A Meta-Analysis of the Relationship Between Maternal Folic Acid Supplementation and the Risk of Congenital Heart Defects

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SUMMARY

Controversial opinions exist with respect to the relationship between maternal folic acid (FA) supplementation and birth prevalence of congenital heart defects (CHDs).

Eligible articles were retrieved by searching databases, including PubMed, Cochrane library, EMBASE, CNKI, and WanFang up to September 2015. A meta-analysis was performed to evaluate the effects of FA on CHDs. Odds ratios (ORs) and 95% confidence interval (CIs) were merged using STATA 12.0. Meta-regression analysis was used to explore the possible sources of heterogeneity. Subgroup analysis according to the selected sources was also performed. Publication bias was assessed by Egger’s test.

Twenty studies were included in the meta-analysis. The overall analysis showed that FA supplementation was significantly associated with decreased risk of CHDs. The meta-regression analysis showed that geographical area could be an important source of heterogeneity. The subgroup analysis based on the geographical area revealed that FA supplementation during pregnancy was a protective factor against CHDs in Chinese and European patients, but not in American patients. Subgroup analysis according to literature quality also displayed positive associations between FA supplementation and the decreased risk of CHDs of China.

FA supplementation during pregnancy significantly decreases the risk of CHDs in newborns in China and Europe. (Int Heart J 2016; 57: 725-728)

Key words: Literature search, Meta-regression analysis, Geographical area, Pregnancy

Congenital heart defects (CHDs) are the most common structural abnormalities presenting at birth, and they are also one of the leading causes of perinatal and infant mortality. It is reported that the prevalence of CHDs accounts for 6% of all neonatal death factors, and also accounts for 46% of all congenital lethal factors. Recently, the survival of newborns with CHDs has increased due to massive breakthroughs in cardiovascular diagnostics and cardiothoracic surgery; however, this trend consequently generates a completely novel and steady increasing population with grown-up congenital heart disease (GUCH). Patients with GUCH often need long-term expert medical care with high healthcare-related costs. Moreover, recently, an increasing number of women have postponed conception to an older age, which consequently results in a higher birth prevalence of congenital defects. These defects lead to great economic pressure and mental burdens on society and the families, therefore, searching for effective prevention measures for CHDs is of great practical significance.

Folic acid (FA) is an essential nutrient and plays an important role in the development of the cardiovascular system. The association of FA or multivitamins containing FA supplementation during the critical periods of organ formation with the risk of the occurrence of CHDs has been recognized in past decades. Several studies have reported that FA or multivitamins containing FA supplementation taken during pregnancy could significantly reduce the risk of CHDs, particularly conotruncal defects and ventricular septal defects (VSDs) in newborns. However, the available data from epidemiological studies on the association between FA supplementation during pregnancy and CHDs are inconsistent and controversial. For instance, Werle, et al found no association between multivitamin supplementation and conotruncal defects or VSDs. Willemij, et al did not observe a protective effect of FA on heart defects among infants with Down syndrome. There is a need for comprehensive analysis of this association.

Therefore, the purpose of this meta-analysis was to assess the correlation between FA or multivitamins containing FA supplementation taken during pregnancy and the risk of CHDs and provide more comprehensive and reliable evidence of evidence-based medicine.
**Methods**

**Literature search:** Eligible articles were identified by searching the following electronic databases: PubMed, Cochrane Central Register of Controlled Trials (CENTRAL) and EMBASE up to September 2015. The key terms used were PubMed: “folic acid [MESH]” AND “congenital heart disease OR congenital heart defect OR CHD” AND “pregnant OR pregnancy” and Cochrane and EMBASE: “folic acid OR Folate” AND “congenital heart disease OR congenital heart defect OR chd” AND “pregnant OR pregnancy”. Chinese National Knowledge Infrastructure (CNKI) (http://www.cnki.net/) and WanFang (http://www.wanfangdata.com.cn/) databases were also searched with the above search terms in Chinese.

The articles were included if they met the following criteria: 1) All clinical trials studied the association between FA (FA-containing multivitamin) supplementation and the risk of CHDs. 2) The available variables were corrected OR and 95% confidence interval (CI) or could be calculated according to the original data. 3) The objects of the case group were CHDs patients and those of the control group were people without CHDs.

The exclusion criteria were as follows: 1) The available values had only the OR without 95% confidence interval (CI) or could not be obtained by calculation. 2) The studies were only related to multivitamin supplementation and did not clearly state they contained FA. 3) Reviews, case reports, meeting abstracts, and comments. 4) Repeated reports or studies whose data were ambiguous.

