

Review

Folic acid supplementation is not the sole factor in determining neural tube defects: The possible role of autoantibodies

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Accepted 6 June, 2012

Neural tube defects (NTDs) are severe but common congenital malformations. Neonates who suffer from NTDs may experience long-term complications throughout their lives. These NTD complications which have been reduced worldwide are primarily due to environmental and genetic factors. Multicenter NTD studies conducted in Malaysia report a prevalence ranging from 0.79 to 1.29 per 1000 live births based on NTD etiologies, such as anti-epileptic drug consumption and maternal folate levels. In addition, intervention studies concluded that daily consumption of 400 µg of folic acid effectively reduced NTD risk; however, this data has not been robustly tested on the entire population due to the inefficiency of the three interrelated folate transport mechanisms and autoantibody generation. In this review, we evaluated the studies indicating that folic acid supplementation may not be the sole factor in reducing NTD incidence and that autoantibodies may have an important role in the NTD etiological pathway.

Key words: Neural tube defect, folic acid, methylenetetrahydrofolate reductase (MTHFR), Malaysia, anti-epileptic, folate transport mechanisms, autoantibodies.

INTRODUCTION

Neural tube defects

Neural tube defects (NTDs) (OMIM identification number: 182940) are severe but common congenital malformations that affect the central nervous system. NTDs are costly diseases that are accompanied by physical disabilities which require special care from a team of experienced experts, including physiotherapists and language therapists (Detrait et al., 2005). Neonates who suffer from NTDs may experience long-term complications throughout their lifespan. As patients grow older, physical disabilities, such as bedsores, obesity and cardiovascular disease, become more challenging

problems (Rasmussen et al., 2008). One study has reported that spina bifida, together with cardiovascular diseases, have increased the number of mortalities in adults (Rendeli et al., 2004).

Moreover, obese children can be treated with type II diabetes drugs, such as metformin to combat metabolic syndromes (Yanovski et al., 2011). Psychologically, 30% of adults inflicted with NTDs are isolated from social activities and exhibit depression (Zoeller, 2007). Furthermore, neurogenic bowel syndrome, which accompanies NTDs, may later progress and worsen, resulting in constipation and fecal incontinence (Bischoff et al., 2009).

Therefore, it is imperative that this disease is prevented with the easiest and most cost-effective measures possible. Many studies suggest that supplementation with folic acid is useful in the prevention of NTDs (Berry et al., 1999; De Wals et al., 2007; EUROCAT, 2009).

This review has been written to challenge the long-standing belief that folic acid supplementation alone is

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beneficial in preventing NTDs.

EPIDEMIOLOGY AND ETIOLOGY

The estimation of total prevalence at birth (live births, stillbirths and malformed fetuses after termination of pregnancy) would be an accurate method to determine the incidence of NTDs (Czeizel, 2005). In this review, we are referring only to isolated NTD since syndromic NTD represent about 10% of NTD cases. In general, there has been a decreasing trend of NTD complications worldwide, with the exception of pregnancies in China, which may be due to environmental and genetic factors. The Eastern people, particularly people from Asian and African descent, are generally less susceptible to NTDs. The Malaysian multicenter NTD studies have reported a difference in NTD prevalence.

For example, the Alor Setar Hospital Kedah reported that the incidence of anencephaly was 1.29 per 1000 live births (Peng and Chuan, 1988), while 10.8% of the present congenital malformations at the University Hospital Kuala Lumpur were identified to result from NTDs (Hayati et al., 2008). In the Kinta district, Perak, the NTD prevalence was 0.79 per 1000 live births (Hayati et al., 2008).

