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Original article

Higher concentration of selenium in placental tissues is associated with reduced risk for orofacial clefts

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SUMMARY

Background & aims: Growing evidence suggests that essential trace element imbalance during pregnancy may contribute to fetal malformations, but the role of essential trace elements in the occurrence of orofacial clefts (OFCs) is unknown. We aimed to examine the association between concentrations of zinc (Zn), manganese (Mn), selenium (Se), cobalt (Co), molybdenum (Mo), and nickel (Ni) in placental tissues and the risk for OFCs in offspring in a rural population in northern China with a high prevalence of OFCs.
Methods: The case–control study subjects were 103 OFC infants and 206 non-malformed infants. The concentrations of selected trace elements in placental tissues were determined using inductively coupled plasma-mass spectrometry. Sociodemographic information was collected from the mothers through face-to-face interviews using a structured questionnaire. The risk for OFCs in association with higher concentrations of the trace elements was estimated using the odds ratio (OR) with its 95% confidence interval (95% CI).
Results: The placental median concentrations of Se and Ni were significantly lower, but those of Mo were significantly higher in OFC cases than in controls (all $P < 0.05$). A Se concentration above the median of all subjects was associated with a 58% reduced risk for OFCs (adjusted OR 0.42, 95% CI 0.23, 0.77) after adjusting for potential confounding factors. The risk for OFCs decreased with increases in placental Se concentrations, with adjusted ORs of 0.45 (95% CI 0.22, 0.92) for the second tertile and 0.22 (95% CI 0.10, 0.49) for the top tertile of Se concentration, with the lowest tertile concentration as the referent ($P_{trend} < 0.001$). No association was observed between placental Zn, Mn, Co, Mo, or Ni concentration and risk for OFC.
Conclusions: The concentration of Se in placental tissues was dose-dependently associated with decreased risk for OFCs in offspring. This finding suggests that maternal Se intake during pregnancy may protect against OFCs in offspring.

1. Introduction

Orofacial clefts (OFCs), the most common craniofacial anomalies in humans, result from the failure of the lip and/or palate to grow or fuse by the 4th to 8th and/or 6th to 12th week of gestation [1]. The etiology of OFCs is multifactorial and involves the environment, genetics, and the interaction between them. It is well documented that maternal nutritional status during pregnancy not only has a profound effect on mothers' health but also affects the organogenesis, growth, and health of the fetuses and newborns [2–4]. Essential trace elements, mainly derived from various foods in diet, are important micronutrients in fetal development [5]. Thus, micronutrient deficiencies during pregnancy may contribute to the occurrence of fetal malformations.

Animal experiments have shown that adequate zinc (Zn) has a protective effect on palate development, while Zn deficiency or

Abbreviations: BMI, body mass index; Co, cobalt; CI, confidence interval; ICP-MS, inductively coupled plasma-mass spectrometry; Mn, manganese; Mo, molybdenum; NTDs, neural tube defects; Ni, nickel; OFCs, orofacial clefts; OR, odds ratio; P_{25}, lower quartile; P_{75}, upper quartile; Se, selenium; Zn, zinc.

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insufficiency during pregnancy can induce cleft palate (CP) [6–9]. No reports are available on teratogenicity in animals regarding the inadequacy or excess of manganese (Mn), selenium (Se), cobalt (Co), molybdenum (Mo), or nickel (Ni). Moreover, there have been no epidemiological studies on the associations among any of these trace elements and OFC risk in offspring. There are reports on the associations between these trace elements and other congenital malformations. For example, Zn, Se, Co, and Ni deficiency and excess of Mn have been reported to be associated with elevated risk for neural tube defects (NTDs) and diaphragmatic hernia [10–16]. Therefore, it is of interest to know whether these trace elements are associated with the risk for OFCs. Knowledge in this area could inform nutritional interventions for OFCs.

The placenta is responsible for the exchange of nutrients and waste products between the fetus and the mother. As a birth biospecimen, the placenta does not block the transport of the aforementioned trace elements to the fetus, and it has the ability to store biochemical information about events during gestation [17–20]. Therefore, placental concentrations of trace elements may be used as a biomarker to reflect the intrauterine environment and maternal nutritional status during pregnancy.

We hypothesized that higher concentrations of selected trace elements in placental tissues may be associated with increased or decreased risk for OFCs in offspring. To test this hypothesis, we measured the concentrations of Zn, Mn, Se, Co, Mo, and Ni in placental tissues of newborns with or without OFCs and examined the association between these trace elements and the risk for OFCs in a rural area of China, where the prevalence rate of OFCs was high, 3.27 per 1000 births in 2003–2004 [21], compared to the average birth prevalence rates ranging from 1.57 to 0.57 per 1000 births among the six continents around the globe during 1950 and 2015 [22].

