

# Maternal serum zinc deficiency in cases of neural tube defect in Gorgan, north Islamic Republic of Iran

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عوز الزنك في أمصال الأمهات في حالات عيوب الأنبوب العصبي، في جرجان في شمال جمهورية إيران الإسلامية  
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الخلاصة: أظهرت دراسات سابقة معدلات مرتفعة من عيوب الأنبوب العصبي في جرجان في شمال جمهورية إيران الإسلامية. وفي هذه الدراسة للحالات والشواهد التي أجريت في عامي 2003 و2004 يقارن الباحثون مستويات الزنك في المصل وغيرها من المتغيرات، لدى 23 من أمهات الولدان المصابين بعيوب الأنبوب العصبي، مع 36 من الأمهات لولدان أصحاء في مستشفى ديزياني في جرجان. وقد وجدوا أن وسطي مستويات الزنك في المصل في الحالات المرضية يبلغ 13.43 ميكرومول/ل (بانحراف معياري 6.3) وفي الشواهد 11.41 ميكومول/ل (بانحراف معياري 6.3). ووجد عوز الزنك في 13 حالة (36.5%) و7 شواهد (19.4%). وقد أظهر تحليل التحوُّف اللوجستي ترابطاً بين وجود عيوب الأنبوب الهضمي وبين عوز الزنك (معدّل الأرجحية 5.56 بفاصلة ثقة 95% مع تراوح معدّل الأرجحية بين 1.51 و16.94).

**ABSTRACT** Previous studies have shown a high rate of neural tube defects (NTD) in Gorgan, northern Islamic Republic of Iran. This case-control study during 2003-04 compared serum zinc levels and other variables in 23 mothers of neonates affected with NTD and 36 mothers with normal healthy neonates in Dezyani hospital in Gorgan. Mean serum zinc levels in the case and control groups were 13.43  $\mu\text{mol/L}$  (SD 6.3) and 11.41  $\mu\text{mol/L}$  (SD 6.3) respectively. Zinc deficiency was found in 13 (36.5%) of the cases and 7 (19.4%) of the controls. Logistic regression analysis showed an association between the presence of NTD and zinc deficiency (OR 5.06; 95% CI: 1.51-16.94).

## Diminution du zinc sérique chez la mère et anomalies du tube neural à Gorgan (nord de la République islamique d'Iran)

**RÉSUMÉ** De précédentes études ont montré un taux élevé d'anomalies du tube neural (ATN) à Gorgan (nord de la République islamique d'Iran). Cette étude cas-témoins réalisée en 2003-2004 a comparé les niveaux de zinc sérique et d'autres variables chez 23 mères de nouveau-nés présentant des ATN et chez 36 mères ayant donné naissance à des enfants en bonne santé à l'hôpital Dezyani de Gorgan. Les taux moyens de zinc sérique dans le premier et le deuxième groupe étaient respectivement de 13,43  $\mu\text{mol/L}$  (E.T. 6,3) et de 11,41  $\mu\text{mol/L}$  (E.T. 6,3). Une carence en zinc a été constatée chez 13 mères (36,5 %) dont le nouveau-né souffrait d'ATN et chez 7 mères (19,4 %) appartenant au groupe témoin. L'analyse de régression logistique a montré un lien entre la présence d'ATN et la carence en zinc (OR 5,06 ; IC 95 % : 1,51-16,94).

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## Introduction

Neural tube defects (NTD) are severe congenital malformations which occur due to abnormalities in neural tube formation [1]. They include spina bifida, anencephaly and encephalocele and are an important cause of perinatal morbidity and mortality [2]. NTD are the second cause of serious congenital malformations in the United States of America after heart malformation [3].

The incidence of NTD varies by geographical zone [4]. The spectrum is wide, ranging from 1 per 100 live births in parts of China to about 1 per 5000 live births in Scandinavian countries [4]. In many countries the prevalence is approximately 1 per 1000 births. In recent years some studies have shown the prevalence of NTD ranging from 1.76 to 5.1 per 1000 in some parts of the Islamic Republic of Iran [5–7]. Furthermore, 2 previous studies reported the prevalence of NTD at 2.88 per 1000 and 3.12 per 1000 in Gorgan in the north of the country [8,9].

The etiology of these anomalies is not completely understood and has often been considered multifactorial, with genetic and environmental as well as nutritional factors playing a role [10–12]. Nutritional factors such as folic acid and zinc deficiency are believed to be important in NTD [13]. Zinc is a trace element required by the organism for multiple biological functions, including cell division, development and differentiation. Zinc is a cofactor for many enzymes in various metabolic pathways, for example in nucleic acid synthesis [13–15]. Studies carried out in different parts of the world have shown the role of zinc deficiency in NTD [14–18].

Faced with a high prevalence of NTD in our region [8,9], we planned this study to determine the role of maternal serum zinc deficiency in NTD in neonates.

## Methods

### Setting

This case-control study was conducted at Dezyani hospital, the main referral hospital with a labour facility in Gorgan, the capital of Golestan province in the north of the Islamic Republic of Iran. The hospital handles more than 6000 deliveries per annum, accounting for the majority of deliveries (70%) in the city. Patients are usually from moderate to low socioeconomic classes.

