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Maternal exposures and risk of oral clefts in South Vietnam

Vu H. A. Dien¹, Christy M. McKinney², Araya Pisek¹, Waranuch Pitiphat³

¹Department of Community Dentistry, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand ²Division of Craniofacial Medicine, Department of Pediatrics, University of Washington, and Center for Child Health, Behavior and Development, Seattle Children's Research Institute, Seattle, USA ³Department of Community Dentistry, Faculty of Dentistry, and Chronic Inflammatory and Systemic Diseases Associated with Oral Health Research Group, Khon Kaen University, Khon Kaen, Thailand

Abstract

Background: Oral clefts are among the most common congenital anomalies. Most studies on risk factors of oral clefts have been carried out in developed countries. We investigated the associations between maternal exposures in the first trimester and oral clefts in South Vietnam.

Methods: We conducted a hospital-based case-control study during October 2014–November 2015. Cases included 170 patients with nonsyndromic cleft lip with or without cleft palate and those with cleft palate only. Controls were 170 children without oral clefts, matched to each case by age and gender. Mothers were interviewed using structured questionnaire. We performed conditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs).

Results: Passive smoking was associated with increased risk of oral clefts in univariate analysis, but not in multivariable analysis (adjusted OR [aOR] = 1.68; 95% CI, 0.53–5.37). No association was observed between liver intake and oral clefts. Compared with nondrinkers, mothers who reported consumption of caffeine-containing beverages were more likely to have an infant with oral cleft (aOR = 5.89; 95% CI, 1.08–32.00). Periconceptional use of folic acid and multivitamins supplementation was associated with reduced risk of oral clefts (aOR = 0.01; 95% CI, 0.00–0.09 and aOR = 0.03; 95% CI, 0.01–0.13, respectively).

Conclusions: The results suggest no associations of maternal passive smoking or liver intake with oral clefts. Periconceptional use of folic acid or multivitamins may protect against oral clefts. Further studies are warranted to examine the roles of caffeine consumption in pregnant mothers on occurrence of oral clefts in offspring.

Keywords

caffeine; folic acid; multivitamins; oral clefts; passive smoking; Vietnam

Correspondence Waranuch Pitiphat, Faculty of Dentistry, Khon Kaen University, 123 Friendship, Highway, Muang District, Khon Kaen, 40002, Thailand. waranuch@kku.ac.th.

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CONFLICT OF INTEREST

None to declare.

1 | INTRODUCTION

Oral clefts are among the most common anomalies in both developed and developing countries. The prevalence of oral clefts worldwide is approximately 1 per 800 newborns and it varies across different countries and races (Rahimov, Jugessur, & Murray, 2012). Oral clefts can affect the entire life of a patient and can have a substantial influence on quality of life of individuals and society.

Etiology of oral clefts involves both environmental and genetic factors. Cigarette smoking, including both active smoking (Lebby, Tan, & Brown, 2010; Little, Cardy, Arslan, & Mossey, 2004; Meyer, Williams, Hernandez-Diaz, & Cnattingius, 2004) and passive smoking (Ayu & Samsudin, 2003; McKinney et al., 2016; Sabbagh et al., 2015), is reported to increase the risk of oral clefts though there is less evidence related to passive smoking. The magnitude of the association observed in previous population-based studies ranged between 1.2–2.0 for active smoking (Little et al., 2004; Wyszynski & Wu, 2002) and 1.0–2.1 for passive smoking (Little et al., 2004; Wang, Guan, Xu, & Zhou, 2009). Evidence is inconsistent for the associations between consumption of coffee and caffeinated beverages in pregnant women and oral cleft risk in infants (Browne, 2006; Bille et al., 2007; Johansen, Wilcox, Lie, Andersen, & Drevon, 2009; Kurppa, Holmberg, Kuosma, & Saxén, 1983; McDonald, Armstrong, & Sloan, 1992; Nehlig & Debry, 1994a).