2. Material and methods

2.1. Study design and participants

We used a case–control study design, which has been described in more detail elsewhere [23]. Briefly, the participants were recruited from five rural counties (Pingding, Taigu, Shouyang, Xiyang, and Zezhou) of Shanxi Province in northern China, where a population-based birth defects surveillance program was established to monitor major external structural birth defects (OFCs, NTDs, congenital hydrocephalus, etc.) in 2003. County healthcare workers made diagnoses of newborns or terminated fetuses with birth defects through physical examinations, supplemented with prenatal ultrasound scans. When a newborn or fetus affected by a major external birth defect was ascertained, a newborn with no congenital malformation was selected as a control to match the case by residence of the mother (the same county), date of last maternal menstrual period (±4 weeks), and newborn sex. Maternal peripheral blood samples, placental tissue, and umbilical cord blood samples were requested after informed consent had been obtained from the mother.

In this study, we included all 103 OFC cases with available placental tissues as the case group (16 OFC cases had other malformations, i.e., NTDs) and 206 newborns that were randomly selected from among 509 non-malformed newborns with available placental tissues as the control group, enrolled from January 2003 through December 2016.

2.2. Questionnaire interview

Participants received face-to-face interviews before discharge (1–10 days after delivery) from the hospital, using a structured questionnaire to collect sociodemographic information by trained healthcare workers. Maternal age was determined at delivery (<25, 25–29, ≥30 years). Educational status was classified into three categories (junior high or lower, high school, college or higher). Folic acid use was defined as having ever taken folic acid supplements or multivitamins containing folic acid during the periconceptional period (1 month before, to 2 months after, conception) (yes/no). Influenza or fever was defined as having a fever 38.5 °C or higher lasting >24 h or having a cold or the influenza during the periconceptional period (yes/no). Passive smoking was restricted to exposure to secondhand smoke on average at least once per week and at least 1 cigarette each time from other people at home or in public places during the periconceptional period (yes/no). Alcohol drinking included use of liquor, beer, or wine during the periconceptional period (yes/no). Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Before data collection, interviewers helped women confirm the exposure period according to the date of the last menstrual period.

2.3. Placenta sample preparation and trace element assessment

Placentas were collected immediately after delivery and stored frozen at −20 °C up to the time of the measurement. After thawing at 4 °C, approximately 6 g wet sample within 3 cm around the point of cord attachment on the fetal portion of the placenta was cut, using a titanium tool to avoid external metal contamination. The specimens were cleansed of blood three times using deionized water, blotted on clean tissue paper, and freeze-dried (ALPHA2-4 LD plus, Christ, Germany) for 24 h to remove water.

Approximately 0.2 g (CP225D, Sartorius, Germany) lyophilized placental tissue was digested with 2.0 mL nitric acid (UP-grade) and 0.5 mL hydrogen peroxide (UP-grade) in quartz digestion vessels under a high-pressure microwave digestion system (Ultra WAVE, Milestone, Italy). The dissolved placental tissues reached 15 mL volume and were diluted four times with deionized water. Finally, the concentrations of Zn, Mn, Se, Co, Mo, and Ni were evaluated via inductively coupled plasma-mass spectrometry (ICP-MS, 7700x, Agilent, USA). A blank solution alongside each of 15 placenta samples, standard material made from pig liver (GBW10051) with known concentrations of trace elements of interest, and certified standard from Chinese national reference materials (GSB 04-1767-2004 for Zn, Mn, Co, and Ni, 100 μg/mL; GSB 04-1737-2004 for Mo, 1000 μg/mL; GSB 04-1751-2004 for Se, 1000 μg/mL) were also simultaneously analyzed on the instrument to check for possible contamination during the digestion procedure and sample operation, to guarantee the accuracy of the results, as well as to generate and validate the standard curves, respectively.

During the whole process, the operators were masked to the group information (i.e., case or control) of the placenta samples. Rhenium (GSB 04-1745-2004) was used as an internal reference to calibrate and check the stability of the ICP-MS. All correlation coefficients of the regression lines for the calibration curves of the six trace elements were greater than 0.999. The limits of detection for Zn, Mn, Se, Co, Mo, and Ni were 0.022 ng/mL, 0.028 ng/mL, 0.328 ng/mL, 0.000 ng/mL, 0.002 ng/mL, and 0.016 ng/mL, respectively. The detection rate was 100% for Zn, Mn, Se, Co, and Mo, but 85% for Ni. Samples with a concentration of Ni lower than the detection limit were recorded as zero in data analyses.

The study was approved by the Institutional Review Board of Peking University (Beijing, China). All participants provided written informed consent.

