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# Maternal Folic Acid Supplementation and the Risk of Oral Clefts in Offspring

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**Introduction:** There is controversial evidence from the literature regarding the protective effect of folic acid supplementation during pregnancy against orofacial clefts. The authors undertook this meta-analysis to assess whether folate supplementation during pregnancy can reduce the risk of nonsyndromic cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO) in infants.

**Methods:** Eligible articles were identified by searching databases, including PubMed, Medline, Scopus, ISI (Web of Knowledge) to September 2017. A meta-analysis was performed to evaluate the effects of maternal supplementation on oral clefts. Odds ratios (ORs) and 95% confidence intervals (CIs) were pooled using Stata software. Publication bias was assessed by the Begg and Egger test. (Registration ID: CRD42018083922)

**Results:** Out of the 1630 articles found in the authors' initial literature searches, 6 cohort studies, and 31 case-control studies were included in the authors' final meta-analysis. The results of the main analysis revealed that maternal folate supplementation was associated with a modest but statically significant decreased risk of all cleft subtypes (OR = 0.69, 95% CI: 0.60, 0.78). Folic acid intake alone was inversely associated with CL/P (OR = 0.73, 95% CI: 0.62–0.85,) but to a lesser extent than CPO (OR = 0.75, 95% CI = 053–1.04). Multivitamin intake had a significant protective effect for CL/P (OR = 0.65 95% CI = 0.55–0.80) as well as CPO (OR = 0.69, 95% CI = 0.53–0.90).

**Conclusions:** Our results indicate that maternal supplementation in early pregnancy reduces the risk of nonsyndromic CL/P and CPO in infants. These data can serve to reassure women planning a pregnancy to consume multivitamins during the periconception period to protect against oral clefts.

**Key Words:** Cleft lip, cleft palate, folic acid, meta-analysis

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Received October 28, 2017.
Accepted for publication January 27, 2018.

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The authors report no conflicts of interest.
Supplemental digital contents are available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jcraniofacialsurgery.com).


ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000004488

## Background

Orofacial clefts (OFC) are one of the most common congenital birth malformations in the oral and maxillofacial area, with a global prevalence ranging from 1/200 to 1/2500 live births depending on ethnic and socioeconomic status.[1] These deformities seriously affect the patient's facial appearance and compromise their psychological and physiological function.[2] Two main categories of oral clefts including cleft lip with or without palate (CL/P) and cleft palate only can be isolated or occur as part of other congenital anomalies such as syndromes, associations, and or disruptions. Management of children with cleft lip and palate should go through a multidisciplinary cleft team who will provide comprehensive treatment beginning at birth and spanning until adulthood. The cleft team usually includes orthodontist, maxillofacial surgeon, plastic surgeon, prosthodontist, speech therapist, audiologist (ENT specialist), and psychologist. Goals of treatment of the child with a cleft lip and palate should include the repairing the birth defect (lip, palate, and nose), achieving normal speech, hearing, functional occlusion, and good dental health.[3,4]

Epidemiological and family-based studies suggest that both genetic and environmental factors are involved in the etiology of nonsyndromic OFC.[5] A variety of environmental factors that could contribute to the occurrence of OFC during pregnancy include: maternal smoking, tobacco use, alcohol drinking, exposure to teratogenic agents (eg, anticonvulsant, aminopterin, corticosteroids), and vitamin and folate deficiency.[6–8]

Addressing folate deficiency as an ethologic factor in OFC has been the subject of considerable studies. A number of animal experiments and human clinical trials have shown that folate deficiency during the critical periods of organ formation can increase the risk of OFC occurrence. Folate is an essential nutrient involved in many vital biological reactions such as DNA and RNA biosynthesis as well as in amino acid metabolism (homocysteine) and methylation. During pregnancy, folate requirements increase to support optimal growth of the fetus and supply sufficient blood volume of the mother.[9,10] The discovery that folic acid (FA) supplementation in early pregnancy can reduce the risk of congenital birth defects is one of the important public health advances of recent years. Strong evidence shows that daily FA supplementation has a significant protective effect in preventing neural tube defects (NTDs).[11] However, the current evidence from epidemiological studies regarding the association between folic acid and oral cleft remains inconsistent and controversial. Several observational and interventional studies have been have been performed in an attempt to investigate the role of folate in the etiology of oral clefts but results have been variable.[12–14]

## Objective

The main objective of this systematic review and meta-analysis according to the PICO (P; population, I; intervention, C; comparison and O; outcome) was to answer to this clinical question: "Does maternal folic acid supplementation during pregnancy have any effect on the prevalence of oral clefts?." The primary outcome in this study was the risk of oral cleft occurrence in infants. There was no secondary outcome measured in this study.