Maternal dietary intake and nutrition status play a significant role in the development of oral clefts (Krapels, Vermeij-Keers, Muller, de Klein, & Steegers-Theunissen, 2006). Intake of liver may be associated with the occurrence of oral clefts (McKinney et al., 2013, 2016; Mitchell, Murray, O'Brien, & Christensen, 2003), but the direction of the association is conflicting. Multivitamins use is reported to reduce the oral cleft risk in most studies (Badovinac, Werler, Williams, Kelsey, & Hayes, 2007; Jia et al., 2011; Johnson & Little, 2008; Loffredo, Souza, Freitas, & Mossey, 2001; Wilcox et al., 2007). Folic acid supplementation alone is shown to decrease the risk of neural tube defects (De-Regil, Pena-Rosas, Fernandez-Gaxiola, & Rayco-Solon, 2015), but its effect on oral clefts remains inconsistent (Badovinac et al., 2007; De-Regil et al., 2015; Johnson & Little, 2008; Rozendaal et al., 2013).

Most studies on risk factors of oral clefts are carried out in developed countries, and there is only one study examining the risk factors for oral clefts in Vietnam (Figueiredo et al., 2015). This was an international, multisite, hospital-based case-control study that investigated parental demographic and environmental factors in relation to oral clefts among underserved populations in Vietnam and three other countries. Because risk factors may vary by place, it is necessary to investigate the etiology of oral clefts across settings to identify risk factors in developing countries. We conducted this study in South Vietnam to examine the associations of oral clefts with behavioral and environmental factors and maternal dietary intake in the first trimester of pregnancy. We focused on only potentially modifiable risk factors that could prevent the occurrence of oral clefts. Our primary hypothesis was that mothers who were passive smokers during pregnancy had a higher odds of having an infant born with oral clefts than mothers who were not.

2 | MATERIALS AND METHODS

2.1 | Study population

This hospital-based matched case-control study recruited 170 pairs of cases and controls from three referral centers serving population from Central and South Vietnam between October 2014 and November 2015. Cases were infants with newly diagnosed oral cleft, aged less than 18 months, who attended the National Hospital of Odonto-Stomatology in Ho Chi Minh City or Ho Chi Minh City Hospital of Odonto-Stomatology. Eligible cases were infants diagnosed with a nonsyndromic cleft lip with or without palate (CL ± P), or cleft palate only (CP). We included infants with or without other diagnosed anomalies not part of a syndrome. All eligible cases who were contacted during the study period agreed to participate. We abstracted medical records to confirm the diagnosis of cleft infants.

Controls were infants born without oral clefts, aged less than 21 months, who attended Tu Du Hospital, the largest maternity hospital serving the region, for routine immunizations. Each control was matched to a case on gender and age within 3 months. We excluded children whose mothers were not Vietnamese or not able to communicate in Vietnamese, and those who were not accompanied by their biological mothers. This study was performed in accordance with the Declaration of Helsinki. Mothers in both groups signed written informed consent before study participation. The study protocol was reviewed and approved by the internal review boards of Khon Kaen University, Thailand (IRB00001189; October 16, 2014), University of Washington, Seattle, USA (IRB48164; September 19, 2014), and University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam (IRB134; April 14, 2014).

We calculated the sample size of this study based on our primary exposure of interest, which is passive smoking. The prevalence of passive smoking in Vietnam was 72.3% (Center for Disease Control and Prevention, 2012). According to a study in Malaysia (Ayu & Samsudin, 2003), the odds ratio (OR) of having oral clefts associated with secondhand smoking was 2.41. Based on a 1:1 ratio of cases and controls, alpha level of 5%, and power of 90%, the required sample size was 170 for cases and 170 for controls.

For our sample size and 5% alpha level, we calculated power using the information on the OR and prevalence of exposure among the controls obtained from previous studies (Johansen et al., 2009; Leite & Koifman, 2009; McKinney et al., 2013; Wilcox et al., 2007). We had 82%–99% power to detect associations between oral clefts and all exposures of interest, except alcohol consumption, which was only 68%.

2.2 | Data collection and variable definitions

The mothers were interviewed using a structured questionnaire to obtain information on socio-demographics, maternal behaviors and medical history. Periconceptional stage is defined as a duration from 4 weeks before conception to the first 3 months after conception. If mothers reported use of folic acid supplement or folic acid-containing multivitamin supplement in the periconceptional stage, we categorized them as having “any folic acid supplement use.” Duration of passive cigarette smoking exposure was categorized using a cutoff of 15 min per day (Ayu & Samsudin, 2003). Maternal smoking, caffeine consumption,

alcohol drinking, and liver intake in the first trimester were classified as any or none. To further explore the association with caffeine consumption, we also calculated the total amount of caffeine mothers had consumed in the first trimester based on the caffeine content, frequency, and the number of drinks they drank for each type of beverages (DeRoo, Wilcox, Drevon, & Lie, 2008). A 100-ml cup of coffee contains approximately 64 mg caffeine. The amount of caffeine in brewed tea and classic coke are 21 mg and 10 mg, respectively (Center for Science in the Public Interest, 2014). We also classified the amount of caffeine into three groups using cutoff points of 100 mg and 500 mg (Johansen et al., 2009) to estimate the dose-response relationship between caffeine intake and oral clefts.