2.4. Statistical analysis

The differences in sociodemographic characteristics between the case and control groups were tested using Chi–square or

Fisher's exact tests. Descriptive statistics for the concentrations of the six trace elements in the two groups were shown as medians along with lower quartile (P_{25}) and upper quartile (P_{75}) due to a skewed distribution. The Mann–Whitney U test was used to compare the differences in the median concentrations of the six trace elements between OFC cases and controls.

The median concentration of a given trace element for all subjects was used as the cutoff value to estimate the associations between a higher concentration of each of the six trace elements and the risk for OFCs in offspring. The risk was evaluated using the odds ratio (OR) with its 95% confidence interval (CI) with unconditional logistic regression. The selection of potential confounders was based on literature and the significant association with outcome and exposure in univariate analyses, including maternal age, BMI, farming occupation, influenza or fever, passive smoking, and alcohol drinking during periconceptional period, gestational age, and previous history of pregnancy affected by birth defects. In dose–response relationship analyses, specific trace element concentrations in all subjects were divided into tertiles. A two-sided P value of <0.05 was considered statistically significant. All data were analyzed using SPSS Statistics 20 (IBM Corp., NY, USA).

3. Results

The characteristics of the cases and controls are presented in Table 1. Almost all (99.7%) of the subjects were of the Han ethnic group, the largest in China. No differences were found between the case and control groups with regard to maternal age, BMI, or education. Active smoking was rare in both groups. OFC infants were more likely than control infants to experience shorter gestation, and their mothers had higher proportions of self-reported previous history of pregnancy affected by birth defects, influenza or fever, or passive smoking during the periconceptional period; however, more control mothers delivered female newborns and worked as farmers (all $P < 0.05$). About half of all mothers reported using folic acid during the periconceptional period; the proportions in each group did not significantly differ.

The concentrations of the six trace elements in placental tissues in cases and controls are shown in Table 2. The concentrations of Se (1.03 μg/g) and Ni (10.20 ng/g) were significantly lower in OFC cases than in controls (Se 1.11 μg/g, Ni 12.89 ng/g), while placental Mo concentrations were significantly higher in the case group (35.9 ng/g) than in controls (32.1 ng/g) (all $P < 0.05$). No significant differences in Zn, Mn, and Co concentrations were found between the groups.

Because concentrations of trace elements may change over the course of a pregnancy, and mean and median gestational age were shorter in OFC cases than in controls, we further examined the correlation between concentrations of trace elements in placental tissues and gestational age in the case group, which had wider variation in gestational age, enabling us to do an analysis by gestation. The concentrations of Zn, Mn, and Mo decreased with gestation, while the concentration of Co increased with gestation (Table 3).

The associations between trace element concentrations in placental tissues and the risk for OFCs are listed in Table 4. In univariate analyses, higher concentrations of Se and Ni were associated with decreased risk while higher concentrations of Mo were associated with increased risk for OFCs. After taking into account potential confounders, higher concentrations of Se were

Table 2
Concentrations of elements in placental tissues (dry weight) of infants with or without orofacial clefts in northern rural China, 2003–2016.

Elements	Median (P_{25}–P_{75})		P[a]
	Controls (n = 206)	Cases (n = 103)	
Zn (μg/g)	68.3 (62.1–75.0)	69.2 (63.0–77.5)	0.224
Mn (μg/g)	0.54 (0.46–0.63)	0.55 (0.48–0.63)	0.133
Se (μg/g)	1.11 (1.02–1.24)	1.03 (0.90–1.13)	<0.001
Co (ng/g)	16.59 (12.47–22.07)	14.61 (11.34–21.20)	0.124
Mo (ng/g)	32.1 (27.3–37.0)	35.9 (31.7–41.8)	<0.001
Ni (ng/g)	12.89 (7.92–20.70)	10.20 (7.27–13.79)	0.011

Abbreviations: Co, cobalt; Mn, manganese; Mo, molybdenum; Ni, nickel; P_{25}, 25th percentile; P_{75}, 75th percentile; Se, selenium; Zn, zinc.
[a] Comparison of median values of controls by Mann–Whiney U test.

Table 1
Characteristics of mothers of infants with or without orofacial clefts in northern rural China, 2003–2016.

