

Association of maternal folic acid supplementation and incidence of non-syndromic cleft lip and palate

Hendry Rusdy¹, Isnandar¹, Indra Basar Siregar¹, Rizkiani Cahya Putri Sinaga²

¹Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

²Undergraduate Student, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

ABSTRACT

Background: Cleft lip and palate as a frequent congenital defect is caused by genetic and environmental factors. Micronutrient folic acid as an environmental factor has shown a role as a cleft lip and palate protective factor in several previous studies. **Purpose:** The purpose of this study was to determine the distribution of adequacy of folic acid supplementation and the association between folic acid supplementation during the first trimester of pregnancy with the incidence of cleft lip and palate at Mitra Sejati Hospital, Medan, North Sumatra, Indonesia. **Methods:** This study used analytical research with a case-control design and questionnaire. A Chi-square test was used to observe the association between the folic acid supplement intake during the first trimester with the incidence of cleft. The p-value ≤ 0.05 was deemed to be significant. **Results:** The study found that 47 mothers (51.09%) had insufficient, while 45 mothers (48.91%) had adequate folic acid supplementation. This study did not show a significant association between supplement use and all cleft incidence ($p > 0.05$), but a significant result was found between inadequate supplementation ($< 400\mu\text{g/day}$) and cleft lip with or without cleft palate occurrence ($p = 0.043$; OR 2.4 [1.022-5.625]). **Conclusion:** The present study showed that most pregnant women did not have sufficient folic acid supplement in the first trimester. Furthermore, inadequate maternal folic acid supplementation ($< 400\mu\text{g/day}$) during the first trimester of pregnancy increased the tendency for cleft lip and cleft palate (with or without cleft lip) to occur significantly.

Keywords: cleft lip and palate; folic acid; 400mcg; first trimester; pregnancy

Correspondence: Hendry Rusdy, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara. Jl. Alumni No. 2, Medan 20155, Indonesia. Email: hendry_rusdy@yahoo.co.id

INTRODUCTION

Cleft lip and palate is one of the most common congenital anomalies in newborn babies, with a worldwide prevalence range from 3.4 to 22.9 per 10,000 births.¹ According to the Indonesia Basic Health Research Report (RISKESDAS), there was a nationally increasing incidence from 2013 to 2018 (0.08 to 0.12%).^{2,3} Cleft occurs as a result of prominent tissues not merging correctly during embryonic lip-palate formation. Most people with clefts will often have speech difficulties and hyper-nasal speech, malocclusion, mouth breathing, abnormal tongue and lip postures, and ear problems like otitis media. This condition, especially cleft palate, affects a baby's nutritional intake and can consequently cause malnutrition.⁴

Cleft can be syndromic or non-syndromic. Cleft is caused by multiple factors, genetic and environmental.

According to the Global Burden of Disease, the worldwide estimated prevalence of non-chromosomal orofacial cleft is 1.25 per 1000 births and 1.36 per 1000 births in Southeast Asia.¹ Environmental factors include maternal nutritional deficiencies, alcoholic beverages, cigarette smoke inhalation, medicine like antiepileptic and antiretroviral, radiation, and toxic chemicals such as pesticides.^{5,6} Nutritional deficiency such as micronutrient deficiency, especially folate/folic acid, has been linked to a higher likelihood of developing cleft in some studies. Folate or folic acid is required for DNA synthesis during embryonic development. Folate or folic acid act as a one-carbon donor in the form of tetrahydrofolate during deoxythymidine monophosphate (dTMP) nucleotide, which is required for DNA synthesis. The increased cell proliferation during pregnancy requires more DNA, and the need for folate also increases.^{7,8}

Several studies have shown an association between folic acid and the occurrence of cleft lip and palate, but other studies have shown no significant association in certain types of cleft.¹ A study conducted by Kelly et al.⁹ and Kunjana¹⁰ showed a significant association between folic acid consumption during the first trimester and cleft, while Song Li et al.¹¹ showed that taking folic acid was consistently associated with a lower risk of giving birth to babies with cleft lip and palate, whereas Neogi et al.¹² showed no significant results. Subsequently, most of the previous studies that showed significant results still used mothers of patients with a family history of clefts, which is a factor that is difficult to prevent.^{9–13} The results of this study motivated the researchers to determine the association between the two events (variables), especially among patients with no family history of cleft lip and palate.