2.3 | Statistical analyses

We compared the characteristics of the cases and matched controls using McNemar's test for categorical variables and paired *t* test for continuous variables. We performed conditional logistic regression to calculate crude and adjusted ORs, corresponding 95% CIs, and *p* values between our exposures of interest and oral clefts. Because of small numbers, all types of oral clefts were combined for our primary analysis. Potential confounders considered included parental education, parental age, family history of oral clefts (Romitti et al., 2007), and social economic status. In multivariable models, paternal educational level was classified into two groups (below 12 years and 12 years or more) and household income was categorized into two groups based on a threshold of 9 million Vietnamese Dong (approximately 405 USD) above which people have to pay personal income tax. Potential confounders were put into the model if they changed the OR more than 10% after adding into the initial model or if they were statistically significant.

We also conducted subgroup analyses by replicating our conditional logistic regression analysis among (1) those with CL $\pm$ P excluding CP since CP cases are often syndromic and have different risk factors; and among (2) those with no family history of CL $\pm$ P/CP since they may have a genetic basis relating to oral cleft. Data analysis was performed in Stata version 12.0 (Stata Corp, College Station, TX). A *p* value of less than 0.05 was set as statistically significant.

To address the impact of uncontrolled confounding, we calculated an E-value, a novel measure that assesses how far off the true risk estimate may be given unmeasured confounding in a study in a sensitivity analysis (VanderWeele & Ding, 2017).

3 | RESULTS

3.1 | General characteristics of the sample

Of the 170 oral cleft cases, the majority (46.5%) was cleft lip (CL), followed by cleft lip and palate (CLP) (28.8%) and CP (24.7%). The cases and controls were different on several socio-demographic characteristics (Table 1). Case infants were less likely to have low birth weight than the controls (5.3% versus 17.1%). There was a higher proportion of cases (15.9%) with family history of oral clefts compared to the controls (1.2%). Case parents tended to be younger, and have lower educational level and lower income than the controls. Forty-one percent of the case mothers had only one child, while this was 72% for control

mothers. The average time ($\pm$ standard deviation) between the maternal interview and the birth month of the index child was slightly longer in cases than in controls (10.7 ± 4.7 months versus 10.3 ± 4.8 months, $p < .001$). No control infants had any other birth defects.

3.2 | Maternal behaviors and medical history

More control mothers reported that they used vitamin and folic acid supplements in the periconceptional stage, compared to cases (Table 2). Control mothers were less likely to be exposed to passive cigarette smoking and caffeinated beverages versus cases. No mothers reported cigarette smoking in the first trimester. There were only seven mothers, all of which were cases, who reported drinking alcohol (Table 2). Medical history in the first trimester of pregnancy, including fever, infection, and history of intended abortion or miscarriage, was reported in the cases more often than in the controls. Other characteristics were similar between the two groups.

3.3 | Associations between maternal exposures and oral clefts

In unadjusted logistic regression analysis, there was a statistically significant positive association between passive smoking and risk of oral clefts (OR = 1.92; 95% CI, 1.20–3.09) (Table 3). However, after controlling for confounding factors, the association became nonstatistically significant (adjusted OR = 1.68; 95% CI, 0.53–5.37). When we analyzed the data based on the number of hours pregnant women exposed to cigarette smoking daily, we found no association between exposure duration to cigarette smoking and the risk of oral clefts (unadjusted OR = 1.11; 95% CI: 0.69–1.76).

