The Impact of Maternal Micronutrient Levels on Risk of Offspring Neural Tube Defects in Egypt

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Abstract: Neural tube defects (NTD) are important causes of infant mortality. Poor nutrition was essential factor for central nervous system deformation. Mothers gave NTD offspring had abnormal serum levels of micronutrients. The present research was designed to study the effect of maternal micronutrient levels and oxidative stress on the incidence of NTD in offspring. The study included forty mothers; twenty of them of 30.9±7.28 years had conceived fetuses with NTD were considered as cases; and twenty mothers of 28.2 ± 7.82 years with healthy neonates. We determined se rum vitamin B_{12} and folic acid by using radioimmunoassays. Also, serum zinc was assessed using atomic absorption spectrophotometry. While serum copper and iron were measured colorimetrically and serum ceruloplasmin was analyzed by radialimmunodiffusion. Cases showed significantly lower levels of folic acid, vitamin B_{12} and zinc ($P \leq$ 0.0005, 0.01, 0.01 respectively) than that of the control. Concentrations of copper, ceruloplasmin, and iron were markedly increased in cases as compared to controls ($P \leq 0.01$, 0.01, and 0.05 respectively). In conclusion, the current study clearly indicated the etiology of NTD cannot be explained with one strict etiologic mechanism, on the contrary, an interaction among maternal nutritional factors and oxidative stress would explain these anomalies. Vitamin B_{12}, folic acid, and zinc supplementations should be considered for further decrease in the occurrence of NTD. Preventing excess iron during pregnancy favors better pregnancy outcomes. Report and Opinion. 2009;1(6):45-50]. (ISSN: 1553-9873).

Key words: Vitamin B_{12}, folic acid, zinc, copper, iron, ceruloplasmin, oxidative stress, neural tube defects.

1. Introduction:

Neural tube defects are important causes of infant mortality and childhood morbidity. The development and closure of the neural tube are normally completed within 28 days after conception, before many women are aware that they are pregnant. It's generally accepted that neutral tube defects are caused by the failure of the neural tube to close, although it has also been suggested that a closed tube may reopen (Campbell and Sohal, 1990). Spina bifida and an encephaly are the most common forms of neural tube defects (Birnbacher *et al*, 2002). Over years, epidemiologic studies have been instrumental in elucidating the causes of NTD in humans. Overall, these studies have suggested that environmental and genetic factors have a joint role in the causation of NTD. Nutrition may interact with a person's genetic make up. Furthermore, the effect of poor nutrition may be magnified in the developing embryo, where active cell proliferation occurs at a time when access of nutrients is limited (Botto *et al*, 1999). Therefore, poor nutrition was suggested to be an important risk factor for central nervous system malformations and women gave birth to babies with NTD had low serum levels of micronutrients (Jiang, 1991). Several groups have reported lower vitamin B concentrations and folate in particular in mothers of infant with NTD (Steegers-Theunissen, 1995). Therefore, folic acid supplementation is recommended for women who are planning to become pregnant as well as pregnant women (Lee et al, 2005 and Michels et al, 2008) because folate nutriture at periconception is critical for preventing NTD (Ryan-Harshman and Aldoori, 2008).Vitamin B_{12} is a part of coenzymes present in all body cells and is essential in the synthesis of DNA. It works closely with folic acid.

Minerals have also been implicated in the pathogenesis of NTD. Lower maternal serum and hair concentrations of zinc were associated with many anomalies (Srinivas *et al*, 2001). Iron deficiency is the most common nutrient deficiency in pregnant women and has been linked to negative impacts on the fetus,
women with NTD affected pregnancies had minimal or no ferritin (Felkner *et al*., 2005). Iron requirements during pregnancy are not easily fulfilled through diet alone, thus, it is recommended that pregnant women take iron supplements (Groenen *et al*., 2004). Copper deficiency during pregnancy is associated with multiple developmental defects that can affect the central nervous system and induce teratogenesis in the offspring (Keen *et al*., 2003 and Beckers

Trapp *et al*., 2006).

Evidence about the preventive effects of nutrients other than folate on the occurrence of NTD is scarce. Also, many studies have examined the impact of single nutrients on NTD risk and the role of nutrients in combination has received much less attention. Therefore, the aim of the current study was to investigate possible nutritive risk factors for the occurrence of NTD and some of their inter-correlations in women with normal and NTD infants at delivery.

2. Subjects and Methods:

(1) Subjects

The present study involved 40 mothers randomly chosen from the Neonatology unit of Maternity Hospital, Ain Shames University; twenty of them had neonates with neural tube defects. The mean age of them was 30.9 ± 7.28 year, their mean gestational age was 37.1 ± 2.92 weeks. The other twenty mothers had healthy neonates. Their mean age was 28.24 ± 7.82 year and their mean gestational age was 38.3 ± 1.12 weeks. Mothers never administered any supplement, during gestation. All studied mothers were subjected to full history with laying stress on their obstetric history including grandparity, gestational age, consanguinity and similar condition in the family. Also, attention was focused on their possible exposures to physical agents such as trauma, hyperthermia, radiation, infections and metabolic disarrangement such as diabetes mellitus or drugs. In addition, a dietary questionnaire was taken.

