



Original Work

Implications of zinc on fetal neural tube defects

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ABSTRACT: Zinc is essential for normal growth and differentiation in all mammalian species and it is reported that folic acid supplementation has reduced the incidence of neural tube defects (NTD). It is still considered one of the important congenital malformations having wide implications. Zinc deficiency has been reported to produce NTD in animals. The present study was undertaken to evaluate zinc status of newborn babies with NTD and their mothers. Blood samples were taken from 287 mothers and their babies having NTD and from 110 controls visiting hospitals and health clinics. Zinc level as $\mu\text{g/ml}$ for blood and serum and $\mu\text{g/g}$ for cell mass were determined on GBC 932 atomic absorption spectrophotometer (Australia) by fluorometry. The mean maternal blood, serum and cell mass concentration in NTD group ($14.56 \pm 1.34 \mu\text{g/ml}$, $0.6 \pm 0.01 \mu\text{g/ml}$, $5.64 \pm 0.35 \mu\text{g/gm}$ respectively) were significantly lower than those of the control mothers ($24.15 \pm 2.95 \mu\text{g/ml}$, $0.72 \pm 0.03 \mu\text{g/ml}$, $7.37 \pm 0.44 \mu\text{g/gm}$ respectively). There is a significant decrease in the concentration of Zinc in newborns having NTD ($15.65 \pm 3.18 \mu\text{g/ml}$, $0.56 \pm 0.08 \mu\text{g/ml}$, $5.11 \pm 0.18 \mu\text{g/gm}$ respectively) as compared with normal newborns ($28.04 \pm 1.1 \mu\text{g/ml}$, $0.59 \pm 0.08 \mu\text{g/ml}$ and $6.08 \pm 0.29 \mu\text{g/gm}$ respectively). Maternal nutritional zinc deficiency in newborns and their mothers is thought to be one of the factors responsible for NTD. However, the lowered zinc concentration may be influencing the causation of NTD. More investigations on zinc status in mothers during antenatal period, especially in the prenatal development and antenatal zinc status including normal babies and NTD babies are required at population level.

KEY WORDS: *Neural Tube Defect; Trace Element; Zinc*

INTRODUCTION

Neural tube defects (NTD) constitute a significant proportion of all congenital malformations in human beings. Extensive work has been done so far but little progress has been made in finding the etiology of some of them (anencephaly & spina bifida) and on zinc deficiency as a basis for generating etiological hypotheses by various epidemiological studies.¹ Association between vitamins especially folic acid, Vitamin B₁₂²⁻⁴ and other trace elements like copper and selenium is also reported to reduce the incidence of NTD, but generally they are not prescribed by the

clinicians/practitioners during pregnancy.⁵ A number of prospective & retrospective human studies have provided evidence that folic acid supplementation to pregnant women lowers the incidence of NTD in the offspring⁶⁻⁸. Folic acid is widely used by pregnant women but NTD is still an important congenital malformation having wide implications. This may be due to the fact that deficiency of folic acid alone is not responsible for all kind of NTD and many other factors (nutritional & genetic) are responsible in the etiology of various kinds of NTDs⁹⁻¹².

The maternal dietary intake of many nutrients affects the prenatal development of the embryo and fetus, and deficiency or excess of certain essential nutrients may cause malformations^{13,14}. The importance of zinc for embryonic development was first established for chick embryos. Turk et al¹⁵

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showed that chicks hatched from hens fed a zinc deficient diet were weak, and died within four days. Blamberg et al¹⁶ also found grossly deformed embryos. A similar range of skeletal defects was observed by Keinholz et al¹⁷. Zinc transfer to fetus is reduced resulting in higher percentage of stillbirths and lower viability of piglets. Reproductive aspects of zinc deficiency in pigs are well documented¹⁸.

Zinc is essential for the growth and development of the fetus and plays a critical role in many cellular reactions, including gene transcription and cell division and differentiation. The inadequate intake of zinc is associated with NTDs in both animals and humans¹⁹. The essentiality of zinc in the formation of the neural tube is further supported by the observation that women with acrodermatitis enteropathica, a disorder of impaired zinc absorption from the intestine, are at high risk for babies with NTDs²⁰.

Zinc deficiency in humans and animals causes teratological, genetic and medical abnormalities. Human requirement of zinc seems to be high during periods of rapid growth, such as embryonic life, infancy, puberty, pregnancy and tissue repair. During gestation, deficiency of this trace element even if transitory, is teratogenic and associated with chromosomal abnormalities and alters cognitive functions²¹. Newborns with NTDs have significantly low level of serum zinc, supporting zinc deficiency as an association of NTDs²²⁻²⁴. Zeyrek et al²⁵ reported that low maternal zinc and high copper during pregnancy may be responsible for NTDs. Over the last three decades, zinc has been recognized to play a vital role in almost all aspects of living systems either directly or indirectly²⁶.

