

Relationship between Plasma Concentrations of Maternal Zinc during Pregnancy and the Risk for Orofacial Cleft

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Abstract

Aim: Recent studies have suggested the occurrence of a variety of abnormalities including oral clefts following the deficiency of nutritional elements. The present study aimed to address the association between plasma concentrations of maternal zinc and the risk of an infant being born with an orofacial cleft. **Materials and Methods:** In this case–control study conducted in Hamadan, Iran, 2015, 48 mothers of children with an isolated cleft lip with or without cleft palate and 48 control mothers of children with no congenital malformations were recruited. The concentration of zinc in the whole blood was measured using flame atomic absorption spectrometry method. Data were analyzed by the use of descriptive and analytical statistics in SPSS version 16. **Results and Discussion:** Plasma level of zinc in a group with orofacial clefts was $16.87 \pm 8.17 \mu\text{mol/L}$, and in the control group was 19.28 ± 8.83 that was statistically similar between the groups ($t = -1.329$, $P = 0.187$). *T*-test showed that the two groups did not have a significant difference in zinc level. The odds ratio (OR) for the case and control group was 1.6, which indicates there was no significant difference in zinc deficiency between the two groups (OR = 1.66, 95% confidence interval = 0.61–4.54, $P = 0.426$). **Conclusion:** Despite lower zinc plasma levels in women of the case group, the difference in concentration of this element was not statistically significant between the two groups. In general, there is no definitive conclusion on whether the deficiency of nutritional elements during pregnancy is the cause of malformations or not, and it is still necessary to further studies.

Key words: Case–control, orofacial cleft, plasma concentrations, pregnancy, zinc

INTRODUCTION

Orofacial clefts are common birth defects in humans that are prevalent with an overall incidence of 0.5–3 for every 1000 children born.^[1] However, the pathogenesis of these phenomena remains unclear, and there was evidence of the roles of etiological effects of genetic and environmental-related factors such as lifestyle, low socioeconomic, and nutritional habits.^[2] Over the years, it has been believed that the mothers' nutritional regimens may play an important role and predictor in the

development of birth defects and a newborn.^[3] In recent years, investigations on the association between maternal

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nutritional habits and the risk for neonatal defect have been focused on the role of folic acid and vitamin supplements. In this regard, it has been shown that folic acid supplements could effectively prevent neural tube defects in those women with a history of giving birth to infants with congenital anomalies.^[4] In addition, recent studies on animal models have shown that zinc deficiency during pregnancy can lead to a variety of abnormalities including oral clefts.^[5] Furthermore, it has been suggested that copper deficiency during embryonic and fetal development may result in numerous gross structural and biochemical abnormalities that may arise through a variety of mechanisms including low maternal dietary copper intake, disease-induced or drug-induced changes in maternal metabolism.^[6] However, the role of zinc deficiency on the development of congenital anomaly in human remains uncertain, and few publications in the medical literature are available in this area. The present study aimed to address the association between plasma concentrations of maternal zinc and the risk of an infant being born with an orofacial cleft.

MATERIALS AND METHODS

The present study was approved by the Ethics Committee at Hamadan University of Medical Sciences with ethical code IR.UMSHA.REC.1394.71. In this case-control study conducted in Hamadan, Iran, 2015, 48 mothers of children with cleft lip and 48 control mothers of children with no congenital malformations were recruited. The maximum interval between delivery and blood sampling for mothers in the cleft group and those in the control group was 5 years because changes in lifestyle up to 5 years cannot affect the amount of plasma material. None of the participants took mineral supplements previously. Furthermore, all participants had body mass index <30 kg/m², none had previous use of alcohol, cigarette, or any teratogenic drugs, and none had a history of the birth of children with congenital malformations. Baseline characteristics were collected by interviewing and using general study questionnaire. The diagnosis of congenital malformations was confirmed by a pediatrician. Blood samples were taken from a vein in the elbow crease the morning after an overnight fast. Whole blood samples were kept frozen in tubes containing heparin until analysis. Immediately after phlebotomy, tubes containing blood samples were placed on ice, and then, centrifuged to separate plasma from blood cells. Phlebotomy was done for the prevention of false high levels of zinc.^[7] Phlebotomy and centrifugation times were recorded for each of the participants for controlling their possible confounding effects at the time of collection and processing. Plasma samples were frozen immediately on

