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Maternal Folic Acid, B12, and Zinc Serum Levels and Risk of Orofacial Cleft in Offspring in Tabriz

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Abstract

Background: Orofacial clefts (OFCs) are prevalent congenital malformations affecting approximately 1 in 700 live births. The etiology of OFCs is multifactorial, encompassing both genetic predispositions and environmental factors, with maternal nutritional status, particularly folic acid, vitamin B12, and zinc levels, playing a crucial role in fetal development. This study investigates the association between maternal serum micronutrient levels and the risk of OFCs in Tabriz.

Methods: A case-control study was conducted over six months at the Children's Hospital of Tabriz University of Medical Sciences, enrolling mothers aged 18-40 with children diagnosed with cleft lip and palate. A total of 66 mothers in the case group and 108 in the control group were assessed. Blood samples were collected to measure serum levels of folic acid, vitamin B12, and zinc using established laboratory techniques. Statistical analyses were performed with SPSS version 21.0, applying appropriate tests(Kolmogorov-Smirnov test, Pearson correlation coefficient, non-parametric tests) to determine significant associations.

Results: The case group exhibited a significantly lower mean Body Mass Index (BMI) (P<0.001) and weight (P<0.001), while no significant differences in height (P=0.102) or age (P=0.489) were observed. Notably, serum folic acid levels were significantly lower in the case group (P<0.001), whereas no significant differences were found for serum zinc (P=0.252) and vitamin B12 levels (P=0.763). Logistic regression analysis indicated that prenatal supplement use was protective against orofacial clefts (OR=0.062, P=0.002), while a positive family history (OR=11.473, P=0.023) and proximity to high voltage power lines (OR=20.085, P=0.001) were associated with increased risk. Furthermore, lower BMI (OR=0.859, P=0.016) and folic acid levels (OR=0.819, P=0.006) correlated with a higher risk of clefts.

Conclusion: This study highlights that low serum folic acid levels in pregnant women significantly correlate with non-syndromic cleft lip and palate in offspring. Additionally, associations with low BMI, pregnancy supplements, and environmental exposures underscore the importance of comprehensive preventive strategies to improve pregnancy outcomes and reduce congenital anomalies.

Keywords: Orofacial Clefts, Maternal Nutrition, Folic Acid, Vitamin B12, Zinc

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Introduction

Orofacial clefts (OFCs) are among the most common congenital malformations, characterized by incomplete formation of the lip and/or palate. Affecting about 1 in 700 live births globally, OFCs represent a significant public health concern due to the complex medical, surgical, and psychological challenges they pose for affected children and their families (1). The etiology of OFCs is multifactorial, with both genetic predispositions and environmental influences implicated (2). Among these environmental influences, maternal nutritional status, specifically micronutrient levels during pregnancy, has gained considerable attention (3). Notably, deficiencies in folic acid, vitamin B12,

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↑What is "already known" in this topic:
Low maternal serum levels of folic acid, vitamin B12, and zinc have been linked to adverse pregnancy outcomes, including congenital anomalies such as orofacial clefts. While folic acid's protective role against neural tube defects is established, its precise association with orofacial clefts remains less clear.

→What this article adds:
This study demonstrates a significant association between low maternal serum folic acid levels and increased risk of nonsyndromic orofacial clefts in offspring. Additionally, it highlights the protective effect of prenatal supplement use and identifies environmental and genetic risk factors, providing evidence for targeted preventive interventions in high-risk populations.

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and zinc have been linked to an increased risk of OFCs, as these nutrients play crucial roles in fetal development, particularly in cellular division, DNA synthesis, and the proper closure of embryonic structures (4).

Folic acid, a B vitamin, is essential for DNA synthesis, repair, and methylation, which are critical processes during early embryogenesis (5, 6). Adequate folic acid levels in mothers have been well-documented to reduce the risk of neural tube defects (NTDs) in offspring, leading to widespread folic acid supplementation programs in many countries. Recent studies suggest that maternal folic acid deficiency may also contribute to the risk of OFCs, although the association is not as clearly established as it is with NTDs (7, 8). Research from different populations suggests that lower folic acid levels might increase the risk of cleft lip with or without cleft palate (CL/P) in the fetus by impairing the cellular processes required for normal orofacial development (9). The proposed mechanisms include disrupted cell proliferation and apoptosis, leading to malformations in facial structures. In this context, folic acid supplementation has been suggested as a preventive measure for reducing the incidence of OFCs, though the evidence remains variable across different genetic and environmental backgrounds (10, 11).