Over the past decades, neighboring countries such as Thailand and Singapore have performed numerous studies on NTDs. From 1994 to 2000, the NTD incidences in Thailand have been inconsistent. The Thai medical statistic unit has reported 1.16, 0.78, 0.66, 0.91, 0.84, 0.66 and 0.42 incidences per 1000 live births over the past seven years (Sirikulchayanonta et al., 2004). Moreover, Singapore claimed to have 4% in NTD births, which accounted for 0.91 per 1000 live births from 1993-2002 (Tan et al., 2007). Pregnancies resulting from first-degree relatives are a major cause of recurrent NTD risks (1 in 30 births), but this frequency decreases with familial relationship; for example, second-degree relatives have an estimated recurrent risk of 1 in 220 births (Toriello and Hinggin, 1983). Similarly, mothers with NTD pregnancies or an NTD child's first-degree relatives are affected in 1 in 70 to 1 in 140 births (Timson, 1972). Unfortunately, the first- and second-degree relatives of the NTD pregnant mother's sister will endure NTD risks as high as 1 in 40 births. Thus, this raises the question of whether genetic factors predispose NTD occurrence. Subsequently, Arata et al. (2000) reported a 0.5 to 1% incidence of bearing an NTD child from an NTD pregnancy; however, this estimated family recurrent risk has not been validated for accuracy or reliability.

Toriello and Hinggin (1983) do not agree on this estimation, as they suggest that environmental factors could also be a major influence in determining the incidence rate of NTDs. If the family recurrence estimate is to be corrected, would a marriage between cousins result in a higher risk of bearing an NTD child?

Drug exposure is typically associated with NTD pregnancy and anti-epileptic drug consumption is known to promote mental retardation (Sener et al., 2006). The risk of syndromic NTD in the offspring of epileptic pregnant women increases after treatment of valproic acid, carbamazepine and phenytoin. Upon exposure to phenytoin, there was a marked decrease in folic acid levels compared with vitamin B₆ levels (Sener et al., 2006), which suggests that NTD development is secondary to folate deficiency *in utero* (Opladen et al., 2010). Alternatively, phenytoin could potentially impair dietary folate deconjugation in the intestine and enhance folate catabolism, which lowers plasma and cerebrovascular fluid (CSF) folate levels. Methylene tetrahydrofolate reductase (MTHFR) (EC 1.5.1.20) irreversibly catalyzes the conversion of 5,10-methylene tetrahydrofolate into 5-methyl tetrahydrofolate (Wei et al., 2012). This conversion is worsened in *MTHFR 677C>T* variants, where pregnant women are at a greater risk of vascular toxicity and teratogenic pregnancy (Sener et al., 2006), and can result in a 3.5-fold risk of major malformations occurring in the child (Kini et al., 2007).

Other studies have also revealed that maternal folate levels can determine NTD occurrence; specifically, high folate levels can lower the risk of NTD (Berry et al., 1999; Olsen and Knudsen, 2008). In addition, lower socioeconomic status often leads to suboptimal folate consumption. A review article by Au et al. (2010) reported that women who lacked a high school equivalent degree and resided in low socioeconomic neighborhoods were prone to NTD pregnancy compared with women who achieved a higher education level. Au et al. (2010) also suggested that mothers with higher education were more likely to be treated with folic acid during the periconceptional period and vice versa. Yet, this observation seems to be a biased statement, as education level should not be a major factor that influences NTD pregnancy formation. Although a higher education level will help mothers to further understand the importance of folic acid in relation to pregnancy, it does not mean that people who receive a lower level of education are incapable of primary prevention. Furthermore, medical administrations should provide mothers with more education to help understand the importance of folic acid fortification during pregnancy.

FOLIC ACID SUPPLEMENTATION

A number of intervention studies have concluded that a daily dose of 400 µg of folic acid is effective in reducing NTD risk (Vitamin Study Research Group Mrc, 1991; Czeizel and Dudás, 1992; Berry et al., 1999; De Wals et al., 2007; Olsen and Knudsen, 2008; EUROCAT, 2009). Subsequently, most westernized medical administrations, such as the United States Public Health Services, has

recommended that women of childbearing age should consume 400 µg of folic acid daily during the periconceptional period (Fohr et al., 2002) due to its ability to alleviate congenital abnormalities (from 30 to 70%) (Fohr et al., 2002; Houcher et al., 2009). Furthermore, one study has reported that NTD-affected pregnant mothers who did not receive any folic acid supplementations exhibited a 2-3% recurrence during pregnancy (Grosse et al., 2008).