### Sample

To estimate the zinc level in mothers of neonates with NTD and healthy mothers with a probability of 95% and power of 80%, sample size was estimated to be  $\geq 21$  for each group. The case group comprised all 23 mothers who gave birth to babies with NTD during the study period (June 2003 to September 2004). For the control group, for every case we selected the subsequent 2 mothers who gave birth to normal, healthy babies. As some of the blood samples haemolysed and some control mothers did not agree to enter the study, the final sample for the control group was 36.

### Data collection

All babies delivered in this hospital during the investigation period were examined after delivery for NTD by a gynaecologist and later the diagnosis was confirmed by a paediatrician. The health of neonates in the control group was assessed clinically by a gynaecologist and paediatrician before these mothers entered the study. A questionnaire covering all relevant clinical and demographic factors was filled out for each case and control infant by the paediatrician and then completed by a nurse during an interview with the mothers. The data included: birth date, sex, birth weight, birth height, gestational age and type of

NTD. Also recorded were: parents' ages, mother's parity, mother's previous history of abortions, family history of congenital anomalies and mother's history of exposure to any type of drugs during the 1st trimester of pregnancy (except for folate, which is routinely given to pregnant women).

A peripheral blood sample was collected from all mothers and the serum samples were analysed for zinc level by spectrophotometric methods (Randox kit, UK). Zinc deficiency was defined as a zinc level < 10.6 µmol/L [19,20].

Ethical consent was obtained for the study from the ethical committee of Gorgan University of Medical Sciences. The mothers' consent was obtained prior to their entering the study.

### Analysis

Student *t*-test, chi-squared, Mann–Whitney and Fisher exact tests were performed for statistical analysis. Odds ratios (OR) and 95% confidence interval (CI) were calculated as part of logistic regression analysis using SPSS, version 11.5, in a saturated model using forward stepwise procedures modelling according to the likelihood ratio.

### Results

We studied 23 mothers with neonates affected with NTD. Age range for the case

mothers was 21–42 years and for the case fathers 22–42 years. The most frequent type of NTD was spina bifida in 14 neonates (61%), followed by anencephaly in 7 (30%) and encephalocele in 2 (9%). The control group was 36 healthy mothers with normal babies. The ages range of the mothers of the controls was 17–37 years and for the control fathers 20–0 years.

Multiparity (4+ pregnancies) was reported in 17.4% of the case mothers and 16.7% of the control mothers. No risk tendency for NTD was found to be related to number of pregnancies ( $\chi^2 = 0.016$ ,  $P = 0.493$ ). A past history of infants with NTD was not reported by any mother in either group. However, mother's history of congenital malformations other than NTD was reported by 11.1% of the cases and 4.3% of the controls (Table 1).

No statistically significant differences were found between the groups for any of the demographic or family history variables (Table 1).

The mean gestational age for the cases was 36.39 weeks [standard deviation (SD) 2.35], range 31–38 weeks, and for controls 38.53 weeks (SD 0.81), range 36–39 weeks ( $P = 0.001$ ) (Table 2).

The mean serum zinc level in mothers of NTD neonates [11.41 µmol/L (SD 6.3), range 2.2–35.4] was lower than in the control group [13.43 µmol/L (SD 6.3) range

Table 1 Demographic characteristics of mothers with neonates affected by neural tube defects (NTDs) and healthy controls

Variable	Cases (n = 23)	Controls (n = 36)	P-value
Maternal age [mean (SD) years]	26.9 (6.1)	25.6 (5.3)	0.38 <sup>a</sup>
Paternal age [mean (SD) years]	30.1 (6.1)	29.0 (6.1)	0.33 <sup>a</sup>
Multiparity [no. (%)]	4 (17.4)	6 (16.7)	0.48 <sup>b</sup>
Previous abortion [no. (%)]	5 (13.9)	3 (13.0)	0.62 <sup>b</sup>
History of births with NTD [no. (%)]	0 (0.0)	0 (0.0)	–
History of births with other congenital malformations [no. (%)]	4 (11.1)	1 (4.3)	0.35 <sup>b</sup>

<sup>a</sup>Student *t*-test; <sup>b</sup>Chi-squared test.

**Table 2 Birth and gestational characteristics of neonates affected by neural tube defects and healthy controls**

Variable	Cases (n = 23)	Controls (n = 36)	P-value
Birth weight [mean (SD) g]	2910 (726)	3460 (490)	0.001 <sup>a</sup>
Birth height [mean (SD) cm]	46.4 (4.75)	49.7 (2.50)	0.006 <sup>a</sup>
Gestational age [mean (SD) weeks]	36.39 (2.35)	38.53 (0.81)	0.001 <sup>b</sup>
Maternal exposure to drugs in 1st trimester [no. (%)]	6 (26.1)	1 (2.8)	0.011 <sup>c</sup>
Low maternal serum zinc level <sup>e</sup> [no (%)]	13 (56.5)	7 (19.4)	0.003 <sup>d</sup>

<sup>a</sup>Student t-test; <sup>b</sup>Mann-Whitney test; <sup>c</sup>Fisher exact test; <sup>d</sup>chi-squared test; <sup>e</sup>< 10.6 µmol/L.

