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

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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), I^2 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 I^2 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 periconception 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

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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

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families and the society at large. It is the first among causes of death due to congenital anomalies in children under 5 years old.[3] It can also be a cause for lifelong disability, morbidity, and increased health care costs in children and adults.[4,5]

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.[6] 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 was 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.[7] The protocol of this review was not registered in PROSPERO.

Search strategies

To perform this systematic review and meta-analysis, all relevant articles were vigorously 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.[8] 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[9] random effects meta-analysis (random effects model).

Assessing heterogeneity and publication bias

Heterogeneity across studies was assessed using I^2 statistic and Cochran Q test (chi-squared statistic). The I^2 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 I^2 ranges between 0% and 100%, where 0% indicates no observed heterogeneity and large values indicate increasing heterogeneity.[10] An I^2 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 ($\leqslant$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 regression[11] and Begg's rank correlation[12] 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,[13–21] and another 9 studies were from Hungary.[22–30] 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;[43,44]1 study from Denmark and Norway;[45] 1 study from Russia;[46] 1 study from Northern Ireland;[47] 1 study from Australia[48] and 1 study from India.[49] Regarding the study design of included studies, 27 (72.97%) are case–control studies.[13–25,28,29,31,33–35,38–42,46–48] The remaining 8 are cohort studies[27,32,36,37,43–45,49] 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

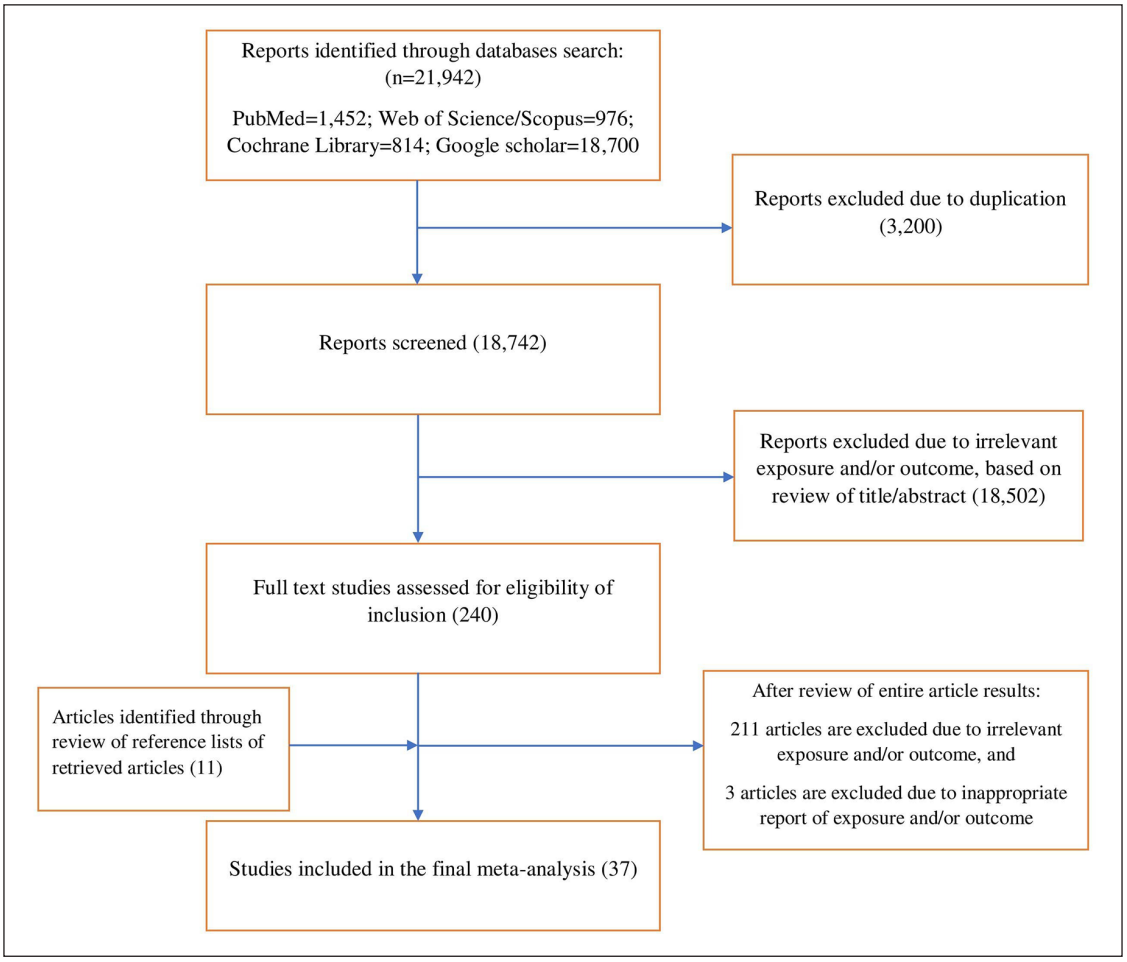

Figure 1. Flow chart revealing the procedures of study selection for the current systematic review and meta-analysis.

