The association between folic acid supplementation and congenital heart defects: Systematic review and meta-analysis

Amsalu Taye Wondemagegn1 and Mekbeb Afework2

Abstract
Introduction: Various trial and epidemiological studies consistently documented the association between maternal folic acid supplementations and neural tube defects. However, existing literatures revealed inconclusive findings about maternal periconceptional folic acid supplementations and the risk of congenital heart defects. Thus, the current systematic review and meta-analysis was aimed to estimate the pooled association between maternal periconceptional folic acid supplementations and congenital heart defects.

Methods: Electronic searches of PubMed, Web of Science/Scopus, Cochrane library and Google Scholar databases were conducted to access the required studies published up to March 2021. Predetermined eligibility criteria were used for study selections. Data extraction were independently done on excel. STATA version 14 software was used to calculate the pooled effect size with 95% confidence intervals (95% CI) of maternal periconceptional folic acid supplementations on congenital heart defects using the DerSimonian and Laird random effects meta-analysis (random effects model). Statistical heterogeneity was checked using the Cochran Q test (chi-squared statistic), I² statistic, and by visual inspection of the funnel plot.

Results: A total of 37 studies of case–control, cohort and randomized controlled trial in nature were included in the review. The finding of the present systematic review and meta-analysis indicated that periconceptional folic acid supplementation significantly decreases the risk of congenital heart defects (risk ratio (RR), 0.79; CI, 0.71, 0.89). Both Cochrane Q test statistic ($\chi^2 = 19.33, p = 0.962$) and I² test statistic ($I^2 = 0.0\%$, $p = 0.962$) did not reveal statistically significant heterogeneity among included studies. In this meta-analysis, traditional funnel plot, Begg’s funnel plot, Egger’s weighted regression ($p = 0.13$) as well as Begg’s rank correlation statistic ($p = 0.676$) revealed no evidence of publication bias.

Conclusion: The present systematic review and meta-analysis found that maternal periconceptional folic acid supplementation was significantly associated with the risk of congenital heart defects. The risk of congenital heart defects was significantly reduced by 21% among those children of mothers who use periconceptional folic acid supplementations in high-income countries. We recommend that a large prospective study be conducted to investigate the association between maternal periconceptional folic acid supplementation and occurrence of congenital heart defect of various types, especially in the developing countries.

Keywords
Folate, folic acid, multivitamin, congenital anomalies, congenital heart defects, association, effects, systematic review

Introduction
Congenital heart defects (CHDs) are structural defects of the heart that are detected prenatally, at birth or later in life. They are the most common congenital anomalies, occurring in between 6 and 13 per 1000, clustering around 8 per 1000 live births worldwide.1,2

CHD is a major public health concern which touches the livelihood of affected children and their care givers, the
families and the society at large. It is the first among causes of death due to congenital anomalies in children under 5 years old. It can also be a cause for lifelong disability, morbidity, and increased health care costs in children and adults.

As a result of its serious and critical impacts on the world’s population, researchers in the area have extensively worked to identify causes for cardiac developmental errors, and there is conclusive acceptance of the opinion that the etiology of CHD is complex and probably lies within the interaction of environmental exposures and inherited factors. Currently, there exists many scientific evidences which demonstrated association between maternal exposures to various environmental factors. These include folic acid, smoking, alcohol, illicit drugs use, caffeine uses and others and the risk of major congenital defects such as, neural tube defects, cleft lip and cleft palate, down syndrome and CHDs in offspring.

Various trial and epidemiological studies consistently documented association between maternal folic acid supplementations and neural tube defects. However, existing literatures revealed contradicting findings between maternal periconceptional folic acid supplementations and the risk of some of other congenital anomalies especially maternal periconceptional folic acid supplementations and the risk of CHDs. There are many published literatures to date which have reported the association between periconceptional folic acid supplements and the risk of CHDs in offspring, in which the finding of those studies is equivocal with report of positive, negative and no association probably due to inadequate sample size. Thus, the current systematic review and meta-analysis aimed to estimate the pooled association between maternal periconceptional folic acid supplementations and occurrence of CHDs using a large sample size.

Methods

The report of the present systematic review and meta-analysis is presented based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. The protocol of this review was not registered in PROSPERO.

Search strategies

To perform this systematic review and meta-analysis, all relevant articles were rigorously searched. Electronic searches of PubMed, Web of Science/Scopus, Cochrane library and Google Scholar databases were conducted for the required studies published up to March 2021. The study findings were accessed using the following Medical Subject Heading Terms (MeSH Terms) and free text terms individually and in combination through “AND,” and “OR” Boolean operators as follows: “Multivitamin” AND “supplementation” OR “folic acid” [MeSH] OR “folic” AND “acid” OR “folic acid” AND “risk” [MeSH] OR “risk” OR “risk of” AND “heart defects, congenital” [MeSH] OR “heart” AND “defects” AND “congenital” OR “congenital heart defects” OR “congenital” AND “heart” AND “defects.” In addition, reference lists of retrieved articles and key review articles were also investigated to identify more eligible studies.