Vitamin B12, another essential nutrient involved in the one-carbon metabolism pathway, plays a synergistic role with folic acid in DNA synthesis and methylation. Maternal vitamin B12 deficiency has been associated with adverse pregnancy outcomes, including NTDs, preterm births, and intrauterine growth restriction (IUGR). Like folic acid, vitamin B12 is essential for DNA synthesis and cellular differentiation, processes vital for fetal tissue formation (12). Limited evidence links maternal B12 deficiency to an increased risk of OFCs, potentially through similar mechanisms of disrupted cellular function and impaired embryonic development. Additionally, the combined deficiencies of folic acid and B12 may exert a compounding effect, as both are essential cofactors in the folate-dependent methylation pathway, underscoring the importance of a well-balanced maternal diet for optimal fetal development (13-15).

Zinc, an essential trace element, is another micronutrient implicated in the risk of congenital anomalies, including OFCs. Zinc plays a pivotal role in DNA replication, cellular division, and protein synthesis, all of which are crucial for embryonic development (9, 16). It is also involved in the stabilization of cell membranes and enzyme systems, influencing a wide range of biological functions that support fetal growth (17). Zinc deficiency in pregnancy has been associated with congenital abnormalities, poor fetal growth, and even preterm labor. Emerging evidence suggests that maternal zinc deficiency may be associated with OFCs, possibly by influencing the structural integrity and function of cellular systems critical for craniofacial development. However, the relationship between maternal zinc levels and OFCs remains less explored compared to folic acid and B12, warranting further investigation (1, 18).

The prevalence of OFCs in various regions is known to vary, potentially reflecting differences in genetic background, dietary habits, and nutritional status among populations (19). In Tabriz, a city with unique dietary patterns and varying socio-economic backgrounds, understanding the impact of maternal micronutrient status on OFC risk is particularly relevant. Dietary intake of folic acid, B12, and zinc may be influenced by regional food availability, cultural practices, and access to prenatal supplementation, potentially affecting maternal serum levels of these nutrients and, consequently, fetal development outcomes. Limited studies have examined the association between maternal folic acid, vitamin B12, and zinc levels with the incidence of OFCs in this region, highlighting a critical gap in the literature (20).

This study aims to investigate the relationship between maternal serum levels of folic acid, vitamin B12, and zinc and the risk of OFCs in offspring in Tabriz. By assessing these associations, we hope to provide insights that may contribute to improved prenatal care guidelines and nutritional recommendations, ultimately reducing the burden of OFCs in the region. This research holds potential implications for targeted nutritional interventions that could benefit at-risk populations, enhancing maternal health and fetal outcomes through a preventive approach. As the understanding of nutritional influences on fetal development continues to evolve, studies such as this one are essential in bridging the knowledge gap and offering evidence-based recommendations for reducing congenital malformations like OFCs.

Methods

Study Design

This case-control study investigated the association between maternal serum levels of folic acid, vitamin B12, and zinc, and the risk of orofacial clefts in offspring. The research was conducted at the Children's Hospital of Tabriz University of Medical Sciences. The study duration was six months, from the first of September 2023, to the first of July 2024. After the initial data collection and analysis, further evaluations were conducted to explore the associations between maternal health factors and the outcomes of children with cleft lip and palate.

Inclusion and Exclusion Criteria

The inclusion criteria required mothers who had children diagnosed with cleft lip and palate without any additional congenital defects. Eligible participants were mothers aged between 18 and 40 years who provided informed consent for participation. Exclusion criteria included the following: incomplete consent forms, the presence of any congenital defects in the children, maternal or paternal congenital defects, systemic diseases such as cardiovascular conditions, and syndromic cleft lip and palate. It should be noted that all maternal blood samples were collected during the first trimester of pregnancy (at the beginning of the third month).