Yet, folic acid supplementation has been shown to be effective in ameliorating the risk of NTD pregnancy only in childbearing-aged women who have received supplementations three months prior to pregnancy (De Wals et al., 2007; EUROCAT, 2009). Ironically, most NTD-affected pregnancies do not have folate deficiencies (Kirke et al., 1993; Cabrera et al., 2008). This can be attributed to the fact that folic acid supplementation is often difficult to be achieved before pregnancy because not all planned pregnancies are successful and because not all pregnancies can be planned. A few un-randomized hospital-based studies in three South America countries have suggested that there were 58, 50 and 50% reduction of NTD cases in Argentina, Brazil and Chile, respectively after years of post- fortification. However, the author suggested this study will only exhibit cause and effect association and are assuming that NTD do not interact with other variables (Lopez-Camelo et al., 2010). And in order to generalize the protective effect of folic acid supplementation on NTD, its effectiveness should be tested on both unadjusted and adjusted variables.

An *in vitro* study using a mouse model with *Axd* mutant and *SELH / Bc* strain indicated that there was a protective effect of folic acid supplementation on NTD occurrences (Haris and Juriloft, 2010). However, the protective effect is conferred in the presence of methionine, as methionine significantly reduced the bioavailability of supplemented folic acid. This is attributed to the fact that folic acid is converted to methionine in one-carbon metabolism pathway and therefore low levels of folic acid will result in no protective effect on NTD (Essien and Wannberg, 1993). The researchers have also suggested that maternal nutrient levels of Purina 5001, which are enriched cereal grains containing isoflavones will affect NTD formation instead of lack of folic acid supplementation (Harris and Juriloff, 2005). However, care should be taken when generalizing this result to human without conducting proper clinical trials.

Currently, folic acid supplementation has become the primary preventative approach for NTD worldwide. Yet, how do these studies relate to folate-responsive etiological pathways? How is dietary folate transported across the blood-CSF barrier for uptake by brain cells?

FOLATE TRANSPORT MECHANISMS

Folate transport mechanisms occur in three interrelated

ways, expressing high-affinity 5-methyltetrahydrofolate (MTHF) folate receptors (FOLR α and FOLR β); low-affinity MTHF reduced folate carriers (RFC), and passively diffuse MTHF at exceptionally high extracellular MTHF concentrations (Opladen et al., 2010). The FOLR α and FOLR β are glycosylphosphatidylinositol-anchored proteins, where FOLR α is expressed on the plasma membrane of the placental microvilli, while FOLR β is significantly expressed in placental and hematopoietic cells (Steinfeld et al., 2009). The FOLR α has a higher binding affinity (in the physiological nanomolar range) than the FOLR β for folic acid (Steinfeld et al., 2009) and are critical for food folate assimilation, distribution and retention in the cells (Selhub, 1994). Both receptors are energy-dependent, and mediate unidirectional plasma MTHF endocytosis against FOLR concentration gradient into the CSF compartment (Holm et al., 1991). With this FOLR-mediated endocytosis, the CSF has a 1.5- to 2-fold higher folate level compared with the bloodstream (Spector and Lorenzo, 1975), which is essential for normal brain development. In contrast, many studies have reported that this endocytic process has a lower FOLR α and FOLR β capacity, as these receptors need to return to the cell surface to function (Nutt et al., 2010). The transmembrane RFC is expressed on the placental microvilli and basolateral plasma membrane. It is a neutral anion exchanger that bidirectionally transports MTHF and antifolate with a higher affinity than oxidized folate through carrier-mediated mechanisms to various cell types, that could ensure optimal level of MTHF and avoiding abnormal neural tube closure (Nutt et al., 2010; Solanky et al., 2010).

Proton-coupled folate transporter (PCFT) present in the intestine is preeminently expressed on the apical brush-border membrane of the duodenum and proximal jejunum (Zhao et al., 2009) for intestinal folate absorption. FOLR-mediated endocytosis is regulated by the brain PCFT in acidified endosomes (pH 6.0 to 6.5) (Yang et al., 2007), where a trans-endosomal pH gradient is a requirement for folate dissociation from its receptor. This is further exocytosed on the apical side of the CSF into the central nervous system (Zhao et al., 2009). As a result of an autosomal recessive disorder in the *PCFT* gene, hereditary folate malabsorption may reduce the export of intestinal folate into the central nervous system, which is essential for growth (Zhao et al., 2009).