Inclusion and exclusion criteria

Articles included in this systematic review and meta-analysis were (1) prospective randomized controlled trials, cohort studies, and case–control studies; (2) demonstrated the association between maternal periconceptional folic acid intake and CHDs overall or any subtypes of CHD in offspring; (3) published in English language; (4) reported risk ratios/odds ratios (RRs/ORs) and associated 95% confidence intervals (CIs) or provided raw data from which RRs/ORs could be calculated; and (5) defined case groups were CHDs patients and those of the comparison/ control groups were people without CHDs overall or any subtypes.

Exclusion criteria for this paper were (1) the reported values of the articles had only the RR/OR without 95% CI or could not be obtained by calculation from given raw data; (2) the reported articles on multivitamin supplementation that didn’t clearly report whether the supplementation contained folic acid; (3) reviews, animal studies, editorials, clinical answers, case reports, meeting abstracts, and commentaries; and (4) studies whose data were vague.

Data extraction

Two authors (A.T.W. and M.A.) independently extracted all necessary data using data extraction template on Microsoft Excel. The following important information were extracted: names of first author, year of publications, study settings, study period, study designs, sample size, case classification data, exposure and outcome information, and adjusted ORs/RRs with corresponding CIs. When no adjusted estimates were available, we extracted a crude estimate. If no estimate of relative risk/OR was provided in a given study, we calculated ORs or RRs and 95% CIs from the raw data presented in the study using standard equations. Controversies during the data extraction process were resolved through discussion and common understanding was created between the two authors.

Quality of the included studies

The quality of the included studies was judged using the Newcastle–Ottawa Scale system. In this system, each study included in this systematic review and meta-analysis was judged on the following three broad parameters: the selection of the study groups, the comparability of the study populations, and the ascertainment of the exposure or outcome of interest for case-control and cohort studies, respectively. The maximum quality scores were expected to be 9 points, and in our review and meta-analysis we demarcated a
high-quality study as one with a quality score greater than or equal to 7, medium quality 5–6 and low-quality study that score below 5.

**Statistical analysis**

Extracted data in Microsoft Excel spreadsheet software were imported onto STATA/SE for windows version 14 software for further analysis. STATA version 14 software was used to calculate the pooled effect size with 95% CI of maternal periconceptional folic acid supplementations on CHDs using the DerSimonian and Laird random effects meta-analysis (random effects model).

**Assessing heterogeneity and publication bias**

Heterogeneity across studies was assessed using I² statistic and Cochran Q test (chi-squared statistic). The I² statistic is the percentage of variation (inconsistency) in the measures of association across studies that is due to heterogeneity rather than chance.10 The value of I² ranges between 0% and 100%, where 0% indicates no observed heterogeneity and large values indicate increasing heterogeneity.10 An I² value of 25%, 50% and 75% is considered respectively as low, moderate and high heterogeneity.10 We conducted subgroup analyses based on study design, study settings, sample size (≤4000 versus >4000), and year of publication (before 2013 versus 2013 and after). Meta-regression analysis was also performed to investigate the possible sources of heterogeneity among subgroups. Finally, we performed sensitivity analyses to explore whether individual study strongly influenced the results of the meta-analysis, by omitting one study at a time.

Publication bias was assessed via visual inspection of traditional and Begg’s funnel plot for asymmetry. In addition, publication bias was also assessed using both Egger’s linear regression11 and Begg’s rank correlation12 methods, and for both tests statistically significant publication bias was declared at p value < 0.05.

**Results**

**Retrieved studies**

Initial search on the stated databases using periconceptional folic acid supplementation outcomes on CHDs yielded a total of 21,942 research findings. We removed duplicate retrievals and 18,742 reports remained. Through initial screening, 18,502 reports were excluded by reviewing their titles and/or abstracts which were found to be irrelevant because of one of the following reasons: the titles and/or abstracts of most of the papers were not directly related to the present topic and the titles and/or abstracts of the remaining studies reported effects of folic acid supplementation on other birth defects. Full text findings of 240 articles and 11 articles identified through review of reference lists of retrieved articles were assessed for eligibility based on predetermined inclusion and exclusion criteria and 214 studies were excluded. Which means, after review of entire content of articles, 211 articles were excluded due to irrelevant of exposure and/or outcome. These papers consider multivitamin use as exposure variable and not explicitly determined folic acid supplementation impact on the outcome variable of interest (CHDs). Another three articles were excluded due to inappropriate reporting of folic acid exposure status and the risk of corresponding outcomes of interest. Finally, 37 studies which fulfilled the inclusion criteria were included in the present systematic review and meta-analysis (Figure 1).