Mothers who consumed caffeinated beverages during the first trimester of pregnancy had a strong increased risk of having an infant with oral clefts (adjusted OR = 5.89; 95% CI, 1.08–32.00) compared to those who did not (Table 3). No linear association was detected between amount of caffeine intake and oral clefts (adjusted OR = 1.00; 95% CI, 0.95–1.05 for 100-mg increase in caffeine intake). In addition, we did not observe a dose-response effect of caffeine consumption on oral clefts when compared daily caffeine intake of >100 mg to <500 mg (adjusted OR = 0.30; 95% CI, 0.01–8.06) and intake of more than 500 mg (adjusted OR = 3.75; 95% CI, 0.59–24.05) to the referent group of 0 to 100 mg (P for trend = 0.29). When examining different types of caffeinated beverages, we observed no association of oral clefts with the consumption of tea, or other caffeinated soft drinks. A statistically significant association between coffee consumption and increased oral cleft risk was found in the unadjusted analysis (unadjusted OR = 3.43; 95% CI, 1.48–7.96). The association was statistically non-significant when controlling for paternal education and maternal folic acid supplementation (adjusted OR = 2.31; 95% CI, 0.28–19.00). There was no association between oral clefts and liver intake in both crude and adjusted analysis (Table 3).

We found a strong reduction in oral clefts among mothers who used folic acid supplement (folic acid only or folic acid-containing multivitamins) within the periconceptional stage (adjusted OR = 0.01; 95% CI, 0.00–0.09) versus nonusers. Similarly, the risk of oral clefts in infants was statistically significantly lower in mothers who used multivitamins in the

periconceptional stage compared to those who did not use (adjusted OR = 0.03; 95% CI, 0.01–0.13) (Table 3).

In subgroup analysis of $CL \pm P$, the results were similar, except that the CI of adjusted ORs for associations between $CL \pm P$ and most exposures were wider (Table 4). In subgroup analyses excluding infants with no family history of oral clefts and their matched controls, we also found consistent results (Table 4).

We assessed the impact of unmeasured confounding for the associations between oral clefts and significant variables in adjusted analyses. The E-value for the estimate and for the upper confidence limit was 199.5 and 21.7 respectively for folic acid use, and 66.2 and 14.9, respectively for multivitamin use. For caffeine consumption, the E-value was 11.3 for the estimate and 1.4 for the CI.

4 | DISCUSSION

To our knowledge, this is the largest case-control study to investigate the risk factors of oral clefts in Vietnam. In contrast to our primary hypothesis, we found no association between maternal passive smoking and oral clefts. Similarly, a null association was observed for liver intake. On the other hand, our results showed that maternal caffeine consumption increased oral cleft risk in infants. Importantly, folic acid or multivitamins supplement use in pregnant mothers during the first trimester substantially reduced the oral cleft risk.

A previous study in Vietnamese population recruited cases from an oral clefts treatment mission of a nonprofit organization in Hanoi during a 4-month period (Figueiredo, 2015). Controls were obtained from women giving birth at maternity wards at the same hospitals. There was a large difference in age between the cases and controls (12.6 months versus 0.2 months), which may have contributed to differential recall between the cases and controls. Our study has overcome such limitations by recruiting cases from participants seeking treatment for oral clefts at major referral centers in Ho Chi Minh City. Each control was matched to a case on age, resulting in only a few months difference between the two groups.

In the unadjusted analysis of our study, pregnant women who were exposed to cigarette smoking 15 min or more per day during the first trimester were almost twice more likely to have increased risk of having an oral cleft infant than those who were exposed less than 15 min per day. The association was not statistically significant in adjusted analysis, which is consistent with earlier studies (Honein et al., 2007; Little et al., 2004). A previous study in Vietnam also found a two-fold increased risk of oral clefts associated with household smoking, which was a proxy for second hand smoking (unadjusted OR = 2.0; 95% CI, 1.1–3.7) (Figueiredo et al., 2015). The association was not significant after controlling for confounding factors (adjusted OR = 1.8; 95% CI, 0.9–3.7), similar to our study. On the other hand, studies in other Asian countries including Malaysia and Thailand found a significant association between passive smoking and increased risk for oral clefts (Ayu & Samsudin, 2003; Lie et al., 2008; McKinney et al., 2016). Differences in methods of measurement of passive smoking across studies may obscure the true association.

