

## SERUM COPPER CONCENTRATION IN NEURAL TUBE DEFECTS PREGNANCIES IN GORGAN-NORTHERN IRAN: A CASE CONTROL STUDY

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### ABSTRACT

**Objectives:** To determine an association between copper deficiency and neural tube defects in Northern Iran, which is reported to have a high prevalence of neural tube defects.

**Methodology:** This hospital based case control study was conducted on 13 mothers with newborns having neural tube defects and 35 healthy controls mothers in Northern Iran during 2005-2006. Serum copper was measured by spectrophotometry.

**Results:** Serum copper level in mothers with NTD affected newborns and mothers with normal newborns was  $15.9 \pm 6.1$  micromol/litter and  $15.2 \pm 6.2$  micromol/litter, respectively. Overall 15.4% of mothers in case group and 25.7 % of mothers in control group had copper deficiency. Logistic regression analysis showed no association between the presence of NTD and copper deficiency (OR =0.5, 95% CI: 0.05-3.2).

**Conclusion:** This study showed that there is no association between the presence of neural tube defects (NTD) and copper deficiency.

**KEY WORDS:** Birth Defect, Neural tube defects, Anencephaly, Copper deficiency, Pregnancy.

Pak J Med Sci July - September 2010 Vol. 26 No. 3 585-588

### How to cite this article:

Golalipour MJ, Mansourian AR, Keshtkar AA. Serum Copper concentration in Neural Tube Defects pregnancies in Gorgan-Northern Iran: A case control study. Pak J Med Sci 2010;26(3):585-588

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\* Received for Publication: December 17, 2009

\* Accepted: April 6, 2010

## INTRODUCTION

Neural tube defects (NTD) are important cause of infant mortality and childhood morbidity.<sup>1,2</sup> Etiology of NTD is considered multifactorial, with genetic, environmental and nutritional factors in all playing some role.<sup>3-5</sup>

Several studies have revealed a high incidence of NTDs in Iran. NTDs occur in about 28-32 per 10000 births in Iran.<sup>6,7</sup> These rates are higher than the incidence of NTDs in United States (9.3 to 14.6/10000),<sup>8</sup> England (17.9/10000)<sup>9</sup> and France.<sup>10</sup>

The findings of randomized trials determined that folic acid supplementation before conception and during early pregnancy decreased the incidence of NTDs more than 50%.<sup>11,12</sup> Although there is some evidence to suggest that the incidence of NTDs could be reduced even further

by larger folic acid supplements, there may be other environmental factors, probably nutritional, that contribute to the etiology of NTDs, because folic acid supplementation and fortification with folic acid have not eliminated all NTDs.<sup>13</sup>

There is considerable inconsistency concerning the relationship between maternal plasma copper (Cu) concentrations and the occurrence of NTDs. Some studies showed that exposure to copper sulfate resulted in a failure of elevation of the neural folds and consequent development of NTDs in mouse embryo<sup>14</sup> and copper – bearing intrauterine devices were related with anencephaly in anecdotal cases.<sup>15</sup> Another study indicated that there is association between high concentration of copper in pregnancy and presence of NTD in newborns.<sup>13</sup> In other hand, some studies showed that copper deficiency rather than the excess copper was related with teratogenesis.<sup>16,17</sup>

Copper has a key role in embryo development and Copper deficiency in embryogenesis period can cause neural tube defects. The causes of copper deficiency can be due to decreasing of enzymatic activity and increasing of Oxidative damages.<sup>18,19</sup> Buamah<sup>17</sup> reported that serum copper concentration was significantly lower in abnormal pregnancies. But Bro reported that there is no association between Copper deficiency in pregnancy and presence of neural tube defects in newborns.<sup>20</sup>

We conducted this study to look for an association between serum copper level and neural tube defects in Gorgan- Northern Iran which is reported to have a high prevalence.

## METHODOLOGY

This hospital based case control study was conducted during 2005-2006 at the Dezyani hospital in Gorgan located in the north of Iran. The study was approved by the Human Research Review Committee at the Golestan University of medical sciences. The cases consisted of 13 mothers with NTDs affected newborns and the controls consisted of 35 mothers with healthy newborns which were chosen at the hospital delivery list immediately after cases. Control to

case ratio was almost 3:1. Mother consent was obtained for the study along with a clearance from Institutional ethical committee. Data were collected regarding the maternal age, exposure to drug during pregnancy and parity.

After delivery NTD and health was diagnosed by gynaecologist and later the diagnosis was confirmed by a paediatrician.

*Serum collections and copper measurements:* Peripheral blood sample was collected from case and control groups maximum two hours after delivery. Serum was separated and analyzed for copper level by spectrophotometric method, using Randox Kit UR.<sup>21</sup> Copper level between 12-24.4 micromol/litter was used as normal. Serum copper level less than 12 micromol/litter was used as the cut-off to label the individual as copper deficient.