Characteristics	Controls (n = 206)[a]	Cases (n = 103)[a]	P[b]
	No. (%)	No. (%)	
Maternal age (years)			0.142
<25	91 (44.2)	44 (42.7)	
25–29	63 (30.6)	41 (39.8)	
≥30	51 (24.8)	17 (16.5)	
Maternal BMI (kg/m^2)			0.918
<18.5	26 (12.6)	12 (11.7)	
18.5–24.9	129 (62.6)	66 (64.1)	
≥25.0	50 (24.3)	23 (22.3)	
Maternal education			0.991
Junior high or lower	134 (65.0)	67 (65.0)	
High school	39 (19.0)	19 (18.5)	
College or higher	33 (16.0)	17 (16.5)	
Maternal farming occupation	165 (80.1)	66 (64.1)	0.002
Newborn male sex	102 (49.5)	64 (62.1)	0.017
Gestational age < 37 weeks	9 (4.4)	26 (25.2)	<0.001
Primigravida	85 (41.3)	45 (43.7)	0.684
Primiparas	107 (51.9)	62 (60.2)	0.170
Previous history of pregnancy affected by birth defects	2 (1.0)	5 (4.9)	0.031
Maternal folic acid use	111 (53.9)	53 (51.5)	0.475
Maternal influenza or fever	30 (14.6)	36 (35.0)	<0.001
Maternal passive smoking	69 (33.5)	58 (56.3)	<0.001
Maternal alcohol drinking	14 (6.8)	12 (11.7)	0.151

[a] Values for some characteristics may not be equal to total numbers of OFC cases or controls because of missing or unknown data. BMI, body mass index.
[b] Comparison between the case and control groups using the Chi–square or Fisher's exact tests.

Table 3
Spearman correlation between concentrations of elements in placental tissues (dry weight) and gestational age in northern rural China, 2003–2016.

Elements	Gestational age[a]			*r*
	20–27 weeks (n = 15)	28–36 weeks (n = 11)	≥37 weeks (n = 77)	
Zn (μg/g)	78.2 (68.4–83.0)	74.2 (66.4–83.0)	67.4 (61.9–74.8)	−0.318[c]
Mn (μg/g)	0.62 (0.53–0.66)	0.64 (0.56–0.71)	0.54 (0.48–0.62)	−0.242[b]
Se (μg/g)	1.04 (0.90–1.10)	1.06 (0.91–1.13)	1.03 (0.90–1.13)	−0.004
Co (ng/g)	10.27 (7.98–11.67)	11.93 (8.40–21.17)	17.18 (13.18–22.28)	0.478[c]
Mo (ng/g)	39.5 (35.6–49.5)	39.2 (35.7–43.3)	35.3 (29.1–39.5)	−0.343[c]
Ni (ng/g)	12.43 (6.92–16.58)	7.84 (6.44–13.36)	10.03 (7.39–13.31)	−0.029

Abbreviations: Co, cobalt; Mn, manganese; Mo, molybdenum; Ni, nickel; Se, selenium; Zn, zinc.
[a] Data are shown as medians (lower quartile–upper quartile).
[b] $P < 0.05$.
[c] $P < 0.01$.

Table 4
Element concentrations in placental tissues (dry weight) and risk for orofacial clefts in northern rural China, 2003–2016.

Elements	Cutoff value[a]	No. of cases/controls	Crude OR[b] (95% CI)	Adjusted OR[b,c] (95% CI)
Zn (μg/g)	≥68.6	54/101	1.15 (0.71, 1.84)	0.90 (0.50, 1.60)
Mn (μg/g)	≥0.54	59/106	1.27 (0.79, 2.04)	1.44 (0.80, 2.59)
Se (μg/g)	≥1.09	38/120	0.42 (0.26, 0.68)	0.42 (0.23, 0.77)
Co (ng/g)	≥15.75	44/111	0.64 (0.40, 1.03)	0.93 (0.52, 1.66)
Mo (ng/g)	≥33.6	65/90	2.20 (1.36, 3.58)	1.42 (0.78, 2.59)
Ni (ng/g)	≥11.39	37/94	0.58 (0.35, 0.97)	0.62 (0.33, 1.16)

Abbreviations: Co, cobalt; CI, confidence interval; Mn, manganese; Mo, molybdenum; Ni, nickel; OR, odds ratio; Se, selenium; Zn, zinc.
[a] Cutoff value: the median of all subjects.
[b] ORs were from an unconditional logistic regression.
[c] Adjusted for maternal age, BMI, farming occupation, flu or fever, passive smoking, and alcohol drinking during periconceptional period, newborn sex, gestational age, and previous history of pregnancy affected by birth defects.

associated with a 58% (95% CI 0.23, 0.77) decreased risk for OFCs. However, the associations for Mo and Ni became nonsignificant (adjusted OR 1.42, 95% CI 0.78, 2.59 for Mo; adjusted OR 0.62, 95% CI 0.33, 1.16 for Ni). No associations were observed between higher concentrations of Zn, Mn, and Co and the risk for OFCs in either univariate or multivariate analyses.

To investigate possible dose–response relationships between concentrations of Se, Ni, and Mo in placental tissues and the risk for OFCs, we further categorized the concentrations of these trace elements according to the tertiles of each trace element in all subjects and calculated the corresponding ORs and adjusted ORs for each category (Fig. 1 and Supplementary Table 1). A decreasing trend in OFC risk with the tertile of Se in placental tissues was observed ($P_{trend} < 0.001$).