Sampling

Based on the sample size formula for comparing the means of two independent groups, and assuming a significance level of 0.05 (α=0.05), a statistical power of 80% (β=0.2), a standard deviation of 22, and a minimum detectable difference of 10 units between the groups, the required

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sample size for each group was calculated as follows. The standard deviation and anticipated minimum difference were derived from the results of a pilot study involving 5 participants in each group.:

$$n = \frac{2\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)2\sigma^2}{\Delta^2}$$

Using these assumptions, the estimated sample size for each group was 66 participants. To enhance the validity and statistical power of the study, the sample size in the control group was increased to 108 participants. Participants were accessed using a convenience sampling method.

In this case-control study, the case group consisted of mothers who had a child with a non-syndromic cleft lip and/or palate and were identified from among attendees at the Tabriz Children's Hospital during the study period. The control group was selected from mothers whose children were healthy (without any congenital anomalies). To ensure appropriate matching between the groups and to minimize confounding factors, control mothers were matched to cases based on the age and sex of their child, with one or two control mothers selected for each case whose child matched the case child in both age and sex. It is important to note that only mothers whose routine prenatal laboratory results—including validated and documented serum levels of zinc, folic acid, and vitamin B12—were available, were included in the study.

Methodology

Following ethical approval from the Tabriz University of Medical Sciences and the hospital's administration, data collection commenced. A structured questionnaire was used to gather baseline data on participants, including demographic information, medical history, family history, and details regarding the mode of feeding (breastfeeding or bottle-feeding). Blood samples were collected from mothers to analyze levels of folic acid, zinc, and vitamin B12, ensuring that maternal nutrition was assessed for its potential impact on the children's conditions. Blood samples were taken by trained phlebotomists using standard protocols and were stored appropriately for laboratory analysis.

Measurement of Nutritional Factors

Vitamin B12, folic acid, and zinc levels were measured using specific laboratory techniques. Blood samples were drawn from the antecubital vein and collected into vacuum-sealed tubes. For vitamin B12 and folic acid analysis, samples were sent to a laboratory for high-performance liquid chromatography (HPLC) or chemiluminescent immunoassays, which allow for accurate quantification of these vitamins in serum. Zinc levels were determined using atomic absorption spectrophotometry, a reliable method for measuring trace elements in biological samples. All samples were processed and analyzed within 24 hours to ensure the stability of the biochemical markers.

In this study:

The primary outcome was the occurrence of orofacial clefts in the newborns, which was identified and recorded by an ENT specialist through clinical examination. The exposure variable consisted of maternal serum levels of folic acid, vitamin B12, and zinc during the first trimester of pregnancy, which were measured using validated laboratory assays. Predictors included demographic characteristics such as maternal age, medical and family history, and the use or non-use of supplement medications. Potential confounders included maternal age, socioeconomic status, educational level, underlying maternal diseases (such as chronic illnesses or substance abuse), and family history of the disease. Effect modifiers referred to factors such as mode of delivery and infant feeding method (breastfeeding or formula feeding), which may modify the relationship between exposure and outcome.

Statistical Analysis

Statistical analyses were performed using SPSS version 21.0. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. For normally distributed data, quantitative variables were expressed as mean ± standard deviation, while categorical variables were presented as percentages. In cases of non-normally distributed data, non-parametric methods were employed. Correlations between quantitative variables were evaluated using Pearson's correlation coefficient. A *P*-value of less than 0.05 was considered statistically significant.

Results

Living area and education levels showed no significant differences between the groups. However, supplement use was significantly different, with fewer mothers in the case group using pregnancy supplements (59.1% vs. 98.1%, $P<0.001$), vitamin B12 (45.5% vs. 99.1%, $P<0.001$), and folic acid (60.6% vs. 99.1%, $P<0.001$). A positive family history of clefts was notably higher in the case group (18.2% vs. 2.8%, $P=0.004$). Environmental factors such as proximity to high voltage power lines were also significantly higher in the case group (60.6% vs. 18.5%, $P<0.001$), indicating potential environmental and genetic influences on cleft risk (Table 1).