# METHODS

## Protocol and Registration

The protocol of this systematic review was registered on the National Institute of Health Research Database (www.crd.york.ac.uk/prospero, Protocol ID and code: CRD42018083922).

**TABLE 1.** The Components of the Target Question and Their Definition According to the Population, Intervention, Comparison, and Outcome Analysis

| Component | Definition |
|---|---|
| Participants | Pregnant woman during the periconception period |
| Intervention | Folic acid consumption |
| Comparisons | No folic acid consumption |
| Outcomes | Oral cleft occurrence |
| Study design | Patient control and cohort studies |

## Data Sources and Search Strategy

We collected the studies that reported the effect of folic acid-containing supplements on oral clefts. We identified potentially relevant published studies by searching Medline, Scopus, ISI (Web of Knowledge) using MeSH headings and text words from their earliest available date to the end of October 2017. The following search expressions were used: ((("Folic Acid"[Mesh]) OR (Folic Acid) OR (Vitamin B9) OR (Vitamin M) OR (Pteroylglutamic Acid) OR (Folvite) OR (Folate)) AND ((("Cleft Lip"[Mesh]) OR (Cleft Lip) OR (Harelip)) OR (("Cleft Palate" [Mesh]) OR (Cleft Palate) OR (Orofacial cleft)))). Review articles and other meta-analyses were reviewed and the reference lists of all retrieved studies were searched to identify any additional articles.

## Inclusion and Exclusion Criteria

We focused on case control and cohort studies which reported the effect of maternal folic acid-containing intake during pregnancy on the risk of nonsyndromic oral clefts in infants. The details of review parameters according to PICO analysis are shown in Table 1. Studies were excluded if: outcome measures and estimates of variance were not presented or could not be estimated, the number of subjects in case and control groups was not presented, information about timing of folic acid consumption was lacking, and infants with syndromic cleft were included. Animal studies, review articles, clinical reports, patient series, and meeting abstracts were excluded as well. There were no restrictions on language of publication.

## Study Selection

We reviewed all abstracts, and each relevant article was marked for further review. We retrieved the full text of the relevant studies, and the studies that met our inclusion criteria were included in our analysis. The references listed in each eligible article were also screened for relevant articles. The search was performed independently by 2 authors.

## Study Quality Assessment

The quality of the eligible literature was assessed using the Newcastle–Ottawa Scale system(NOS). In this scoring system each observational study was judged based on 3 domains: selection of study group, comparability of groups, and ascertainment of exposure/outcome. The maximum score that a study could be awarded was 9 points, and scores of less 5, 6 to 7, and 8 to 9 were regarded as high, medium, and low risk of bias, respectively.

## Data Extraction

Two authors independently extracted the study characteristics, quality, and data from included studies using predetermined data extraction forms. The following data were collected from each study: the year of publication, the country in which the study was performed, study design, the number of subjects in patients and

control groups, type of cleft, whether folic acid was consumed as a part of a multivitamin or alone as a folic acid supplement, and timing of supplement intake relative to conception and adjusted estimates and their corresponding 95% CIs, and confounding factors that could influence the effect estimate.

Timing of any supplement intake was divided into 2 categories including the periconception period and during pregnancy. Periconception period had different descriptions in each study. We defined it as the period 3 months before conception to the end of the first trimester of pregnancy. Data were extracted separately for CL/P and cleft palate only (CPO). When CL/P and CPO were not distinguished in a study, the data was reported for oral cleft as a group in the analysis. Any disagreements in data extraction were resolved by discussion with a third investigator.

## Statistical Analysis

Meta-analysis was performed to assess the strength of association between maternal folic acid intake and fetal oral clefts by the odds ratios (ORs) with corresponding 95% confidence intervals (CIs). We extracted reported numbers for the study groups, and the contributing crude odds ratio and associated 95% CI were calculated by means of Stata/SE 14.1 software for each study.

The $Q$ test and $I^2$ were used to assess heterogeneity of effect size estimates across studies. If there was evidence of substantial heterogeneity ($P$ value $< 0.10$ or $I^2 \geq 50\%$) the summary OR estimation was calculated with a random-effect model. Otherwise, the fixed-effect model was applied.

## Investigation of Publication Bias

Publication bias was evaluated by means of visual inspection of the funnel plot as well as the Begg correlation test and Eager liner regression analysis. All statistical analyses were conducted using Stata14.1 software and $P < 0.05$ was considered significant.