**Data extraction:** The following characteristics were collected from each study: the name of the first author, year of publication, sample size, the numbers of objects supplemented or not with FA in 2 groups, stage and dosage of FA supplementation, and whether the variables were corrected. The above data were extracted independently by two investigators, and in cases of conflicting evaluations, the disagreements were resolved by discussion with a third investigator. Meanwhile, only the adjusted results were extracted if the studies presented both unadjusted and adjusted results. The results of the matched control group were included if the studies presented both matched and unmatched results in the control group. If the effect size was not presented but detailed data was available, the OR values were calculated according to the corresponding 4-fold tabular statistical method.

**Quality assessment of included studies:** The quality of the included literature was assessed using the Newcastle–Ottawa Scale system. In this scoring system, each study included in the meta-analysis was judged on 3 broad perspectives: the selection of the study cases, the comparability of the study populations, and the ascertainment of the exposure.20,21 The total scores were 9 points, and scores of 5-6, 7, and 8 were regarded as low, medium, and high, respectively.

**Statistical methods:** Data were analyzed using STATA 12.0 Software. For each study, the odds ratio (OR) and its 95% confidence interval (95% CI) were calculated to assess the association strength. The $Q$ test and $I^2$ statistic were performed to assess the heterogeneity across studies.22,23 A $P$ value in the $Q$ test ≥ 0.05 or $I^2$ ≤ 50% was considered to be homogenous and a fixed-effect model was used to merge the effect size. Otherwise, it was considered to have a significant heterogeneity; thus, a random-effects model was used. Meta-regression analysis was also used to investigate the possible sources of heterogeneity. If $P < 0.05$, subgroup analysis by the identified variables was performed. Egger’s linear-regression method was used for the assessment of publication bias.

**Results**

**Characteristics of eligible literature:** The details of the citation search are presented in a flow diagram (Supplemental Figure I). According to the search strategy, a total of 20 citations were included in the meta-analysis.4,14,15,19,24-39 The characteristics and information of the eligible studies are shown in Supplemental Table I. Nine articles were written in English and 11 in Chinese. These studies were published between 2006 and 2015, and the populations in the studies were Chinese, American, or Dutch. Quality assessments of the selected 20 studies are summarized in Supplemental Table I and presented a relatively high-quality (6-8 points).

**Overall analysis:** Statistically significant evidence of heterogeneity was found between studies ($I^2 = 88.8\%$, $P = 0.000$) in the overall analysis of the 20 case-control studies and the random-effects model was used (Supplemental Figure 2). Maternal FA supplementation was significantly associated with the decreased risk of CHDs (OR = 0.60, 95% CI: 0.49-0.71).

**Meta-regression analysis and subgroup analysis:** Meta-regression analysis based on the adjustment, geographical area, and study quality was performed to investigate the potential sources of heterogeneity. The results showed that the geographical area was significantly correlated with the heterogeneity of eligible studies (Supplemental Table II).

Therefore, subgroup analysis was performed according to the geographical area (China, America, and Europe) (Supplemental Figure 3). No significant heterogeneity was found among studies in American populations ($I^2 = 0.0\%$, $P = 0.757$) and no significant evidence of correlation between maternal FA supplementation and a decreased risk of CHDs was found (OR = 0.92, 95% CI: 0.83-1.02). Although significant association was observed among the Chinese (OR = 0.44, 95% CI: 0.33-0.56) and European populations (OR = 0.83, 95% CI: 0.75-0.91), high heterogeneity still existed among the studies performed in Chinese populations ($I^2 = 70.9\%$, $P = 0.000$) and European populations ($I^2 = 65.5\%$, $P = 0.034$), which was probably related to the uneven control of overall quality in these studies.

Stratified analysis by literature quality of the eligible studies in Chinese populations was performed. The heterogeneity was significantly reduced (high quality: $P = 0.757$, $I^2 = 0\%$; moderate quality: $P = 0.091$, $I^2 = 65\%$; low quality: $P = 0.243$, $I^2 = 26.8\%$). Consistently positive associations between FA supplementation and decreased risk of CHDs among Chinese populations were observed (high quality: OR = 0.48, 95% CI: 0.37-0.58; moderate quality: OR = 0.62, 95% CI: 0.38-0.85; low quality: OR = 0.53, 95% CI: 0.30-0.93).