Next, we investigated correlations between the consumption frequencies of certain foods by mothers during the periconceptional period and concentrations of trace elements in placental tissues (Supplementary Table 2). Only Co was positively correlated with higher consumption of bean or bean products and pickled vegetables.

The results did not change materially when cases with other malformations were excluded for all trace elements, except Ni. The association between Ni concentrations in placental tissues and the risk for isolated OFCs became significant before (Crude OR 0.41, 95% CI 0.23, 0.74) and after (adjusted OR 0.34, 95% CI 0.16, 0.70) adjusting for potential confounders (Supplementary Table 3). However, in dose–response analyses, only the top tertile of Ni was associated with a decreased risk for isolated OFCs (Supplementary Table 4).

4. Discussion

In this study, we used placental concentrations of Zn, Mn, Se, Co, Mo, and Ni as an internal exposure marker to investigate the association between exposure to these trace elements and the risk for OFCs in offspring. We found that higher Se concentrations in placental tissues were associated with reduced risk for OFCs in a dose-dependent manner. The associations between placental Mo and Ni concentrations and OFC risk became nonsignificant after adjusting for potential confounders, and no associations between placental Zn, Mn, and Co concentrations and OFC risks were observed. Our results support the hypothesis that insufficiency or deficiency of certain trace elements during pregnancy may increase the risk for OFCs in offspring.

Placental concentrations of trace elements vary with the population studied. In our study, the concentrations of Zn in the

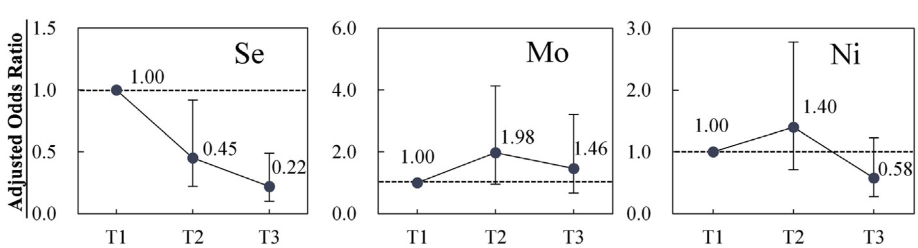

Fig. 1. Adjusted odds ratios of orofacial clefts in association with the tertiles of selenium (Se), molybdenum (Mo), and nickel (Ni). The three concentration levels categorized by tertile of all subjects were recorded as T1 (lowest), T2 (second), and T3 (top).

case and control groups were similar to reports from Germany and Bangladesh (in both populations, concentrations were 66 μg/g) [24,25]; higher than the concentrations reported from Japan (48.1 μg/g), India (50.5 μg/g), and the Czech Republic (54.6 μg/g) [19,26,27]; and approximately two-thirds of the concentrations reported in a study from Sweden (93.2 μg/g) [28]. Median Mn concentrations for both groups in our study were about the same as those in a report from Bangladesh (0.53 μg/g) [25], but higher than those reported in healthy Swedish pregnant women (0.07 μg/g) [28]. The placental medians of Se, Co, and Mo concentrations in OFC cases and controls in our study were far greater than those reported in Sweden (0.16 μg/g, 2.71 ng/g, and 5.71 ng/g, respectively) [28], but our values for Se were similar to those reported in a previous study on a Japanese population (1.04 μg/g) [19] and lower than those reported in Bangladesh (1.4 μg/g) [25]. The median concentrations of Ni in placental tissues in both groups in our study were lower than the concentrations of Chinese women living in a natural area (14.30 ng/g) (Supplementary Table 5) [29].

We also found that placental concentrations of Zn, Mn, and Mo decreased but Co increased with gestation. Similarly, a previous study found that Zn contents in placenta were significantly higher during the first trimester than at term [30]. A study from Sweden found that women with a gestational age over 281 days at birth were more likely to have higher Co and Mo concentrations in their placental tissues [28]. In our study, higher Zn concentrations and lower Co concentrations were also observed in the shorter gestational age subgroup of the case group. Thus, the association between Zn concentrations and risk for OFCs may be underscored, while the protective effects of Co on the development of lip and/or palate may be overestimated. Further investigations to establish whether women could potentially benefit from the optimization of Zn and Co status are needed. To the best of our knowledge, no study has investigated the potential correlation between Mn concentrations in placental tissues and pregnancy duration. Because the concentrations of Se did not change with gestational age, adjustment for gestational age had little impact on the association observed in univariate analyses.

