Serum Copper and Zinc Levels in Mothers and Cord Blood of their Newborn Infants with Neural Tube Defects: *A Case-control Study*

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Manuscript received: February 28, 2008; Initial review completed: May 14, 2008; Revision accepted: October 3, 2008.

Objectives: To measure the cord blood and maternal serum levels of folic acid, vitamin B_{12}, zinc, copper, selenium and lead in infants born with neural tube defect (NTD), and to examine a possible relationship between the nutriture of these micronutrients and occurrence of neural tube defect.

Design: Case-control study.

Methods: Maternal serum and cord blood samples were obtained at delivery from 70 healthy mothers and 74 mothers who had a newborn with NTD.

Results: The mean (± SD) maternal serum zinc level in the NTD group was significantly lower than that of the control group (835.6 µg/L ±333.8 µg/L *vs.* 1035.7 µg/L ± 299.8 µg/L, *P*=0.004, respectively). The mean maternal and cord serum copper levels in the NTD group were significantly higher when compared to the control group (2831.1 µg/L ± 1017 µg/L *vs.* 2402 µg/L ± 744.2 µg/L; *P*=0.03; and 789.8 µg/L *vs* 517.2 µg/L, *P*<0.001, respectively). There was a negative correlation between the cord levels of folic acid and copper in the NTD group with the respective maternal serum levels (*r*=-0.289; *P*=0.018).

Conclusions: High maternal serum levels of copper and lower level of zinc during pregnancy associated with NTD in newborn.

Keywords: *Copper, Folic acid, Micronutrients, Neural tube defects, Zinc.*

Published online 2009 April 1. PII: S097475590800132-1

Neural tube defects (NTD) are one of the most common forms of human congenital malformations(1). NTD occur in 1-6.5 per 1,000 births, with marked geographic and ethnic variations(2,3). Comparatively, the incidence of NTD at 9.5/1,000 births in our region is somewhat high(4). The etiology of NTD is multifactorial involving nutritional deficiencies, genetic predisposition and environmental factors(5). Nutritional factors appear to be an important contributor to the etiology of NTD. Although it is known that folic acid deficiency has a definite place in the etiology, supplementation and fortification with folic acid have not eliminated all NTD.

The role of vitamin B_{12} and other trace elements such as copper (Cu) and zinc (Zn) is still uncertain and there is a limited amount of published literature on this topic. Cu is an important component of proteins essential for neural function. The role of copper in the development of NTD is plausible because of its participation in oxidative stress(6-9). Also, zinc is an essential nutrient for normal cellular growth and differentiation in all species and may be especially necessary for closure of the human neural tube. We investigated the cord blood and maternal serum levels of folic acid, vitamin B_{12}, Zn, Cu, selenium (Se) and lead (Pb) in infants born with NTD to examine a possible relationship between the nutriture of these micronutrients and occurrence of NTD.

Accompanying Editorial: Pages 665-667.

METHODS

The study was carried out at Sanliurfa Maternity Hospital and the Maternity and Obstetrics Clinic of Harran University Medical School. Seventy-four newborns (gestational age ≥20 weeks) with NTD (excluding spina bifida occulta) formed the cases and seventy healthy infants born in the same period and from the similar socioeconomic group were used as controls. Babies having possibility of infection and a positive C-Reactive Protein (CRP) level were excluded from the study. Informed consent was obtained from the families and approval was obtained from the local Ethics Committee. A record was taken of the age of the mother, consanguinity of parents. medical history, the number of pregnancies, abortions, history of previous NTD, use of medication, smoking, proximity to radiation during pregnancy, and results of intrauterine ultrasono-graphy (USG). The gestation period, birthweight and length were recorded.

Umbilical cord blood samples and maternal venous samples were collected within 30 minutes after the birth and serum was separated. After immediate centrifugation, serum was transferred to deionized tubes, stored and frozen at –80ºC until determination of Se, Pb, Zn and Cu concentrations was done.

Serum Se and Pb concentrations were determined by a SpectrAA 250 Plus Zeeman Atomic Absorption A spectrometer with a graphite furnace GTA-97 (Varian, Australia) with deuterium background correction using standard method(10,11). Serum Zn and Cu levels were determined by Atomic Absorption Spectrometer (Varian Spectr AA 250 Plus, Australia). Serum samples were diluted (1:5) with ultra deionized water. Cu and Zn values were expressed in mg/L. Serum iron concentration was determined by colorimetric method with a commercial kit (Boehringer Mannheim, Germany) using an automatic analyzer (Hitachi 911, Boehringer Mannheim, Germany). Serum folic acid and vitamin B_{12} levels were determined by commercial kits (Roche Diagnostic, Germany) with an automatic hormone analyzer (Elecsys 2010, Germany). CRP levels were determined by an immunoturbidimetric assay.