**Description of the background characteristics of included studies for the systematic review and meta-analysis**

The publication year of included studies in this systematic review and meta-analysis ranged from 1995 to 2021. Out of 37 included studies, almost 50% were conducted in the United States and Hungary. Specifically, 9 studies were obtained from the United States,13–25 and another 9 studies were from Hungary.26–30 The remaining 7 studies were from China;31–37 3 studies from Netherlands;38–40 2 studies from the United States and Canada;41,42 2 studies from Norway;47,48 1 study from Denmark and Norway,41 1 study from Russia;46 1 study from Northern Ireland;47 1 study from Australia38 and 1 study from India.49 Regarding the study design of included studies, 27 (72.97%) are case–control studies.13–25,28,29,31–33,35–37,38–42,46–48 The remaining 8 are cohort studies42,36,37,39,43,45,49 and 2 randomized controlled trial (RCT) studies.26,30 The sample size of included studies ranged from 407 with the use of case–control study design in the United States and Canada to 894,927 participants with the use of cohort study design in Norway. In case–control studies, the number of cases ranged from 77 in Hungary to 10,593 in China, and the number of controls ranged from 184 in the United States and Canada to 887,580 in Norway. The overall period of exposure of study participants for folic acid supplementation of included studies were 3 months before and until 3 months after conception. The majority (30.3%) of included studies exposure period for folic acid were 1 month before conception through first trimester of pregnancy followed by exposure during first trimester of pregnancy (26.5%), and 20.6% of included studies exposure period were 3 months before through first trimester of pregnancy (Table 1). Majority (30 or 81.1%) of included studies reported the risk of folic acid exposure to CHDs with corresponding 95% CI, and from the remaining 7 articles included, the crude risk is calculated from raw data. Overall, included studies in the present meta-analysis revealed negative, no and positive association between folic acid supplementation and risk of CHDs of various type (Table 1). Most of the studies included in this meta-analysis reported association between folic acid supplementation and risk of overall CHDs...
alone as well as with specific types of CHDs, while others revealed the association with specific types of CHDs alone (Table 1).

Findings of the association between periconceptional folic acid supplementation and CHDs

The pooled relative risk of overall CHDs among those children born from mothers who had periconceptional folic acid supplementation were 0.79 (0.71, 0.89) compared with those born from mothers without having periconceptional folic acid supplementations in the random effects model. In general, the finding of the present systematic review and meta-analysis found periconceptional folic acid supplementation significantly decreases the risk of CHDs by 21% (RR, 0.79; CI, 0.71, 0.89) (Figure 2).

Results of heterogeneity and publication bias of this meta-analysis

Analysis of included studies did not reveal statistically significant heterogeneity using both Cochrane Q test statistic ($\chi^2 = 20.13$ (df=36), $p = 0.985$) and $I^2$ test statistic ($I^2 = 0.0\%$, $p = 0.962$) Figure 2). On a visual observation, almost the effect estimates of CHDs were distributed symmetrically on traditional funnel plot (Figure 3), and this indicated that there was no evidence of publication bias. Moreover, to ascertain this Begg’s funnel plot (Figure 4) with Begg’s rank correlation test was conducted, and the result of the test statistics revealed that there was no significant bias with Kendall’s score of 70 and $p = 0.36$. More importantly, Egger’s weighted regression test statistic was conducted, and this test revealed that there was no significant evidence of publication bias with $r = -0.34$ (95% CI = −0.81, .13) and $p = 0.16$.

Subgroup analysis

Although statistically significant heterogeneity was not observed in the overall analysis, subgroup analysis was performed based on the year of publication, study settings, study design and sample size. Similar with the overall analysis, the subgroup analysis of included studies did not reveal significant heterogeneity (Table 2). In this random effect model, studies done in 2013 and after revealed significant effect size, while the studies done before 2013 did not reveal
Table 1. Summary characteristics of included studies in this systematic review and meta-analysis.

| Study        | Publication year | Study settings/country | Study design | Sample size | Exposure duration                                      | Effect size | 95% CI     | Types of CHDs | Confounders adjusted |
|--------------|------------------|------------------------|--------------|-------------|------------------------------------------------------|-------------|------------|---------------|---------------------|
| Shaw et al.  | 1995             | USA                    | Case–control | 688         | 1 month before until 2 months after conception      | 0.53        | 0.34, 0.85  | CTDs          | Yes                 |
| Czeizel AE   | 1996             | Hungary                | RCT          | 4862        | 1 month before conception through 2nd missed menstrual period | 0.48        | 0.23–1.03  | CHDs          | No                  |
| Czeizel AE   | 1998             | Hungary                | RCT          | 4862        | 3 months before pregnancy through 2nd missed menstrual period | 0.42        | 0.19–0.98  | CHDs          | No                  |
| Scanlon et al.| 1998             | USA/ Washington DC     | Case–control | 805         | Pre-conceptional supplementation                     | 1.08        | 0.69–1.7   | OTDs          | Yes                 |
| Werler et al.| 1999             | USA & Canada           | Case–control | 864         | Periconceptional use (28 days before through 28 days after LNMP) | 1.2         | 0.8, 1.8   | VSDs          | Yes                 |
| Botto et al. | 2000             | USA                    | Case–control | 3987        | 3 months before conception through 1st 3 months of pregnancy | 0.76        | 0.6, 0.97  | CHDs          | Yes                 |
| Correa et al.| 2003             | USA                    | Case–control | 6307        | 3 months before conception through 1st 3 months of pregnancy | 0.45        | 0.24, 0.84 | OTDs          | Yes                 |
| Czeizel et al.| 2004             | Hungary                | Cohort       | 6112        | 28 days before conception through 1st 3 months of gestation | 0.6         | 0.38, 0.96 | CHDs          | Yes                 |
| Bower et al. | 2006             | Australia              | Case–control | 1053        | 1 month before conception to 1st 3 months of gestation | 1.24        | 0.84, 1.82 | CHDs          | No                  |
| Meijer et al.| 2006             | USA & Canada           | Case–control | 407         | 1st 3 months of pregnancy                             | 0.95        | 0.61, 1.47 | CHDs          | Yes                 |
| Thomas et al. | 2008             | India                  | Cohort       | 462         | 1st trimester of pregnancy                            | 1.49        | 0.63, 3.49 | CHDs          | No                  |
| Malik et al. | 2008             | USA                    | Case–control | 7014        | 1 month before conception through 1st 2 months of conception | 1           | 0.9, 1.12  | CHDs          | Yes                 |
| Smedts et al.| 2008             | Netherlands            | Case–control | 600         | 1 month prior to conception through 1st 2 months of conception | 1.3         | 0.9, 1.9   | CHDs          | Yes                 |
| Shaw et al.  | 2010             | USA                    | Case–control | 1001        | 2 months before through 2 months after conception     | 1.03        | 0.66, 1.4  | TGAs          | No                  |
| Van Beynum et al. | 2010       | Netherlands            | Case–control | 3012        | 4 weeks before conception to 8 weeks after            | 0.74        | 0.62, 0.88 | CHDs          | Yes                 |