Teratogenic effects of caffeine have been demonstrated when administered at high levels in animal studies (Nehlig & Debry, 1994b). Whether caffeine has teratogenic effects in humans is still controversial. Caffeine and its metabolites can cross the placenta and reach the fetus (Scott, 1983). Caffeine may increase the level of homocysteine in plasma (Grubben et al., 2000; Urgert, van Vliet, Zock, & Katan, 2000), which has been shown to be associated with increased oral cleft risk (Abdulla, Tellis, Athikari, & Kudkuli, 2016; Blanco, Colombo, Pardo, & Suazo, 2016; Kumari, Ali, Sukla, Singh, & Raman, 2013; Wong et al., 1999). Homocysteine is an intermediate product in the biosynthesis of the amino acids cysteine and methionine. Homocysteine can follow two major pathways: remethylation to form methionine, or transsulfuration to form cystathionine. Caffeine is a methylxanthine, which might take action as vitamin B6 antagonist and disturb the vitamin B6-dependent transsulfuration pathway, resulting in the increased level of plasma homocysteine (Grubben et al., 2000; Verhoef, Pasman, Van Vliet, Urgert, & Katan, 2002; Urgert et al., 2000). Therefore, intake of caffeinated beverage may be an additional risk factor for hyperhomocysteinemia, predisposing to oral cleft.

Previous studies of maternal caffeine intake and oral clefts are inconclusive (Bille et al., 2007; Collier, Browne, Rasmussen, Honein, & National Birth Defects Prevention Study, 2009). A systematic review of seven articles published in 2006 reported no association between caffeine intake in pregnancy and risk of congenital anomalies in offspring (Browne, 2006). A large case-control study in the US found a significant increased risk for oral clefts associated with moderate level of caffeine consumption (100–199 mg/day), but no association was presented for the higher level of consumption (Collier et al., 2009). A Norwegian study reported a link between coffee consumption and increased risk of CL $\pm$ P, in a dose-response manner (Johansen et al., 2009). The investigators also examined total caffeine intake for all beverages, but the result was significant only in the unadjusted analysis. Our study revealed that pregnant women who drank any type of caffeine-containing beverages had a higher risk of having an oral cleft infant than those who did not. Analysis for coffee consumption also showed similar trend for positive association, although it was not statistically significant in adjusted analysis.

Nevertheless, our results on caffeine consumption should be interpreted with caution. Although the observed association was strong, the confidence interval was wide due to a limited sample size. These results may thus be a chance finding or affected by residual confounding.

Vietnam is an ideal place to investigate the influence of folic acid supplementation in pregnancy as presently there is no national program of either folic acid supplementation or folate fortification in staple foods. Folic acid is an essential nutrient for embryogenesis through one-carbon metabolism, which plays an essential function in synthesis of DNA and RNA. Folate deficiency may disturb cellular multiplication and differentiation in embryogenesis (Krapels et al., 2006; Wu, Imhoff-Kunsch, & Girard, 2012). Our investigation found a very strong preventive effect of folic acid supplement in mothers during the periconceptional stage on oral clefts in infants. The results are consistent with a meta-analysis of 12 case-control studies and 5 prospective studies which reported an advantage effect of folic acid-containing supplement consumption in pregnancy on the oral

cleft risk (Badovinac et al., 2007). Another meta-analysis found an 18% reduction in the risk of CL $\pm$ P associated with folic acid supplementation, but no significant association for CP (Johnson & Little, 2008). A subsequent prospective cohort study in China (Li et al., 2012) and a case-control study in Norway (Wilcox et al., 2007) similarly showed a lower risk of CL $\pm$ P associated with folic acid use, but did not observe a reduced risk of CP. Our study found a protective effect of maternal folic acid supplementation for all oral clefts, as well as for CL $\pm$ P. But with limited number of CP cases, we did not have adequate power to examine the effect of folic acid use among CP cases.

Evidence suggests that vitamins (i.e., riboflavin as vitamin B2, folate, cobalamin as vitamin B12) and minerals (i.e., zinc, iron) involved in molecular-biological process and palatogenesis, that are essential for normal embryogenesis (Cetin, Berti, & Calabrese, 2010; Krapels et al., 2006). Our findings showed an extremely low risk of oral clefts among women who took multivitamins within the periconceptional stage, compared to those who did not.