Folate in food varies widely and tends to be unstable and easily damaged by heat during the cooking process.¹⁴ Folic acid supplements, which have a more stable form than folate, have been recommended for pregnant women to get enough folate during foetal development. Since the facial development process starts from the fourth week, the role of folate in normal lip and palate formation is most important in the first trimester of pregnancy.^{7,15,16} The WHO recommends that all pregnant women take a folic acid supplement of 400µg daily.¹⁷

In Indonesia, the government has determined that every pregnant woman is to be given a minimum of 90 tablets of 400µg folic acid supplements.¹⁸ According to the Indonesian Basic Health Research Report,³ around 87.6% of pregnant women have received folic acid supplements in Indonesia, and only 70.9% in North Sumatra. It is necessary to evaluate folic acid supplementation in this area, especially in hospitals that handle cleft lip and palate cases, such as the Mitra Sejati Hospital in Medan, North Sumatra. Mitra Sejati Hospital sees many patients with cleft lip and palate, handling approximately 144 cases per year. We wanted to see a comparison of the sufficient folic acid supplementation in biological mothers of children with cleft lip and palate with mothers of children without the condition, to see if the two groups differed significantly. Data on the distribution of adequacy of maternal folic acid supplementation was then needed to assess the association between the two variables. Therefore, this case-control analysis is needed to assess the association of sufficient folic acid with the incidence of cleft lip and palate in North Sumatra, especially in Mitra Sejati Hospital, Medan.

MATERIALS AND METHODS

This was a retrospective case-control study conducted in a hospital. This study took place between July and August 2021 at Mitra Sejati Hospital, Medan, North Sumatra, Indonesia, as one of the referral hospitals for cleft lip and palate care and was part of Smile Train, a nongovernmental

organization that provides free treatment for orofacial clefts. This study was approved by Mitra Sejati Hospital (No. 993/UN5.2.1.6/SSA/2021) and the Research Ethics Commission of Universitas Sumatera Utara (No. 685/KEP/USU/2021).

According to the standard of selection (purposive sampling), 92 patients (46 cases) were enrolled in the study. Cases were paediatric patients presenting cleft lip only (CL), cleft lip and palate (CLP), and cleft palate only (CPO), not associated with any other birth defects or syndromes (non-syndromic cleft lip/palate patients). The exclusion criteria included children whose biological mother was dead, who had been adopted, who had a family history of cleft, and children who were not the first child born with a cleft. Controls were hospitalised paediatric patients without a congenital disorder or systemic disease. All patients were paediatric patients aged 18 years and under (according to the definition of a child).

Mothers of paediatric patients were debriefed by the same researcher. The questionnaire was used to investigate information on demographic characteristics, such as the first-trimester maternal antenatal visits, the first three months and preconception period (at least one month before pregnancy) maternal folic acid supplement use, folic acid supplement dosage daily consumption, and cleft incidence among first- and second-degree family relatives. They were asked to give more details about the folic acid supplement brand, dosage, duration and when they used the supplement. Adequacy of folic acid supplementation was divided into sufficient and insufficient. Women who consumed at least 400µ/day during the first trimester were added to the sufficient supplementation group, and those who had never and those who were not consistent in taking supplements were placed in the insufficient group. If the mother during the first trimester consumed at least 400µ of folic acid most days but ever missed it, then the mother was still categorised as a subject with insufficient supplementation. All mothers of the patients in the study agreed to participate. Only the data from one pregnancy was provided by each woman for this study. This study was conducted using secondary data in the form of medical record status at Mitra Sejati Hospital between the years 2017 to 2021.