6.2–38.2], but not statistically significantly so ( $P = 0.092$ ). Significantly more of the cases ( $n = 13$ , 56.5%) than the controls had zinc deficiency ( $n = 7$ , 19.4%) (serum zinc < 10.6 µmol/L) ( $P = 0.003$ ) (Table 2).

Medications consumed during pregnancy in the case group were antibiotics and analgesics for urinary tract infections (4), propranolol for bicuspid valve prolapse (1) and anti-hyperthyroid drugs (1) (Table 2). In the control group 1 mother had been exposed to antibiotics. This difference was significant ( $P = 0.007$ ).

Logistic regression analysis of the significant variables showed an association between the presence of NTD and zinc deficiency (OR 5.06; 95% CI: 1.51–16.94;  $P = 0.008$ ) and drug consumption during the 1st trimester (OR 13.12; 95% CI: 1.31–130.97;  $P = 0.028$ ) (Table 3).

## Discussion

The results of this study indicated that there was an association between NTD in neonates and zinc deficiency in mothers. Our findings are similar to results from research in Canada [15], France [21], California [14] the Netherlands [22] and elsewhere [23–25]. Our findings are also comparable with studies in neighbouring Turkey. In a case-control study, Cengiz et al. found that serum zinc levels in mothers with NTD

were significantly lower than in controls [26]. Cavdar et al. showed that maternal serum and hair zinc concentrations in the NTD group were lower than those in the control group [16]. Other researchers, however, reported no relation between zinc deficiency and NTD [27–30]. One study showed a significant relation between maternal nutrition during pregnancy and NTD [31].

The diversity of functions in the organism has yielded diverse hypotheses to explain the association between zinc deficiency and NTD. Abnormal synthesis of nucleic acids and proteins, abnormal polymerization of tubulin, chromosome defects, excessive cell death and an increase in cell membrane lipid peroxidation in humans may be due to zinc deficiency [32]. A promising finding for the prevention of NTD has been the discovery of the protective effect of

**Table 3 Logistic regression analysis of the variables included in the study**

Risk factor	Adjusted OR	95% CI	P-value
Low maternal serum zinc level	5.06	1.51–16.94	0.008
Maternal exposure to drugs in 1st trimester	13.12	1.31–130.97	0.028

OR = odds ratio; CI = confidence interval.

folic acid [33–37], leading to recommendations for folate supplementation in the diet of pregnant women. It has been noted that the periconceptional use of folate supplements also reduced the incidence of NTD [38]. In a systematic review of case–control studies, a moderate association between low maternal vitamin B12 status and the risk of fetal NTD was noted [39].

Although folic acid supplementation programmes have been implemented for pregnant women in many countries, NTD continue to be one of the most frequent congenital malformations worldwide. This directed us to presume that not all NTDs are related to folic acid, and that other genetic and nutritional factors may play an important role. According to the MRC Vitamin Study Research Group report, only 70% of NTDs were prevented by supplemental folic acid, and that zinc deficiency could also play a role [14,15,23,40,41].

Recently, Velie et al. studied the periconceptional use of vitamins, minerals and food supplements in mothers of 430 babies with NTD and 429 controls. They found a protective effect of zinc on NTD [14]. Folic acid and zinc deficiencies have been proposed elsewhere as possible causes of NTD [42]. Therefore administration of zinc during pregnancy is at least as important as folic acid [43]. An American study showed that higher preconceptional intake of nutrient zinc decreased the risk of NTD [23].

Other studies have also indicated a moderate association between low maternal B12 status and the risk of fetal NTD [39,44]. Afman et al. suggested that vitamin B12 supplementation might therefore be warranted for prevention of NTD [44].

Genetic factors have also been hypothesized. Some researchers in the Eastern Mediterranean Region have reported that consanguinity in parents of babies with NTD is high [45,46].

The other significant difference between the cases and controls in the present study was the consumption of medications in the 1st trimester of pregnancy, a factor that is widely accepted as a cause in congenital malformations. A study in Turkey reported a significant relation between medication during pregnancy and NTD [31]. We also observed a shorter gestational time in cases than in controls and a lower weight and length in cases that were probably a result of the congenital malformations.

## Conclusion

In this study, the first of its kind in this region, we found an association between NTD and low zinc levels, adding to the evidence about the importance of nutritional and maternal health factors in the etiology of this disease. In addition to folic acid supplementation, zinc supplementation should be considered for the further decrease in the recurrence and occurrence of NTD.

In our opinion, it would be better to select the study group at 12–16 weeks of pregnancy after the diagnosis of NTD in the fetus with ultrasonography because the zinc concentration in mothers could change during the next 2 trimesters. Further studies in humans are needed to clarify the metabolic interrelations between folic acid and zinc to provide more solid support for supplementation programmes during gestation.

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## Correction

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The name of the third author in Arabic should read جواد علي اللواتي , not جواد أحمد اللواتي .