## Sensitivity Analyses

We planned to conduct sensitivity analyses to detect whether a specific study strongly influenced the overall effect estimate by removing 1 study at a time from our pooled analysis.

# RESULTS

## Selection of Studies

A total of 1630 potentially relevant articles were identified by searching the electronic database. After removing duplicates, 1403 articles remained for title and abstract review. From the remaining 1403 relevant articles and 2 hand searched articles, we identified 31 case control and 6 cohort studies on the association between folic acid consumption during pregnancy and oral cleft risk in infants. These studies were published between 1958 and 2016 (Fig. 1).

## Study Characteristics

Table 2 summarized characteristics of 32 case controls. In the case group, 3824 woman had consumed folic acid and 23,221 did not.[3,13,15–43] The control group consisted of 9319 supplement users and 1,140,867 nonusers. The total number of infants with clefts was 27,045. Details of the 6 included cohort studies are reported in Table 3.[44–49] A total of 231 infants with oral clefts were born to 264,690 women who did not consume a FA supplement during pregnancy. The length of the follow-up period ranged from 11 to 17 years. From these eligible studies, 19 were conducted in Europe, 9 in the United States, 6 in China, and 3 in other regions (Thailand and Australia).

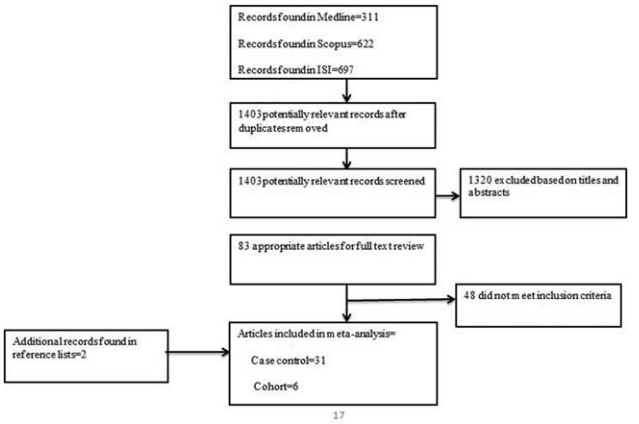


**FIGURE 1.** Process for identifying studies assessing the association between maternal folate intake during pregnancy and the risk of oral clefts in infants.

## Quality Assessment

The case control and cohort studies scored between 4 and 9 from a possible 9 stars on NOS. (See Supplemental Digital Content, Table E1, http://links.lww.com/SCS/A303)

## Synthesis of Results and Outcome

### Maternal Folate Supplementation and Risk of Oral Clefts

The combined data of all included studies provided evidence for a significant decrease in the risk of all cleft subtypes with maternal folate supplementation (OR = 0.69, 95% CI: 0.60, 0.78). There was evidence of significant heterogeneity across the studies ($I^2 = 80.5\%$, $P = 0.000$), thus the random effect model was applied.

### Subgroup Analysis

In this meta-analysis, we conducted several subgroup analyses based on the cleft type; supplement intake, geographic region, and timing of supplementation as follows

### Effect of Folic Acid Intake Alone on Oral Clefts

Seventeen studies and 14 studies reported data on the effect of folic acid intake alone supplementation on CL/P and CPO respectively. Analysis of pooled OR data yielded a significant correlation for CL/P (OR = 0.73, 95% CI: 0.62–0.85) but no significant association was found for CPO (OR = 0.75, 2 95% CI = 053–1.04) (Fig. 2).

### Effect of Multivitamin Intake on Oral Clefts

Seventeen studies and 14 studies mentioned that women consumed FA as part of a multivitamin and reported data on CL/P and CPO respectively. The subgroup analysis indicated that multivitamin intake was associated with a significant decreased risk for CL/P (OR = 0.65 95% CI = 0.55–0.80) and CPO (OR = 0.69, 95% CI = 0.53–0.90) (Fig. 3).

The seven studies that analyzed oral clefts as a group, showed a significant decreased risk in mothers who consumed FA alone supplements (OR = 0.54, 95% CI: 0.32, 0.91, $P = 0.00$, $I^2 = 92.6.1\%$) but no such significant correlation was found for those consumed multivitamins (OR = 0.72, 95% CI: 0.22, 2.32, $P = 0.00$, $I^2 = 95.9\%$) (See Supplemental Digital Content, Figure E2, http://links.lww.com/SCS/A303).