The maternal ages were divided into five groups respectively: <19; 20–24; 25–29; 30–34; and ≥35 years of age. Folic acid supplementation was divided into none/any use during the first trimester, adequate folic acid consumption (≥400µg/day) during the first trimester, none/any pre-pregnancy use (preconception).

An IBM SPSS Statistics for Windows version, 20.0 (Armonk, New York, USA) software application was used to analyse the data, and the descriptive statistics of epidemiology were presented by frequency and percentage in the table. The association of two variables was analysed using the Chi-square test. The analyses were done as Odds Ratio (ORs) with 95% confidence intervals (CI), and a *p* value of <0.05 was deemed significant.

RESULTS

After collecting >150 medical records as the population, several of these were excluded due to exclusion criteria and maternal unavailability as study subjects. Then, 92 subjects were obtained that matched the inclusion criteria. The study subjects, as the parents of cases and control patients, gave informed consent; hence, they filled out the questionnaire honestly. The study subjects filled out the questionnaire, assisted by the researcher’s explanation beforehand.

In conformity with the requirements of selection, 92 patients (46 cases and 46 controls) opted to participate in this study. Table 1 represents the frequency of cleft-control status based on gender. Cleft lip with cleft palate showed the highest number of cases (73.91%), followed by cleft lip only (19.57%), and cleft palate only (6.52%) (Table 1). Male patients were the more dominant part of sample (55.43%), compared to girls (44.57%). There were 24 boys and 19 girls in cleft and palate cases.

There is no significant difference in the incidence of all cleft between boys and girls, and in this study the number of cases in boys was only slightly higher than in girls (Table 2 and Figure 1). However, cleft palate only was not found in boys in this study (Figure 1). Cleft lip only (CL) were

recorded for 20.93% of CL/P (cleft lip with or without cleft palate) cases. The association of gender with the occurrence of cleft is depicted in Table 2.

The distribution of cases and control status by maternal characteristics can be seen in Table 3. The average maternal age in the case group was 28.46, and 26.78 years in the control group. A maternal age between <19 years and ≥35 years at birth was more common in the case group (28.26%) than in the control group (13.04%). There was

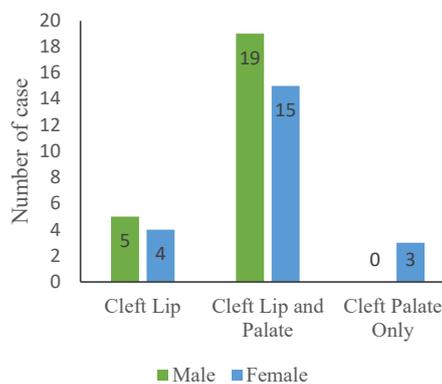


Figure 1. Number of cleft cases by gender.

Table 1. Distribution of case-controls status by gender

	Male N (%)	Female N (%)	Total (%)
Cases	24 (52.17)	22 (47.83)	46
CL	5 (10.87)	4 (8.7)	9 (19.57)
CL+P	19 (41.3)	15 (32.61)	34 (73.91)
CPO	-	3(6.52)	3 (6.52)
Controls	27 (58.7)	19 (41.3)	46
Total	51 (55.43)	41 (44.57)	92

Notes: CL/P: cleft lip with or without cleft palate; CPO: cleft palate only

Table 2. Chi-square test results of gender and occurrence of cleft

Gender	CL/P				All Cleft				Controls N (%)
	n	OR	95% CI	p	n	OR	95% CI	p	
Male	24	0.889	0.383-2.060	0.784 ^a	24	0.768	0.337-1.750	0.529 ^a	27 (58.7)
Female	19				22				19 (41.3)

Notes: ^aStatistical evaluation by the Pearson’s Chi-Square; p-value <0.05 statistically significant