dry ice after preparation, and dry ice was replaced on a continuous basis. Samples which showed hemolysis were excluded and blood extraction was redone. Freezing temperatures were fixed at -80° until transferred to the location of elements analysis. The concentration of zinc in the whole blood was measured using flame atomic absorption spectrometry method (Atomic Absorption, PerkinElmer Company, USA). Results were presented as mean ± standard deviation for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Continuous variables were compared using *t*-test or Mann-Whitney U-test, whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the groups. Categorical variables were, on the other hand, compared using chi-square test or Fisher's exact test when >20% of cells with expected count of <5 were observed. For the statistical analysis, the statistical software SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used. *P* = 0.05 or less was considered statistically significant.

Table 1: Baseline characteristics in the study population

Characteristics	(n=48), n (%)		P
	Study group	Control group	
Parity status			
Primipar	25 (52.1)	28 (58.3)	0.541
Multipar	23 (47.9)	20 (41.7)	
Occupational status			
Housewife	39 (81.3)	37 (77.1)	0.612
Employed	9 (18.7)	11 (22.9)	
Educational level			
Primary and secondary school	31 (64.6)	26 (54.2)	0.299
High school	9 (18.8)	10 (20.8)	
College degree	8 (16.6)	12 (25.0)	
Type of delivery			
Normal vaginal	15 (31.3)	12 (25.0)	0.493
Cesarean section	33 (68.7)	36 (75.0)	

RESULTS

The study groups including women with neonates who suffered orofacial clefts and control ones were matched for baseline characteristics such as status of parity, occupational state, educational level, and type of delivery [Table 1]. About 48% and 41.7% were multipara, cesarean section in 68.7% and 75.0%, occupational employment in 18.7% and 29.0%, and having college degree in 16.6% and 25.0%, respectively, with no significant differences.

Plasma level of zinc in group with orofacial clefts was 16.87 ± 8.17 $\mu\text{mol/L}$ and in the control group was $19/28 \pm 8/83$ that was statistically similar between the groups ($t = -1.329$, $P = 0.187$) [Table 2].

As indicated in Table 3, a significant difference was found in plasma concentration of zinc between the groups adjusted for women's age in group with orofacial clefts, the mean level of zinc was 11.82 ± 5.02 $\mu\text{mol/L}$ in women older than 30 years; however, the other group with the same age range had the zinc

level of 22.2 ± 8.27 $\mu\text{mol/L}$ that was lower in former group. However, no difference was found in plasma concentration of zinc between the group with orofacial clefts and the healthy neonates group considering mothers age range <30 years (18.2 ± 8.36 $\mu\text{mol/L}$ vs. 18.67 ± 8.92 $\mu\text{mol/L}$, $P = 0.810$). Besides, no significant difference was observed in plasma concentration of zinc between the two orofacial clefts group and control group adjusting for history of pregnancy.

The odds ratio (OR) for the case and control group was 1.6, which indicates that zinc deficiency in the case group was greater than the control group; hence, there was no significant difference in zinc deficiency between the two groups according to the confidence intervals (CIs) (OR = 1.66, 95% CI = 0.61–4.54, $P = 0.426$) [Table 4].