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=−.34 (95% CI=−.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.[21]	1995	USA	Case–control	688	1 month before until 2 months after conception	0.53	0.34, 0.85	CTDs	Yes
Czeizel AE[30]	1996	Hungary	RCT	4862	1 month before conception through 2nd missed menstrual period	0.48	0.23–1.03	CHDs	No
						0.29	0.08–1.05	VSDs & CTDs	
Czeizel AE[26]	1998	Hungary	RCT	4862	3 months before pregnancy through 2nd missed menstrual period	0.42	0.19–0.98	CHDs	No
						0.29	0.09–0.97	CTDs	
Scanlon et al.[19]	1998	USA/ Washington DC	Case–control	805	Pre-conceptional supplementation	1.08	0.69–1.7	OTDs	Yes
						0.73	0.39–1.36	TGAs	
Werler et al.[42]	1999	USA & Canada	Case–control	864	Periconceptional use (28 days before through 28 days after LNMP)	1	0.7, 1.5	CTDs	Yes
						1.2	0.8, 1.8	VSDs	
Botto et al.[14]	2000	USA	Case–control	3987	3 months before conception through 1st 3 months of pregnancy	0.76	0.6, 0.97	CHDs	Yes
						0.46	0.24,0.86	OTDs	
						0.59	0.38,0.94	Septal defects	
						0.61	0.38, 0.99	VSDs	
						0.5	0.14, 1.79	ASDs	
						1.09	0.21, 5.66	AVSDs	
Correa et al.[15]	2003	USA	Case–control	6307	3 months before conception through 1st 3 months of pregnancy	0.45	0.24, 0.84	OTDs	Yes
						0.6	0.37, 0.97	Septal defects	
Czeizel et al.[27]	2004	Hungary	Cohort	6112	28 days before conception through 1st 3 months of gestation	0.6	0.38, 0.96	CHDs	Yes
						0.26	0.09, 0.72	VSDs	
Bower et al.[48]	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.[41]	2006	USA & Canada	Case–control	407	1st 3 months of pregnancy	0.95	0.61, 1.47	CHDs	Yes
						0.7	0.21, 2.32	CTDs	
						1.46	0.77, 2.74	VSDs	
						0.94	0.48, 1.85	ASDs	
Thomas et al.[49]	2008	India	Cohort	462	1st trimester of pregnancy	1.49	0.63, 3.49	CHDs	No
Malik et al.[18]	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.[40]	2008	Netherlands	Case–control	600	1 month prior to conception through 1st 2 months of conception	1.3	0.9,1.9	CHDs	Yes
						1.5	0.97,2.2	OTDs	
						1	0.6,1.8	Non-OTDs	
Shaw et al.[20]	2010	USA	Case–control	1001	2 months before through 2 months after conception	1.03	0.66, 1.4	TGAs	No
						0.91	0.56, 1.26	Tetralogy of Fallot	
Van Beynum et al.[38]	2010	Netherlands	Case–control	3012	4 weeks before conception to 8 weeks after	0.74	0.62,0.88	CHDs	Yes
						0.56	0.43, 0.74	Septal defects	
						0.69	0.47, 1.03	CTDs	
						1.28	0.33, 4.95	AVSDs	

(Continued)