(Continued)
| Study                   | Publication year | Study settings/country | Study design | Sample size | Exposure duration                                      | Effect size | 95% CI      | Types of CHDs | Confounders adjusted |
|------------------------|------------------|------------------------|--------------|-------------|-------------------------------------------------------|-------------|-------------|---------------|---------------------|
| Hobbs et al.           | 2011             | USA                    | Case–control | 667         | Intake during pregnancy                                 | 1.03        | 0.96, 1.12  | CHDs          | Yes                 |
| Bean et al.            | 2011             | USA                    | Case–control | 1118        | Before conception or during 1st 4 weeks of conception  | 0.79        | 0.39, 1.19  | AVSDs         | No                  |
| Obermann-Borst et al.  | 2011             | Netherlands            | Case–control | 591         | 4 weeks before conception to 10 weeks after            | 0.79        | 0.39, 1.19  | AVSDs         | No                  |
| Lupo et al.            | 2012             | USA                    | Case–control | 4760        | 1 month before conception through 1st trimester of pregnancy | 0.95        | 0.83, 1.1   | CHDs          | No                  |
| Csáky-Szunyogh et al.  | 2013             | Hungary                | Case–control | 1500        | 1st trimester of pregnancy                              | 0.54        | 0.39, 0.73  | CTDs          | Yes                 |
| Csáky-Szunyogh et al.  | 2013             | Hungary                | Case–control | 4195        | 1st trimester of pregnancy                              | 0.76        | 0.63, 0.97  | VSDs          | Yes                 |
| Csáky-Szunyogh et al.  | 2013             | Hungary                | Case–control | 771         | 1st trimester of pregnancy                              | 0.53        | 0.38, 0.75  | LVOT          | Yes                 |
| Vereczkey et al.       | 2013             | Hungary                | Case–control | 38228       | 2nd month of pregnancy                                  | 0.53        | 0.34, 0.84  | AVSDs         | Yes                 |
| Li et al.              | 2013             | China                  | Case–control | 780         | 3 months prior to conception to 2 months after conception | 0.47        | 0.32, 0.7   | CHDs          | Yes                 |
| Csáky-Szunyogh et al.  | 2014             | Hungary                | Case–control | 1150        | 1st trimester of pregnancy                              | 0.57        | 0.45, 0.73  | VSDs          | Yes                 |
| Czeizel et al.         | 2015             | Hungary                | Case–control | 8962        | 1st trimester of pregnancy                              | 0.63        | 0.4, 0.98   | ASDs          | Yes                 |
| Leirgu et al.          | 2015             | Norway                 | Cohort       | 517784      | Before and during pregnancy                            | 0.99        | 0.86, 1.13  | CHDs          | Yes                 |
| Jin et al.             | 2015             | China                  | Cohort       | 8729        | 1st trimester of gestation                              | 0.618       | 0.39, 0.98  | CHDs          | No                  |
| Liang et al.           | 2016             | China                  | Cohort       | 5381        | Before and during pregnancy                            | 0.579       | 0.38, 0.89  | CHDs          | No                  |
| Study          | Publication year | Study settings/country | Study design | Sample size | Exposure duration                                      | Effect size | 95% CI     | Types of CHDs | Confounders adjusted |
|---------------|------------------|------------------------|--------------|-------------|-------------------------------------------------------|-------------|------------|---------------|---------------------|
| Mao et al.    | 2017             | China                  | Cohort       | 10087       | Before and during pregnancy                           | 0.73        | 0.46, 1.14 | CHDs          | Yes                 |
|               |                  |                        |              |             |                                                       | 0.6         | 0.36, 1.01 | TGAs          |                     |
|               |                  |                        |              |             |                                                       | 0.54        | 0.31, 0.95 | Septal defects |                     |
|               |                  |                        |              |             |                                                       | 0.67        | 0.39, 1.14 | PDA           |                     |
|               |                  |                        |              |             |                                                       | 0.59        | 0.31, 1.1  | ASDs          |                     |
| Kovalenko et  | 2018             | Russia                 | Case–control | 49463       | During pregnancy                                      | 1.14        | 0.84, 1.55 | VSDs          | Yes                 |
| al.           |                  |                        |              |             |                                                       | 0.6         | 0.36, 1.01 | CHDs          |                     |
| Øyen et al.   | 2019             | Denmark & Norway       | Cohort       | 197213      | 4 weeks before through 8 weeks after conception      | 1.08        | 0.93, 1.25 | CHDs          | Yes                 |
|               |                  |                        |              |             |                                                       | 0.98        | 0.59, 1.64 | CTDs          |                     |
|               |                  |                        |              |             |                                                       | 1.03        | 0.84, 1.26 | Septal defects |                     |
| Qu et al.     | 2019             | China                  | Case–control | 29204       | 3 months before through 1st trimester of pregnancy   | 0.65        | 0.44, 0.96 | CHDs          | No                  |
|               |                  |                        |              |             |                                                       | 0.58        | 0.3, 1.13  | VSDs          |                     |
|               |                  |                        |              |             |                                                       | 0.83        | 0.36, 1.93 | ASDs          |                     |
|               |                  |                        |              |             |                                                       | 0.43        | 0.17, 1.12 | PDA           |                     |
|               |                  |                        |              |             |                                                       | 1           | 0.2, 4.96  | AVSDs         |                     |
|               |                  |                        |              |             |                                                       | 1.5         | 0.24, 9    | Tetralogy of Fallot |                     |
| Dolk et al.   | 2020             | Northern Ireland       | Case–control | 1208        | 1st trimester of pregnancy                            | 0.86        | 0.57, 1.29 | CHDs          | Yes                 |
| Gildestad et  | 2020             | Norway                 | Cohort       | 894927      | Before and during pregnancy                           | 1.05        | 0.98, 1.12 | CHDs          | Yes                 |
| al.           |                  |                        |              |             |                                                       | 0.69        | 0.62, 0.76 | CHDs          |                     |
|               |                  |                        |              |             |                                                       | 0.15        | 0.1, 0.23  | CTDs          |                     |
|               |                  |                        |              |             |                                                       | 0.14        | 0.06, 0.36 | AVSDs         |                     |
|               |                  |                        |              |             |                                                       | 0.33        | 0.18, 0.58 | LVOT          |                     |
|               |                  |                        |              |             |                                                       | 0.84        | 0.75, 0.95 | Septal defects |                     |
|               |                  |                        |              |             |                                                       | 0.85        | 0.74, 0.98 | VSDs          |                     |
|               |                  |                        |              |             |                                                       | 0.84        | 0.71, 0.99 | ASDs          |                     |
| Qu et al.     | 2021             | China                  | Case–control | 15297       | 3 months before to 1st trimester of pregnancy         | 0.7         | 0.6, 0.8   | CHDs          | Yes                 |

