Efficacy of Periconceptional High-Dose Folic Acid in Isolated Orofacial Cleft Prevention: A Systematic Review

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Abstract

Background The effectiveness of folic acid in prevention of neural tube defects has been well established. Periconceptional supplementation of folic acid in low doses has been shown to be effective in some studies on its efficacy in prevention of occurrence of clefts. There are few studies on high-dose folic acid for prevention of occurrence and recurrence of clefts in high risk cases and the overall consensus based on these is not available.

Objectives The aim of this review is to assess whether high-dose folic acid supplementation during the periconceptional period reduces the risk of occurrence of nonsyndromic clefts and recurrence in high-risk cases.

Search Methods Search was conducted in the various databases and trial registers. There were no restrictions in the search with regards to language, study setting, or date of publication.

Results The search yielded four studies—one randomized control trial, two prospective control trials, and a case–control surveillance on screening 401 articles. The three case–control studies were specifically on recurrence of clefts in high risk cases. The heterogeneity of the studies prevented conduction of a meta-analysis. But results of the studies demonstrate a strong association between high-dose folic acid and isolated nonsyndromic cleft lip with or without cleft palate (CL±CP). But such an effect is weak with regards to isolated cleft palate (CP).

Conclusions With the limited evidence available, our conclusion is that high-dose folic acid probably has a role in prevention of recurrence of isolated CL±CP in high-risk individuals, but not CP.

Keywords

- periconception
- high-dose folic acid
- cleft lip
- cleft palate

Introduction

Orofacial cleft is a common congenital anomaly of the craniofacial region—the incidence being between 1 in 300 live births to 1 in 1,000. A child born with a cleft has to undergo several surgical procedures over a period of time for correction of the deformities and in addition to this, there are other hospital visits required for associated issues. This situation is financially and emotionally draining resulting in

significant reduction in the quality of life of the patients and their family members.^{1,2} Therefore, prevention of clefts is of utmost importance as a public health issue.

The cascade of processes leading to development of normal lip and palate are intricate, and prone to disruption at various stages resulting in specific deformities. Orofacial cleft includes cleft of the lip with or without palate and cleft palate alone. These two entities have differences in embryonic origin and differences in the recurrence rates.

The etiology of clefts is linked to genetic and environmental factors. Of the environmental factors, one which can be easily manipulated is the folic acid link.

A study of susceptibility genes for cleft lip and palate has attempted to correlate specific gene variants with specific environmental risk factors.³ Thus, some genetic variants are associated with a background risk which is manifested when exposed to a particular environmental factor. The genes found to be associated with folic acid in this way are *MTHFR* and *RFC1*.⁴⁻⁷

The recommended dose of folic acid in females of childbearing age for prevention of neural tube defects is 400 micrograms daily before conception to week 12 of pregnancy. In those with high risk of neural tube defects, the dose is 5 mg (British National Formulary). Low doses of folic acid have been shown to prevent neural tube defects (NTD) and high doses the recurrence of NTD.⁸ There is no such evidence based folic acid dose prescribed for oral cleft occurrence or recurrence. Recurrence which is the occurrence of clefts in members of affected families is higher by 40 times that of the general population.

There have been several studies reporting the effects of multivitamins containing folic acid on clefts. Wilcox et al⁹ reported reduced risk of isolated cleft lip with or without cleft palate by a third with folic acid (400 micrograms) supplementation during early pregnancy, but no protection against cleft palate alone. In a case-control study to determine the association between nonsyndromic clefts and folic acid, Little et al have concluded that higher intake of folate does not appear to prevent clefts¹⁰ while a study in Netherlands demonstrated that periconceptional maternal folic acid supplement was beneficial to reduce the risk for CLP.¹¹ A Cochrane's review on periconceptional folic acid on a regular dosage¹² showed no evidence of any preventive effects on cleft palate. A recent meta-analysis by Jahanbin et al¹³ have shown that periconceptional multivitamin containing folic acid has a protective effect on oral clefts.