(2) Blood sampling and analytical methods

Venous blood samples were withdrawn immediately after delivery from fasting mothers; serum was separated by centrifugation and stored at -20°C for analysis. Simultaneous quantitative determinations of vitamin B_{12} and folate were done by radioimmunoassays (RIAs). ICN pharmaceticals simul TRAC, SNB radioassay kit, was used (Kubasik *et al*., 1979). Vitamin B_{12} and folate tracers, binders and standards were supplied in combined form. The pteriyoglutamic acid form of folate is used as both standard and tracer in an incubation mixture at pH 9.5. The two tracers [^{57}Cr] for vitamin B_{12}, and [^{125}I] for folate produce energies at levels which were easily separated by two-channel gamma counter. Serum zinc measurement was done by flame atomic absorption spectrophotmetry (Smith *et al*., 1979). Serum copper and iron levels were determined colorimetrically (Abe *et al*., 1989 and Williams *et al*., 1977; respectively) using commercially available kits. Sentinel CH. Italy and Elitech diagnostic, respectively. Moreover, ceruloplasmin concentrations were estimated by using radial immunodiffusion (RID) plates obtained from Biocientifica S.A. Argenting according to Verbruggen (1975).

(3) Statistical methods

All statistical analysis is performed with the use of SPSS computer program. Mean ± SD differences between cases (mothers had NTD neonates) and control (mothers had healthy neonates) were analyzed with a student's two tailed t. test. A probability value $P < 0.05$ was considered to be statistically significant, while that corresponding to $P < 0.01$ was considered to be highly significant.

3. Results

Table (1) indicates mean values of serum folic acid and vitamin B_{12} in mothers of healthy and NTD offspring. It shows that serum vitamin B_{12} concentrations in cases were significantly decreased than those in control ($P < 0.01$) and the percentage of decrease was 35.9. However, folic acid levels were significantly reduced in cases by 31.4%. Serum mean values of zinc, copper, ceruloplasmin and iron are represented in table (2). Mothers with NTD infants exhibited an extreme decrease in zinc level ($P < 0.01$) by 36.3% indicating a significant association between zinc level and presence of NTD. By contrast, the concentrations of copper, ceruloplasmin and iron were markedly increased ($P < 0.01$, 0.01 and 0.05 respectively). The percentages of increase were 31.8, 31.9 and 18.2 respectively. It is obvious from the percentages that those of copper and ceruloplasmin were nearly the same. Results indicated that there was a strong association between copper, ceruloplasmin and iron levels and incidence of NTD.

Table 1. Serum Folic Acid and Vitamin B_{12} Concentrations (Means ± S.D.) in Mothers of Healthy and NTD Offspring

Groups	Folic acid (ng/ml)	Vitamin B_{12} (pg/ml)
Mothers of healthy offspring (n=20)	10.5 ± 0.668	1070 ± 387
Mothers of NTD offspring (n = 20)	7.2 ± 0.346	685.5 ± 103**

** High significant difference at $P < 0.01$.
***Very high significant difference at $P<0.0005$.

Table 2. Comparison of Serum Trace Elements and Ceruloplasmin Levels (Means±S.D.) Between Mothers of Healthy and NTD Offspring

Groups	Zinc µg/dL	Copper µg/dL	Ceruloplasmin mg/dL	Iron µg/dL
Mothers of healthy offspring (n=20)	74.1 ± 4.1	95.6 ± 31.6	42.6 ± 13.8	84.4 ± 16.7
Mothers of NTD in offspring (n = 20)	47.2± 9.5**	126**±26.5	56.2**±17.4	99.8*± 27.8

* Significant difference at $P < 0.05$.
** High significant difference at $P < 0.01$.

4. Discussion

Neural tube defects are the most common congenital anomalies of the central nervous system resulting from failure of the neural tube to close in the first trimester. They result from multiple intrinsic and extrinsic factors (Botto *et al*., 1999). NTD are considered as important causes of infant mortality and childhood morbidity. Women of childbearing age are currently advised to consume 0.4 mg of folic acid per day to prevent NTD (Williams, 1995). In addition to folate, other nutrients such as vitamin B_{12}, zinc, and methionine may also influence NTD risk. Accordingly, this study was designed to evaluate micronutrient status in women with normal and NTD offspring at delivery.

Fetal growth and development are characterized by widespread cell division. Adequate folate is critical because of its role in DNA and RNA synthesis. Our data revealed that serum folic acid levels showed statistically significant reduction in mothers had NTD neonates as compared to those with healthy ones. This finding has been pointed out in different studies (Steegers - Theunissen, 1995 and Boyles *et al.,* 2008). But Weekes *et al*., (1992) and Villarreal *et al*., (2001) have pointed out that folic acid exhibited no change in NTD mothers. This discrepancy may be resulted from sampling variations. However, abnormal folate metabolism affects gene expression resulting NTD (Dunlevyl *et al.,* 2007 and Boyles *et al.,* 2008). Moreover, folate deficiency can lead to hyperhomocysteinemia (Van-wersh *et al*., 2002), which is a known risk factor for NTD (Vollset *et al*., 2000). Besides, an approximate doubling of the risk of spina bifida has been associated with mutations in the gene for methylenetetrahydrofolate reductase which can be prevented by folic acid supplementation (Michels *et al.,* 2008).