Table 1. (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.[16]	2011	USA	Case–control	667	Intake during pregnancy	1.03	0.96, 1.12	CHDs	Yes
Bean et al.[13]	2011	USA	Case–control	1118	Before conception or during 1st 4 weeks of conception	0.79	0.39, 1.19	AVSDs	No
						0.62	0.25, 0.99	ASDs	
						0.79	0.43, 1.15	VSDs	
Obermann-Borst et al.[39]	2011	Netherlands	Case–control	591	4 weeks before conception to 10 weeks after	0.79	0.5, 1.1	CHDs	No
Lupo et al.[17]	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.[25]	2013	Hungary	Case–control	1500	1st trimester of pregnancy	0.54	0.39, 0.73	CTDs	Yes
Csáky-Szunyogh et al.[23]	2013	Hungary	Case–control	4195	1st trimester of pregnancy	0.76	0.63, 0.97	VSDs	Yes
Csáky-Szunyogh et al.[24]	2013	Hungary	Case–control	771	1st trimester of pregnancy	0.53	0.38, 0.75	LVOT	Yes
Vereczkey et al.[29]	2013	Hungary	Case–control	38228	2nd month of pregnancy	0.53	0.34, 0.84	AVSDs	Yes
Li et al.[31]	2013	China	Case–control	780	3 months prior to conception to 2 months after conception	0.47	0.32, 0.7	CHDs	Yes
						0.39	0.25, 0.61	Septal defects	
						0.55	0.33, 0.89	CTDs	
Csáky-Szunyogh et al.[22]	2014	Hungary	Case–control	1150	1st trimester of pregnancy	0.65	0.47, 0.9	ASDs	Yes
Czeizel et al.[28]	2015	Hungary	Case–control	8962	1st trimester of pregnancy	0.57	0.45, 0.73	VSDs	Yes
						0.63	0.4, 0.98	ASDs	
						0.53	0.17, 0.94	Tetralogy of Fallot	
						0.47	0.26, 0.86	TGAs	
Leirgu et al.[43]	2015	Norway	Cohort	517784	Before and during pregnancy	0.99	0.86, 1.13	CHDs	Yes
						0.99	0.8, 1.22	CTDs	
						1.19	0.78, 1.81	AVSDs	
						1.02	0.78, 1.32	LVOT	
						0.97	0.72, 1.29	RVOT	
						1.19	1.1, 1.3	Septal defects	
						1.3	1.08, 1.56	ASDs	
						1.16	1.06, 1.27	VSDs	
Jin et al.[36]	2015	China	Cohort	8729	1st trimester of gestation	0.618	0.39, 0.98	CHDs	No
Liang et al.[37]	2016	China	Cohort	5381	Before and during pregnancy	0.579	0.38, 0.89	CHDs	No

(Continued)

Table 1. (Continued)

Study	Publication year	Study settings/ country	Study design	Sample size	Exposure duration	Effect size	95% CI	Types of CHDs	Confounders adjusted
Mao et al.[32]	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 al.[46]	2018	Russia	Case–control	49463	During pregnancy	1.14	0.84, 1.55	VSDs	Yes
Øyen et al.[45]	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.[33]	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.[47]	2020	Northern Ireland	Case–control	1208	1st trimester of pregnancy	0.86	0.57, 1.29	CHDs	Yes
Gildestad et al.[44]	2020	Norway	Cohort	894927	Before and during pregnancy	1.05	0.98, 1.12	CHDs	Yes
Qu et al.[34]	2020	China	Case–control	15297	1st trimester of pregnancy	0.69	0.62, 0.76	CHDs	Yes
						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.[35]	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.

