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Research Article

Differences in Folate and Vitamin B12 Serum Levels Between Mothers Whose Children were Diagnosed with Non-syndromic Cleft Lip and Cleft Lip with Palate

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Abstract.
Non-syndromic (NS) cleft lip (CL) and cleft lip with palate (CLP) are abnormalities in the orofacial area that are not accompanied by abnormalities in the head and neck and have normal physical growth and cognitive development. Cleft lip and CLP are caused by two factors, genetic and environmental factors which include deficiencies of micronutrients such as folate and vitamin B12. This study aimed to determine the differences in folate and vitamin B12 serum levels between mothers whose children were diagnosed with NS CL and CLP. An analytical observational study was conducted on folate and vitamin B12 serum levels from 44 mothers whose children were diagnosed with NS CL and CLP. Data were analyzed by t-test for folate serum levels and Mann Whitney for vitamin B12 serum levels. All mothers whose children were diagnosed with non-syndromic CL and CLP, respectively, had normal serum folate levels. Meanwhile, most of the mothers suffered from vitamin B12 deficiency, both mothers whose children were diagnosed with CL (91.6%) and mothers whose children were diagnosed with CLP (93.75%). The mean folate serum level between CL was slightly higher than CLP (p-value = 0.711) but showed no statistical difference in folate levels in the two groups. The median B12 levels between CLP were slightly higher (p-value = 0.394), which indicated that the B12 levels between the two groups were not statistically significant. There were no differences in folate and vitamin B12 serum levels between mothers whose children were diagnosed with non-syndromic cleft lip and cleft lip with palate.

Keywords: Folate, B12, CL, CLP

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1. INTRODUCTION

Non-syndromic CL and CLP are cases that do not have developmental abnormalities other than cleft lip. The incidence of non-syndromic CL and CLP cases is more common, which is about 93-95% of total syndromic CL cases, which is only about 5-7% (1). Asian ethnic groups have the highest prevalence, which is 1 in every 500 births, while the prevalence in Indonesia ranges from 1 in every 700 births (2). CL and CLP are caused by two factors, the interaction between the genetic factors and the environmental factors.

Nutritional imbalances, such as deficiency of vitamin B12, folate, zinc (Zn), and vitamin A, have been reported as risk factors for cleft lip and palate (3).

Differences between folate and vitamin B12 with CL have been studied several times. Research by Kunjana (2017) concluded that folate deficiency in the first trimester will increase the risk of cleft lip by 13 times (4). Warubania (2017) also found that 71% of cleft lip patients had mothers who had folate deficiency during pregnancy. Van Rooij (2003) concluded that low serum vitamin B12 levels in pregnant women will increase the risk of their child developing cleft lip (5). Research conducted by Blanco et al. (2016) showed that the most influential micronutrient in increasing the risk of CL or CLP was vitamin B12 (6).

In view of the role of folate and vitamin B12 in CL and CLP as found in those previous studies, this study was conducted to determine differences of folate and vitamin B12 serum levels between mothers whose children diagnosed with non-syndromic CL and CLP.

2. MATERIALS AND METHODS

This study used an analytic observational method involving a sample of all secondary data (medical records) from mothers whose children diagnosed with non-syndromic CL and CLP at CLP Center University of Muhammadiyah Malang, Indonesia. The sample of this research was taken by purposive sampling technique. This research was conducted at the CLP Center of the University of Muhammadiyah Malang, Indonesia, lasting for 30 days from April 2021 to May 2021.

3. RESULTS

Results showed that 12 and 32 mothers whose children diagnosed with non-syndromic CL and CLP, respectively, had normal serum folate levels. Meanwhile, most of the mothers suffered from vitamin B12 deficiency, both mothers whose children were diagnosed with CL (91.6%), and mothers whose children were diagnosed with CLP (93.75%) (table 1).

With the Shapiro Wilk test, the Follid Acid CL and CLP data were normally distributed (sig $>$ 0.05) so that a parametric test was performed, while the B12 CL and CLP data were found not normally distributed (sig $<$ 0.05) so that the test was continued with a non-parametric test.

Table 1: Data of folate and vitamin B12 serum levels in mothers whose children diagnosed with CL and/or CLP at CLP Center University of Muhammadiyah Malang.