Statistical analysis of the data was performed with SPSS Version 11.0. Differences in demographic data between the study group and control group were compared by Chi-square test (or Fisher's exact test, if the predicted number of subjects in any category was less than five). Median levels of trace elements were compared between the study group and the control group women and their newborns with the use of Student's *t*-test and Mann-Whitney U test according to distribution normality. Presence of NTD was considered as the dependent factor in multivariate logistics regression analysis. Independent factors included in the analysis as dichotomous variables were parity, history of a previous NTD, history of abortions, multivitamin use, maternal smoking, and infection in pregnancy. The correlations within the groups between folic acid, vitamin B_{12} and trace elements were assesed by Pearson's rank correlations (r_p). Statistical signi-ficance was defined for P values of less than 0.05.

RESULTS

The parental characteristics of patients and control subjects are shown in ***Table* I**. Of the infants with NTD, 44 (59.5%) had anencephaly, 3 (4%) had encephalocele, and 27 (36.5%) were diagnosed with spina bifida. A prenatal diagnosis had been made on 43 (58.1%) of the babies with NTD.

The mean (± SD) Zn level of the mothers who gave birth to infants with NTDs was significantly lower when compared to the control group (835.6 ±333.8 *vs.* 1035.7±299.8; *P*=0.004). However, the mean maternal and cord serum Cu levels in the NTD group were significantly higher when compared to the control group (2831.1±1017 *vs.* 2402±744.2, *P*=0.03 and 789.8 *vs.* 517.2, *P*<0.001, respectively). In comparison with the control group, the ratio of Cu/Zn in the mothers of the babies with NTD was found to be significantly higher (3.8±2.1 *vs.* 2.6±1.2, *P*=0.001) (***Table* II**). A statistically significant nega-tive relationship was determined between the levels of Cu and folic acid in the cord blood of the new-borns with NTDs and their mothers (*r*=–0.401; *P*=0.001 and *r*= –0.289; *P*=0.018, respectively) (***Fig.* 1** and ***Fig.* 2**).

TABLE I BASELINE CHARACTERISTICS OF THE STUDY AND CONTROL GROUPS

	Case (*N*=74)	Control (*N*=70)	OR (95%CI)	*P* value
Age (years)*	28.8±7.3 (17-49)	25.8±5.8 (17-39)		0.03
Maternal age groups				
<20	6	8	0.7 (0.2-2.3)	0.50
20-35	53	58	0.5 (0.2-1.3)	0.10
>35	15	4	4.1 (1.3-13.3)	0.01
Newborn gender (M/F)	34/40	26/44	1.4 (0.7-3.0)	0.28
Gestational age (weeks)*	34.2±6.2 (20-41)	39.3±0.8 (37-41)		<0.001
Birth order*	3.9±2.8 (1-13)	3.3±2.3 (1-10)		0.22
History of previous NTD, *n* (%)	5 (6.8)	1 (1.4)	5 (0.6-43.9)	0.11#
History of abortion, *n* (%)	14 (18.9)	8 (11.4)	1.8 (0.7-5.1)	0.21
Multivitamin use, *n* (%)	6 (8.1)	2 (2.8)	3 (0.5-22.4)	0.16
Maternal smoking, *n* (%)	8(10.8)	14(20)	0.5 (0.2-1.4)	0.12
Infection in pregnancy, *n* (%)	4 (5.4)	3(4.3)	1.3 (0.3-5.9)	0.53#