A cohort-controlled study by Czeizel et al¹⁴ of multivitamin intervention, no significant effect was found for low dose of folic acid on prevention of oral clefts. But high dose of folic acid (6 mg) was found to prevent some orofacial clefts.¹⁵ Most studies—case-control and observational—have reported on doses of folic acid less than 1mg and many have not reported the dose of folic acid used in their studies. There is a dilemma regarding whether low dose or high dose of folic acid is required in prevention of orofacial clefts.

A systematic review and meta-analysis by Johnson and Little¹⁶ has shown no strong evidence regarding association between folic acid and oral clefts. The dosage of folic acid in the various studies ranged from 0.5 to 10 mg and no dose dependent difference was found in their analysis. But they have concluded that multivitamins in early pregnancy may have a protective effect on clefts, especially CL ± CP.

We postulate that a high dose of folic acid in the periconceptional period is required to reduce the occurrence and recurrence of isolated nonsyndromic cleft.

Methods

Protocol was registered on the PROSPERO register of systematic reviews (CRD 42018090927).

PRISMA-P guidelines for the conduct of systematic review and meta-analysis protocol were followed.

Search Strategy

The following electronic databases were searched:

- Cochrane, Medline, EMBASE, LILACS BIREME (Latin American and Caribbean Health Science Information database).
- Ongoing trials: World Health Organization International Clinical Trials Registry Platform, U.S. National Institutes of Health Ongoing Trials Register.
- The metaRegister of Controlled Trials.
- The US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov.
- The Australian New Zealand Clinical Trials Registry.
- The World Health Organization International Clinical Trials Registry Platform.
- The EU Clinical Trials Register.

There were no restrictions in the search with regards to language, study setting, or date of publication.

Study Selection

Studies were selected based on the following criteria: human studies assessing the effectiveness of administration of high-dose folic acid in prevention of nonsyndromic cleft lip with or without cleft palate (CL ± CP) and cleft palate (CP) were included. Studies that did not meet the inclusion criteria, review articles, case reports, editorials, and letters were excluded.

The population of interest was females of reproductive age group who were at high-risk of having a child with nonsyndromic CL ± CP or isolated CP.

A three stage review process was followed. During the initial stage the titles were reviewed by two reviewers and the articles not relevant to the review were excluded. In the second stage, the abstracts of the selected articles were reviewed against the inclusion criteria (► Fig. 1: PRISMA-P flow diagram). The final stage consisted of detailed review of the full texts selected by both reviewers. Discrepancies that arose were dealt with by discussion.

Data Extraction

We designed data extraction forms to record authorship, year of publication, and details of study based on inclusion criteria. Two review authors extracted data independently. The following details were recorded when available.

- Trial methods: method of randomization, allocation, sample size, blinding methods, and losses at follow-up.
- Participants: country of origin, year of study, setting, sample size, inclusion, and exclusion criteria.
- Intervention: dosage of folic acid supplemented and duration of supplementation.

Fig. 1 Study selection PRISMA-P flow diagram.

- Control: no supplementation or low-dose folic acid supplementation.
- Outcomes: Occurrence or recurrence of CL $\pm$ CP or isolated CP.

In instances where there is more than one study from the same population by the same team of researchers, the more recent and relevant study has been included.

Dealing with Missing Data

In trials with missing data, we contacted the principal investigator by electronic mail.

Data Synthesis

Assessment of Risk of Bias

The assessment of the risks of bias was done using Cochrane's tool for assessing risk of bias as described in section 8.5 of the Cochrane Handbook for Systematic Reviews of Interventions. Two reviewers independently performed the assessment and any disagreements were resolved through discussion with the third reviewer.

Assessment of risk of bias performed for the RCT is as follows.

Sequence Generation

Generation of the randomization sequence was done using permuted blocks of random size—low risk of bias.