Nutrition Status	Folate		B12	
	CL	CLP	CL	CLP
Normal	12	32	1	2
Possible deficiency	-	-	-	7
Deficiency	-	-	11	23

Difference test of folate serum levels between CL and CLP using independent t-test with the following results:

Table 2: The folate serum Levels difference test between CL and CLP.

Group	Mean ± SD	Sig.
CL (n=13)	33.14 ± 3.64	0.711
CLP (n=32)	31.78 ± 10.22	

The mean folate serum level between CL was slightly higher than CLP but the result of p-value = 0.711 showed that there was no statistical difference in folate levels in the two groups.

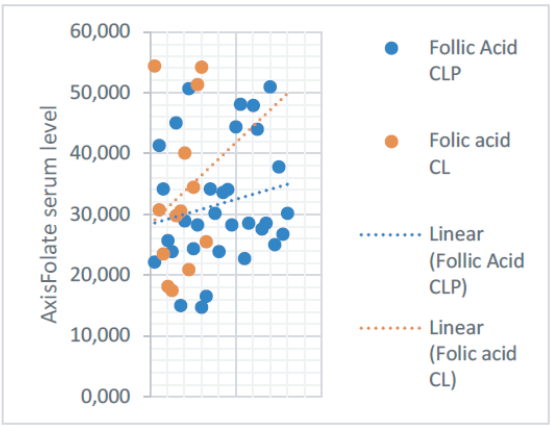

Figure 1: Scatter plot of the folate serum levels on CL and CLP.

The B12 level difference test between CL and CLP using Mann Whitney with the following results:

Table 3: The Vitamin B12 Serum Levels difference test between CL and CLP.

Group	Median (Min-Max)	Sig.
CL (n=13)	113.50 (82.39 – 255.35)	0.394
CLP (n=32)	116.72 (100.44 – 443.24)	

Table 3 shows the median B12 levels between CLP were slightly higher with p-value = 0.394 which indicated that the B12 levels between the two groups were not statistically significant.

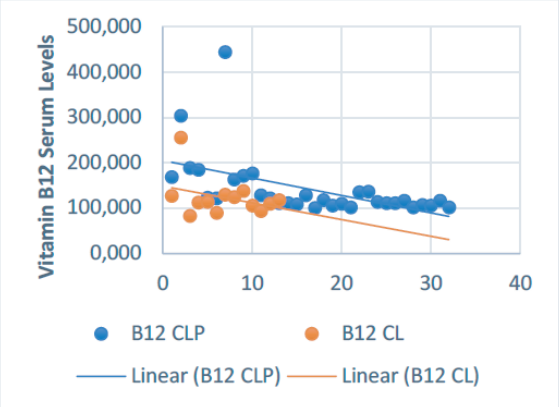

Figure 2: Scatter plot of the vitamin B12 serum levels on CL and CLP.

4. DISCUSSION

4.1. Mean Serum Level of Folate in Mothers with NS CL and CLP Children

Mean serum level of folate in mothers with children diagnosed with NS CL and CLP were 33.95 nmol/L and 31.783 nmol/L, respectively. The normal level of folate is 13.5-45.3 nmol/L or 6-20 ng/ml, so it can be interpreted that on average mothers who had children diagnosed with non-syndromic CL and CLP had normal levels of folate.

A case study conducted by Angulo-Castro et al. (2017) stated that folate levels in mothers or pregnant women affect the incidence of CL and CLP in infants (7). Based on the data analysis, there were 15 children with CL (37.5%) and 4 children with CLP (83.3%) with an OR of 3.27 and a p-value of 0.00 in mothers who did not take folate during pregnancy. Another study was a retrospective study conducted by Jamilian et al. (2017) showing that from 187 children diagnosed with non-syndromic CL and CLP, 130 mothers did not take folate during pregnancy (69.5%) (8). Based on data analysis on risk factors that can increase non-syndromic CL and CLP, risk factors such as not taking folate during pregnancy have an OR of 7.3489 with a p-value of 0.001. The difference in the incidence of CL and CLP was related to the effect of folate as a cofactor for the action of MTHFR enzyme which is protective in some populations, but not effective in others (9). Meanwhile, a meta-analysis study conducted by Blanco et al. (2016) stated that folate did not have a significant effect on the incidence of non-syndromic CL and

CLP, but this could also be due to the insufficient number of samples which then affected the results of the study (6).

