to be better than the predicted spinal level.[121] Nevertheless, in utero fetal surgery is technically challenging, requires a multi disciplinary team approach, and is not without risks to both mother and fetus.[122] In 2003, the Management of Myelomeningocele Study was introduced as an unblinded, randomized controlled trial to evaluate outcomes from in utero versus neonatal surgery for

spina bifida. Three centers conducted this research in the US, namely, the University of California at San Francisco in San Francisco, California, The Children's Hospital of Philadelphia in Philadelphia, Pennsylvania, and Vanderbilt University Medical Center in Nashville, Tennessee.[123] The trial was stopped for efficacy of prenatal surgery after the recruitment of 183 of a planned 200 patients since it significantly improved patient outcomes. One of the measured primary outcomes of the study was a composite of fetal or neonatal death or the need for placement of a ventriculoperitoneal (VP) shunt by the age of 12 months. Another primary outcome was a composite of mental development and motor function at 30 months. Only 40% of infants receiving in utero surgery required a VP shunt at 12 months of age compared with 82% of infants who had postnatal surgery. Prenatal surgery also resulted in significant improvement at 30 months in the composite score for mental development, and motor function (p=0.007). Nevertheless, in utero surgery was associated with both fetal and maternal morbidity, namely increased risk of preterm delivery and uterine dehiscence at delivery.

Primary prevention. Folic acid supplementation. It has long been suspected that diet has a role in the causation of NTDs. The possibility that folic acid might be involved was raised in 1964.[124,125] Smithells hypothesized that undernutrition could be the underlying factor in the origin of NTDs,[126] and tested the effect of diet supplemented with a multivitamin containing 0.36 mg of folic acid in a first intervention trial.[127] Two later reports detailing the results of this intervention study were published separately for the Yorkshire region of the UK,[128] and Northern Ireland,[129] which found 91% and 83% reduction in NTDs recurrence. A randomized multicenter double-blind prevention trial was conducted by the Medical Research Council (MRC) in the UK in women at high risk of having a pregnancy with NTDs, because of a previous pregnancy.[130] The study found a 72% protective effect of folic acid use (relative risk 0.28, 95% confidence interval 0.12-0.71). This was followed by other studies supporting the importance of periconceptional folic acid supplementation.[131] A Chinese-US study[132] exploring the efficacy of 0.4 mg folic acid reported a 79% reduction in the risk of NTDs in areas with high rates of NTDs (6.5 per 1000), while this reduction was 41% in areas with low rates of NTDs (0.8 per 1000). A Cochrane review[133] of 5 trials of supplementation involving 6105 women concluded that periconceptional folate supplementation substantially reduces the incidence of NTDs (risk ratio [RR] 0.28, 95% confidence interval [CI] 0.15 to 0.52). Folic acid also had a significant protective effect for reoccurrence (RR 0.32, 95% CI 0.17 to 0.60).

Several programs were implemented globally to prevent folic acid preventable birth defects and other folate deficiency diseases. These aimed at promoting periconceptional (namely, 2-3 months before and until 3 months after conception) folic acid supplementation through daily multivitamin intake or consumption of folate (**Table 1**).[134] The target is to enable all women capable of becoming pregnant to take 0.4 mg of folic acid daily rich foods.[135,136] An evidence-based study[137] by the Genetics Committee of the Society of Obstetricians and Gynecologists of Canada recommended that women should be advised to maintain a healthy diet by using foods containing excellent to good sources of folic acid, which include fortified grains, spinach, lentils, chick peas, asparagus, broccoli, peas, Brussels sprouts, corn, and oranges (level III-A). However, it was stated that diets were unlikely to provide alone levels similar to folate-multivitamin supplementation (level III-A).

Table 1 - Recommendations regarding folic acid supplementation for the prevention of neural tube defects (NTD).*

Category	Dose of folic acid	Form of supplementation (and additional notes)
Women capable of becoming pregnant	400 µg (0.4 mg) daily	Folic acid supplement, multivitamins and/or fortified foods (particularly important before conception and through the first trimester of pregnancy) (The total daily dose should not exceed 1000 µg [1 mg], unless prescribed by a physician, because of potentially masking timely detection of vitamin B12 deficiency)
Women who have a prior NTD-affected pregnancy, a first degree relative with an NTD, or are themselves affected	4000 µg (4 mg) daily	Supplementation should start at least one month and preferable 3 months before conception.