Allocation

Allocation concealment present—low risk of bias.

Blinding

Double blinding done—subjects, investigators, and researchers were blinded to randomization assignment—low risk of bias.

Incomplete Outcomes Data

Attrition and exclusions and the reasons for these have been reported. The changes in the recruitment such as reducing the length of participation and including isolated cleft palate

early in the study and not later have all been mentioned in the publication—low risk of bias.

Selective Outcome Reporting

A study protocol has been published. The inclusion criteria in the protocol mentioned all cases of nonsyndromic cleft lip with or without cleft palate, both unilateral and bilateral, excluding cases with recognized syndromes and cleft palate only. In the overall analysis of outcome results these are included. But results after exclusion are also provided—low risk of bias.

Other Bias

As a clear study protocol has been published and all variations from the protocol and study period are stated in the article no other bias was detected.

Results

The study selection PRISMA-P flow diagram is given in ► Fig. 1. The search yielded 401 articles, out of which four studies were selected based on the inclusion criteria—a single RCT,¹⁷ two prospective case-control trials,^{18,19} and a single case-control surveillance—Hungarian Case-Control Surveillance of Congenital Abnormalities^{15,20} on effect of periconceptional high-dose folic acid on prevention of isolated nonsyndromic clefts. The three case-control studies were on the prevention of recurrence of clefts, while the HCCSCA was on congenital abnormalities in general, and with clefts, was regarding occurrence rather than recurrence of clefts.

CL with or without CP was used as an inclusion criterion for two of the studies, but in effect only one study (Tolarova and Harris) has reported only on CL $\pm$ CP. All others have included isolated CP also in the analysis. ► Table 1 shows analysis of the results of the three case-control studies.

Czeizel et al had conducted two interventional studies earlier and the low dose of folic acid in the multivitamin supplemented was reported to be ineffective in preventing clefts. The HCCSCA (► Fig. 2) like the other two studies was on several congenital anomalies of which cleft was one. Use of high-dose folic acid is reported to bring about some reduction in both CL $\pm$ CP and CP.

We came across a study protocol of a community based randomized clinical trial on effect of high-dose folic acid on prevention of congenital malformations in Italy and Netherlands.²¹ Contacting the principal investigator by electronic mail revealed that the analysis of the trial is still ongoing.

Discussion

The double blinded randomized control trial (RCT) by Wehby et al¹⁷ on the effect of periconceptional folic acid on prevention of recurrence of isolated cleft was conducted during the period 2004 to 2009. This involved 2,508 females and the dose of folic acid given to the study population was 4 mg while the controls received the usual dose present in a multivitamin which is 0.4 mg. The outcome was based on 234 live births. The recurrence rates for oral clefts were