CI: confidence interval; CTDs: conotruncal defects; CHDs: congenital heart defects; RCT: randomized controlled trial; VSDs: ventricular septal defects; OTDs: outflow tract defects; TGAs: transposition of great arteries; ASDs: atrial septal defects; AVSDs: atrioventricular septal defects; LVOT: left ventricular outflow tract defects; RVOT: right ventricular outflow tract defects; PDA: patent ductus arteriosus; LNMP: last normal menstrual period.
significant effect size. Case–control studies were found to reveal statistically significant effect size and the remaining cohort and RCT studies did not reveal significant effect size. The present meta-analysis also revealed the different effect size with different sample size, and the higher the sample size the more precise is the effect size. Finally, the setting in which the included studies conducted was an important variable that contributed for the effect size differences in the random effect model. Studies conducted in Hungary and China revealed statistically significant effect size (Table 2).

**Discussion**

Up to our efforts, this systematic review and meta-analysis is the comprehensive research work which revealed the pooled relative risk of periconceptional folic acid supplementation...
on CHDs by incorporating studies conducted from 1995 up to March, 2021.

The overall, as well as most of the subgroup analysis, results of the present systematic review and meta-analysis found that periconceptional folic acid supplementation significantly decreased the risk of occurrence of CHDs in the offspring (RR, 0.79; CI, 0.71, 0.89). In this meta-analysis, all of the included studies were obtained from America, China, European countries, and Russia. This is probably due to the fact that the practice of maternal periconceptional folic acid supplementations were seen in 46% in China; around 51% in America; about 78% in Europe; and about 46% in Asia. No reported figure was found regarding periconceptional folic acid supplementation practice in the countries of the continent of Africa and others.50 Therefore, the result could better be applied for the aforementioned countries.

The findings of this meta-analysis revealed that through supplementation of folic acid immediately before and in the early period of pregnancy, the risk of CHDs of various type could be reduced by 21%.