**The data are given as mean±SD (range); # Fisher's exact test.*

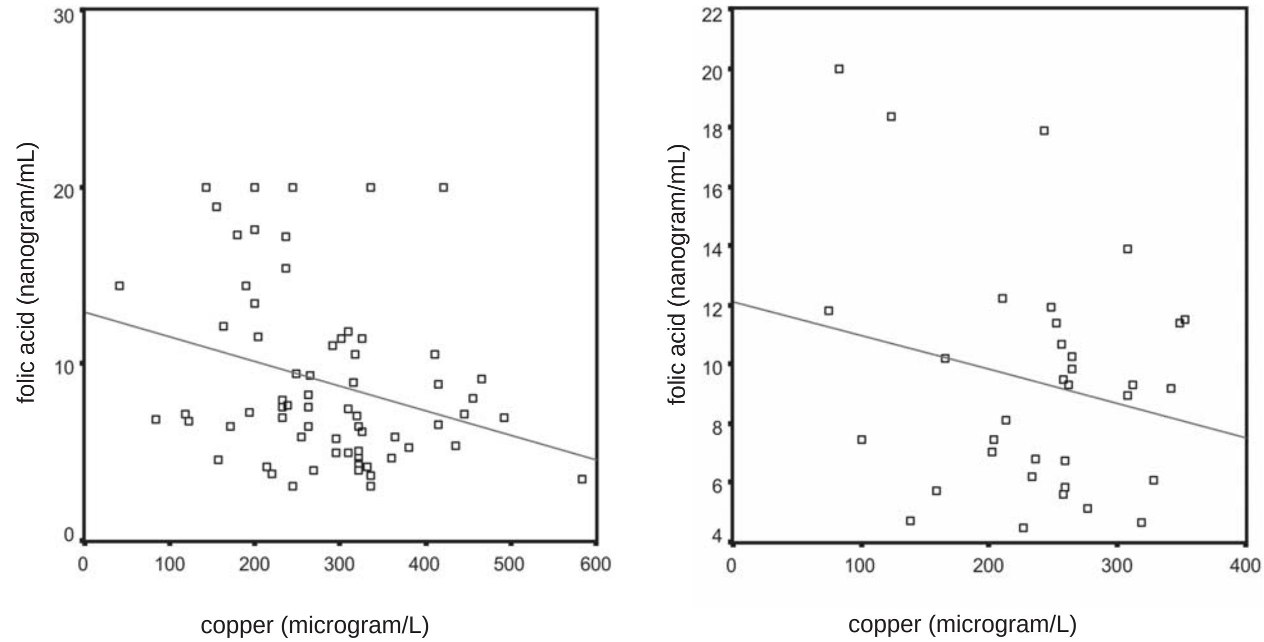
TABLE II SERUM LEVELS OF FOLIC ACID, B_{12} AND TRACE ELEMENTS IN MOTHERS AND NEWBORNS (Mean±SD)

	Mothers			Newborns		
Element	NTD (*N*=74)	Control (*N*=70)	*P* value	NTD (*N*=74)	Control (*N*=70)	*P* value
Folic acid (ng/mL)	9.0±4.9	9.4±3.9	0.66	18.0±7.0	15.9±3.3	0.10
Vitamin B_{12} (pg/mL)	217.8±125.5	261.6±154.3	0.12	309.1±182.4	407.3±391.7	0.10
Copper (µg/L)	2831.1±1017.0	2402±744.2	0.03	789.8	517.2	<0.001
Zinc (µg/L)	835.6±333.8	1035.7±299.8	0.004	1390.7±504.4	1294.1±345.7	0.33
Selenium (µg/L)	46.8±26.4	47.6±20.6	0.87	42.2±21.9	39.9±20.0	0.60
Lead (µg/L)	155.0±150.4	125.4±126.5	0.35	182.2±177.8	164.5±161.0	0.63
Iron (µg/dL)	63.1±15.9	66.2±19.0	0.61	82.3±19.0	87.0±20.7	0.50
Copper/Zinc	3.8±2.1	2.6±1.2	0.001	1.0±1.1	0.7±1.3	0.32