METHODOLOGY

In the present study 287 newborns with NTD (Meningomyelocele-132, Meningocele-47, Lipomenigocele-15, Spina bifida occulta-41 and Encephalocele-52) have been studied and their mothers served as our cases and 110 apparently normal full term babies and their mothers served as controls selected from the same population from the year 1997-2000. A detailed maternal obstetric history, age and socio-economic status were noted, and newborns with NTD examined carefully with the help of a pediatrician. Maternal socio-economic status was classified according to Kuppaswamy socio-economic status score²⁷.

Venous blood (5.0 ml each) from all newborns and their mothers was drawn from the antecubital vein, using stainless steel needles (disposable syringes) and collected in trace element free plastic vials. All necessary precautions were taken to avoid contamination. One hour after collection, the blood was centrifuged and the clear serum was transferred to plastic vials. The sera were stored frozen at -20°C until analyzed.

The blood from each case was divided into three groups. In group (I) whole blood, in group (II) blood serum and in group (III) packed cell mass was subjected to extraction of zinc. All the samples were digested in long necked round bottom flasks with triple acid (conc. HNO₃, 70% perchloric acid and conc. H₂SO₄ . 10:3:1). This process was executed by heating the contents till most of the triple acid mixture evaporated from the flask. The contents of each flask were then washed with triple distilled water and were stored in plastic vials at 4°C for further analysis. The zinc levels as ng/ml were determined by GBC 932 spectrophotometer²⁸. All the data were given as arithmetic means, SD, SEM and ranges. The 95% confidence intervals (CI) for the population means were also shown. T-test was used to compare the Zinc levels of the subjects²⁹.

RESULT

The result of the Zinc concentration in blood, cell mass and serum in the mothers having NTD newborns and the mothers having normal newborns are shown in **Table 1**. The mean value of Zinc concentration of control group is 24.15 ± 2.95 µg/ml for blood, 7.37 ± 0.44 µg/gm for cell mass and 0.719 ± 0.035 µg/ml for serum as compared to the mothers of NTD newborns who showed a zinc level of 14.56 ± 1.34 µg/ml, 5.64 ± 0.35 µg/gm and 0.606 ± 0.0163 µg/ml respectively. All the values were significantly lower (p<0.05) in the mothers of newborns having NTD.

The concentration of zinc was also significantly lower in newborns with NTD and is summarized in **table 2**. The zinc concentrations in blood of normal newborns, in cell mass and serum was 28.04 ± 1.11 µg/ml, 6.08 ± .288 µg/gm and 0.596 ± 0.08 µg/ml, in comparison to NTD newborns having zinc concentration 15.65 ± 3.18; 5.11 ± 0.178 and 0.556 ± 0.079, respectively. The average age of mothers in proband was 24.52 ± 0.22 and control 25.65 ± 0.39 respectively and varies significantly.

Table 1: Maternal Zinc Status of Healthy and Newborns with NTD

Analysis	Mothers of Newborns with NTD	Control Mothers with Normal Newborns
Blood Zinc (µg/ml)		
Mean ± SEM (SD)	14.56 ± 1.34 (5.99)	24.15 ± 2.95 (13.2)*
95% CI	11.93 to 17.18	18.46 to 29.83
Range	6.15 - 32.73	3.65 - 46.48
Cell Mass Zinc (µg/g)		
Mean ± SEM (SD)	5.64 ± 0.357 (1.6)	7.37 ± 0.44 (2.0)*
95% CI	4.93 to 6.34	6.44 to 8.29
Range	0.256 - 3.57	2.14 - 11.87
Serum Zinc (µg/ml)		
Mean ± SEM (SD)	0.606 ± 0.016 (0.02)	0.719 ± 0.035 (0.16)*
95% CI	0.575 to 0.638	0.648 to 0.789
Range	0.17 - 1.96	0.268 - 1.30