Existing research findings are not sufficient regarding mechanism of how folic acid could reduce birth defects including CHDs in offspring. However, it is suggested that folic acid is a vital nutrient important for nucleic acid (DNA) synthesis/mitosis and methylation during series of cell division that happen in embryonic and fetal developmental periods. During conception, depleted folate level can impair cellular growth and division in the embryo and fetus or placenta. Existing evidences appear to reveal how reduced folate adversely affects the development of the heart. Reduced folic acid leads to reduced availability of tetrahydrofolate (reduced bioactive forms of folic acid) which acts

**Table 2.** Subgroup analysis of 37 included studies in this systematic review and meta-analysis by considering year of publication, study settings, study design and sample size.

| Variables used for subgroup analysis | Random effect size with (95% CI) | I² (%) | p value |
|-------------------------------------|---------------------------------|-------|---------|
| Year of publications                |                                 |       |         |
| Before 2013                          | 0.87 (0.72, 1.05)               | 0.0%  | p=0.995 |
| 2013 and after                       | 0.75 (0.65, 0.86)               | 0.0%  | p=0.985 |
| Study settings                       |                                 |       |         |
| USA                                 | 0.92 (0.74, 1.15)               | 0.0%  | p=0.970 |
| Hungary                             | 0.58 (0.44, 0.77)               | 0.0%  | p=0.998 |
| China                               | 0.66 (0.54, 0.82)               | 0.0%  | p=0.984 |
| Othersa                             | 0.98 (0.79, 1.22)               | 0.0%  | p=0.995 |
| Study design                        |                                 |       |         |
| Case–control                        | 0.75 (0.66, 0.85)               | 0.0%  | p=0.984 |
| Cohort                              | 0.96 (0.76, 1.21)               | 0.0%  | p=0.906 |
| RCT                                 | 0.45 (0.15, 1.36)               | 0.0%  | p=0.906 |
| Sample size                         |                                 |       |         |
| ⩽4000                               | 0.77 (0.63, 0.94)               | 0.0%  | p=0.967 |
| >4000                               | 0.80 (0.70, 0.92)               | 0.0%  | p=0.844 |

*aIncludes Netherlands, Denmark & Norway, Norway, Northern Ireland, Russia, Australia and India.*
on folate receptors on the surface of dividing cells. Reduced availability of tetrahydrofolate is in turn associated with methyl tetrahydrofolate (most reduced folate in human RBC or serum) which leads to fewer methyl groups which are important for DNA synthesis and methylation of developing cells. In addition, reduced availability of methyl groups leads to slowed or stopped epithelial-mesenchymal transformation of cardiac neural crest cells, slowed or stopped cardiac neural crest cell migration and inadequate cardiac neural crest cell mass. Defective cardiac neural crest cell migration was associated with abnormal cardiac development in an experimental animal study. In addition, this experimental animal study found that depletion in folate level was associated with impaired folate receptors in the region of dorsal neural tube cell surfaces which result in significant reduction in proliferation of neural crest precursors, and finally failure of cardiac neural crest cells to migrate into the primordial heart.

More importantly, folic acid supplemented during pregnancy may had an effect similar with that of nutraceuticals which are food (or part of food) that offers medicinal or health benefits, including the prevention and treatment of diseases. It has been reported that selected nutraceuticals are effective in preventing the development of cardiovascular disease. According to existing evidences, the mechanism by which nutraceuticals could decrease the risk of cardiovascular diseases is (1) by modification of plasma lipid profile, that is, by reduction of total cholesterol level and low-density lipoprotein cholesterol, and (2) due to antihypertensive and antidiabetic effects of selected nutraceuticals which in turn may reduce the risk of cardiovascular diseases.

Like other systematic review, this systematic review and meta-analysis has its own limitation. Therefore, these limitations should be considered before the interpretation of results. The first limitation of this study was only English articles or reports were considered to conduct this review; thus, our finding may be affected by those findings published in other languages. In addition, the nature of design and the adequacy of sample size of some of included studies might affect the estimated report. Furthermore, in this meta-analysis all of the included studies were reported from developed countries such as the United States and countries especially in Europe and Asia. The major strength of the present systematic review and meta-analysis is use of the largest sample size which has high statistically significant power to reveal the association between maternal periconceptional folate supplementations and the relative risk of acquiring CHDs.

Conclusions and recommendations
The present systematic review and meta-analysis found that maternal periconceptional folic acid supplementation was significantly associated with the risk of CHDs in relatively high-income countries and the risk is reduced by 21% among those with periconceptional folic acid supplementations. However, the status of periconceptional folic acid supplementation and its impact on birth outcomes among mothers living in relatively poor socioeconomic settings like Africa is not determined. Moreover, it is suggested that a significant proportion of women of reproductive age, particularly those living in developing countries, do not use folic acid containing foods or eat folic acid fortified foods. Thus, we recommend large scale cohort study to be conducted to investigate the effect of maternal periconceptional folic acid supplementation on the occurrence of CHD of various types among mothers living in poor socioeconomic settings/countries. It is suggested that investigation of maternal periconceptional folic acid supplementation effect on CHD in developing countries needs to consider the role of anti-aging gene Sirt 1 because Sirt 1 gene is vital for DNA methylation and expression of developmental genes and its expression in the developing world populations may determine the outcomes of maternal periconceptional folic acid supplementations.

Authors’ contribution
A.T.W.: Conception of research protocol, study design, literature review, data extraction, data analysis, interpretation and drafting the manuscript. M.A.: data analysis, reviewing the manuscript, data extraction and quality assessment. All authors have read and approved the manuscript.

Availability of data and material
Data will be available upon request of the corresponding author.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval
Ethical approval was not sought for the present study because this article is a review, not a clinical trial or animal experiment.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

Informed consent
Informed consent was not sought for the present study because this article is a review, not a clinical trial or animal experiment.