DISCUSSION

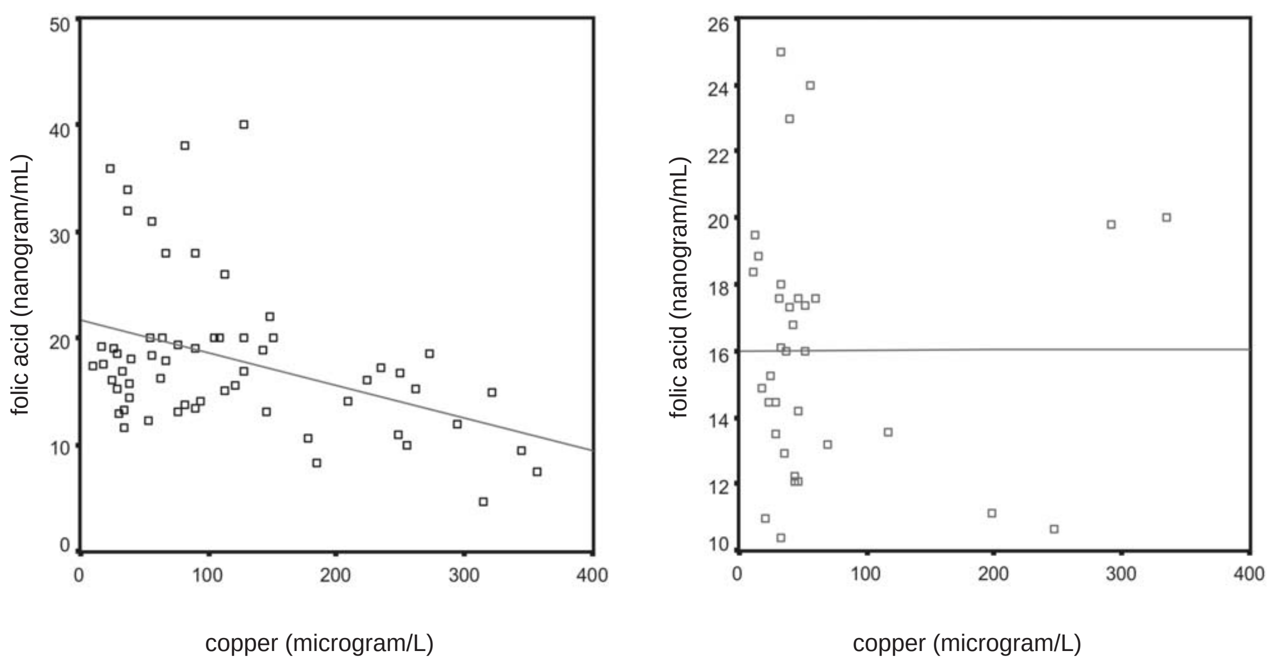
In our study, both the newborn infants with NTDs and their mothers had high levels of serum Cu and low levels of Zn when compared to the control group. However, the levels of folic acid, vitamin B_{12}, Se, Pb and Fe were not significantly different between the two groups.

Our study results showed that in comparison with the control group, the mothers who had given birth to babies with NTD had high levels of serum copper and low levels of zinc. This is in line with the findings of Cengiz, *et al*.(7). This difference between the two groups may be related to the levels of copper and zinc in the environment and their nutrition. It is known that serum levels of copper and zinc could be high in mothers from an area where the soil has high levels of these elements(12). However, the role played in the development of NTD by the level of Cu in the water supply is controversial(8). Copper and zinc levels are closely related to nutrition. The poor socioeconomic conditions of Sanliurfa and the consumption of yeast-free bread are seen as the reasons for the widespread lack of Zn(13).



(*a*) Cases (*b*) Controls

FIG. 1 *The levels of serum copper and serum folic acid in the mothers of chilren with NTD and the controls.*



(*a*) Cases (*b*) Controls

FIG. 2 *The levels of serum copper and serum folic acid in cord blood of the infants with NTD and the controls.*

Furthermore, there are important interactions between trace elements and vitamins at the level of intestinal absorption. When there is a decrease in Zn, the increase in Cu absorption is the reason for the increased level of serum copper. Zn-Cu interaction during intestinal absorption may be the answer for

WHAT IS ALREADY KNOWN?

- Nutritional factors are important in the pathogenesis of neural tube defects.

WHAT THIS STUDY ADDS?

- Mothers giving birth to infants with neural tube defects have high serum levels of copper and low serum levels of zinc. There is a negative correlation between the high level of serum copper and the level of serum folic acid in the mothers of infants with neural tube defects.

the relative increase in Cu in patients with NTDs in our series. The level of serum copper increases and that of zinc decreases in inflammation(14). NTD itself gives rise to inflammation explaining the pathology of serum copper and zinc levels in our study. An earlier study has shown that the level of serum copper in pathological pregnancies was significantly higher in comparison to normal pregnancies(12).