4.2. Mean Serum Level of Vitamin B12 in Mothers with Non-Syndromic CL and CLP Children

Mean serum level of vitamin B12 in mothers whose children were diagnosed with non-syndromic CL and CLP were 126.376 pg/ml and 141.737 pg/ml, respectively. The normal level of vitamin B12 is 200-900 pg/ml, so it can be interpreted that the average mothers who has a child diagnosed with non-syndromic CL and CLP has an abnormal or deficient serum vitamin B12 level.

Vitamin B12 deficiency is one of the factors that can cause non-syndromic CL and CLP because vitamin B12 affects homocysteine in folate cycle (10). Elevated homocysteine is a strong risk factor for embryonic toxicity and the development of congenital defects, especially in the form of CL and/or CLP (11). A research conducted by Munger (2021) stated that there was a close difference between deficiency or low levels of vitamin B12 with the incidence of CL and or CLP (12). The study stated that a person deficient in vitamin B12 had a 6.5 higher risk of developing CL and CLP than someone who had normal vitamin B12 levels. A case study conducted by Chattopadhyay et al. (2021) found that of 81 children diagnosed with CL and/or CLP, 29 children (35.80%) had low serum vitamin B12 levels or were deficient with a p-value of 0.051 (13).

4.3. Difference of Folate and Vitamin B12

A study by Kunjana (2017) concluded that folate deficiency in the first trimester increased the risk of cleft lip by 13 times (4). Warubania (2018) also found that 71% of cleft lip children had mothers who had folate deficiency during pregnancy (14). Van Rooij (2003) concluded that low serum vitamin B12 levels in pregnant women increased the risk of their child developing cleft lip (5).

The authors had conducted this study using secondary data sources in the form of medical records at the CLP Center of the University of Muhammadiyah Malang, Indonesia, from April to May 2021 with a total sample of 46 mothers and their children who had been diagnosed with CL and or CLP. The mean folate serum level between CL was slightly higher than CLP but the result of p value = 0.711 showed that there was no statistical difference in folate levels in the two groups. The median B12 levels

between CLP were slightly higher with p value = 0.394 which indicated that the B12 levels between the two groups were not statistically significant.

4.4. Most Affecting Risk Factors in Increasing the Incidence of CL and CLP

The following studies discussed the most important risk factors of the increase of CL and/or CLP incidence. A study by Angulo-Castro et al. (2017) found that the following factors increased the incidence of CL and CLP: mothers who did not consume folate in the first trimester of pregnancy with an OR of 2.6 and a p-value of 0.02; active or passive smoking during pregnancy with an OR of 2.05 and a p-value of 0.01; and alcohol abuse during pregnancy with an OR of 1.90 and a p-value of 0.03 (7). A case study by Jamilian et al. (2017) stated that the risk factors for CL and CLP included a family history of being affected by CL and CLP with an OR of 7.4; not taking folate during pregnancy with an OR of 7.3; and inbreeding with an OR of 3.2 (8). A study conducted by Blanco et al. (2016) showed that the most influential micronutrient in increasing the risk of CL or CLP was vitamin B12 with a p-value of 0.012, then folate with a p-value of 0.039, and finally homocysteine with a p-value of 0.831 (6). Based on the three studies, it can be concluded that the most important risk factor in increasing the incidence of CL and CLP is the presence of a family history of CL and CLP.

In particular, several studies of nutrients (other than folate) involved in one-carbon metabolism have found a strong association between the risk of non-syndromic CL and CLP with vitamin B12 intake. Vitamin B12 also works in a cycle with folate. Normal folate levels can increase the risk of developing non-syndromic CL and CLP, especially non-syndromic CLP if the vitamin B12 levels are low. Vitamin B12 will affect the folate cycle so that the folate cycle is not optimal. Homocysteine will increase and resulting in apoptosis as well as disrupted homeostasis. So if folate levels are normal while vitamin B12 levels are deficient, newborn can have a risk of developing non-syndromic CL and CLP, and vice versa (15).

5. CONCLUSION

Based on the results of the study, it can be concluded that mothers whose children were diagnosed with non-syndromic CL and CLP had a normal mean serum folate level, had a low or deficient mean serum vitamin B12 level, and there was no difference of folate and vitamin B12 serum levels between those mothers.

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