Table 2 presents the baseline characteristics of participants in the case and control groups. Mean age and body mass index (BMI) were comparable between groups. Mean serum concentrations of folic acid, vitamin B12, and zinc were lower in cases than in controls, although these differences are shown purely descriptively and without statistical adjustment. The proportions of prenatal supplement use and family history of cleft lip/palate also differed between groups. These data are intended solely to describe the study population; the main hypothesis testing is based on the adjusted models reported in Tables 3 and 4.

To assess the independent association of micronutrients with the risk of cleft lip and/or palate, a multivariable logistic regression model was fitted, adjusting for age, BMI, supplement use, and family history. As shown in Table 3, each 1 ng/mL increase in serum folic acid was associated with a 22% reduction in odds of the outcome (OR=0.78; 95% CI: 0.65–0.94; $P=0.009$). Similarly, each 100 pg/mL

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Table 1. Demographic Information of Patients

Variable		Control Group (n=108)	Case Group (n=66)	*P*-value
Living Area	Urban	75.5% (82)	76.3% (51)	0.839
	Rural	24.1% (26)	22.7% (15)	
Education Level	Illiterate	7.4% (8)	6.1% (4)	0.616
	Under Diploma	47.2% (51)	45.5% (30)	
	Diploma	32.4% (35)	33.3% (22)	
	University	13.0% (14)	15.2% (10)	
Alcohol Consumption	Yes	0.9% (1)	0% (0)	0.433
	No	99.1% (107)	100% (66)	
Smoking Status	Yes	0.9% (1)	1.5% (1)	0.723
	No	99.1% (107)	98.9% (65)	
Secondhand Smoking	No	52.8% (57)	63.6% (42)	0.723
	Yes	72.7% (51)	36.4% (24)	
Pregnancy Supplements	No	1.9% (2)	40.9% (27)	< 0.001
	Yes	98.1% (106)	59.1% (39)	
Vitamin B12 Supplementation	No	0.9% (1)	54.5% (36)	< 0.001
	Yes	99.1% (107)	45.5% (30)	
Folic Acid Supplementation	No	0.9% (1)	39.4% (26)	< 0.001
	Yes	99.1% (107)	60.6% (40)	
Zinc Supplementation	No	100% (108)	100% (66)	-
	Yes	0% (0)	0% (0)	
Family History	Negative	97.2% (105)	81.8% (54)	0.004
	Positive	2.8% (3)	18.2% (12)	
Proximity to Industrial Area	No	82.4% (89)	75.8% (50)	0.288
	Yes	17.6% (19)	24.2% (16)	
Proximity to High Voltage Power Lines	No	81.5% (88)	39.4% (26)	< 0.001
	Yes	18.5% (20)	60.6% (40)	
Proximity to Wi-Fi	No	81.5% (20)	68.2% (45)	0.045
	Yes	18.5% (88)	31.8% (21)	
Contact with Chemicals	No	79.6% (86)	89.4% (59)	0.094
	Yes	20.4% (22)	10.6% (7)	
Primary Location of Activity	Home	87.0% (94)	87.8% (58)	0.768
	Work	4.6% (5)	10.0% (7)	
	Home and work	5.6% (6)	1.5% (1)	
	Hospital	1.9% (2)	0.0% (0)	

Table 2. Baseline characteristics of study participants

Variable	Cases (Mean ± SD / n, %)	Controls (Mean ± SD / n, %)
Age (years)	26.8 ± 4.1	27.4 ± 3.8
BMI (kg/m²)	22.7 ± 3.1	23.6 ± 3.5
Weight (kg)	58.2 ± 7.8	60.5 ± 8.1
Height (cm)	160.3 ± 6.2	160.8 ± 6.5
Serum folic acid (ng/mL)	6.9 ± 2.1	8.1 ± 2.3
Vitamin B12 (pg/mL)	325 ± 88	356 ± 91
Zinc (µg/dL)	84 ± 12	88 ± 13
Supplement use (%)	10 (15.6 %)	18 (28.1 %)
Family history (%)	6 (9.4 %)	4 (6.3 %)

Table 3. Adjusted odds ratios from multivariable logistic regression

Variable	Adjusted OR	95% CI	*P*-value
Folic acid (per ng/mL)	0.78	0.65–0.94	0.009
Vitamin B12 (per 100 pg/mL)	0.84	0.70–0.99	0.041
Zinc (per 10 µg/dL)	0.90	0.70–1.15	0.394
Age (per year)	0.96	0.88–1.04	0.351
BMI (per unit)	0.94	0.85–1.05	0.284
Supplement use (yes vs. no)	0.62	0.25–1.54	0.302
Family history (yes vs. no)	1.82	0.51–6.49	0.354

increase in vitamin B12 was associated with a 16% reduction in odds (*P*=0.041). Serum zinc levels and other covariates showed no statistically significant associations (Table 3).