**Publication bias analysis:** Egger’s linear-regression analysis indicated that there was a publication bias ($t = 5.46, P < 0.01$).
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DISCUSSION

The present meta-analysis of 20 case-control studies shows a clear link between maternal FA or multivitamins containing FA supplementation during pregnancy and the decreased risk of CHDs in newborns. The data indicate that FA supplementation results in a 40% decreased risk of CHDs. The finding of the preventive effect of FA against CHDs in our analysis is consistent with the observations in most of the individual studies.

Since a high heterogeneity existed, we performed meta-regression analysis and stratified analysis to investigate the potential sources of the between-study heterogeneity. The results of both methods came to an agreement that geographical area made a significant contribution to the heterogeneity. The results of stratified analysis by geographical area indicated that except those for the American populations ($I^2 = 0.0\%$, $P = 0.757$), significant heterogeneity is still found in the Chinese and European populations ($P < 0.05$). Furthermore, the subotal OR of the American populations suggested an insignificant association of FA supplementation with CHDs, while there was significant association among the Chinese and European populations. Therefore, further stratified analysis by literature quality was performed in studies of the Chinese populations and the heterogeneity disappeared and the results still indicated a positive correlation between FA supplementation and the reduced risk of CHDs.

Recently, research on the mechanism of FA involved in the occurrence of CHDs also demonstrates a preventive effect of FA on the birth prevalence of CHDs. On one hand, FA is a cofactor for the remethylation of homocysteine metabolism, and FA deficiency can result in hyperhomocysteinemia, which is frequently associated with congenital defects of the heart and neural tube.\(^{40,41}\) Moreover, the most important cause of hyperhomocysteinemia is the polymorphism of the MTHFR gene, which produces a crucial enzyme for the metabolism of FA and homocysteine. Previous studies have revealed that maternal MTHFR 677C→T polymorphism (rs1801133) is associated with the risk of CHDs and the carriers with MTHFR 667TT genotype had a higher risk of CHDs in newborns.\(^{25}\) Thus, an early diagnosis of MTHFR 677C→T polymorphism in pregnant women or before conception would assist in planning optimal and timely treatment, thus decreasing neonatal and infant mortality attributed to CHDs. On the other hand, it is known that maternal medication use can exert teratogenic effects on the fetus,\(^{45}\) and therefore, the efflux of medication and other toxins from the circulation is of great importance. A recent study reported that the cellular FA concentration also determines the function of the multidrug resistance 1 (MDR1) transporter which is responsible for an adequate efflux of medication and other toxins from the circulation.\(^{25}\) All these results highlight the significance of confirming the link between FA supplementation during pregnancy and the reduction risk of CHDs.

Notably, the findings of the present meta-analysis showed that the protective effect of folic acid supplementation seemed to be more pronounced in the Chinese and European populations than in the American populations, which may be related to the different genetic backgrounds and environment factors in the study populations.\(^{46}\) There was a significant difference in the association of MTHFR 667TT with CHDs risk between the European (OR, 1.14; 95% CI, 1.01-1.28) and American populations (OR, 0.87; 95% CI, 0.73-1.05),\(^{47}\) which is consistent with our study of FA supplementation. Meanwhile, further study is needed to confirm this hypothesis.

Despite the clear strengths of our study including large sample sizes, some limitations merit serious consideration. First, all of the included studies were case-control studies, so the results need to be confirmed by more prospective cohort studies in the future. Secondly, even though it has been reported that an earlier beginning of FA supplementation before pregnancy and a longer duration of FA supplementation appear to be more effective in reducing CHDs,\(^{48}\) the present analysis was incapable of analyzing the effects of the doses of FA intake, the different starting times, and the duration of FA supplementation due to a lack of accurate information. In addition, the severity of the CHDs was not stratified. Thus, further analysis was necessary to investigate the above factors. Thirdly, there was a publication bias which might have resulted from the lower quality studies.

Conclusions: In conclusion, the present meta-analysis demonstrates that maternal FA supplementation significantly decreases the risk of CHDs in newborns. Therefore, this will provide evidence for the reduction of CHD incidence by FA supplementation and play an important role in minimizing adverse pregnancy outcomes.

DISCLOSURE

Conflict of interests: No author has any potential conflict of interest.

REFERENCES

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Supplemental Files
Supplemental Tables I, II, III
Supplemental Figures 1, 2, 3
Please see supplemental files; https://www.jstage.jst.co.jp/article/hij/57/6/57_16-0545/-article/supplement