**TABLE 2.** Characteristics of Case-Control Studies Reporting Maternal Folate Consumption in Pregnancy and Occurrence of Oral Clefts

| Study | Country | Cleft Type | Supplementation | Exposure Period* | Quality (Points) | Adjustments |
|---|---|---|---|---|---|---|
| Saxén 1975[17] | Finland | CL/P, CPO | Multivitamin | During pregnancy | 5 | No |
| Hill1988[3] | Great Britain | Oral cleft | FA,Multivitamin | During pregnancy | 4 | No |
| Shaw1995[18] | Finland | CL/P, CPO | FA | −1 to + 3 mo | 5 | No |
| Hayes 1996[19] | US and Canada | CL/P, CPO | FA | −1 to + 3 mo | 7 | No |
| Czeizel 1996[27] | Hungarian | CL/P, CPO | FA | −1 to + 3 mo | 6 | No |
| Werler 1999[28] | US and Canada | CL/P, CPO | Multivitamin | −1 to + 3 mo | 7 | Maternal age, maternal education, maternal race, planned pregnancy, nausea, and vomiting during first lunar month |
| Loffredo 2000[15] | Brazil | CL/P, CPO | Multivitamin | During pregnancy | 6 | Pollution, heredity, maternal epilepsy, maternal hypertension, intake of antihypertensive, contraceptives |
| Itikala 2001[29] | US | CL/P, CPO | Multivitamin | −1 to + 3 mo | 7 | Maternal age, education, sex of baby, smoking status, flu, epilepsy, diabetes, family history |
| Beaty 2001[30] | Maryland | CL/P,CPO | Multivitamin | −3 to + 3 mo | 6 | No |
| Kallen 2002[31] | Sweden | CL/P, CPO | FA | During pregnancy | 5 | No |
| deWalle 2002[32] | Netherland | CL/P, CPO | FA | −1 to + 3 mo | 8 | Race, sex, alcohol use, smoking |
| Vanrooji 2003[33] | Netherland | CL/P | FA | −1 to + 3 mo | 6 | No |
| Mitchell 2003[16] | Denmark | CL/P, CPO | Multivitamin | During pregnancy | 6 | No |
| Vanrooji 2004[34] | Netherland | CL/P | FA | −1 to + 3 mo | 6 | No |
| Bower2006[35] | Australia | CL/P, CPO | FA | −1 to + 3 mo | 6 | No |
| Krapels 2006[20] | California | CL/P, CPO | FA | −1 to + 3 mo | 5 | No |
| Shaw 2006[36] | Netherland | CL/P, CPO | Multivitamin | −3 to + 3 mo | 7 | No |
| Chevrier 2007 [37] | France | CL/P, CPO | FA | During pregnancy | 5 | Center, maternal geographic origin, child's sex |
| Bille2007[38] | Denmark | Oral cleft | FA | First 3 mo | 8 | Period of birth, maternal age, race, smoking, alcohol use |
| Wilcox2007[39] | Norway | CL/P, CPO | FA | −1 to + 3 mo | 9 | Mother's education, smoking, alcohol use dietary folate periconceptional multivitamin use |
| Little 2008[40] | UK | CL/P, CPO | FA, multivitamin | −1 to + 3 mo | 7 | Age, education, race, planned pregnancy, nausea and vomiting during pregnancy |
| Wang 2009[26] | China | Oral cleft | FA, multivitamin | −1 to + 3 mo | 8 | Age, education, race |
| Mirilas 2011[21] | Greece | Oral cleft | FA | First 3 mo | 7 | No |
| Jia 2011[41] | China | CL/P, CPO | FA, multivitamin | First 3 mo | 9 | maternal age, race, smoking, alcohol use |
| Ibarralopez2013[22] | Mexico | Oral cleft | FA | First 3 mo | 7 | Race, sex, alcohol use, smoking |
| Mckinny2013[42] | Thailand | CL/P | FA | −1 to + 3 mo | 7 | Race, sex, alcohol use, smoking |
| Rozendaal2013[13] | Netherland | CL/P, CPO | FA, multivitamin | −1 to + 3 mo | 7 | No |
| Lin 2014[24] | China | Oral cleft | FA | First 3 mo | 7 | No |
| Hao 2015[23] | China | CL/P, CPO | FA, multivitamin | −1 to + 3 mo | 6 | No |
| Xu, L 2015[25] | China | Oral cleft | FA, multivitamin | First 3 mo | 6 | No |
| Mckinny 2016[43] | Netherland | CL/P | Multivitamin | First 3 mo | 7 | No |

CL/P, cleft lip with or without cleft palate; CPO, cleft palate only; FA, folic acid.
*The number represents the month according to being pregnant or not. Minus means the month before conception. The positive is the month after conception.