Table 1 Study characteristics of case-control trials

Studies	Wehby (2013)	Tolarova and Harris (1995)	Briggs (1976; continuation of peer study)
Type of study	Randomized double blind control trial	Nonrandomized prospective case-control	Prospective case-control study
Year	2004–2009	1976–1992	1958–1976
Study location	Six craniofacial clinics in Brazil	Department of Medical Genetics of the Czechoslovak Academy of Sciences, Prague	Saint Barnabas Medical Center, Livingston, New Jersey
Type of cleft	Isolated oral cleft recurrence (CL ± CP and CP)	Recurrence of oral cleft (CL ± CP)	Recurrence of oral cleft (CL ± CP and CP)
Number of patients in study population	1,257	221 pregnancies at risk of child with cleft	228 pregnant mother at risk of child with cleft
Dose of folic acid in study population	4 mg	10 mg	5 mg
Number of controls	1,251	1,901 pregnant women at risk of child with cleft	417 pregnant mother at risk of child with cleft
Dose of folic acid in controls	0.4 mg	Nil	Nil
Time of administration	Before planned conception and continued up to first trimester	2 months before planned conception and continued up to 3 months	Before planned conception and continued up to 5th month of pregnancy
Cleft cases in study population	3 cases CL = 1 CP = 1 CL + CP = 1	3 out of 214 informative pregnancies	7 cases CL + CP = 3 CP = 3
Cleft cases in controls	3 cases of CL + CP	77 out of 1,901 Informative pregnancies	20 cases CL + CP = 15 CP = 5
Method of statistical analysis used	Fischer's exact test and Student <i>t</i> -test	Chi-square and Fischer's exact test	Not mentioned
Recurrence risks	Oral cleft recurrence was 2.9% in 0.4 mg group and 0.8% in 4 mg group (CP excluded)	65% decrease in cleft recurrence found in supplemental group	Incidence rate in study group for both CL ± CP and CP was lower than the control group (3.1% vs. 4.8%)
Adverse effects	Nil	Nil	Nil
Additional comments	Oral cleft recurrence was 2.9% in 0.4 mg group and 1.6% in 4 mg group when both CL ± CP and CP included. Recurrence rate was similar between the two groups	Best efficacy of supplementation was found in the subset with unilateral cleft (3.51% vs. 0.6%), a decrease by 82.6%. No difference in the subgroup of female probands with bilateral cleft	Incidence of 1.9% in the treated group for CL + CP (1.9% vs. 5.5%). Incidence of 6% in the treated group for CP (6% vs. 3.5%)

Abbreviations: CL, cleft palate; CP, cleft lip.

found to be similar between the two groups (2.9% in the 0.4 mg and 2.5% in the 4 mg group) when cases of cleft lip with or without cleft palate as well as isolated cleft palate in the study groups were included. This shows a wide difference from the historic recurrence rate postfolic acid fortification (6.3%; $p = 0.0009$ when both folic acid groups are combined). But isolated cleft palate was not in the inclusion criteria of the study and excluding this, the recurrence rate is 1.6%. Apart from this, one of the cases was diagnosed as Van-der-Woude's syndrome (VWS), and hence excluded, as this is the most common syndromic form of oral cleft with a 50% recurrence risk. The recurrence rate was significantly low in

4 mg group when cleft lip with palate alone was considered (0.8%), while this was 2.9% in the 0.4 mg group.

The researchers have concluded that the small sample limits their ability to arrive at a formal statistical inference to compare the effect of high- and low-dose folic acid on cleft recurrence.

No compromise in fetal growth was observed in any of the groups.

The Hungarian Case-Control Surveillance of Congenital Abnormalities from 1980 to 1996 regarding high-dose folic acid in the periconceptional period was a study on various congenital anomalies. Clefts was not the outcome

Fig. 2 Hungarian Study flow chart.

of primary interest, it was only one of the outcomes being evaluated and included isolated cleft lip with or without cleft palate.

The cases were obtained from the Hungarian Congenital Abnormality Registry (HCAR) and consisted of 22,843 malformed offspring and control group from the National Birth Registry consisting of 38,151 cases.

The dose of folic acid was not recorded in 50% of the pregnant women. But in the validation study based on 600 pregnant women, the dose of folic acid used was found to be 3 mg in 22.5%, 6 mg in 68.6%, and 9 mg in 8.9%. There were 1,374 cases of CL ± CP and 601 cases of CP.

Though the multivitamin with low dose (0.8 mg) of folic acid in the intervention trials¹⁴ by Czeizel et al did not show reduction in orofacial clefts, the high dose in the HCCSCA demonstrated some preventive effect on clefts especially cleft palate.

The nonrandomized case-control study by Tolarova and Harris was specifically on recurrence of nonsyndromic isolated CL ± CP. 221 pregnancies in females at risk of CL ± CP were the study population. They were given multivitamin with 10 mg folic acid from at least 2 months before conception to 3 months after. The control group consisted of 1,901 females at risk of CL ± CP with no supplementation. The incidence

was 1.4 and 4.05% in the supplemented and control groups respectively, indicating a risk reduction of 65.4%. Best results were found with unilateral cleft (3.5 vs. 0.61%), a decrease by 82.6%.