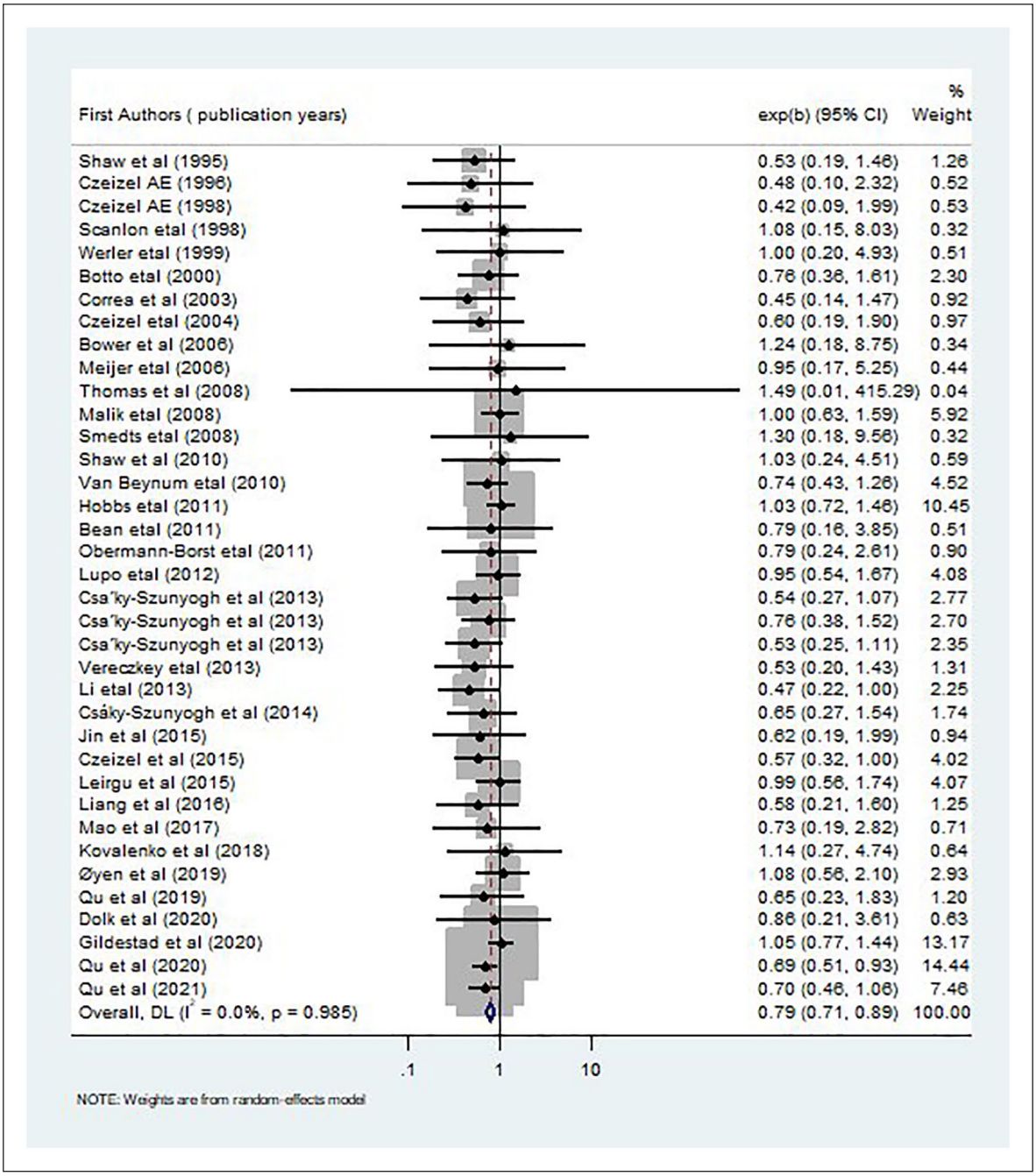

Figure 2. Forest plot of 37 included studies, which reveal the association between periconceptional folic acid supplement and CHDs. The size of the square is proportional to the precision of the study-specific effect estimates, and the bars indicate the corresponding 95% CIs. The diamond is centered on the summary effect size of all included studies, and the width indicates the corresponding 95% CI.

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

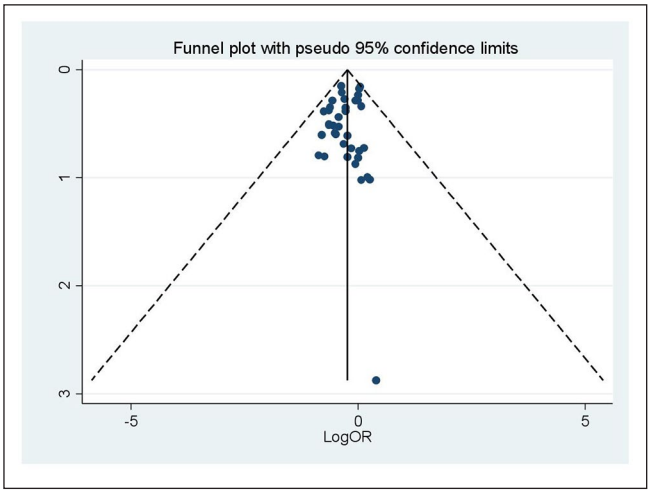

Figure 3. Traditional funnel plot of 37 included studies of periconceptional folic acid supplementations' impact on CHDs; the horizontal line refers the effect estimate and the vertical line refers the expected 95% confidence intervals.

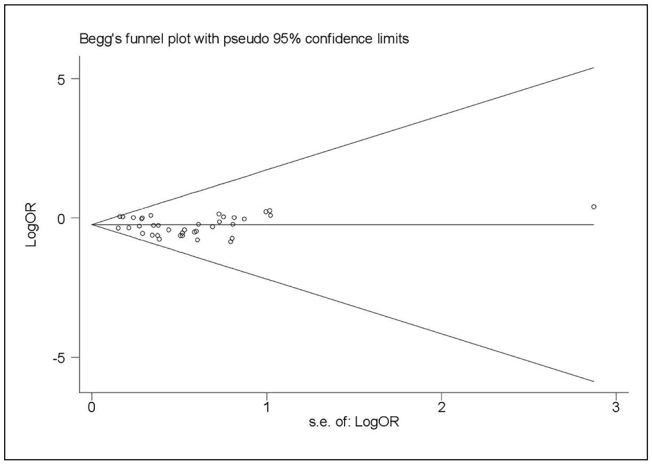

Figure 4. Begg's funnel plot of 37 included studies of periconceptional folic acid supplementations impact on CHDs; the horizontal line in the plot refers to the natural logarithm of effect estimate and the vertical line refers the expected 95% confidence intervals.

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)	I^2 (%), 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
	Others[a]	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

[a]Includes Netherlands, Denmark & Norway, Norway, Northern Ireland, Russia, Australia and India.

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[51] 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

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.[52] 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.[53] 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)[54] 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[54,55] 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 periconception 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.

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