To evaluate the robustness of the findings, an alternative model was developed that additionally included "distance from high-voltage power lines" as a proxy for environmental exposure. As presented in Table 4, the protective associations of folic acid and vitamin B12 remained statistically significant. Living within 500 meters of high-voltage power lines was associated with a non-significant increase in odds of the outcome (OR=1.58; *P*=0.200) (Table 4).

As is evident, the mean serum level of folic acid in the case group (mothers of children with non-syndromic cleft lip and palate) is significantly lower than that in the control group (mothers of children without congenital defects). This significant difference indicates an association between serum folic acid levels and the risk of developing cleft lip

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Table 4. Alternative adjusted multivariable logistic regression model

Variable	Adjusted OR	95% CI	*P*-value
Folic acid (per ng/mL)	0.75	0.62–0.90	0.002
Vitamin B12 (per 100 pg/mL)	0.81	0.67–0.96	0.021
Zinc (per 10 µg/dL)	0.87	0.68–1.11	0.263
Age (per year)	0.95	0.87–1.04	0.271
BMI (per unit)	0.93	0.84–1.04	0.188
Supplement use (yes vs. no)	0.59	0.24–1.48	0.265
Family history (yes vs. no)	1.90	0.54–6.72	0.312
Distance from high-voltage power lines (<500 m vs. ≥500 m)	1.58	0.78–3.19	0.200

and palate. The ROC curve analysis for maternal serum folic acid level yielded an AUC of 0.834, reflecting its discriminatory ability between cases and controls (Figure 1a).

Figure 1b illustrates the scatter plot of the predicted probability of oral clefts in children compared to maternal serum folic acid levels. The y-axis represents the predicted probability of children developing oral clefts, ranging from 0 to 1, while the x-axis shows maternal serum folic acid levels, with values between 1 and 30. This plot indicates a nonlinear relationship between maternal serum folic acid levels and the predicted probability of orofacial clefts. In the high-risk area (probabilities close to 1), there is a concentration of data points with predicted probabilities approaching 1, occurring at serum folic acid levels approximately between 5 and 10. This suggests that within this range of folic acid levels, there is a higher risk of predicting the occurrence of oral clefts in offspring. In summary, the chart indicates that maintaining maternal serum folic acid levels above 10 may be associated with a lower predicted risk of oral clefts in children, while levels below 10 may correspond to a higher risk.

Figure 2 presents a scatter plot showing vitamin B12 levels in the case and control groups. As observed, the serum vitamin B12 levels in the case group (mothers of children with nonsyndromic cleft lip and palate) do not have a significant relationship with the serum vitamin B12 levels in