Table 3. Maternal characteristics with non-syndromic cleft lip/palate

Maternal characteristic	CL/P	CPO	All Cleft N (%)	Control N (%)	Total N (%)
Age at birth					
< 19	3	0	3 (6.52)	2 (4.35)	5 (5.43)
20-24	12	1	13 (28.26)	14 (30.43)	27 (29.35)
25-29	9	1	10 (21.74)	18 (39.13)	28 (30.43)
30-34	10	0	10 (21.74)	8 (17.4)	18 (19.57)
≥35	9	1	10 (21.74)	4 (8.7)	14 (15.22)
First trimester antenatal care					
None	7	1	8 (17.4)	7 (15.22)	15 (16.3)
Any Visit	36	2	38 (82.6)	39 (84.78)	77 (83.7)
First trimester folic acid use					
None	13	1	14 (30.43)	7 (15.22)	21 (22.83)
Any Use	30	2	32 (69.57)	39 (84.78)	71 (77.17)
Periconception daily use					
<400µg/day	27	1	28 (60.87)	19 (41.3)	47 (51.09)
≥400µg/day	16	2	18 (39.13)	27 (58.7)	45 (48.91)
Preconception (before pregnant)					
<400µg/day	41	3	44 (95.65)	45 (97.83)	89 (96.74)
>400µg/day	2	0	2 (4.35)	1 (2.17)	3 (3.26)

Table 4. Chi-square test result of folic acid supplement consumption and occurrence of cleft

	CL/P				All Cleft				Controls N (%)
	N	OR	95% CI	p	N	OR	95% CI	p	
Folic Acid Use									
None	13				14				7 (15.22)
Any Use	30	2.414	0.858-6.795	0.149 ^b	32	2.438	0.878-6.764	0.082 ^a	39 (84.78)
≥400µg/day									
No	27				28				19 (51.09)
Yes	16	2.398	1.022-5.625	0.043 ^{a*}	18	2.211	0.960-5.088	0.061 ^a	27 (48.91)

Notes: ^a Statistical evaluation by the Pearson's Chi-Square; ^b Statistical evaluation by; * *p*-value ≤ 0.05 statistically significant are displayed in bold

no significant difference in the number of mothers who had visited antenatal care in the case group (82.6%) and the control group (84.78%).

It was found that 77.17% of subjects had obtained folic acid supplements during the first trimester, while another 22.83% of subjects had never done so. Subjects who had consumed folic acid supplements during this period were more common in the control group (84.78%) than in the cases group (69.57%). Only 48.91% of subjects had sufficient daily doses of folic acid. Subjects who had ≥400µg/day were more common in the control group (58.7%) than in the CL/P group (37.21%) and the all cleft group (39.13%). Only 3.26% of subjects who took adequate folic acid supplements before pregnancy, and none of the subjects from the cleft palate only group had taken folic acid supplements before pregnancy.

Table 4 shows that the association between the history of taking folic acid supplements during the first trimester and the incidence of all cleft and CL/P was not significant in this study. The association of the history of maternal folic acid use during the first trimester with the incidence of all cleft was assessed, but no statistically significant results were found. However, a significant association was found (*p*=0.043) between the adequate intake of folic acid supplements (≤400µg/day) in the first three months and the incidence of CL/P with $\chi^2 = 4.108$ which showed a significant association (>3.841) and contingency coefficient = 0.21.

DISCUSSION

This study used secondary data from Mitra Sejati Hospital medical records to reduce face-to-face interactions during the COVID-19 pandemic and to facilitate the identification of samples that could meet the required criteria. Subjects and patients in this study came from Medan and surrounding areas in the North Sumatra region. No study has been conducted to examine the relationship between adequate folic acid intake during the first trimester and cleft without family history in North Sumatra, Indonesia.

