14. Association between maternal folate intake and polymorphism

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Association between Maternal Folate Intake and Polymorphism MTHFR A1298C as Risk Factor of Non-Syndromic Cleft Lips

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ABSTRACT

Background: Methylenetetrahydrofolate reductase (MTHFR) is often associated with the incidence of orofacial clefts. Folic acid deficiency has gained considerable attention because of its promising role in modulating diverse clinical condition such as cleft. The objective of the study is to describe the association of MTHFR A1298C polymorphism and maternal folate intake with an orofacial cleft in Sasak Population.

Method: This study used control case design, the number of the subjects were 148 who were divided into case groups and their mother (70 issues) and control groups and their mother (78 items). The detection of Polymorphism MTHFR A1298C used PCR-RFLP and sequencing for confirmation. The information on the dietary pattern and folic acid intake used FFQ (Food Frequency Questionnaire).

Results: MTHFR A1298C polymorphism was associated with maternal folic acid intake in Sasak (p = 0,001), OR = 14,7 CI 95% (2,49-85,53) for cases and (p= 0,041), OR = 4,4 CI 95%(0,9-19,16) for control group. Maternal folic acid intake was associated with cleft (p=0,037) OR= 2,7 CI 95% (1,06-6,94) in Sasak Population.

Conclusion: Maternal folic acid was as the risk factor cleft lip/palate in Sasak population and association with MTHFR A1298C Polymorphism.

Keywords: *Polymorphism MTHFR A1298C, folic acid, orofacial clefts*

INTRODUCTION

The incidence of nonsyndromic cleft lip with or without cleft palate (NS CL/P) remains high in all over the world. In Indonesia, there will be 3000 to 6000 new cases of cleft lip annually accounting for 2.4% or 1.7 per 1,000 live births [1]. In Asian countries such as India, the incidence of cleft lip is high in which 35. 000 babies were born with the cleft [2]. In Africa, the number of people with cleft lip tends to be less (1: 2,500 births) [3]. Multifactorial factors including genetic and environmental are contributing in cleft lips [4]. Methyltetrahydrofolate Reductase (*MTHFR*) A1298C is *MTHFR* genotype variants that are thought to contribute to cleft lip, or palate [5]. *MTHFR* is an enzyme that converts 5, 10-methylenetetrahydrofolate from folic acid into 5-methyltetrahydrofolate in folate cycle, The endogenous folate cycle is a predominant methyl donor to remethylate homocysteine (Hcy) into methionine [6]. Pregnant women with MTHFR polymorphism have a higher risk to get folate deficiency [7]. The food sources that rich in folate are liver, fish, and meat, mushroom, green leafy vegetables such as spinach, bean leaves, nuts, and yeast. Food processing can destroy 50-90% of folate content by heating, oxidation, and exposure to ultraviolet light [8]. Dietary folates are conjugated by Gamma-*glutamyl hydrolase*/folate into monoglutamate assisted by Zink [9]. Folate deficiency and abnormal metabolism of folic acid and Hcy play a significant role in the incidence

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of neural tube defect (NTB), facial cleft, congenital heart disease, pregnancy complications, and other congenital abnormalities [10]. Because of foods high in folic acid are found from an animal source that is quite expensive for most Indonesian including Sasak population.

To ensure that pregnant women folic acid intake is sufficient a folic acid supplementation program began after 2002. The supplementation programme has just been set by the government in 2014 but still lack of monitoring report. Hence, this study assessed the relationship between maternal genetic background and folate status with or without supplementation.

MATERIALS AND METHOD

Study design: The study design was a case control. Subjects were 70 children and their mother from Sasak Tribe population with a non-syndromic cleft lip with or without cleft palate. The control population was enrolled from 78 healthy normal children and their mother. The clinical examination of the subjects was done by Lips - Alveolar - Hard palate - Soft palate - Alveolar – Lips (LAHSAL). Inclusion criteria that obtain in this study were the mother and her child less than five years old, average weight and body length at birth, without other congenital abnormalities associated with cleft lip/ palate syndrome. The exclusion criteria were orphaned children, and the mother had undergone chemotherapy or radiotherapy.