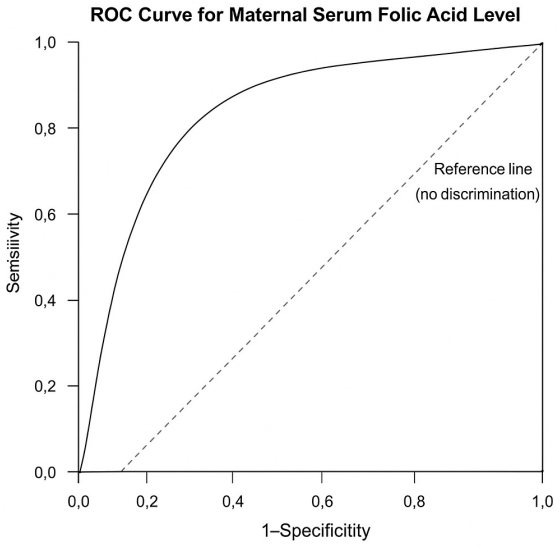

Figure 1a. Comparison of Serum Folic Acid Levels in Case and Control Groups

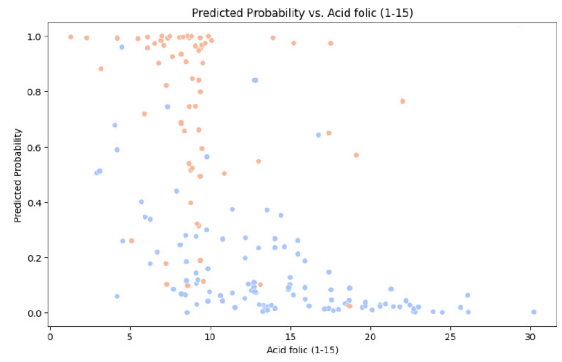

Figure 1b. The scatter plot of the predicted probability of oral clefts in children compared to maternal serum folic acid levels

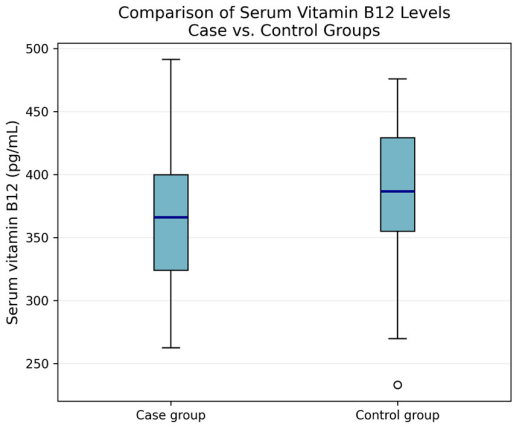

Figure 2. Comparison of B12 Levels in Case and Control Groups

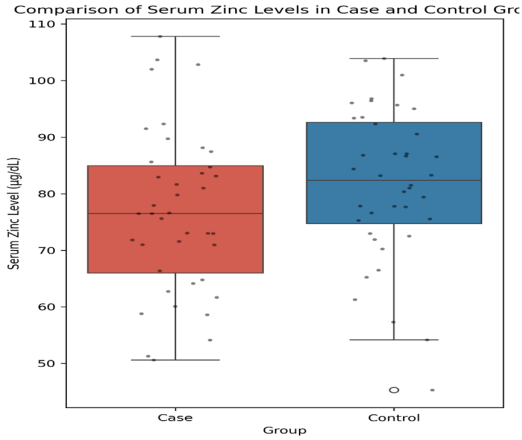

Figure 3. Comparison of Zinc Levels in Case and Control Groups

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the control group (mothers of children without congenital defects).

Figure 3 presents a scatter plot of zinc levels in the case and control groups. As shown, the serum zinc levels in the case group (mothers of children with nonsyndromic cleft lip and palate) do not exhibit a significant relationship with the serum zinc levels in the control group (mothers of children without congenital defects).

Discussion

The discussion of this study highlights the significant association between maternal serum folic acid levels and the occurrence of non-syndromic cleft lip and palate in offspring, while also examining other factors such as body composition, environmental exposure, and prenatal supplement use. The lower mean body mass index (BMI) and weight observed in the case group compared to the control group suggest that body composition may play a role in the risk of developing cleft lip and palate, though mechanisms linking lower BMI and weight to orofacial anomalies remain speculative. This section explores potential mechanisms, the role of folic acid, and the implications of these findings on public health (21, 22).

The significant difference in BMI and weight between the case and control groups points to a potential influence of maternal nutritional status on orofacial clefts. Lower BMI and weight in mothers of affected children might indicate nutritional deficiencies or altered metabolism, which could affect fetal development (23). Several studies suggest that low maternal BMI may correlate with reduced nutrient stores, which could compromise nutrient availability to the fetus. In particular, folic acid absorption and metabolism might be less efficient in women with lower BMI, potentially contributing to lower serum levels. The observed BMI differences raise questions about optimal maternal health status and suggest that specific weight and BMI recommendations could be beneficial for at-risk populations (24, 25). The observed association between lower BMI and an increased risk of oral clefts (OFC) warrants a more thorough etiological investigation. It is plausible that low BMI might directly contribute to the pathophysiology of OFCs, or it could reflect pre-existing nutritional deficiencies that impact fetal development. To elucidate this, it is necessary to determine whether these BMI differences existed prior to pregnancy or if they are solely attributable to pregnancy-related changes. Further research, considering maternal nutritional status and the timing of BMI variations, is crucial for clarifying the precise mechanism of this association (26, 27).