ORCID iD
Amsalu Taye Wondemagegn https://orcid.org/0000-0002-2476-4377

References
1. Moore JP and Aboulhosn JA. Introduction to the congenital heart defects: anatomy of the conduction system. Card Electrophysiol Clin 2017; 9(2): 167–175.
2. Shaw GM, Strickland MJ, Riehle-Colarusso T, et al. Prevalence of congenital heart defects in metropolitan Atlanta, 1998-2005. *J Pediatr* 2008; 153(6): 807–813.

3. Cucu IA and Chiffriuc MC. Congenital heart disease: global burden and challenges to eliminate health disparities. *Ann Public Health Reports* 2018; 2(1): 26–29.

4. Centers for Disease Control Prevention. Hospital stays, hospital charges, and in-hospital deaths among infants with selected birth defects—United States. *MMWR* 2003; 2007: 25–29.

5. Waitzman NJ, Romano PS and Scheffler RM. Estimates of the economic costs of birth defects. *Inquiry* 1994; 31(2): 188–205.

6. van der Bom T, Zomer AC, Zwinderman AH, et al. The changing epidemiology of congenital heart disease. *Nature Reviews Cardiology* 2011; 8(1): 50–60.

7. Moher D, Liberati A, Tetzlaff J, et al. The PG: preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLOS Med* 2009; 6(7): e1000097.

8. Wells GA, Shea B, O’Connell Da Peterson J, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. *Oxford* 2000; 2000: 79559024.

9. DerSimonian R and Laird N. Meta-analysis in clinical trials. *Control Clin Trial* 1986; 7(3): 177–188.

10. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327(7414): 557–560.

11. Egger M, Smith GD, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315(709): 629.

12. Begg CB and Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; 50(4): 1088–1101.

13. Bean LJ, Allen EG, Tinker SW, et al. Lack of maternal folic acid supplementation is associated with heart defects in Down syndrome: a report from the National down syndrome project. *Birth Defects Res A: Clin Mol Teratol* 2011; 91(10): 885–893.

14. Botto LD, Mulinare J and Erickson JD. Occurrence of congenital heart defects in relation to maternal multivitamin use. *Am J Epidemiol* 2000; 151(9): 878–884.

15. Correa A, Botto L, Liu Y, et al. Do multivitamin supplements attenuate the risk for diabetes-associated birth defects. *Pediatrics* 2003; 111(5 Pt.2): 1146–1151.

16. Hobbs CA, MacLeod SL, Jill James S, et al. Congenital heart defects and maternal genetic, metabolic, and lifestyle factors. *Birth Defects Res A Clin Mol Teratol* 2011; 91(4): 195–203.

17. Lupo PJ, Symanski E, Langlois PH, et al. Maternal occupational exposure to polycyclic aromatic hydrocarbons and congenital heart defects among offspring in the national birth defects prevention study. *Birth Defects Res A Clin Mol Teratol* 2012; 94(11): 875–881.

18. Malik S, Cleves MA, Honcin MA, et al. Maternal smoking and congenital heart defects. *Pediatrics* 2008; 121(4): e810–816.

19. Scanlon KS, Ferenz C, Loffredo CA, et al. Preconceptional folate intake and malformations of the cardiac outflow tract. *Epidemiology* 1998; 9(1): 95–98.

20. Shaw GM, Carmichael SL, Yang W, et al. Periconceptional nutrient intakes and risks of conotruncal heart defects. *Birth Defects Res A Clin Mol Teratol* 2010; 88(3): 144–151.

21. Shaw GM, O’Malley CD, Wasserman CR, et al. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. *Am J Med Genetic* 1995; 59(4): 536–545.

22. Csáky-Szunyogh M, Vereczkey A, Urbán R, et al. Risk and protective factors in the origin of atrial septal defect secundum—national population-based case-control study. *Cent Eur J Public Health* 2014; 22(1): 42–47.

23. Csáky-Szunyogh M, Vereczkey A, Kósa Z, et al. Association of maternal diseases during pregnancy with the risk of single ventricular septal defects in the offspring—a population-based case-control study. *J Matern Fetal Neonatal Med* 2013; 26(8): 738–747.

24. Csáky-Szunyogh M, Vereczkey A, Kósa Z, et al. Risk factors in the origin of congenital left-ventricular outflow-tract obstruction defects of the heart: a population-based case-control study. *Pediatr Cardiol* 2014; 35(1): 108–120.

25. Csáky-Szunyogh M, Vereczkey A, Kósa Z, et al. Risk and protective factors in the origin of conotruncal defects of heart—a population-based case-control study. *Am J Med Genet A* 2013; 161A(10): 2444–2452.

26. Czeizel AE. Periconceptional folic acid containing multivitamin supplementation. *Europ J Obstet Gynecol Reprod Biol* 1998; 78(2): 151–161.

27. Czeizel AE, Dobó M and Varga H. Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows a reduction in certain congenital abnormalities. *Birth Defects Res A Clin Mol Teratol* 2004; 70(11): 853–861.

28. Czeizel AE, Vereczkey A and Szabó I. Folic acid in pregnant women associated with reduced prevalence of severe congenital heart defects in their children: a national population-based case-control study. *Eur J Obstet Gynecol Reprod Biol* 2015; 193: 34–39.

29. Vereczkey A, Kósa Z, Csáky-Szunyogh M, et al. Isolated atrioventricular canal defects: birth outcomes and risk factors: a population-based Hungarian case-control study, 1980-1996. *Birth Defects Res A Clin Mol Teratol* 2013; 97(4): 217–224.