In the subgroup analysis by geographic region, we observed no evidence of positive association for studies conducted in Australia (OR = 0.81, 95% CI: 0.45–1.47) and Thailand (OR = 0.87, 95% CI = 0.57–1.32). In contrast, we found a significant association among an American population (OR = 0.68, 95% CI = 0.56–0.83) in which a moderate heterogeneity was detected ($I^2 = 46\%$, $P$ value = 0.031). Although significant association was found between the Chinese (OR = 0.54, 95% CI: 0.39–0.74) and

**TABLE 3.** Characteristics of Cohort Studies Reporting Maternal Folate Consumption in Pregnancy and Occurrence of Oral Clefts

| Study | Country | Cleft Type | Supplementation | Exposure Period* | Quality (Points) | Adjustments |
|---|---|---|---|---|---|---|
| Conway 1958[45] | United States | CL/P | Multivitamin | −3 to + 2 mo | 7 | Maternal age, education, sex of baby, smoking status, flu, epilepsy, diabetes, family history |
| Brigg 1976[44] | United States | CL/P, CPO | Multivitamin | First 3 mo | 5 | No |
| Tolarova 1995[46] | Czechoslovakia | CL/P | Multivitamin | −2 to + 3 mo | 7 | No |
| Czeizel 2004[47] | Hungary | CL/P, CPO | Multivitamin | −1 to + 3 mo | 5 | No |
| Li 2015[48] | China | CL/P, CPO | FA | −1 to + 3 mo | 7 | No |
| Czeizel 1999[49] | Hungary | CL/P, CPO | Multivitamin | −1 to + 3 mo | 5 | No |

CL/P, cleft lip with or without cleft palate; CPO, cleft palate only; FA, folic acid.
*The number represents the month according to being pregnant or not. Minus means the month before conception. The positive is the month after conception.

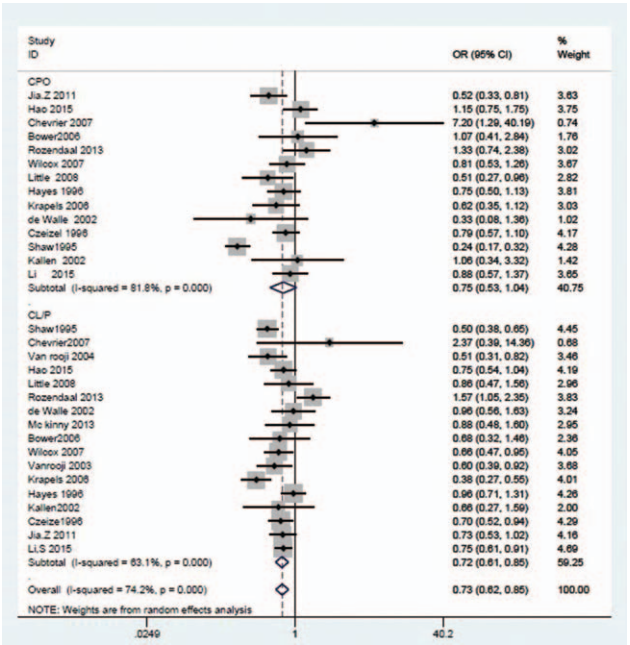


**FIGURE 2.** Odds ratios for the association between maternal folic acid alone consumption during pregnancy, by cleft subtypes (cleft lip with or without cleft palate, cleft palate only). Meta-analysis random-effects estimates were used.

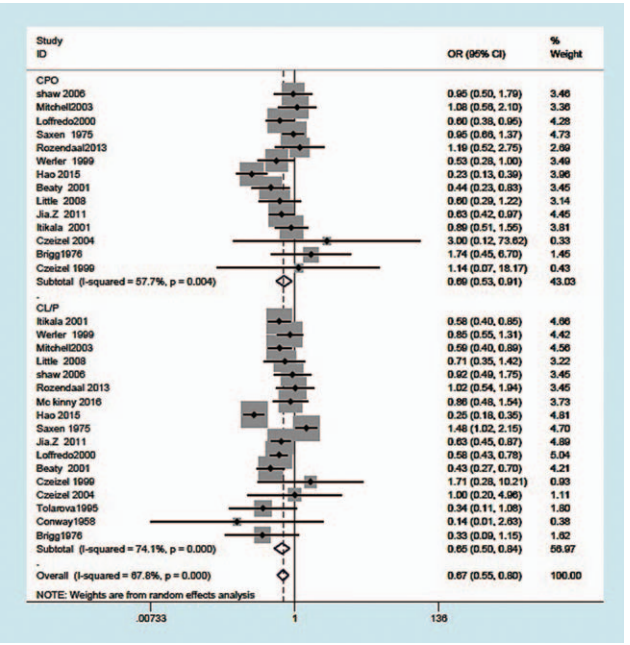


**FIGURE 3.** Odds ratios for the association between maternal multivitamin consumption during pregnancy, by cleft subtypes (cleft lip with or without cleft palate, cleft palate only). Meta-analysis random-effects estimates were used.