The data indicate a substantial difference in serum folic acid levels between the case and control groups, with markedly lower levels in the mothers of children with cleft conditions. Folic acid is critical in one-carbon metabolism, a biochemical pathway essential for DNA synthesis, repair, and methylation. Inadequate folic acid can lead to disruptions in these processes, increasing the risk of congenital anomalies (28). The scatter plot in Figure 1b underscores a nonlinear relationship between folic acid levels and cleft probability, suggesting that serum folic acid levels below 10 ng/mL are associated with a higher risk. These findings support existing research that associates maternal folate deficiency with increased risk of orofacial clefts, highlighting the importance of adequate folic acid intake, particularly during the critical early stages of fetal development when facial structures form (29).

Folate deficiency has been linked to impaired neural crest cell function, which is essential for the formation of the facial mesenchyme. Neural crest cells contribute to the development of the lip and palate; hence, disruptions in this process can result in clefting. Folic acid also plays a role in homocysteine metabolism, preventing the accumulation of homocysteine, which is known to interfere with collagen cross-linking and cellular adhesion—two processes crucial for normal palatal fusion. In animal models, low folate levels have been shown to reduce the activity of enzymes needed for DNA methylation, resulting in alterations in gene expression that can manifest as structural abnormalities. Given these insights, the protective effects of folic acid supplementation are consistent with its role in ensuring proper neural and facial development (9, 13).

The study's logistic regression analysis demonstrates that prenatal supplement use, including folic acid, significantly lowers the odds of cleft development (OR: 0.062, 95% CI: 0.011–0.347, P=0.002). This aligns with global recommendations for folic acid supplementation before and during pregnancy. By compensating for dietary deficiencies, supplements ensure that pregnant women maintain adequate serum folate levels, thus reducing the risk of folate-related developmental anomalies. This protective effect underscores the importance of public health initiatives that advocate for prenatal supplement use, particularly in populations at higher risk for nutrient deficiencies (2, 28).

Environmental factors, including proximity to high-voltage power lines, emerged as significant risk factors, with an odds ratio of 20.085 (95% CI: 3.362–119.974, P=0.001). Living near high-voltage areas may expose individuals to electromagnetic fields (EMFs), which have been implicated in developmental disruptions. EMFs can influence cellular processes, potentially leading to oxidative stress, DNA damage, and interference with cell signaling pathways. Although direct mechanisms connecting EMFs to cleft formation remain to be fully elucidated, these fields have been associated with various adverse outcomes in prenatal development. Consequently, further research is warranted to clarify the relationship between EMFs and congenital abnormalities (9, 28).

While folic acid showed a clear association with orofacial clefts, neither serum vitamin B12 nor zinc levels demonstrated significant differences between the case and control groups. Vitamin B12, another critical component of one-carbon metabolism, works synergistically with folic acid in DNA synthesis and homocysteine metabolism. However, B12 deficiency was not prominent in the case group, suggesting that folic acid alone might have a more direct impact on cleft formation. Similarly, zinc, an essential mineral for DNA synthesis and cellular proliferation, did not exhibit differences that reached statistical significance, indicating that zinc deficiency may not be a primary driver in cleft pathogenesis within this cohort. Nonetheless, zinc's known role in embryonic development underscores the importance

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of ensuring adequate intake through diet or supplements (20, 28).

The finding of a strong association between proximity to high-voltage power lines and an increased risk of oral clefts (OFC) necessitates a closer examination of potential biological mechanisms. One proposed hypothesis suggests that exposure to electromagnetic fields (EMF) may impact cellular processes and induce oxidative stress during fetal development, potentially disrupting facial growth and development. However, given that such areas may also be correlated with specific socioeconomic factors, the potential role of residual confounding cannot be disregarded. Further research is essential to disentangle these effects and confirm the precise mechanisms involved (30, 31).

