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Research Article

The Polymorphism of Gene *Reduced Folate Carrier* 1 (RFC1) A80G Not Difference Between NSCLP Case and Control in Sumatera Utara, Indonesia

Budi Yulhasfi Febrianto[1], Eddy Sutrisno[2], Frank Bietra Buchari[2], Utama Abdi Tarigan[2], Hidayat[3]

[1]Department of Surgery, Faculty of Medicine, Universitas Sumatera Utara - H. Adam Malik General Hospital, Medan
[2]Divsion of Plastic Surgery, Department of Surgery, Faculty of Medicine, Universitas Sumatera Utara - H. Adam Malik General Hospital, Medan
[3]Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara

Abstract: Cleft lip is the second most frequent congenital anomalies in Indonesia after Down Syndrome with the prevalence among children aged 24-59 months. The etiology of non syndromic cleft lip with or without cleft palate has not yet been defined. Some studies have investigated the involvement of genetic and environmental factors. Some genes involved in the folate metabolism have been recently examined in order to discover the genetic factors in the cleft lip etiology. This research intends to compare polymorphism in the Reduced Folate Carrier 1 A80G between subject with non syndromic cleft lip and palate (NSCLP) and control subject in the population of Sumatera Utara. In this case control study, 62 patients NSCLP and 61 controls underwent DNA isolation and genotyping process using Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP).The distribution of genotype and allele variants between the case and control were not significantly different (p=0.271, p=0.809). In Conclusion, This research obtained the data that polymorphism RFC1 A80G mutant variant did not difference between case with NSCLP and control in the population of Sumatera Utara.

Keywords: (*NSCLP, Human RFC1 gene, polymorphism, CLO, CPO*)

INTRODUCTION

The case of cleft lip most frequently found is cleft lip with or without cleft palate which may be experienced by 1 in 500 births in the population of Asia and Indian-North American, intermediate in the Caucasian population (1:1000) and the lowest in the African population (1:2500).[1] The prevalence of cleft lip in Indonesia according to the data of RISKESDAS 2013 was 0.08% with Sumatera Utara, the prevalence 0.07%.[2] The data from one of the private hospitals in Medan obtained 102 patients with cleft lip during 2016.[3]

Non syndromic cleft lip and palate is a complex end result of the genetic and environmental factors. In human embryology, there are stages of gene expression, cell migration, cell transformation, and apoptosis.[4] Disorders of any component that regulate the process either by genetic or environmental factors may predispose to non syndromic cleft lip and palate. [5]

One of the components required in the process of embryogenesis is folic acid. Several studies have found that low levels of folic acid are predisposing factors to NSCLP. This is not only influenced by intake or supplementation of folic acid but also in the process of folic acid metabolism.[5,6] Folate undergoes a metabolism consisting of absorption, modification, transport and interconversion. One of the disturbances in the metabolism process can reduce folate levels in the blood and have a clinical impact of NSCLP. One of the proteins that play an important role in the process of folate metabolism is the Reduced Folate Carrier 1 (RFC1) protein that plays a 2-way transport of 5-methyltetrahydrololate and thiamine monophosphate into intracellular and red blood cells and maintains folate homeostasis in the event of down regulation in folate deficiency. The RFC1 protein also known as Folate Transporter (FOLT) is coded by the human solute carrier family genes 19, member 1 (SLC19A1) or also called the RFC1 gene mapped at the end of the long arm of chromosome 21 (21q22.2-q22.3)[7–9]

The polymorphism of this gene is widely investigated for its function as a folate uptake transporter and its relation to disease risk. In several studies of polymorphism of RFC1 A80G with folic acid levels found individuals with AA genotype had higher plasma folate levels than individuals variant AG and GG, [10,11] but this relationship was not found in other studies.[9] *The purpose of this study was to comparing proportion polymorphism in RFC1 A80G between subject with non syndromic cleft lip and palate (NSCLP) and*

control subject in the population of Sumatera Utara.

MATERIAL AND METHOD

This study is a case-control study that has earned the approval of the ethics committee. The case subjects in this study were Non Syndromic Cleft Lift and Palate (NSCLP) patients treated for Labioplasty at the Acuplast Hospital and RSUP Haji Adam Malik Medan from July to September 2017. The number of case and control groups were 62 and 61 respectively obtained by using concecutive sampling method and have signed informed concent for genotype examination.

The DNA of the subject was extracted from a blood sample using the Wizard® Genomic DNA purification kit (Promega Corporation, USA) refered to the procedure in accordance with the manufacturer's instructions. Then the DNA was amplified by the PCR method following the procedure used by Lakkakula et al with the forward and reverse primer sequences used as follows 5 - AGC GGT GA GA GA GGT-3 and 5 - GGA GGT AGG GGG TGA TGA AG-3. The DNA chain was denatured in the incubation process at 94 ° C for 5 minutes, followed by 35 chain reaction cycles (94^{o}C for 30 seconds, annaling at 61°C for 30 seconds and 72°C for 30 seconds) followed by the final stage at 72^{o}C for 5 minutes. The PCR product was then electrophoresed with agarose 2% which was stained with ethidium bromide and obtained DNA fragment 140bp. PCR products were cleaved with PCR-RFLP techniques using restriction enzyme HaeII for 4 hours at 30°C and electrophoresis on agarose gel 3% stained with ethidium bromide.