Our findings suggest that a higher concentration of Se in placental tissues is associated with a reduction in the risk for OFCs. To the best of our knowledge, no report in the literature is available on the relationship between maternal or fetal Se exposure and the risk for OFCs. Studies conducted in Saudi Arabia and Turkey found that mothers of infants with NTDs had significantly lower serum Se during the first and second trimester than mothers of healthy infants [13,31], which suggests that Se is protective against fetal NTDs. The protective role of Se may result from its important biological function as an antioxidant. Pregnancy is an exceptional condition of oxidative stress, resulting from enhanced demand for various nutrients and oxygen in the body of the mother and the developing fetus [32]. Oxidative stress may be involved in the pathophysiology of OFCs [13,33]. Se is a constituent of selenoproteins, which are part of the defense system against oxidative stress [34]. Therefore, maternal Se deficiencies can be considered a contributory factor to the impairment of the functional activities of selenoproteins, resulting in oxidative stress and adversely affecting lip/palate development.

We found no association between placental Zn concentrations and the risk for OFCs in offspring. This is consistent with three previous studies, which reported Zn concentrations in maternal plasma and toenails were not associated with the risk for OFCs [35–37]. However, studies conducted in The Netherlands, the Philippines, and Poland have reported that lower maternal red blood cells, plasma, serum, and whole blood Zn are a risk factor for OFCs in offspring [38–41]. The type of biological specimens used for Zn measurement may contribute to the differences in findings. We also observed that placental Mn concentrations were not associated with risk for OFCs. Stoll et al. [35] reported that mothers who had conceived OFC babies had Mn concentrations in plasma at 6–13 weeks of pregnancy that were similar to mothers who had conceived healthy babies. The results of an ecological study were also suggestive of no association between Mn concentrations in drinking water and the prevalence of OFCs [42]. Our findings are in line with these studies. Animal experiments have found that Co has a potential role in the prevention of CP occurrence in mice [43,44]. However, there has been no evidence of the protective effects of Co on OFCs in humans. In our univariate analyses, higher Mo concentrations in placental tissues were associated with elevated risk for OFCs; however, the associations became nonsignificant after adjusting for potential confounders. More studies are needed on this topic.

In this study, higher Ni concentrations in placental tissues were not associated with risk for total OFCs but were protective for isolated OFCs in the top tertile concentration. OFCs with other malformations are considered syndromic and are more likely to be affected by genetic factors [45], while isolated OFCs are multifactorial, for which environmental factors may play a major role. A study conducted on the same population found that Ni in maternal hair is protective against fetal NTD risk [10]. Thus, higher concentrations of Ni may be protective against OFC risk, but more studies are needed to confirm this finding.

Some limitations of this study need to be addressed. First, we did not measure selenoenzymes (e.g. glutathione peroxidase) activity in placental tissues. This is because the placental tissues were collected years ago and stored at −20 °C. Enzyme activity would not reflect the activity at the time of delivery or pregnancy termination. Second, we did not measure Se concentration or glutathione peroxidase activity in plasma/serum. Plasma or serum has a short turnover, Se concentration or enzyme activity in plasma/serum can only reflect the status of past days or weeks. Third, trace element concentrations in placental tissues may not precisely represent the concentrations of these trace elements at the critical window of lip and palate development. Forth, we measured total metal concentrations, which confined our ability to determine the effects of their different forms or valence states.

The current study also had several strengths. The primary one was the use of placenta as the biospecimen for trace element assessment. Unlike serum and maternal hair, the placenta may reflect a longer period of past exposure and is not subject to ambient air or other environmental contamination. In addition, the healthcare workers who participated in this study were trained to standardize the face-to-face interviews, which should help minimize recall error. Finally, the group statuses of the subjects were blind to researchers during sample preparation and assessment procedures, helping to reduce the possible introduction of bias.

5. Conclusions

This study suggests that higher concentrations of Se in placental tissues are associated with decreased risk for OFCs in a dose-dependent pattern. Maternal dietary intake of Se during pregnancy may protect against OFCs in offspring. The possible protective role of high concentrations of Ni requires further investigation. No associations were found between the placental concentrations of Zn, Mn, Co, and Mo and the risk for OFCs. Further studies are warranted to replicate the findings of the present study and to elucidate the underlying mechanisms of the association that was observed.

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Statement of authorship

Xin Pi and Yihui Wei participated in sample preparation and assessment procedures. Xin Pi conducted the data analysis and drafted the manuscript. Aiguo Ren conceptualized the study, supervised the implementation, and revised the manuscript. Zhiwen Li, Lei Jin, Jufen Liu, Yali Zhang, and Linlin Wang participated in the implementation of the study and provided critical comments on the manuscript. All authors reviewed the manuscript and approved the final version for submission.

Conflict of Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2018.11.002.