*Recommended by The American College of Medical Genetics.[134]

It has been demonstrated that the lowest risk of having a child with NTD was related to a red blood cell folate concentration equal or higher than 906 nmol/L.[138] However, 8-12 weeks are needed to reach this level after the previously recommended 0.4 mg folic acid supplementation. Using a higher dose of 0.8 mg folic acid for 4.2 ± 3.5 weeks resulted in the necessary level of red blood cell folate concentrations.[139] Thus, the daily recommended intake of folate advised for women of childbearing age is 0.70 mg[140] or 0.80 mg.[141] Czeizel et al[131] recommended, as optimum, a daily dose of 1.0 mg of folate/folic acid for all pre-pregnant and pregnant women, via the consumption of 0.2-0.3 mg folate through diet and supplementation with 0.7-0.8 mg folic acid.

The major dietary sources of folates are fresh and frozen green leafy vegetables, liver, wheat bread, citrus fruits and juices, and legumes, such as beans (including chickpeas and broad beans). In anticipation of conception, about 3.5-fold increase in folate intake is needed every day to achieve the necessary folate consumption (estimated as 0.66-0.70 mg).[131] This would require consuming 500 g raw spinach, 900 g boiled spinach, or 900 g raw broccoli, that is, about 15 servings of broccoli each day.[131] Hence, increase in folate intake through diet alone does not seem to be practical. However, broad beans (faba beans), one of the ancient cultivated legumes, which form a staple diet along the Nile Valley, has a commendably high folate content. Fresh broad beans provide 0.423 mg (106% recommended daily allowance) of folate per 100g.[142]

Awareness of the benefits of folic acid is still not optimum even in advanced industrialized countries. In a study from USA,[143] women whose pregnancies were unintended; who were black, Hispanic, or from other racial ethic groups; who entered prenatal care after the first trimester; and who did not receive higher education (obtained a high school education or less), were less aware of folic acid. Promotion of folic acid as preventive from NTDs has been ongoing for 10 years in Ireland without a concomitant reduction in the total birth prevalence of NTDs.[144] The reason for this was due to the fact that periconceptional intake did not rise above 24% although the proportion of women who took folic acid during pregnancy increased from 14-83% from 1996 to 2002. In this study,[144] the main barrier to periconceptional uptake was attributed to the lack of pregnancy planning. In Canada,[145] the preconceptional use of supplements containing folic acid was 61% among Canadian-born mothers. Significantly lower rates were found among those who migrated from Caribbean and Latin America, Northern Africa and the Middle East, and China, and the South Pacific.

The Danish National Board of Health recommends folic acid supplementation from planned pregnancy until 3 months after conception.[146] Whereas 82% had knowledge of folic acid supplementation, only 51% followed the national recommendations. There was statistically significant correlation between higher educational level and knowledge about folic acid supplementation and its implementation in accordance with the national recommendations. A recent report[147] on preconceptional folic acid supplementation in France found that only 14.8% of women used folic acid before pregnancy. Supplementation was more frequent in women with higher educational levels and those needing medical monitoring or treatment before conception, primiparae, and French citizens.

In studies from Iraq,[148] Nigeria,[149] and Sudan,[43] none of the NTD's mothers had periconceptional folic acid supplementation. Around 88% of college students in the Western Region of Saudi Arabia were reported to be unaware of the importance of folic acid in preventing NTDs.[150] In 2006, only 18% of women in Izmir of Turkey were reported to have heard of folic acid. Their level of knowledge significantly increased following a regional health education campaign.[151] Consequently, fortification of staple foodstuffs is considered to be the only reliable and practical means of primary prevention of NTDs. Beginning in 1998, The Food and Drug Administration (FDA) required the addition of folic acid to all enriched breads, cereals, flours, corn meal, pasta products, rice and other cereal grain products sold in the United States.[152] Food fortification proved its efficacy in primary prevention of NTDs. A declining population prevalence of NTDs by 30-50% was observed following folic acid fortification in many countries.[153-163] A significant decrease (26%) in the rate of NTDs followed fortification in the US, although the percentage of folic acid preventable cases is probably higher (50-60%) due to the likelihood of incomplete ascertainment of antenatally detected NTDs cases.[162] A study on new evidence published since 1996 on the benefits of folic acid supplementation in women of childbearing age, also supported that folic acid-containing supplements reduce the risk for NTD-affected pregnancies.[164] Moreover, blood folate data from the National Health Nutrition Examination Surveys (NHNES) have documented improvements in the folate status of the US population after folate fortification was implemented.[165]

In Chile (one of several South America countries who fortify wheat flour with folic acid) NTDs decreased by 31% during the 2000-2001 biennium, corresponding to the birth of the peri conceptionally

fortified infants.[157] In Canada, where food fortification with folic acid was mandated in November 1998, the rates of NTD fell by 78% after the implementation of folic acid fortification, from an average of 4.36/1000 births during 1991-1997 to 0.96/1000 birth during 1998-2001.[166] Since 1996, voluntary fortification of food with folate has been allowed in Australia and New Zealand for the purpose of preventing NTD.[167] However, folic acid supplement use was found to be strongly correlated with socioeconomic and educational states, whereas consumption of voluntary fortified foods was not. Although a decrease of around 30% in NTDs was observed in the non-Aboriginal population, no change has been seen in the Aboriginal population. Based on these findings, the Australian and New Zealand Food Regulation Ministerial Council agreed that mandatory fortifications of food with folate should be considered as a priority.[167] Fortification became mandatory in Australia from September 2009 and required Australian millers to add folic acid to wheat flour for bread-making purposes.[168]