No difference was found in female probands with bilateral cleft.

Publication by Briggs¹⁹ is a continuation of the Peer et al²² study, as a prospective case–control study on effect of 5 mg of folic acid during the periconceptional period on recurrence of oral clefts.

The incidence in the pregnancy after having had a child with isolated cleft and subsequent pregnancies are available separately. The study group consisted of 228 women with previous cleft children who were supplemented with vitamins and the control group 417 women with previous clefts who were not supplemented. Of the 228, 161 had children with CL $\pm$ CP and the recurrence was 3 (1.9%). There were 67 with cleft palate alone and the recurrence was 4 (6%), a total recurrence rate of 3.1%. The incidence in the control group was 5.5%, 3.5%, and 4.8% for CL $\pm$ CP, CP alone, and total respectively.

The figures show an impressive effect when the deformities are taken separately in that there is a significant reduction in CL $\pm$ CP in the supplemented group. The recurrence rate for CP alone is considerably high. There is an overall reduction in recurrence of 1.7% when compared with the unsupplemented group.

The dose of folic acid in the high dose studies varied widely. The inclusion criteria, study target as in CL $\pm$ CP and isolated CP, method of supplementing folic acid—alone or in combination with other vitamins, and variations in control group management as in no folic acid or low-dose folic acid are some of the heterogeneities when comparing the four studies. Due to the various heterogeneities in the studies a meta-analysis is not justifiable.

Of the four studies available to date, the most recent one itself is almost 10 years and the other three more than 25 years, demonstrating the paucity of such studies in current times.

The studies were conducted in Europe, United States, and South America. There are none from Asian or African countries. Since the incidence of cleft is maximum in Asian countries, absence of studies from this continent is a significant drawback.

Conclusions

There are several gaps in the evidence regarding the efficacy of high-dose folic acid in prevention of oral clefts. From the data obtained from the available studies (Level 1b evidence for the RCT and Level 3b evidence for the case–control studies based on Oxford Centre for Evidence-based Medicine Levels of Evidence), there does seem to be a strong association between high-dose folic acid administration and prevention of isolated CL $\pm$ CP. With regard to isolated CP, the link appears to be very weak. The fact that these two entities are etiologically different could be the reason for the difference in behavior.

None of the studies have reported any adverse side effects from supplementation of high-dose folic acid during the periconceptional period.

Considering the high-cost related to management of cleft cases and psychological and social burden associated, it might be justifiable to supplement high-dose folic acid during periconceptional period to high risk females to prevent recurrence of clefts.

Multicenter randomized controlled trials in different continents with large number of cases and controls assessing the efficacy of specifically high-dose folic acid rather than multivitamins containing folic acid in prevention of occurrence and recurrence of orofacial clefts are required. The dose of folic acid for the recurrence prevention studies need to be standardized to 5 mg as done for neural tube defects. It might be prudent to conduct numerous trials in Asian countries like India as numbers are easily achievable due to the very high incidence rates of cleft in this continent. The studies should be done separately on CL $\pm$ CP and isolated CP as there is a significant difference in behavior in response to folic acid supplementation from studies so far.

Protocol Registered with PROSPERO

(International prospective register of systematic reviews).
Registration number: CRD42018090927.

Conflict of Interest

None.