RFLP products were visualized under UV light to group genotype variants. The fragment 140bp showed allele A and allele G seen on fragments 76 bp and 64bp.

STATISTICAL ANALYSIS

The data of genotype and allele variant frequency is displayed in the form of sum and percentage data. Differences in the proportion of genotype varieties and alleles in case and control groups were analyzed using chi-square, if not eligible, then analyzed by alternate assays of Mann-Whitney and Fisher.

RESULTS

DNA amplification to detect RFC1 polymorphism

Results of PCR-RFLP gene RFC1 exon 2 A80G in this study can be seen in figure 1. allele A seen on Fragment 140bp and allele G were seen on fragment 76 bp and 64bp. subjects with genotype AA seen at no 8,9,10, where the ribbon appears only at 140bp. Subjects with AG were clearly visible on 13 and 19 where the bands appeared in fragments 140 bp, 76 bp and 64bp, subject to G / G genotype seen at no 1, where the bands are seen only in fragments 76 bp and 64 bp.

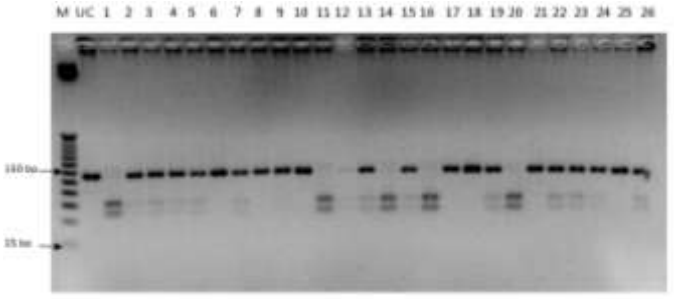

Figure 1. electrophoresis of PCR-RFLP RFC1products Caption: Lane 1 is a DNA marker with an increase of 25 bp. Lane 2 (UC) is an undigested PCR productsseen in a DNA fragment 140 bp. In the Lane subsequently appeared result of subjects 1-26, subjects having allele A seen in Fragment 140bp and G allele seen in fragments 76 bp and 64bp.

Distribution of RFC1 polymorphism

The proportion of RFC1 exon 2 A80G gene polymorphism in case study and control subjects of this study can be seen in Table 1. In genotype distribution group AA 30.6%, AG 51.6%, GG 17.7% compared to control group AA 36.1 %, AG 37.7% and GG 26.2%.
The genotype distribution between the NSCLP and control cases was not significantly different (p = 0.271). The allele G frequency between the control group and the NSCLP cases was also not that different [45.1% (55/67) versus 43.5% (54 / 70); p = 0.809). However, there were significant differences in genotype distribution between subgroup of NSCLP and control cases (p = 0.044)

Table 1. Distribution of Genotype and frequency of Allele RFC1 A80G in NSCLP

	Control %	Overall Clefts (%)
AA	22 (36,1)	19 (30,6)
AG	23 (37,7)	32 (51,6)
GG	16 (26,2)	11 (17,7)
P value	0,271*	
A alelle	67 (54,9)	70 (56,5)
G alelle	55 (45,1)	54 (43,5)
P value	0,809*	

*Tested by Chi-square
** Tested by Mann-Whitney

DISCUSSION

Orofacial cleft, the most common defect in humans, results from the interaction between genetic and environmental factors. Several previous studies have found the role of low levels of folic acid as the etiology of cleft lip because folic acid is involved in DNA methylation process, an important process for regulating gene expression, also contributes to the conversion of uracil to thymidine and is [9]needed for DNA synthesis and repair.[4]

Folic Acid undergoes metabolism process consisting of absorption process, modification, transportation and interconversion, if there is interference in one of the process it

will influence the level of folate in blood.[12] Nowadays, researchers have investigated the association of single nucleotide polymorphism (SnPs) with the occurrence of a disease or drug side effects due to amino acid substitution caused and affecting the activity of the protein produced.[7,13,14]

This study was compare the polymorphism of RFC 1 gene between Subject with NSCLP and control subject. This gene SNPs locates on the long arm of chromosome 21 (21q22.2-q22.3) exon 2 which causes the amino acid substitution of histidine to arginine (H27R; rs1051266) due to substitution of nucleotide 80 adenine to guanine (A80G). These RFC1 proteins play a role in regulating folate transport to red blood cells, in a previous study found that subjects with RFC1 alleles G experienced a decrease in plasma folic acid and increased levels of homocysteine due to negative regulation caused by the RFC1 protein in allele G patients.[9,14]

In this study, we found did not difference proportion variants genotype and allele between subject with NSCLP and control subject .The findings of this study also resemble the results of Lakkakula et al's study in India that examined 142 non-syndromic cleft and 141 control subjects, who found a comparison of genotype variant distributions between case and control groups was not statistically different.[11]

In conclusion, in this case-control study there was not difference the polymorphisme RFC1 A80G between subject with NSCLP and control in the Population of Sumatra Utara.

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