Despite how efficient the three interrelated folate transport mechanisms are, NTDs represent a multifactorial disease, and recent studies suggest that autoantibodies may play an important etiological role.

AUTOANTIBODIES ON THE FOLATE RECEPTOR

There are two types of autoantibodies or immunoglobulins in normal human serum; these are the antigen-driven immunoglobulins and natural immunoglobulins (Bille et al., 2010). The antigen-driven immunoglobulins,

such as immunoglobulin E, interact with a primary epitope in a high-affinity manner, whereas the natural immunoglobulins have a multitude of polyactivity toward both self and foreign antigens (Bille et al., 2010). To date, the causes of autoantibody generation are still ambiguous. A recent study (Ramaekers et al., 2008) speculates that autoantibodies can be stimulated by cow milk, which may block the human folate receptor. This hypothesis is consistent with that of Berrocal-Zaragoza et al. (2009), who similarly suggest that autoantibodies block the human folate receptor and result from milk consumption. One possible explanation may be the biological plausibility of a high structural homology of the folate receptor protein between humans and bovines in which direct bovine antigenic autoantibodies bind to the human folate receptor (Berrocal-Zaragoza et al., 2009). In addition, Ramaekers et al. (2007) reported that NTD pregnant mothers generate autoantibodies, such as the human immunoglobulin M (IgM) and immunoglobulin G (IgG). These circulating serum autoantibodies bind to FOLR α that are expressed on the choroid plexus epithelial cell membrane and acts as a competitive inhibitor of MTHF, thus preventing MTHF flow into the spinal fluid compartment. Blockage of these autoantibodies from binding to the placental membrane has reduced the amount of maternal MTHF that penetrates into the cerebrum of the embryo, which increases the risk of NTD during embryonic development.

A recent study investigated the association between autoantibodies and the FOLR α in a Norwegian population (Boyles et al., 2011). They revealed a significantly enhanced NTD risk of increasing autoantibody binding to the FOLR α , which is consistent with another previous study (Rothenberg et al., 2004). Despite the small sample size, a reliable study was conducted by Boyles et al. (2011), where they collected blood samples from the pregnant mother at a critical time point (17 weeks of gestation) during neural tube closure. In this study, Boyles et al. (2011) were capable of detecting self-reactive antibodies that were previously undetectable and demonstrated that these antibodies significantly increased during pregnancy. Cabrera et al. (2008) also supported these claims with the findings that NTD-affected pregnancies have high concentrations of IgM and IgG in the mid-gestational serum compared with normal pregnancy which suggests that these immunoglobulins might be NTD risk factors.

Furthermore, Ramaekers et al. (2008) speculated that maternal autoantibodies play an indirect role in NTD embryo development and postulate that these autoantibodies hinder folate transportation to the blastocyst during the maternal-embryonic trophoblast interface. During a normal pregnancy, the chorionic villi cytotrophoblasts form barriers to the IgG and IgM that prevent these immunoglobulins from reaching the embryo during neural tube closure (Cabrera et al., 2008). Yet, it is possible that a specific interaction between the

autoantibodies and placenta-specified epitopes could initiate the maternal-embryonic trophoblast interface immune responses, which prevent folate from binding to the placental folate receptor, resulting in an NTD pregnancy.

CONCLUSION AND FUTURE PERSPECTIVES

Although several studies have shown that folic acid supplementation is protective of NTD occurrences, there are few studies that confirm its role in preventing NTD pregnancy. This may be due to various enzymes that cooperatively function in the MTHFR metabolic pathway. Moreover, autoantibodies also hinder MTHF uptake during normal embryo development. Hence, we conclude that folic acid supplementation may not be the sole factor in reducing the incidence of a mother having an NTD baby and that autoantibodies may also contribute to the etiology. A combination of vitamin B₆, vitamin B₁₂, folic acid supplementation with enzyme cofactors may be a more appropriate alternative treatment than folic acid supplementation alone for women of childbearing age. Thus, further studies are necessary to test this hypothesis.

ACKNOWLEDGEMENTS

We acknowledge the financial support from a Research University grant (1001/PPSP/815073). The first author was supported by MyBrain 15 (MyPhD) scholarship.

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