References

[1] Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. Lancet 2009;374:1773–85.
[2] Allen LH. Multiple micronutrients in pregnancy and lactation: an overview. Am J Clin Nutr 2005;81:1206s–12s.
[3] Hong J, Park EA, Kim YJ, Lee HY, Park BH, Ha EH, et al. Association of antioxidant vitamins and oxidative stress levels in pregnancy with infant growth during the first year of life. Public Health Nutr 2008;11:998–1005.
[4] Steegers-Theunissen RP, Steegers EA. Nutrient-gene interactions in early pregnancy: a vascular hypothesis. Eur J Obstet Gynecol Reprod Biol 2003;106:115–7.
[5] Lewicka I, Kocylowski R, Grzesiak M, Gaj Z, Oszukowski P, Suliburska J. Selected trace elements concentrations in pregnancy and their possible role - literature review. Ginekol Pol 2017;88:509–14.
[6] Hurley LS. Teratogenic aspects of manganese, zinc, and copper nutrition. Physiol Rev 1981;61:249–95.
[7] Hurley LS, Swenerton H. Congenital malformations resulting from zinc deficiency in rats. Proc Soc Exp Biol Med 1966;123:692–6.
[8] Brownie CF, Brownie C, Noden D, Krook L, Haluska M, Aronson AL. Teratogenic effect of calcium edetate (CaEDTA) in rats and the protective effect of zinc. Toxicol Appl Pharmacol 1986;82:426–43.
[9] Quinn PB, Cremin FM, O'Sullivan VR, Hewedi FM, Bond RJ. The influence of dietary folate supplementation on the incidence of teratogenesis in zinc-deficient rats. Br J Nutr 1990;64:233–43.
[10] Yan L, Wang B, Li Z, Liu Y, Huo W, Wang J, et al. Association of essential trace metals in maternal hair with the risk of neural tube defects in offspring. Birth Defects Res 2017;109:234–43.
[11] Zeyrek D, Soran M, Cakmak A, Kocyigit A, Iscan A. Serum copper and zinc levels in mothers and cord blood of their newborn infants with neural tube defects: a case-control study. Indian Pediatr 2009;46:675–80.
[12] Dey AC, Shahidullah M, Mannan MA, Noor MK, Saha L, Rahman SA. Maternal and neonatal serum zinc level and its relationship with neural tube defects. J Health Popul Nutr 2010;28:343–50.
[13] Hammouda SA, Abd Al-Halim OA, Mohamadin AM. Serum levels of some micronutrients and congenital malformations: a prospective cohort study in healthy saudi-arabian first-trimester pregnant women. Int J Vitam Nutr Res 2013;83:346–54.
[14] Martin I, Gibert MJ, Pintos C, Noguera A, Besalduch A, Obrador A. Oxidative stress in mothers who have conceived fetus with neural tube defects: the role of aminothiols and selenium. Clin Nutr 2004;23:507–14.
[15] Liu J, Jin L, Zhang L, Li Z, Wang L, Ye R, et al. Placental concentrations of manganese and the risk of fetal neural tube defects. J Trace Elem Med Biol 2013;27:322–5.
[16] Yang W, Shaw GM, Carmichael SL, Rasmussen SA, Waller DK, Pober BR, et al. Nutrient intakes in women and congenital diaphragmatic hernia in their offspring. Birth Defects Res A Clin Mol Teratol 2008;82:131–8.
[17] Iyengar GV, Rapp A. Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 2: essential minor, trace and other (non-essential) elements in human placenta. Sci Total Environ 2001;280:207–19.
[18] Iyengar GV, Rapp A. Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: toxic trace elements in placenta and placenta as a biomarker for these elements. Sci Total Environ 2001;280:221–38.
[19] Sakamoto M, Yasutake A, Domingo JL, Chan HM, Kubota M, Murata K. Relationships between trace element concentrations in chorionic tissue of placenta and umbilical cord tissue: potential use as indicators for prenatal exposure. Environ Int 2013;60:106–11.
[20] Ryan JG, Davis RK, Bloch JR. The placenta as a research biospecimen. J Obstet Gynecol Neonatal Nurs 2012;41:834–45.
[21] Li Z, Ren A, Liu J, Zhang L, Ye R, Li S, et al. High prevalence of orofacial clefts in Shanxi Province in northern China, 2003-2004. Am J Med Genet A 2008;146a:2637–43.
[22] Panamonta V, Pradubwong S, Panamonta M, Chowchuen B. Global birth prevalence of orofacial clefts: a systematic review. J Med Assoc Thai 2015;98(Suppl 7):S11–21.
[23] Ren A, Qiu X, Jin L, Ma J, Li Z, Zhang L, et al. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. Proc Natl Acad Sci U S A 2011;108:12770–5.