The Indonesian government has required folic acid supplements for pregnant women to be at least 90 tablets.¹⁸ The WHO recommendations state that both pregnant women and women who are planning a pregnancy should use a folic acid supplement of 400µg/day from at least approximately four weeks before conception until 12

weeks after conception.¹⁷ Folic acid supplements in the form of blood-boosting tablets (Tablet Tambah Darah) can be obtained freely at the nearest community health hub (Puskesmas) and (Posyandu). Usually, folic acid supplements are given by health workers during antenatal care visits (ANC).^{18,19} This study showed that 16.3% of the subjects never visited antenatal care during the first trimester of their pregnancy. A further 22.83% of subjects never took supplements during the first trimester. Kelly et al.⁹ have shown that mothers with a low income; mothers in lower occupational class; multigravida mothers aged less than 18 years, and mothers with very low education were less likely to take supplements.

There was a difference in the number of subjects who had ever taken supplements (77.17%) with subjects who consumed at least 400µg/day (48.91%). This shows that 36.62% of the subjects who had taken supplements during the first trimester were not disciplined enough to take them every day. These mothers' indiscipline was influenced by a low motivation to take supplements for a long time and the presence of nausea/vomiting, which is common in early pregnancy and makes it difficult for mothers to take supplements. The human chorionic gonadotropin (hCG) hormone produced by the placenta stimulates the production of progesterone and oestrogen, which triggers the production of unused hydrochloric acid and a slowdown in gastrointestinal emptying.²⁰

The results show that the highest incidence of cleft is cleft lip with cleft palate (73.91%), followed by cleft lip only (19.57%) and cleft palate only (6.52%). These results are supported by other studies which still show that CL/P are the most common, whereas cleft lip only and cleft palate only differ from place to place.^{1,4,11,13}

Table 4 shows the association of adequate folic acid supplement intake with the occurrence of non-syndromic cleft lip/palate (*p*=0.043) and an odd ratio (OR= 2.4 [95%CI 1.022-5.625]) which means that mothers who do not take or consume folic acid supplements less than 400µg/day have 2.4 times greater tendency to have a child with cleft lip with or without cleft palate than those with adequate supplementation during the first trimester. Then it became less significant when related to all incidences of clefts in which cleft palate only cases were included (*p*=0.061). This is probably because the small number of CPO cases does not represent the CPO group and is difficult to test statistically separately. It may also be influenced by the adequacy of other vitamins not measured in this study, such as B₆ and

B₁₂, which are required during folic acid metabolism and any folate antagonist drugs such as phenytoin.^{7,15,21}

There was no association between gender and the occurrence of non-syndromic cleft lip and/or cleft palate in this study, although other studies have suggested that boys tend to have cleft lip and cleft palate (with or without cleft lip).^{1,4,6,11,13} However, the results in this study are supported by Eshete et al.,²² Golalipur et al.,²³ and Taghavi et al.²⁴ In this study, the association of folic acid supplement intake with cleft palate only could not be assessed because the number of cases was small, making it difficult to test statistically.

The association of supplement intake before pregnancy could not be tested because the number of mothers who had taken folic acid supplements before pregnancy was very small. This study also shows that mothers who gave birth at an older age showed an increased risk of giving birth to children with a cleft lip and palate. The study's limitations were that it did not look at cases of stillborn babies; and we did not gather details on maternal smoking history, other maternal vitamin supplement consumption, drug use, alcohol consumption, consumption of foods high in folate or maternal malabsorption disease. Moreover, smoking and drinking habits during pregnancy were uncommon among Indonesian women.

In conclusion, this study showed a significant association between the consumption of folic acid supplements of at least 400µg/day during the first trimester with the incidence of non-syndromic cleft lip with or without cleft palate in cases with no family history. The odd ratio was 2.4, which means that mothers who did not consume supplements with folic acid of at least 400µg/day during the first trimester had a 2.4 tendency times to have children with cleft lip only, or cleft lip and cleft palate. And in this study, gender did not show an association with the incidence of non-syndromic cleft lip and palate.

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