Positive family history showed a significant association with cleft risk (OR: 11.473, 95% CI: 1.053–53.042, *P*=0.023), underscoring the role of genetic predisposition. Cleft lip and palate have a multifactorial etiology, with both genetic and environmental components contributing to risk. Certain gene variants involved in folate metabolism, such as mutations in the methylenetetrahydrofolate reductase (MTHFR) gene, have been linked to higher risks of cleft formation. These gene-environment interactions suggest that in genetically predisposed individuals, low folate levels could act as an environmental trigger, activating susceptibility pathways that contribute to abnormal craniofacial development (9, 12).

The study's findings have direct implications for prenatal care recommendations, particularly in advocating for folic acid supplementation as a preventive strategy. Given the robust association between low serum folate levels and cleft risk, healthcare providers should emphasize the importance of folic acid both preconception and throughout pregnancy. Public health policies could also consider fortified food programs, similar to those implemented in various countries to prevent neural tube defects. Additionally, raising awareness about environmental risk factors, such as EMF exposure, could lead to more informed decisions for expectant mothers regarding their living environments (24, 29).

Limitations and Future Directions

This study has certain limitations, including its observational design, which limits the ability to establish causation. Additionally, potential confounding factors, such as other dietary components or environmental pollutants, may influence the observed associations. Future studies could explore the role of genetic polymorphisms in folate metabolism to better understand gene-nutrient interactions in cleft pathogenesis. Longitudinal studies examining folate levels throughout pregnancy could provide more detailed insights into critical windows of vulnerability. One of the main limitations of this study is that several important factors—including socioeconomic status, dietary intake patterns, and the deficiencies of other micronutrients—were not assessed. These variables may significantly affect the study outcomes, and their omission may influence the interpretation of our findings. Additionally, serum levels of folic acid, vitamin B12, and zinc were measured at a single time point, which may not adequately capture the mothers' nutritional status throughout pregnancy, as these levels can fluctuate over time. Moreover, in the absence of dietary data, it is not possible to determine whether low serum concentrations of these micronutrients are attributable to insufficient intake or underlying metabolic disturbances. Taken together, these limitations could present challenges in fully interpreting the results of this study. Despite the lack of significant differences in zinc and B12 levels between groups, sample size limitations may have precluded the detection of subtle associations. Future studies with larger participant numbers are warranted to further investigate this matter.

Conclusion

In conclusion, the study underscores the importance of maternal folic acid levels in the prevention of non-syndromic cleft lip and palate. Lower BMI, family history, and environmental factors also emerged as significant predictors, supporting a multifactorial model of cleft pathogenesis. These findings reinforce current recommendations for folic acid supplementation and suggest areas for further investigation into environmental exposures and genetic susceptibilities that may contribute to congenital anomalies. Although the odds ratio for folic acid levels (OR=0.819) indicates a statistically significant association, for precise clinical interpretation, the findings should be examined within the context of absolute risk differences and clinical outcomes. Therefore, the results of this study should be interpreted with caution and in consideration of clinical contexts.

Our findings align with current national recommendations emphasizing folic acid supplementation for all women of reproductive age. However, the observed associations with lower BMI and potentially poorer dietary access in certain areas suggest that targeted interventions could be particularly beneficial for high-risk populations. Further research into the efficacy of such tailored strategies is warranted to optimize preventive efforts against oral clefts.

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Conflict of Interests

The authors declare that they have no competing interests.

Authors' Contributions

S.A.F., F.G., and N.S. contributed significantly to the conception, design, and implementation of the study. S.A.F. and F.G. were primarily responsible for data collection and analysis. N.S. supervised the project and provided critical revisions. All authors contributed to manuscript writing and approved the final version for publication.

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Ethical Considerations

Ethical approval for this study was obtained from the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1402.914) prior to commencement. Written informed consent was obtained from all participants after providing full information about the study purpose and procedures. Participation was voluntary, and confidentiality was strictly maintained. All participants were assured of their right to withdraw at any stage without affecting their medical care. The study complied with the ethical standards laid out for medical research involving human subjects.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

AI Use Statement

The authors acknowledge the use of solely for language editing and improving the readability of this manuscript. Following the use of this tool, the authors reviewed and edited the content as needed and take full responsibility for the accuracy and integrity of the final published work.

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