30. Czeizel AE. Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. *Am J Med Genetics* 1996; 62: 179–183.

31. Li X, Li S, Mu D, et al. The association between periconceptional folic acid supplementation and congenital heart defects: a case-control study in China. *Prev Med* 2013; 56(6): 385–389.

32. Mao B, Qiu J, Zhao N, et al. Maternal folic acid supplementation and dietary folate intake and congenital heart defects. *PLoS ONE* 2017; 12(11): e0187996.

33. Qu P, Li S, Liu D, et al. A propensity-matched study of the association between optimal folic acid supplementation and birth defects in Shaanxi province, Northwestern China. *Sci Report* 2019; 9(1): 5271.

34. Qu Y, Lin S, Zhuang J, et al. First-trimester maternal folic acid supplementation reduced risks of severe and most congenital heart diseases in offspring: a large case-control study. *J Am Heart Assoc* 2020; 9(13): e015652.

35. Qu Y, Lin S, Bloom MS, et al. Maternal folic acid supplementation mediates the associations between maternal socioeconomic status and congenital heart diseases in offspring. *Prev Med* 2021; 143: 106319.

36. Jin L, Qiu J, Zhang Y, et al. Ambient air pollution and congenital heart defects in Lanzhou, China. *Environ Res Lett* 2015; 10(7): 074005.
37. Liang Q, Gong W, Zheng D, et al. The influence of maternal exposure history to virus and medicine during pregnancy on congenital heart defects of fetus. Environ Sci Pollut Res Int 2017; 24(6): 5628–5632.

38. van Beynum IM, Kapusta L, Bakker MK, et al. Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: a registry-based case-control study in the northern Netherlands. Eur Heart J 2010; 31(4): 464–471.

39. Obermann-Borst SA, Isaacs A, Younes Z, et al. General maternal medication use, folic acid, the MDR1 C3435T polymorphism, and the risk of a child with a congenital heart defect. Am J Obstet Gynecol 2011; 204(3): 236e1–8.

40. Smedts HPM, Rakhshandehroo M, Verkleij-Hagoort AC, et al. Maternal intake of fat, riboflavin and nicotinamide and the risk of having offspring with congenital heart defects. Eur J Nutr 2008; 47(7): 357–365.

41. Meijer WM, Werler MM, Louik C, et al. Can folic acid protect against congenital heart defects in down syndrome. Birth Defects Res A Clin Mol Teratol 2006; 76(10): 714–717.

42. Werler MM, Hayes C, Louik C, et al. Multivitamin supplementation and risk of birth defects. Am J Epidemiol 1999; 150(7): 675–682.

43. Leirgul E, Gildestad T, Nilsen RM, et al. Periconceptional folic acid supplementation and infant risk of congenital heart defects in Norway 1999-2009. Paediatr Perinat Epidemiol 2015; 29(5): 391–400.

44. Gildestad T, Bjorge T, Haaland ØA, et al. Maternal use of folic acid and multivitamin supplements and infant risk of birth defects in Norway, 1999-2013. British J Nutrition 2020; 124(3): 316–329.

45. Øyen N, Olsen SF, Basit S, et al. Association between maternal folic acid supplementation and congenital heart defects in offspring in birth cohorts from Denmark and Norway. J Am Heart Assoc 2019; 8(6): e011615.

46. Kovalenko AA, Anda EE, Odland JO, et al. Risk factors for ventricular septal defects in Murmansk County, Russia: a registry-based study. Int J Environ Res Public Health 2018; 15(7): 1320.

47. Dolk H, McCullough N, Callaghan S, et al. Risk factors for congenital heart disease: the Baby Hearts study, a population-based case-control study. PLoS ONE 2020; 15(2): e0227908.

48. Bower C, Miller M, Payne J, et al. Folate intake and the primary prevention of non-neural birth defects. Aust N Z J Public Health 2006; 30(3): 258–261.

49. Thomas SV, Ajaykumar B, Sindhu K, et al. Cardiac malformations are increased in infants of mothers with epilepsy. Pediatr Cardiol 2008; 29(3): 604–608.

50. Toivonen KL, Lacroix E, Flynn M, et al. Folic acid supplementation during the preconception period: a systematic review and meta-analysis. Prev Med 2018; 114: 1–17.

51. Scholl TO and Johnson WG. Folic acid: influence on the outcome of pregnancy. Am J Clin Nutr 2000; 71(Suppl.): 129S–303S.

52. Rosenquist TH. Folate, homocysteine and the cardiac neural crest. Dev Dyn 2013; 242(3): 201–218.

53. Rosenquist TH, Chaudoin T, Finnell RH, et al. High-affinity folate receptor in cardiac neural crest migration: a gene knockdown model using siRNA. Dev Dyn 2010; 239(4): 1136–1144;

54. Scicchitano P, Cameli M, Maiello M, et al. Nutraceuticals and dyslipidaemia: beyond the common therapeutics. J Functional Foods 2014; 6: 11–32.

55. Sosnowska B, Penson P and Banach M. The role of nutraceuticals in the prevention of cardiovascular disease. Cardiovasc Diagn Ther 2017; 7(Suppl. 1): S21.