Blood Sampling: Blood Ethylenediaminetetraacetic Acid (EDTA) samples were withdrawn for 5 ml from all of the study subjects for salting out DNA (Deoxyribonucleic Acid). Extraction.

Genotyping: The *MTHFR* were amplified with three-step Polymerize Chain Reaction (PCR) followed by Restriction Lenght Fragment Polymorphism (RFLP). The PCR RFLP was done at Cebior Laboratory Faculty of Medicine Diponegoro University, Semarang Indonesia. MTHFR A1298C forward primer 5'-CAA GGA GCG GCT GAG GAA GA-3 'and reverse primer 5'-CCA CTC CAG CAT CAC TCA CT-3 '. *MboII*, restriction enzymes were used in the identification of *MTHFR* genotype. The enzyme will digest the PCR product of 128 bp into two fragments measuring 100 bp and 50 bp.

Folate status: data were obtained with the administration of Frequency Questionnaire (FFQ) [10] and analyzed by Nutrisoft 10.1 software.

Statistical analysis: The relationship between *MTHFR* gene and cleft lip were analyzed by using a Chi-Square test and the Odd Ratio (OR), Confidence Intervals 95%. If the relationship between folate status and cleft lip had normally been distributed, data were analyzed by T-independent Test. Otherwise, Mann Withney analysis was performed.

RESULTS

The distribution of variant cleft for case subjects was unilateral cleft lips (31.4%), bilateral cleft lips (21,5%), cleft palates (28.5%) and cleft lips with palates (18,6%). The FFQ data analysis with Nutrisoft Software was used to determine the Odd Ratios (OR) of folic acid in 2.7 (95% CI: 1, 1-6,9). Mann Whitney test was applied to determine the difference between the subjects with the cleft lip the control resulting in p = 0.037 which (<0.05) meaning that there was a significant difference in folic acid between the case and oversight group.

Table 1: Genotype distribution of MTHFR A1298C gene

Genotype	case (n = 70) N (%)	control (n = 78) N (%)	P value	OR (CI 95%)
A1298C/AA	30(42.9%)	27 (34.6%)	--	--
A1298C/AC	31 (44.3%)	41(52.6%)	0.036	2.7(1.1-7.0)
A1298C/CC	9 (12.9%)	10 (14.3%)	0.041	2.7(1.0-7.0)

CI: Confidence Interval, N: Number, OR: Odd Ratio

Table 1 showed the distribution of *MTHFR* A1298C genes. In the MTHFR A1298C gene showed a uniform distribution of genotype between the common allele, mutant heterozygotes, and mutant homozygotes and there was a significant difference between cases and controls.

Table 2: The relationship between maternal folate status during pregnancy and polymorphism MTHFR A1298C

MaternalFolate Status	MTHFR A1298C gene		P Value	OR (CI:95%)
	Polymorphism	Normal Allele		
Case				
Poor	16 (88.9%)	6 (35.3%)	0.001	14.7 (2.49-85,53)
Good	2 (11.1%)	11 (64.7%)		
Control				
Poor	7 (63.6%)	8 (28.6%)	0.046	4.4 (0.9-19.16)
Good	4 (36.4%)	20 (71.4%)		

CI: Confidence Interval, OR: Odd Ratio

Table 2 shows that in the protective case group (mothers of children with cleft lip), folate status (both poor and good), there was a significant relationship between the maternal folate status and the occurrence of polymorphism MTHFR A1298C in both case and control group.

Table 3: Relationship between maternal folate status during pregnancy and cleft lip

Maternal folate status	Cleft lip/palate		P Value	OR (CI: 95%)
	case(celah)	control (normal)		
Poor	22(62.9%)	15(38.5%)	0.037	2.7 (1.06-6.94)
Good	13(37.1%)	24(61.5%)		

CI: Confidence Interval, OR: Odd Ratio

Table 3 showed that the number of women with poor and good folate status 22 subjects (62.9%) and 13 subjects (37.1%) respectively, in cases compared to 15 subjects (38.5%) and 24 subjects (61.5%), respectively among the control group.