The result is similar to that of previous studies on the consumption of multivitamins (Johnson & Little, 2008; Loffredo et al., 2001; Xu et al., 2015). Indeed, the observed association between multivitamins and folic acid supplementations and oral clefts may be affected by socioeconomic and lifestyle factors related to vitamin use. Our control parents had higher educational level and higher income than the case parents (Table 1). The control mothers were therefore more likely to have prenatal visits and receive a prescription of folic acid supplement or multivitamins during pregnancy. They might also be more knowledgeable about what they should and should not do when being pregnant. Additional analysis (data not shown) confirmed an association between income and vitamin supplements. Socioeconomic status could therefore partly explain the associations of oral clefts with the use of folic acid supplement and multivitamins in our study.

We further performed sensitivity analysis to evaluate how robust our observed associations were to unmeasured confounding. For folic acid use, the E-value was 199.5 for the estimate and 21.7 for the confidence limit. These indicate that an unmeasured confounder would need to be associated with both oral clefts and folic acid by an OR of 199.5 fold each in order to explain away the observed association between folic acid use and oral clefts. In addition, the OR representing the associations of unmeasured confounder with folic acid and oral clefts must be as strong as 21.7 to move the CI to include the null. The possibility for a confounding as strong as 21.7 is very low as the effect size of three-fold or more are not particularly common in biomedical and social sciences research (VanderWeele & Ding, 2017). The robust results were also found for multivitamin use. For caffeine consumption, the E-value of 11.3 for the estimate is quite robust; however, the E-value of 1.4 for the CI indicates that substantial confounder associations with caffeine intake and oral clefts could potentially move the CI to include 1.

In this study, we found no association of oral clefts with maternal liver consumption in the first 3 months after conception. Our result is similar to that of an earlier study in Denmark (Mitchell et al., 2003), which found no connection between CL $\pm$ P and liver meals, as well as liver paste sandwiches. Recently, a study in Northeast Thailand showed that mothers who

reported of having liver intake during the first trimester had lower risk of having an oral cleft infant than those who did not (McKinney et al., 2013). Interestingly, a subsequent study from the same authors found the opposite result that liver intake increased oral cleft risk (McKinney et al., 2016). These conflicting findings may indicate that nutritional deficiency or over-nutrition may alter maternal nutrient metabolism involved in molecular-biological process and palatogenesis (Krapels et al., 2006). The null association between liver intake and oral clefts observed in our study may be due to the small percentage of mothers who reported eating liver in the first trimester in both cases and controls (10% versus 6.5%). Therefore, the presence of exposure in our study was not large enough to see the difference between two groups. We also did not collect information on detailed liver intake (amount, frequency, type) which may have impacted our ability to detect associations.

Our study has limitations. Ideally in hospital-based case-control studies, the controls should be recruited from non-cases in the same hospital where the cases were admitted. However, in Vietnam oral clefts examination and treatment are usually performed in odonto-stomatology hospitals. We recruited cases in two major odonto-stomatology hospitals in Ho Chi Minh city, but had to recruit controls from a maternity hospital because children at such early age (less than 21 months) usually do not visit odonto-stomatology hospitals except for diagnosis and treatment of craniofacial anomalies. All three hospitals in this study are major referral hospitals where most patients come from all over Central and South Vietnam. We also sampled the controls from children who visited well-child clinic for routine immunization as this was independent of the exposures of interest to avoid selection bias. Given that mothers need not travel far to reach an immunization clinic, controls may have been from the city population and not representative of the broader geographic area from which the cases arose. This may explain the apparent differences in socioeconomic characteristics between the cases and controls and might have affected our results.

Another limitation was the small numbers of mothers who reported drinking alcohol or smoking cigarette. Thus, the associations between oral clefts and these potential risk factors could not be determined in this study. Finally, as with any case-control study, recall bias is a concern. The investigators have used several strategies to minimize recall bias, which could be viewed as our strengths. First, we limited the infant's age in our inclusion criteria to shorten the time for mothers to recall. Second, we selected cases diagnosed, on average, before the first year of life. Had cases been diagnosed for a long time ago, mothers might recall their newly adopted behaviors rather than their behaviors in early pregnancy when the cleft developed (Margetts, 2003). All interviewers were well-trained and we did not emphasize any particular exposure during the interview.

5 | CONCLUSIONS

Within the limitations of our study, the results suggest no associations of maternal passive smoking and liver intake with oral clefts. We found that folic acid and multivitamin supplementations in the periconceptional stage decrease oral cleft risk. Recommendation of taking folic acid supplement periconceptionally should be made for Vietnamese women who have planned to become pregnant. Our findings indicate an association between caffeine

consumption and increased risk for oral clefts. Further studies are warranted to examine the roles of caffeine consumption in pregnant mothers on the occurrence of oral cleft in infants.