European populations (OR = 0.76, 95% CI: 0.64, 0.90), high heterogeneity existed among the studies performed in Chinese ($I^2 = 91\%$, $P = 0.00$) and European populations ($I^2 = 75.4\%$, $P = 0.00$) (Table 4).

## Maternal Folic Acid Intake in Periconception Period

The maternal supplementation period varied widely in the 37 eligible studies included in the meta-analysis. Because oral clefts are reported to occur in the critical period around conception and through the first trimester,[50–52] we carried out a meta-analysis limited to those studies that restricted exposure period to 3 months before conception to the first trimester of pregnancy; thus, 6[15–17,37,53,54] studies were excluded. The result showed a surprisingly lower OR in the subgroup that consumed a multivitamin for cleft subtypes: (CL/P: OR = 0.60, 95% CI 0.45–0.81; CPO: OR = 0.65, 95% CI = 0.46–0.92; slightly lower OR was observed in the group that consumed FA without other vitamin supplements for CL/P (OR = 0.70, 95% CI = 0.58–0.86) and CPO (OR = 0.66, 95% CI = 0.46–0.96) as well.

## Recurrence Studies

The researchers in 3 cohort studies[44–46] included women who had previously given birth to a child with a cleft. All these studies compared women receiving a multivitamin containing FA supplement with women receiving no supplement. (The dose of FA included in the supplements ranged from 0.5 to 10 mg per day). The meta-analysis was conducted with no evidence of heterogeneity ($I^2 = 0.00$, $P$ value = 0.18) and showed a decreased risk for CL/P (OR = 0.33, 95% CI: 0.15–0.73). Only 1 study presented results for CPO that showed an increase in risk (OR = 1.70, 95% CI = 0.45–6.99) (Fig. 4).

## Publication Bias

Figure 5 shows the funnel plot created by plotting the OR to SE (log [OR]) for all included studies. There was some evidence of publication bias as Begg correlation test was statically significant ($P$ value $\leq 0.019$). However, Eager linear regression yielded the contrary finding since $t = 1.61$, $P < 0.11$.

## Sensitivity Analysis

We conducted sensitivity analysis to explore whether an individual study strongly influenced the results by discarding 1 study at a time from our pooled analysis. Sensitivity analysis demonstrated that pooled ORs did not change significantly related to the overall ORs, suggesting the robustness of our results. Furthermore, we created a Galbraith plot to graphically assess the sources of heterogeneity for total studies. (See Supplemental Digital Content, Figure E2, http://links.lww.com/SCS/A305). A total of 13 studies[3,13,17–26,37] contributing to the high heterogeneity were identified. Once the outlying studies were excluded, the heterogeneity was effectively removed ($I^2 = 0.0\%$, $P = 0.50$); however, the new corresponding pooled ORs were not substantially altered (OR = 0.69, 95% CI: 0.65–0.74).

# DISCUSSION

It has been suggested that the facial defects associated with folate deficiency is a consequence of problems during neural tube closure and palate development.[51,52] Evaluation of the role of vitamins, especially FA in the prevention of orofacial clefts has been a special interest of many researchers in recent years.[55] From a scientific perspective, randomization is the ideal study design to assess causal relationships. As it is well known that FA consumption can prevent approximately 50% to 75% of NTDs,[56] performing a randomized controlled trial to investigate the