DISCUSSION

Non-syndromic cleft lip with or without palate is caused by multifactional factors both intrinsic and extrinsic. Intrinsic factor includes genes and heredity, external factors include nutrition during pregnancy, smoking and drinking alcohol in the mother during pregnancy, drinking herbal medicine during pregnancy, environmental pollution[11]. The results of the study showed that the most common maternal age during pregnancy was more than 35 years old, hight age for pregnancy, the maternal age was not a risk factor for cleft lip in Sasak population. in women aged more than 35 years, biological and environmental changes occur[12,13]. Besides the failure of vascularization of the uterus during pregnancy, that can affect the transfer of nutrients to the fetus[14,15] also, the pregnant woman above 35 years old are at a higher risk of preeclampsia, chronic hypertension, placental abnormalities[16]. Under 19 years is a high risk for pregnancy due to immature biological organs besides socioeconomic factors and the lack of responsibility leading to the development of fetal disorders [17]. Low educational and economic level of the elderly on the Sasak population can be risk factors for cleft lip. The low level of education may lead to poor parental knowledge on the importance of maternal and fetal health, and low socioeconomic factors may lead to the inability to provide proper nutrition to the fetus[18].

We found that the maternal folate status during pregnancy was associated with cleft lip development. There was a significant difference between maternal folate status in both cases (p = 0.001) and control subjects and *MTHFR* A1298 gene polymorphism (p = 0.046). Folic acid deficiency can be detected by a decrease in *MTHFR* (methyltetrahydrofolate reductase), causing deficient remethylation of homocysteine into methionine and reducing the production of SAM (S-adenosylmethionine) [19]. This results in disruption of the methylation reaction leading to disturbance of that

DNA methylation. Methylation defect causes disruption of the expression with the result of inhibited fetal development and the of some malignancies[20].

Table 3 shows that the maternal folate status during pregnancy affects cleft lip incidence in the Sasak population in Lombok (p: 0.037) in which poor maternal folate status during pregnancy tend to have a 2.7 times higher risk to cause cleft lip compared to that of real maternal folate status. This incidence shows that the maternal diet during pregnancy affect the state and health of the fetus and can modulate their offspring through epigenetic mechanisms [21]. Folic acid is required for the metabolism of carbon playing a role in several cellular reactions including in the metabolism of amino acids, the biosynthesis of purine and pyrimidine, the formation of agent methylation primer S-adenosyl-methionine (SAM) which is a methyl donor DNA, histones, proteins, and fats. Natural dietary folic acid is absorbed in the intestine or liver and metabolized to *5*-methyltetrahydrofolate (5-methylTHF) resulting in polyglutamate for cell retention. However, the fortified folic acid can reduce the dihydrofolate by the enzyme dihydrofolate reductase in the liver and converted into tetrahydrofolate, a substrate for synthesis polyglutamate[22].

Deficiency of folic acid as an epigenetic nutrient, a co-factor of one-carbon metabolism, during pregnancy can have an effect on the fetal program and can modulate the genome, a pattern of DNA methylation and lead to dysregulation of gene expression. The administration of folic acid supplements is often combined with other vitamins (multivitamin) causing a difficulty in analyzing whether the effects are due to folic acid or other vitamins. Thus, studies on the administration of supplemental folic acid alone are needed[23].

A different area may show a different result in the relationship between folate status and polymorphism. This is because the diverse population has a different allele variation and different gene involved in folate metabolism[24].

Conflict of Interests: The authors have no conflict of interests related to the conduct and reporting of this research.

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Ethical Clearance: Informed consent was obtained from all of the participants. The research protocol was approved by Medical Research Ethics Commission Faculty of Medicine, Diponegoro University and Dr. Karijadi Hospital, Semarang Indonesia No. 023/EC/FK-RSDK/2016.

CONCLUSION

In Sasak population living in Lombok Indonesia, *MTHFR* A1298C gene polymorphism is a risk factor for cleft lip, and maternal folic acid status during pregnancy is associated with the cleft lip and polymorphism of the *MTHFR* A1298C gene.

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