**p*<0.05

Table 2: Zinc Status in Normal Newborns and those with NTD

Analysis	Newborns with Neural Tube Defect	Normal Newborns
Blood Zinc (µg/ml)		
Mean ± SEM (SD)	15.65 ± 3.18 (14.26)	28.04 ± 1.11 (4.9)*
95% CI	8.97 to 22.32	25.71 to 30.38
Range	2.51 - 34.22	14.74 - 32.64
Cell Mass Zinc (µg/g)		
Mean ± SEM (SD)	5.11 ± 0.178 (0.8)	6.08 ± 0.29 (1.3)*
95% CI	4.75 to 5.46	5.50 to 6.65
Range	2.89 - 8.25	2.44 - 11.25
Serum Zinc (µg/ml)		
Mean ± SEM (SD)	0.556 ± 0.079 (0.35)	0.596 ± 0.08 (0.40)*
95% CI	0.401 to 0.710	0.407 to 0.787
Range	0.031 - 1.221	0.023 - 1.528

**p*<0.05

DISCUSSION

The present study has demonstrated that the concentration of zinc is significantly lower in

proband as well as their mothers as compared to the control. Zinc is an essential trace element and is a cofactor for many enzymes in various metabolic pathways. Several enzymes that plays significant

parts in nucleic acid metabolism are zinc dependent. In humans zinc deficiency has been reported to be associated with growth retardation^{30,31,10}. Physiological changes during pregnancy increase nutritional requirements. During pregnancy iron is commonly supplemented and it seems that iron does interfere with zinc absorption in the intestine³². It is also reported that zinc absorption tends to increase during pregnancy³³ and it is possible that some other trace elements interfere. Folic acid supplementation is generally recommended decreasing the incidence of NTD². Zinc deficiency in animals has been shown to produce CNS malformations³⁴.

Zinc deficiency during early pregnancy in rats is also reported to produce abnormal blastocyst³⁵, increase the rate of reabsorption and a high incidence of congenital malformation and teratogenicity, particularly fetal neural tube defects such as anencephaly³⁶. Thus it is reasonable to assume that zinc deficiency produces a similar set of changes in humans as observed in rats in most of the earlier studies and suggests a basic common metabolic mechanism of trace elements in mammals in general. As such it is difficult to say anything pertaining to the mechanism whether it is terata or necrosis or both responsible for such congenital malformation in humans. Pregnant women suffering from acrodermatitis enteropathica (AE), an inborn error of zinc metabolism, exhibit high frequency of fetal deaths and malformed infants particularly with neural tube defects³⁶⁻³⁹. The pathogenesis of AE is the result of impaired intestinal zinc absorption and such patients exhibit low serum lipid and arachidonic acid increased IgA and defective prostaglandin synthesis^{38,40}. The malformation observed in the present study can be attributed to the metabolic alterations that are the result of deficient zinc supply to the developing fetus.

The development of the mammalian fetus depends on the constant supply of nutrients from the mother⁴¹. Thus the maintenance of pregnancy implies an increased ingestion of zinc to meet increasing demands of the trace elements of fetus⁴². As the gross need of fetal growth especially in the 3rd trimester of pregnancy are often in excess of the assimilative capacities of the mother and thus imply a net maternal loss of these nutrients⁴³, if the nutrient deficient diets with low level of trace elements are continued during pregnancy. The meagre stored zinc level in maternal tissue is possibly responsible for the low supply across the placenta to the developing fetus possibly lead to congenital anomalies. The low level of zinc during pregnancy also leads to the manifestation of Cd toxicity⁴⁴ or toxicity of Pb, Hg and certain drugs and alcohol⁴⁵. It is, therefore, suggested that a constant supply and monitoring of zinc is a must during pregnancy.

The mechanisms responsible for these defects or abnormal development are thought to be based on depression of nucleic acid synthesis¹³. Prolongation of the mitotic interval and reduction in the number of neural cells early in development could combine to produce a variety of malformations. The specific nature of the defect would then depend on the state of presumptive area of the primitive tube when any given developmental process began. Defects in the closure of neural tube and other problems in differentiation of CNS were observed in early chick embryo explants cultured in zinc deficient media⁴⁶. In addition, mesodermal differentiation and growth are also altered.

In the present study, both the newborn babies with NTDs and their mothers had significantly low levels of Zn when compared to the control group. The results showed that in comparison with the control group, the mothers who had given birth to babies with NTD had low levels of zinc. This is in line with the findings of Cengiz et al⁴⁷. This difference between the two groups may be related to the levels of zinc in the environment and their nutrition. There are important interactions between trace elements and vitamins at the level of intestinal absorption. Although in normal pregnancies, possibly because of the placental transfer to the baby, serum zinc level decreases⁴⁸, in the present study the serum level of Zn in the mothers who had infants with NTD was significantly lower in comparison to the control group.

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 significant role in its development. The results of the present study indicate that Zn supplements may be important in the prevention of NTD. Large scale prenatal zinc supplementation trials are therefore recommended for further confirmation as the association between zinc deficiency and NTD needs further investigation.

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