*Statistical analysis:* Categorical data were compared by Mann\_Whitney U and Fisher's exact Test. Presence of neural tube defect was considered as the dependent factor in multivariable logistics regression analysis. Independent factors included in the analysis were parity, history of abortions, maternal drug exposure and copper deficiency as dichotomous variables. Data were analyzed using SPSS 11.5 and STATA SE/8.  $P < 0.05$  was the criterion for a significant difference.

## RESULTS

Baseline characteristics of the two groups are depicted in Table-I. Serum copper level (mean±SD) in mothers with NTD affected newborns and mothers with normal newborns was  $15.9 \pm 6.1$  micromol/litter and  $15.2 \pm 6.2$  micromol/litter, respectively. Overall 15.4% of mothers in case group and 25.7 of mothers in control group in this study had copper deficiency. Logistic regression analysis showed no association between the presence of NTD and copper deficiency (OR =0.5, 95% CI: 0.05-3.2).

## DISCUSSION

This study indicated that there is no association between NTD and copper deficiency. These findings are similar to the results from other

researchers such as Bro et al in Denmark.<sup>20</sup> McMichael et al<sup>22</sup> from Australia. McMichael in a case-control study from Australia showed that there is no association between NTD and copper deficiency. Bro et al in a cohort study in Denmark has also reported that there is no association between copper deficiency and Neural tube defects. On the other hand, our findings are not similar with results from other studies such as Cengiz et al in Turkey<sup>13</sup>, Buamah et al in England<sup>17</sup> and Morton in South Wales.<sup>23</sup> Buamah et al in England<sup>17</sup> reported that serum copper concentrations in mothers are significantly lower in the pregnancies with anencephaly or subsequent spontaneous abortions. Morton et al from South Wales<sup>23</sup> reported that there is an association between mothers copper deficiency and presence of NTD in newborns. In contrast with Buamah and Morton studies,<sup>17,23</sup> Cengiz et al in Turkey<sup>13</sup> reported that serum copper level in mothers are significantly higher in the pregnancies with anencephaly compared to controls.

Evidence about Cu in prenatal development and pregnancy is controversial. O'shea and Kufman<sup>14</sup> reported that exposure to copper sulfate generally resulted in the failure of elevation of the neural folds and consequent development of NTDs in mouse embryos. Except for some anecdotal cases of fetal NTDs in pregnant women with a Cu intrauterine device in place, there are no consistent reports of abnormalities in the offspring of mothers with untreated Wilson's disease, which is usually characterized with spontaneous abortions.<sup>15</sup> Saner and Yuksel<sup>24</sup> reported that maternal serum Cu levels were significantly higher in NTD pregnancies than healthy controls in which serum and hair Zn levels were significantly low. Our per-

vious study<sup>25</sup> which indicated mothers with NTDs affected newborns had zinc deficiency in comparison with healthy mothers, but in recent study we did not measure the serum zinc level, but one possible mechanism can be related to zinc-copper interactions during intestinal absorption which could be the answer for the relative increase in Cu in patients with NTDs in our cases.

The mechanism of Zn's anti-Cu action is unique, it induces intestinal cell metallothionein, which binds Cu and prevents its transfer into blood. As intestinal cells die and slough, the contained Cu is eliminated in the stool. Thus, Zn prevents the intestinal absorption of Cu.<sup>26</sup> A reversed mechanism (increased absorption) in a Zn-deficient woman may be responsible for increased Cu levels, as observed in our small series.

However, this study had sample size limitation which leads the statistical tests power to be low. We recommend conducting a cohort study with larger sample size for assessing the relationship between maternal serum copper and zinc in prenatal and antenatal intervals. Despite our limitations in this study, we were able to show that there is no association between neural tube defect and serum copper level in mothers.

#### ACKNOWLEDGEMENT

The authors thank the research deputy of Gorgan University of Medical Sciences for the financial support. The authors also wish to thank the director, the manager, the infant ward of Dezyani hospital, and Mrs. Hajizade for their assistance. We are also grateful to Dr. Arezo Mirfazelei the neonatologist at Dezyani hospital.

Table-I: Maternal characteristics in case and control groups

	Cases (n=13)	Controls (n=35)	P-value
Maternal age: year (mean±SD)	24.3±5.8	25.4±5	0.56 †
Paternal age: year (mean±SD)	26.5±79.9	27.9±4.7	0.13 †
Gestational age: week (mean±SD)	34.7±5.3	38.1±1.5	0.048 †
Maternal exposure during 1st trimester (drug) (%)	2 (15.4)	2 (5.7)	0.294 ‡

† based on Mann-Whitney U test, ‡ based on Fisher exact test, \*two independent samples 't' test

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