The issue of food fortification has been complicated since the precise dose of folate to be protective against NTDs has not been determined. A study[169] on total folate and folic acid intake from foods and dietary supplements in the United States (US) found that 53% of the US population used dietary supplements in 2003-2006. However, 29% of non-Hispanic black women had inadequate intakes, whereas 5% exceeded the Tolerable Upper Intake Level. There is neither evidence of increased risk of colorectal cancer following folic acid fortification,[170] nor that fortification caused harm in individuals.[171] In a recent meta-analysis[172] of data on 50,000 individuals in the randomized trials neither folic acid nor multivitamins showed a higher risk for overall and site-specific cancer incidence.

In Saudi Arabia, mandatory fortification of flour was adopted by the National Flour Mills Organization starting from 2001 (Year 1421 in Hijri Calendar) with the minimum requirement of 1.653 gram of folic acid for each kilogram of flour.[56] To evaluate the effect of flour fortification with folic acid, Safdar et al[56] compared, in a study from the Western Region, the incidence of NTDs at King Abdul-Aziz University Hospital (KAUH), Jeddah between the eras before flour fortification (1997-2000) and afterwards (2001-2005). They observed a decline in NTDs from 1.9/1000 live births in the former period to 0.76/1000 live births in the period following fortification (2001-2005). They attributed the relatively high incidence after fortification, compared with other countries, to the fact that fortification in Saudi Arabia was carried out only in flour, while it included all cereals and grains in the US.[56,173] Another contributing factor was that none of the women in their study were receiving folic acid before conception.[56] In the most recent study from Riyadh,[58] the prefortification of flour with folic acid incidence of NTDs was 1.46/1000 compared to postfortification incidence of 1.04/1000 (p=0.103). After excluding syndromic, genetic, and chromosomal causes from calculation of the incidence, there was a significant reduction following fortification from 1.46 to 0.81 per 1000 livebirths (p=0.0088).

In Brazil, fortification of wheat and corn flour with 150 µg/100g of folic acid became mandatory in June 2004.[174] A study in the city of Recife found no significant difference between global prevalence of NTDs in the pre- and post-fortification periods (0.72 and 0.51/1000 live births). This was partly explained by the low frequency of NTDs in the population, which did not allow temporal analysis of NTDs prevalence in the studied population to highlight the group of pregnant women who could have benefited from the fortification. Another explanation could be the consumption of inadequate amounts of fortified food by this population due to local diet habits characterized by low consumption of wheat and corn flour or by the low socioeconomic level of a considerable portion of the population. This Brazilian study highlighted the importance of considering the staple food in future programs of food fortification with folic acid. For example, millet rather than wheat, corn, or rice constitutes the staple food for a large population in India, the Sahel of Africa (Mali, Burkina Faso, Nigeria, Niger, Mali, Chad, and Sudan) and the African Horn (Ethiopia, Eritrea, and Somalia).

For socioeconomic logistics, antenatal diagnosis of NTDs is grossly lacking in low and middle-income countries.[175] A study from Nigeria[176] found that 90% of NTD cases had been delivered at home and without antenatal care. In a more recent study[177] on CNS congenital malformation, including NTDs, 30% of pregnancies were unbooked. Obstetric ultrasonography made a positive diagnosis of CNS anomaly in only 14%. In the Southern Region of Saudi Arabia, a study[55] on NTDs showed that 83% of mothers had attended antenatal care (ANC), but 70% of these presented for ANC from the twelfth week of pregnancy. An obligatory folic acid fortification of food was, therefore, adopted in several countries as a means of population-based intervention to reach women with unplanned pregnancies and those socially disadvantaged.[178] This had a major impact on NTDs in all countries where this has been reported.[179-181] Current fortification programs

are preventing around 22,000 or 9% of the estimated folic acid preventable spina bifida and anencephaly cases.[179]

In conclusion, although the incidence of NTDs has fallen over recent decades in the US and Europe, it still remains high in several less-developed countries, with NTDs incidence ranging between >1 and 11/1000 birth. For socioeconomic logistics, antenatal diagnosis of NTDs is grossly lacking in low and middle-income countries. The pace of preventing NTDs can be accelerated if more countries, especially those in the developing world, adopt fortification of the staple food in their communities (wheat, maize, rice or millet) to increase a woman's daily average consumption of folate.

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