**TABLE 4.** Results of Stratified Analysis for Overall Studies

| Subgroup Analysis | Number of Studies | Summary OR (95% CIs) | *P* Value for Heterogeneity | $I^2$ (%) | P-value for Differences |
|---|---|---|---|---|---|
| Summary pooled crude OR estimate | 37 | 0.68 (0.603, 0.78) | 0.00 | 80.5% | 0.000* |
| Summary pooled adjusted OR | 16 | 0.75 (0.62, 0.91) | 0.000 | 67.4% | 0.004* |
| design | | | | | |
| Case control | 31 | 0.68 (0.59, 0.78) | 0.000 | 82.8% | 0.000* |
| Cohort | 6 | 0.76 (0.63, 0.90) | 0.486 | 0.0% | 0.002* |
| Geographic region | | | | | |
| United States | 9 | 0.68 (0.56, 0.83) | 0.051 | 46.0% | 0.000* |
| Europe | 19 | 0.75 (0.63, 0.89) | 0.00 | 75.4% | 0.002* |
| China | 6 | 0.54 (0.39, 0.74) | 0.000 | 91.9% | 0.000* |
| Thailand | 2 | 0.87 (0.57, 1.31) | 0.96 | 0.0% | 0.51 |
| Australia | 1 | 0.81 (0.44, 1.47) | 0.47 | 0.0% | 0.49 |
| Cleft subtypes | | | | | |
| CL/P | 30 | 0.68 (0.59, 0.79) | 0.000 | 69.5% | 0.000* |
| CPO | 24 | 0.72 (0.57, 0.89) | 0.000 | 73.7% | 0.003* |
| Oral cleft | 7 | 0.59 (0.36, 0.98) | 0.000 | 94.1% | 0.042* |
| Supplementation | | | | | |
| Folic acid alone | 25 | 0.68 (0.58, 0.80) | 0.000 | 81.8% | 0.000* |
| Folic acid + other vitamins | 21 | 0.68 (0.55, 0.84) | 0.000 | 79.4% | 0.000* |
| Exposure period | | | | | |
| Periconception period | 31 | 0.64 (0.56, 0.74) | 0.000 | 80.8% | 0.000* |
| During pregnancy | 6 | 0.90 (0.71, 1.14) | 0.001 | 65.3% | 0.41 |

* $P < 0.05$.

effects of periconceptional folic acid intake on oral clefts would be unethical.

With the exception of the association between FA alone supplementation and CPO, the present meta-analysis showed a clear link between maternal FA supplementation and decreased risk of oral clefts in newborns. This finding was consistent across most of the subgroup analyses. The combined data of overall studies indicated that maternal FA alone resulted in a 28% decreased risk of CL/P and 25% risk of CPO. Woman who took FA as a part of a multivitamin were 33% less likely to have a child with CL/P and 31% less likely to have a child with CPO, compared with woman did not. The observed risk reduction is consistent with a previous meta-analysis performed in 2008.[57] As expected, the increased number of eligible studies in our review yielded to a narrower CI in comparison with previous meta-analysis.

In the current study, a multivitamin containing FA showed a more pronounced protective effect on CL/P and CPO than folic acid alone. There was a controversy in the result of previous observational studies about whether FA alone or FA containing multivitamins is better to prevent oral clefts.[8,47,58] In the studies on the association of multivitamin and oral clefts, the reported preventive effectiveness of multivitamin ranged from 0.18 to 3.0. The justification of the stronger effect of multivitamin is that the additive or synergic effect of B group vitamins such as folic acid, B2, B6, can strengthen the efficacy of multivitamins.

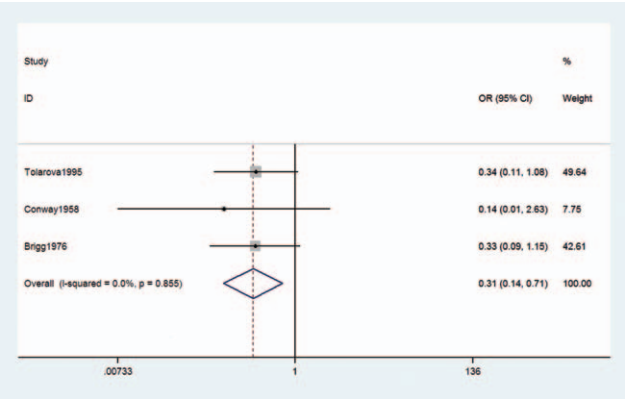


**FIGURE 4.** Fixed effects meta-analysis for recurrence studies of cleft lip with or without cleft palate showing odds ratios and 95% confidence interval.