Maternal vitamin B_{12} levels were significantly lower in cases as compared to control mothers. Our results are in accordance with those of Kirke *et al*., (1993) and Wald *et al*., (1996). Many clinical studies have been generated cobalamin metabolism in an effort to identify the biochemical and the genetic bases of neural tube defects. Cobalamin is a part of coenzymes present in all body cells and is essential in the synthesis of DNA. Maternal cobalamin shortage resulting in a mild hyperhomocysteinemia that was also associated with spina bifida (Steegers-Theunissen *et al*., 1991 and Mills *et al*., 1996). Where the conversion of homocysteine to methionine is a critical step for neural tube closure. This methionine synthase pathway is dependent on folate coenzyme and vitamin B_{12} dependent enzyme (Gerhard and Duell, 1999). As for genes, initial findings indicate that the genetic contribution to NTD is likely to be complex. An approximate doubling of the risk of spina bifida has been associated with homozygosity for a common mutation in the gene for methylenetetrahydrofolate reductase (MTHFR), the C677T allelic variant (Rozen, 1997) which encodes an enzyme with reduced activity. Reduced maternal blood levels of vitamin B_{12} increased this mutation (Christensen *et al*., 1999). However, the reduced levels of vitamin B_{12} may be due to low dietary intake, as it is present only in animal sources, and/or its malabsorption in cases. Consequently, increasing maternal vitamin B_{12} is of benefits to reduce NTD (Kirke *et al*, 1993).

Pronounced zinc depletion has been reported in women with NTD fetuses (Jiang, 1991 and Cengiz *et al*., 2004). In the present study, we observed that zinc changes are consistent with those of the previous authors. Zinc has the critical role in DNA synthesis and regulation of gene expression by binding to DNA and influencing the transcription of specific genes. Also, it is a major regulatory ion in the metabolism of cells especially in tissues with a high cellular turnover (Black, 1998). Moreover, zinc affects folate status as a cofactor for methionine synthase and □-glutamyl hydrolase (Groenen *et al*, 2004). Therefore, it is conceivable that zinc decreased levels may contribute in the risk of NTD.

It is revealed from our data that cases had markedly higher serum copper concentrations as compared to control mothers. Our results are

consistent with previous reports (Cengiz *et al*., 2004). Besides, they demonstrated a negative correlation existed between serum zinc and copper levels in mothers with NTD enfants. There is increasing evidence that significant interactions occur between and among essential nutrients at levels that are not considered to be toxic. High dietary zinc caused a pronounced copper depletion in rats even when adequate copper was included in the diets (Sundaresan *et al*., 1996). Consequently, the decreased levels of zinc in our cases were responsible for enhancing intestinal copper absorption with concomitant increase in its serum levels. About 90% of plasma copper is found in ceruloplasmin in normal mammals and highly significant correlations have been demonstrated between ceruloplasmin level and copper level. Our finding that high serum ceruloplasmin level was accompanied to increased serum copper levels in cases is in agreement with the previous authors (Davis and Mertz, 1987). Moreover, serum ceruloplasmin concentration was strongly influenced by an interaction between zinc and copper. As dietary zinc increased with adequate copper levels, serum ceruloplasmin concentrations decreased (Sundaresan *et al*, 1996).

There is abundent evidence showing that maternal oxidative stress may interfere with neural tube closure (Marzullo & Fraser, 2005, Zhaow-Mosley *et al*., 2006 and Benko & Brodland, 2007), via inhibiting the expression of Pax-3 gene that is essential for such closure (Chang *et al*., 2003 and Loeken, 2006). Therefore, the increase in copper and ceruloplasmin levels in our study may reflect a compensatory defense mechanism of the body against oxidative stress (Johnson *et al*., 1992).

Our data revealed that the iron level was significantly higher in cases than in control. Although iron is an essential nutrient for growth and development, high tissue iron concentrations have been associated with the development and progression of several pathological conditions (Fraga & Oteiza, 2002). Excess iron is a highly effective promoter of lipid peroxidation and free radical generation which result in molecular, cellular, and tissue damages (Kontoghiorghes *et al*., 2000). However, the increase in iron level may be explained by the competitive interactions between iron and zinc (Fairweather-Tait & Thouthon, 1989). It's noticed that the increase in iron concentration was followed by a rise in ceruloplasmin concentration, as ceruloplasmin is an important extracellular antioxidant that function coordinately with transferrin by its ferroxidase activity to promote iron binding and prevention of metal-catalyzed free radical reactions (Johnson *et al*., 1992).

From the foregoing results, it could be concluded that deficiencies of vitamin B_{12}, folic acid and zinc,
in addition to loss of oxidant/antioxidant balance may participate in the higher incidence of NTD pregnancies. The risk of excess supplementation by iron and its potential adverse effects should be considered. Factors other than dietary ones would explain these anomalies.

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