DISCUSSION

The results of our study showed that plasma levels in women with children with cleft palate and noncleft palate compared to control women cannot be considered as a significant risk factor for this congenital defect. Similarly, Shaw *et al.*^[7] reported no significant association between orofacial cleft risk and maternal zinc intake in case and control mothers in the United States. In contrast, the Dutch research group^[8] also reported that mean erythrocyte zinc concentrations of case mothers having a child with orofacial cleft were significantly lower than control mothers. Furthermore, Hozyasz *et al.*^[9] investigated the relationship between concentrations of maternal zinc and the risk of an infant being born with an orofacial cleft, and indicated that the mothers with a whole blood zinc concentration of 47.1 $\mu\text{mol/L}$ or less had a 2.5 times higher risk of having a child with an orofacial cleft than those with a higher concentration. Moreover, it has been suggested that zinc is a constituent of >70 enzymes that play a vital role in fetal development.^[10,11] The contrary results in the studies may be due to some potential reasons such as considering mothers in the different age subgroups (as shown in our survey that the relationship between plasma zinc level and appearance of abnormality can be affected by mothers' age), ignoring different confounders affecting this relationship (nutritional habits, underlying disorders related to the studied elements, mistakenly recalling use of supplement drugs within pregnancy, or the methodological

Table 2: Comparison of zinc plasma concentration ($\mu\text{mol/L}$) in two groups of case and control in women

Group	n	Zn plasma concentration ($\mu\text{mol/L}$), mean \pm SD	P
Case	48	16.87 ± 8.17	0.187 df=94 T=-1.329
Control	48	19.28 ± 8.83	

Independent-sample *t*-test. SD: Standard deviation

Table 3: Plasma level of zinc in two study groups adjusted for age and parity

Characteristics	n=48		P
	Study group	Control group	
Age (years)			
≥ 30	11.82 ± 5.02	22.2 ± 8.27	<0.001
<30	18.2 ± 8.36	18.67 ± 8.92	0.810
Parity			
Primipara	17.09 ± 7.71	19.75 ± 9.22	0.263
Multipara	16.64 ± 8.81	18.38 ± 8.42	0.513

Table 4: Estimated risk of an oral-facial cleft in two groups according to the zinc concentration

Group	n	95% CI	Odds ratio	Zn plasma concentration		Group
				11 ($\mu\text{mol/L}$)	11 ($\mu\text{mol/L}$)	
Case	48	0.612–4.537	1.66	12	36	Case
Control	48			8	40	Control

CI: Confidence interval

heterogeneity including different time points in assessing maternal zinc status.

In recent years, some experimental studies demonstrated that the consumption of zinc supplementations could ameliorate fetal malformations and even mortality that is caused by different genetic and environmental triggers. In this regard, the use of these supplements leads to dealing with pollution, bacterial lipopolysaccharides, and a protein deficient diet.^[12] Furthermore, it seems that the use of these supplements results in downregulating some polymorphisms in the zinc transporter genes so that the deficit in stored body zinc by the limitation of dietary intake of this element may result in unfolding of the mutation effects.^[13-15] One probable reason for our insignificant relationship between plasma level of zinc and developing orofacial cleft may be due to the absence of these gene polymorphisms in our studied women. It, therefore, seems reasonable to test mutations in genes that are involved in the metabolism of zinc as candidate genes involved in the orofacial cleft occurrence.

Study limitations

Our study had some potential limitations. One limitation is the retrospective nature of the study and the impossibility of controlling all confusing factors. In this study, only plasma level of zinc was considered as a sole indicator of zinc status. It is well recognized that zinc plasma level may not be a dependable indicator of zinc status because it is affected by many factors.^[16] Nutritional status of other nutrients as well as genetic and environmental factors should be taken into account for evaluating such associations that were ignored in our survey.

CONCLUSION

Despite lower zinc plasma levels in women of the case group, the difference in the concentration of this element was not statistically significant between the two groups. Along with the current study, some of the investigations in the literature were unable to show the increased risk of developing orofacial cleft malformations due to small sample sizes or ignoring different genetic and environment confounding factors affecting this risk estimation. In general, based on current information and comparing the results of studies conducted in this field, there is no definitive conclusion on whether the deficiency of nutritional elements in humans during pregnancy is the cause of malformations or not, and it is still necessary to study which are well controlled and minimized by a number of confusing factors.

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