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TABLE 1
Socio-demographic characteristics of infants and their parents by case and control status

Characteristics	Case (n = 170)		Control (n = 170)		p value ^a
	n	%	n	%	
Infant					
Male	95	55.9	95	55.9	1.00
Age in months, mean (SD)	10.6 (4.6)		10.1 (4.7)		.34
Low birth weight, < 2,500 grams	9	5.3	29	17.1	<.001
Gestational age, weeks					
Normal term, 37 to 41	133	84.2	151	89.4	.20
Preterm, < 37	22	13.9	18	10.7	
Post-term, > 41	3	1.9	0	0.0	
Family history of oral clefts	27	15.9	2	1.2	<.001
Twins	3	1.8	9	5.3	.15
Mother					
Married	166	97.6	170	100.0	.13
Age, years					
18–24	64	37.7	28	16.5	<.001
25–29	61	35.9	54	31.8	
30–34	33	19.4	62	36.5	
>=35	12	7.0	26	15.2	
Highest education attained					
Elementary	21	12.3	2	1.2	<.001
Secondary	69	40.6	19	11.2	
High school	54	31.8	79	46.5	
Technical college	10	5.9	10	5.9	
College or university	16	9.4	60	35.2	
Working during the first trimester	106	62.3	136	80.0	<.001
Household income, VND					
<5,000,000	72	42.4	1	0.6	<.001
5,000,000–10,000,000	77	45.3	34	20.0	

Characteristics	Case (n = 170)		Control (n = 170)		p value ^a
	n	%	n	%	
10,000,000–18,000,000	15	8.8	68	40.0	
>18,000,000	6	3.5	67	39.4	
Total number of children					
1	69	40.6	123	72.4	<.001
2	76	44.7	39	22.9	
3	25	14.7	8	4.7	
Father					
Age, years					
<24	30	17.7	11	6.5	<.01
25–29	56	32.9	42	24.7	
30–34	57	33.5	65	38.2	
≥35	27	15.9	52	30.6	
Highest education attained					
Elementary	23	13.5	3	1.8	<.001
Secondary	70	41.2	16	9.4	
High school	45	26.5	60	35.3	
Technical college	10	5.9	9	5.3	
College or university	21	12.4	81	47.7	

SD, Standard Deviation; VND, Vietnamese Dong.

^aMcNemar's test for categorical variables and paired t-test for continuous variables.

Maternal behaviors and medical history during the first trimester of pregnancy by case and control status

TABLE 2

Characteristics	Case (<i>n</i> = 170)		Control (<i>n</i> = 170)		<i>p</i> value ^a
	<i>n</i>	%	<i>n</i>	%	
Mother's behavior (Yes/No with Yes shown)					
Any vitamin use	142	83.5	169	99.4	<.001
Any multivitamins use	16	9.4	126	74.1	<.001
Any folic acid supplement use	27	15.9	162	95.3	<.001
Any liver intake	17	10.0	11	6.5	.26
Any herb use for menstrual regulation	4	2.3	0	0.0	.13
Passive smoking (>15 minutes)	67	39.4	43	25.3	.006
Any maternal smoking	0	0.0	0	0.0	Not estimable
Any alcohol drinking	7	4.1	0	0.0	.02
Any caffeine consumption	68	40.0	17	10.0	<.001
Mother's medical history (Yes/No with Yes shown)					
Nausea	84	49.4	94	55.3	.27
Fever	57	32.5	7	4.1	<.001
Infection	28	16.5	1	0.6	<.001
Diabetes	1	0.6	0	0.0	1.00
Gestational diabetes	0	0.0	5	2.9	.06
Seizure	1	0.6	0	0.0	1.00
History of abortion in prior pregnancy	14	8.2	3	1.8	<.01
History of miscarriage in prior pregnancy	12	7.1	3	1.8	.02

^aMcNemar's test.