[24] Schramel P, Lill G, Hasse S, Klose BJ. Mineral- and trace element concentrations in human breast milk, placenta, maternal blood, and the blood of the newborn. Biol Trace Elem Res 1988;16:67–75.
[25] Kippler M, Hoque AM, Raqib R, Ohrvik H, Ekstrom EC, Vahter M. Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. Toxicol Lett 2010;192:162–8.
[26] Reddy YS, Y A, Ramalaksmi BA, Kumar BD. Lead and trace element levels in placenta, maternal and cord blood: a cross-sectional pilot study. J Obstet Gynaecol Res 2014;40:2184–90.
[27] Fiala J, Hruba D, Rezl P. Cadmium and zinc concentrations in human placentas. Cent Eur J Public Health 1998;6:241–8.
[28] Fagerstedt S, Kippler M, Scheynius A, Gutzeit C, Mie A, Alm J, et al. Anthroposophic lifestyle influences the concentration of metals in placenta and cord blood. Environ Res 2015;136:88–96.
[29] Guo Y, Huo X, Li Y, Wu K, Liu J, Huang J, et al. Monitoring of lead, cadmium, chromium and nickel in placenta from an e-waste recycling town in China. Sci Total Environ 2010;408:3113–7.
[30] Kantola M, Purkunen R, Kroger P, Tooming A, Juravskaja J, Pasanen M, et al. Accumulation of cadmium, zinc, and copper in maternal blood and developmental placental tissue: differences between Finland, Estonia, and St. Petersburg. Environ Res 2000;83:54–66.
[31] 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;97:225–35.
[32] Chao HC. Impact of maternal selenium supplementation on neonates. Pediatr Neonatol 2012;53:327–8.
[33] Knott L, Hartridge T, Brown NL, Mansell JP, Sandy JR. Homocysteine oxidation and apoptosis: a potential cause of cleft palate. In Vitro Cell Dev Biol Anim 2003;39:98–105.
[34] Rayman MP. Selenium and human health. Lancet 2012;379:1256–68.
[35] Stoll C, Dott B, Alembik Y, Koehl C. Maternal trace elements, vitamin B12, vitamin A, folic acid, and fetal malformations. Reprod Toxicol 1999;13:53–7.
[36] Munger RG, Tamura T, Johnston KE, Feldkamp ML, Pfister R, Carey JC. Plasma zinc concentrations of mothers and the risk of oral clefts in their children in Utah. Birth Defects Res A Clin Mol Teratol 2009;85:151–5.
[37] McKinney CM, Pisek A, Chowchuen B, DeRouen T, Muktabhant B, Pradubwong S, et al. Case-control study of nutritional and environmental factors and the risk of oral clefts in Thailand. Birth Defects Res A Clin Mol Teratol 2016;106:624–32.
[38] Krapels IP, Rooij IA, Wevers RA, Zielhuis GA, Spauwen PH, Brussel W, et al. Myo-inositol, glucose and zinc status as risk factors for non-syndromic cleft lip with or without cleft palate in offspring: a case-control study. Bjog 2004;111:661–8.
[39] Tamura T, Munger RG, Corcoran C, Bacayao JY, Nepomuceno B, Solon F. Plasma zinc concentrations of mothers and the risk of nonsyndromic oral clefts in their children: a case-control study in the Philippines. Birth Defects Res A Clin Mol Teratol 2005;73:612–6.
[40] Hozyasz KK, Ruszczynska A, Bulska E. Low zinc and high copper levels in mothers of children with isolated cleft lip and palate. Wiad Lek 2005;58:382–5.

[41] Hozyasz KK, Kaczmarczyk M, Dudzik J, Bulska E, Dudkiewicz Z, Szymanski M. Relation between the concentration of zinc in maternal whole blood and the risk of an infant being born with an orofacial cleft. Br J Oral Maxillofac Surg 2009;47:466–9.
[42] Sanders AP, Desrosiers TA, Warren JL, Herring AH, Enright D, Olshan AF, et al. Association between arsenic, cadmium, manganese, and lead levels in private wells and birth defects prevalence in North Carolina: a semi-ecologic study. BMC Public Health 2014;14:955.
[43] Kasirsky G, Sherman WT, Gautieri RF, Mann Jr DE. Cobalt-cortisone interrelationships in the induction and inhibition of cleft palate in mice. J Pharm Sci 1969;58:766–7.
[44] Mitala JJ, Mann Jr DE, Gautieri RF. Influence of cobalt (dietary), cobalamins, and inorganic cobalt salts on phenytoin- and cortisone-induced teratogenesis in mice. J Pharm Sci 1978;67:377–80.
[45] Marazita ML. The evolution of human genetic studies of cleft lip and cleft palate. Annu Rev Genomics Hum Genet 2012;13:263–83.