Although in normal pregnancies, possibly because of the placental transfer to the baby, serum zinc level decreases(15), in our study the serum level of Zn in the mothers who had infants with NTD was significantly lower in comparison to the control group. This difference in serum zinc levels between the cases and controls is unlikely to be related to the difference in gestational age seen in our study. Previous studies have not detected any difference in the serum zinc level of mothers of preterm and full-term babies(16). It appears that the low level of serum Zn in the mother affects the development of NTD. Furthermore, high intake of zinc may provide protection against Cu toxicity by preventing excess Cu uptake. Zinc also removes Cu from its binding site, where it may cause free radical formation(17). Zn may also play a role by influencing copper metabolism in the development of NTD. There might also be a relationship between genetic and metabolic processes involving the pathology of the serum copper and zinc levels of the mothers of babies with NTD. Furthermore, we established a negative correlation between the high level of serum Cu and the level of serum folic acid in the mothers of infants with NTD. It is plausible that a high level of Cu plays a role in etiology of NTD by having a negative effect on the folic acid nutriture.

In conclusion, as the etiology of NTD is thought to be multifactorial, a lack or an excess of trace elements and the interactions between vitamins and trace elements may play a role in its development. Our results indicate that Zn supplements and attention to the high serum copper level may be important in the prevention of NTD. Large scale prenatal zinc supplementation trials are therefore recommended to further confirm this association.

Contributors: All authors contributed to the study design, collection of data, analysis and drafting the manuscript.

Funding: None.

Competing interest: None stated.

REFERENCES

1. Frey L, Hauser WA. Epidemiology of neural tube defects. Epilepsia 2003; 44 (suppl. 3): 4-13.
2. Aqrabawi HE. Incidence of neural tube defects among neonates at King Hussein Medical Centre, Jordan.East Mediterr Health J 2005; 11: 819-823.
3. Feuchtbaum LB, Currier RJ, Riggle S, Roberson M, Lorey FW, Cunningham GC. Neural tube defect prevalence in California (1990–1994): Eliciting patterns by type of defect and maternal race/ ethnicity. Genet Testing 1999; 3: 265-272.
4. Zeyrek D, Iscan A, Sevinc E, Yildiz F, Mil Z, Karazeybek H. The prevalence of neural tube defects in Sanliurfa. New Med 2004; 21: 252-255 (in Turkish).
5. Rengasamy P. Etiology, pathogenesis and prevention of neural tube defects. Congenit Anom 2006; 46: 55-67.
6. O'Shea KS, Kaufman MH. Influence of copper on the early post-implantation mouse embryo: an in vivo and in vitro study. 1979; 186: 297-308.
7. Cengiz B, Soylemez F, Ozturk E, Cavdar AO. Serum zinc, selenium, copper, and lead levels in women with second-trimester induced abortion resulting from neural tube defects: a preliminary study. Biol Trace Elem Res 2004; 97:225-235.

8. Morton MS, Elwood PC, Abernethy M. Trace elements in water and congenital malformations of the central nervous system in South Wales. Br J Prev Soc Med 1976; 30: 36-39.

9. Burkitt MJ. A critical overview of the chemistry of copper-dependent low density lipoprotein oxidation: roles of lipid hydroperoxides, alpha-tocopherol, thiols and ceruloplasmin. Arch Biochem Biophys 2001; 394: 117-135.

10. Milde D, Novak O, Stuzka V, Vyslouzil K, Machacek J. Serum levels of selenium, manganese, copper, and iron in colorectal cancer patients. Biol Trace Elem Res 2001; 79: 107-114.

11. Fernandez FJ. Micromethod for lead determination in whole blood by atomic absorption, with use of graphite furnace. Clin Chem 1975; 21: 558-561.

12. Alebic-Juretic A, Frkovic A. Plasma copper concentrations in pathological pregnancies. J Trace Elem Med Biol 2005; 19: 191-194.

13. Kocyigit A, Koc A, Erel O, Vural H, Atas A. The hematological parameters and serum levels of some trace elements in children who eat bread made without or with yeast. New J Med 1998; 15: 206-210 (in Turkish).

14. Shenkin A. Trace elements and inflammatory response: implications for nutritional support. Nutrition 1995; 11 (1 Suppl):100-105.

15. Tamura T, Goldenberg RL, Johnston KE, DuBard M. Maternal plasma zinc concentrations and pregnancy outcome. Am J Clin Nutr 2000; 71: 109-113.

16. Bahl L, Chaudhuri LS, Pathak RM. Study of serum zinc in neonates and their mothers in Shimla hills (Himachal Pradesh). Indian J Pediatr 1994; 61: 571-575.

17. Powell SR. The antioxidant properties of zinc. J Nutr 2000; 130: 1447S-1454S.