TABLE 3

Associations between maternal exposures of interest and oral clefts in infants

Case (<i>n</i> = 170)		Control (<i>n</i> = 170)		Unadjusted OR (95% CI)	<i>p</i> value	Adjusted OR (95% CI)	<i>p</i> value
<i>n</i>	%	<i>n</i>	%				
Passive smoking >15 minutes ^a							
No	103	60.6	127	74.7	Reference	Reference	.38
Yes	67	39.4	43	25.3	1.92 (1.20–3.09)	1.68 (0.53–5.37)	
Any caffeine ^b							
No	102	60.0	153	90.0	Reference	Reference	.04
Yes	68	40.0	17	10.0	7.38 (3.52–15.43)	5.89 (1.08–32.00)	
Any liver ^c							
No	153	90.0	159	93.5	Reference	Reference	.28
Yes	17	10.0	11	6.5	1.55 (0.72–3.30)	2.58 (0.46–14.49)	
Any folic acid ^d							
No	146	85.9	13	7.7	Reference	Reference	<.001
Yes	24	14.1	157	92.3	0.01 (0.00–0.05)	0.01 (0.00–0.09)	
Any multivitamins ^e							
No	154	90.6	44	25.9	Reference	Reference	<.001
Yes	16	9.4	126	74.1	0.04 (0.01–0.10)	0.03 (0.01–0.13)	

OR, Odds Ratio; CI, Confidence Interval.

^a Adjusted for household income, maternal caffeine consumption, and maternal folic acid supplement consumption.^b Adjusted for paternal educational level and maternal folic acid supplement consumption.^c Adjusted for household income and maternal folic acid supplement consumption.^d Adjusted for paternal educational level, household income, and maternal history of fever.^e Adjusted for maternal age, household income, and maternal history of termination of pregnancy and infection.

TABLE 4

Association between maternal exposures of interest and oral clefts in subgroups

Case		Control		Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
n	%	n	%				
Infants excluding cases and matched controls with cleft palate only (<i>n</i> = 128 for each group)							
Passive smoking >15 min ^a							
No	74	57.8	96	75.0	Reference	Reference	.20
Yes	54	42.2	32	25.0	2.22 (1.27–3.88)	3.15 (0.54–18.36)	
Caffeine ^b							
No	72	56.3	115	89.8	Reference	Reference	.12
Yes	56	43.7	13	10.2	9.60 (3.82–24.11)	5.99 (0.62–58.18)	
Liver ^c							
No	113	88.3	119	93.0	Reference	Reference	.18
Yes	15	11.7	9	7.0	1.67 (0.73–3.81)	5.35 (0.45–63.56)	
Folic acid ^d							
No	110	85.9	10	7.8	Reference	Reference	.78
Yes	18	14.1	118	92.2	0.02 (0.00–0.08)	Not estimable	
Multivitamins ^e							
No	116	90.6	31	24.2	Reference	Reference	<.001
Yes	12	9.4	97	75.8	0.02 (0.01–0.09)	0.03 (0.01–0.17)	
Infants excluding cases and matched controls with family history of oral clefts (<i>n</i> = 142 for each group)							
Passive smoking ^a							
No	84	59.2	101	71.1	Reference	Reference	.43
Yes	58	40.8	41	28.9	1.68 (1.02–2.76)	1.59 (0.50–5.09)	
Caffeine ^b							
No	84	59.2	128	90.1	Reference	Reference	.08
Yes	58	40.8	14	9.9	7.29 (3.31–16.05)	4.79 (0.86–26.78)	
Liver ^c							
No	127	89.4	134	94.4	Reference	Reference	.12

	Case		Control		Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
	n	%	n	%				
Folic acid ^d								
No	122	85.9	11	7.8	Reference	<.001	Reference	<.001
Yes	20	14.1	131	92.2	0.01 (0.00–0.06)		0.01 (0.00–0.11)	
Multivitamins ^e								
No	129	90.9	38	26.8	Reference	<.001	Reference	<.001
Yes	13	9.1	104	73.2	0.03 (0.01–0.10)		0.02 (0.00–0.14)	

OR, Odds Ratio; CI, Confidence Interval.

^a Adjusted for household income, maternal caffeine consumption, and maternal folic acid supplement consumption.

^b Adjusted for paternal educational level and maternal folic acid supplement consumption.

^c Adjusted for household income and maternal folic acid supplement consumption.

^d Adjusted for paternal educational level, household income, and maternal history of fever.

^e Adjusted for maternal age, household income, and maternal history of termination of pregnancy and infection.