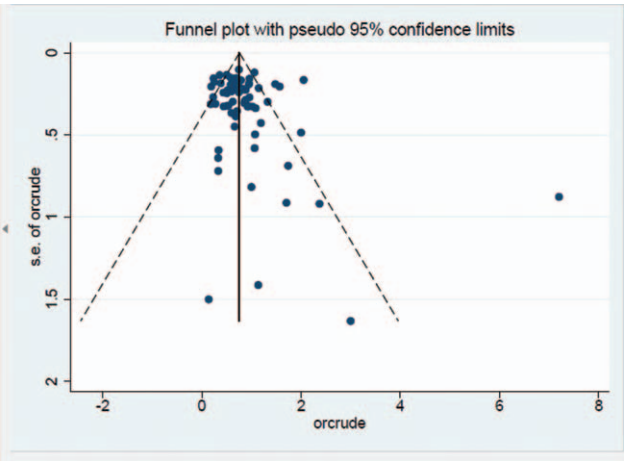


**FIGURE 5.** Funnel plot for assessing publication bias.

The subgroup analysis investigating timing of supplementation intake suggested that women starting FA intake before pregnancy and continuing during the first 3 months of pregnancy had a lower risk of having a child with CL/P and CPO, while no significant risk reduction was found in women who did not consume FA in the periconception period. According to the mentioned preventive effect of multivitamins, it can be suggests that multivitamins, particularly when started prior to pregnancy, can prevent 40% and 35% of patients with CL/P and CPO, respectively. Early supplementation is an indicator of good primary health care just like not smoking or drinking alcohol during pregnancy. It may also be a marker for planned pregnancy. The vast majority of women may not recognize their pregnancy until after 4th week gestation[59] and as a result, they cannot take advantage of the supplementation during the critical gestational period. Our data emphasize the need for an educational campaign to suggest the start of FA supplement intake to women who decide to have a baby.

In our meta-analysis, we could not suggest an optimal dose of periconceptional FA supplement because most women had taken supplements containing at least 0.4 mg folic acid, which is widely recommended by various experts for preventing NTDs.[11,60]

In the meta-analysis conducted on the recurrence studies, the results demonstrated a reduction in the risk of cleft recurrence with maternal multivitamin consumption. The results of the recurrence studies should be interpreted cautiously, because these intervention studies were not randomized, with some serious flaws such as lacking a real control group, small sample sizes, and wide CIs. Wehby et al[61] in a recent randomized clinical trial assessed the effect of high dose (4 mg) versus low dose (0.4 mg) folic acid on isolated cleft recurrence. They reported that the recurrence rate was the same between the 2 studied groups and no elevated perinatal risk was observed in the high dose compared with the low dose group. This finding suggests that the high dose of FA supplementation may be a safe intervention against oral cleft recurrence.

The results of stratified analysis by geographical region indicated that significant heterogeneity was still found in the Chinese and European populations ($P < 0.05$). Furthermore, according to the subtotal OR of the Chinese and American population it seems that the protective effect of FA supplementation is more pronounced in these 2 populations than in the others, which may be related to the different genetic backgrounds and environment factors.

Our study has several important strengths. We systematically searched the literature using multiple electronic databases and the reference lists from the retrieved articles to identify any additional pertinent studies with no language limitation. Thus, our study included data for 27,276 patients, which is enough to perform statistical analysis to investigate the potential association between maternal FA supplementation and the risk of oral cleft. We carried out a quality assessment based on the criteria from the NOS,[62] so that all of the included studies, except 1,[54] received an appropriate score, suggesting no risk of bias. Another strength is that, although there was evidence of heterogeneity in our overall analysis, we conducted a number of sensitivity, subgroup, and Galbraith plot analyses to identify the source of heterogeneity. When we excluded outlying studies, the heterogeneity was notably removed, whereas the corresponding pooled OR was not materially altered, indicating that the overall results were statistically robust.

Despite the clear strengths of our study, including large sample sizes, this study had some limitation that merit consideration. First, the majority of eligible literature was patient control studies that were susceptible to selection and information biases. Furthermore, these studies displayed considerable heterogeneity that limits our ability to determine whether these outcomes indicate a true relationship. One reason contributing to heterogeneity could be explained by some confounding factors, such as maternal age, race, level of education, economic and social status, obstetric history, pregnancy planning, alcohol consumption, smoking, dietary folate intake, and medications. These factors may lead to clinical heterogeneity, as crude ORs from individual studies were extracted for meta-analyses; our pooled results may be confounded. It is worth mentioning that we conducted meta-analysis base on adjusted ORs, the heterogeneity was reduced to some degree, but significant heterogeneity still existed. Second, according to Begg correlation test, our results were affected by publication bias to some extent. This can be due to under reporting of studies with no evidence of decreased risk for FA supplementation and oral cleft.

## CONCLUSIONS

In summary, the findings of the present meta-analysis showed the protective effect of periconceptional multivitamin containing folic acid on oral clefts. Our finding regarding risk reduction of specific types of oral cleft seems important from a public health aspect. At present, we can recommend the daily use of a multivitamin supplement including 0.4 to 0.8 mg of folic acid with a healthy diet and lifestyle for women at the reproductive age, who want have a baby without oral clefts. More prospective studies, particularly in developing countries, are required to further investigate the association between maternal folate supplementation and the risk of oral clefts.

## ACKNOWLEDGMENT

The authors thank the Vice-Chancellor of Research at Mashhad University of Medical Sciences for the financial support provided for this project.

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