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References

- 1 Wehby GL, Ohsfeldt RL, Murray JC. Health professionals' assessment of health-related quality of life values for oral clefting by age using a visual analogue scale method. *Cleft Palate Craniofac J* 2006;43(4):383–391
- 2 Wehby GL, Cassell CH. The impact of orofacial clefts on quality of life and healthcare use and costs. *Oral Dis* 2010;16(1):3–10
- 3 Stuppia L, Capogreco M, Marzo G, et al. Genetics of syndromic and nonsyndromic cleft lip and palate. *J Craniofac Surg* 2011;22(5):1722–1726
- 4 Little J, Gilmour M, Mossey PA, et al. ITS MAGIC Collaboration. Folate and clefts of the lip and palate—a U.K.-based case-control study: Part II: Biochemical and genetic analysis. *Cleft Palate Craniofac J* 2008;45(4):428–438
- 5 Shaw GM, Todoroff K, Finnell RH, Rozen R, Lammer EJ. Maternal vitamin use, infant C677T mutation in MTHFR, and isolated cleft palate risk. *Am J Med Genet* 1999;85(1):84–85
- 6 Jugessur A, Wilcox AJ, Lie RT, et al. Exploring the effects of methylenetetrahydrofolate reductase gene variants C677T and A1298C on the risk of orofacial clefts in 261 Norwegian case-parent triads. *Am J Epidemiol* 2003;157(12):1083–1091
- 7 Chevrier C, Perret C, Bahau M, et al. Fetal and maternal MTHFR C677T genotype, maternal folate intake and

- the risk of nonsyndromic oral clefts. *Am J Med Genet A* 2007;143A(3):248–257
- 8 MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 1991;338(8760):131–137
 - 9 Wilcox AJ, Lie RT, Solvoll K, et al. Folic acid supplements and risk of facial clefts: national population based case-control study. *BMJ* 2007;334(7591):464
 - 10 Little J, Gilmour M, Mossey PA, et al. ITS MAGIC collaboration. Folate and clefts of the lip and palate—a U.K.-based case-control study: Part I: Dietary and supplemental folate. *Cleft Palate Craniofac J* 2008;45(4):420–427
 - 11 van Rooij IALM, Ocké MC, Straatman H, Zielhuis GA, Merkus HM, Steegers-Theunissen RP. Periconceptional folate intake by supplement and food reduces the risk of nonsyndromic cleft lip with or without cleft palate. *Prev Med* 2004;39(4):689–694
 - 12 De-Regil LM, Peña-Rosas JP, Fernández-Gaxiola AC, Rayco-Solon P. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. *Cochrane Database Syst Rev* 2015;2015(12):CD007950
 - 13 Jahanbin A, Shadkam E, Miri HH, Shirazi AS, Abtahi M. Maternal folic acid supplementation and the risk of oral clefts in offspring. *J Craniofac Surg* 2018;29(6):e534–e541
 - 14 Czeizel AE, Dobó M, Vargha P. 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
 - 15 Czeizel AE. The primary prevention of birth defects: Multivitamins or folic acid? *Int J Med Sci* 2004;1(1):50–61
 - 16 Johnson CY, Little J. Folate intake, markers of folate status and oral clefts: is the evidence converging? *Int J Epidemiol* 2008;37(5):1041–1058
 - 17 Wehby GL, Félix TM, Goco N, et al. High dosage folic acid supplementation, oral cleft recurrence and fetal growth. *Int J Environ Res Public Health* 2013;10(2):590–605
 - 18 Tolarova M, Harris J. Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology* 1995;51(2):71–78
 - 19 Briggs RM. Vitamin supplementation as a possible factor in the incidence of cleft lip/palate deformities in humans. *Clin Plast Surg* 1976;3(4):647–652
 - 20 Czeizel AE, Tímár L, Sárközi A. Dose-dependent effect of folic acid on the prevention of orofacial clefts. *Pediatrics* 1999;104(6):e66
 - 21 Bortolus R, Blom F, Filippini F, et al. Italian and Dutch folic acid trial study groups. Prevention of congenital malformations and other adverse pregnancy outcomes with 4.0 mg of folic acid: community-based randomized clinical trial in Italy and the Netherlands. *BMC Pregnancy Childbirth* 2014;14(1):166
 - 22 Peer LA, Gordon HW, Bernhard WG. Effects of vitamins on human teratology. *Plast Reconstr Surg* 1964;34:358–362