Difference in DNA Methylation between Cleft Lip and Cleft Lip and Palate

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Abstract

It is suspected that environmental exposure to non-syndromic oral clefts, which includes cleft lip (CL), cleft lip and palate (CLP) has an effect on epigenetic mechanisms, particularly deoxyribonucleic acid (DNA) methylation. DNA methylation will be expressed during facial morphogenesis and have an impact on facial development. This study aimed to observe differences in DNA methylation between CL and CLP, between CL mothers and CLP mothers, and correlation between CL and CL mothers, CLP and CLP mothers.

This observational study used a sample of 13 patients diagnosed with CL and 14 patients diagnosed with CLP and their respective mothers. The test was performed using ELISA MethylFlashTM Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric).

The median DNA methylation at CLP was 1.92 (0.23 - 14.07) and CL was 1.71 (0.08 - 8.47) (p 0.752 > 0.05)). Median DNA methylation in CLP mothers was 0.997 (0.03 - 6.14) and in CL mothers 0.72 (0.23 - 6.16) (p 0.798 (p> 0.05). Correlation test for DNA methylation of CLP with CLP mother r = -0.259 and (p = 0.394 >0.05). Correlation test for DNA methylation of CL patients with CL mothers revealed r = -0.492 and (p = 0.087 > 0.05).

The results of this study showed no difference in methylation between CL and CLP. This study found that DNA methylation between CL mothers and CLP mothers was relatively the same. This study also found no correlation between DNA methylation of CL and CL mothers, and between CLP and CLP mothers.

Key words: *cleft lip, cleft lip and palate, DNA methylation*

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Introduction

The non-syndromic oral cleft (NS), which includes cleft lip (CL), cleft lip and palate (CLP), and cleft palate (CP), consists of a variety of disorders affecting the lips and oral cavity.[1] One of the most common congenital disorders is NS cleft lip with/without cleft palate (CL/P), affecting 1 in 700 live births worldwide.

This deformity has a long-term effect on the patient's health and social integration. Residual defects due to scarring and abnormal facial development will cause functional and psychosocial problems for the sufferer.[1,2] Abnormalities of NS CL/P follow a multifactorial model supported by heritability studies with estimated genetic contributions varying from 45% to 85%, depending on the population.[3] There were several factors that influence the incidence of CL and CLP, ie. genetic factors, deficiency of vitamin B6, vitamin B12, folic acid and iron, as well as other factors, such as environmental exposure like organic solvents, air pollution, cigarettes and agricultural chemicals.[4,5]

Environmental exposure may have an effect on epigenetic mechanisms, particularly deoxyribonucleic acid (DNA) or histone methylation.[6] Methylation of DNA has an effect of changing gene activity without altering gene sequences and can be inherited.[7] Methylation of DNA will be expressed during facial morphogenesis and have an impact on facial development, so that DNA methylation can determine the type of cleft in CL and CLP.[8] Biologically, epigenetic changes may involve in the incidence of CL and CLP and their severity. However, there are very few studies on this in humans.

This study aimed to observe differences in DNA methylation between CL and CLP patients, between mothers of CL and CLP and correlation between CL patients and CL mothers, CLP patients and CLP mothers.

Methods

This observational study has passed the ethical test with clearance letter no E.5.a/037/KEPK-UMM/III/2020. This study was an analytical study with a cross sectional design and was conducted at Cleft Lip and Palate (CLP) Center, Faculty of Medicine, University of Muhammadiyah Malang. This study used a sample of 13 patients diagnosed with CL and 14 patients diagnosed with CLP and their respective mothers. Written informed consent was obtained from the mothers. Examination was performed using ELISA MethylFlashTM Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric) Base Catalog # P-1030.

Results

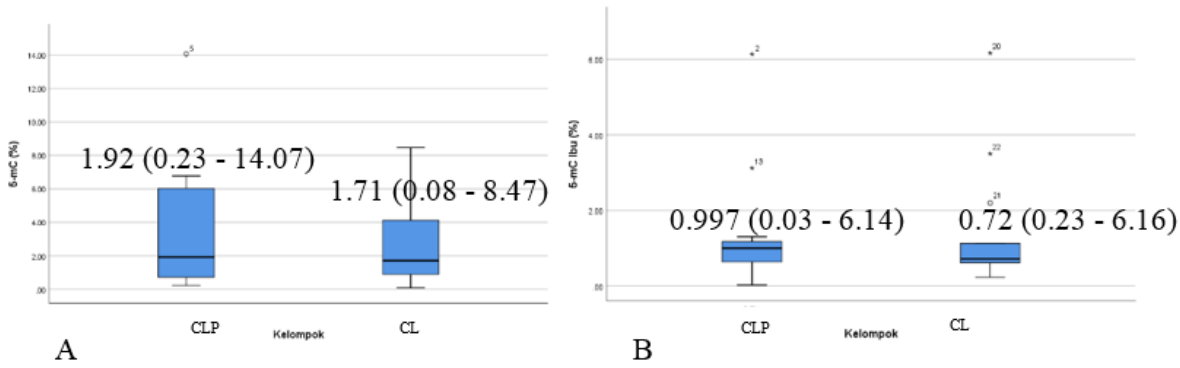

Figure 1. (A) Median DNA methylation in CLP and CL groups; (B) Median DNA methylation in CLP mothers and CL groups

The normality test results revealed normality of DNA methylation data in CL and CLP groups, while in CL mothers and CLP groups the normality test was not met, so the test was carried out with non-parametric statistical Mann-Whitney test. Likewise, the correlation test of DNA methylation in CL and CLP with DNA methylation of the mothers was performed non-parametrically using Spearman Rank correlation test.

Calculations yielded a median DNA methylation value in the CLP patient group of 1.92 (0.23 - 14.07) and in CL patient group of 1.71 (0.08 - 8.47). The Mann-Whitney statistical test obtained a p-value of 0.752 (p> 0.05), which indicated that there was no significant difference in DNA methylation. This test proved that DNA methylation in CL and CLP patient groups was relatively the same.

The calculation resulted in median value of DNA methylation in CLP mother groups of 0.997 (0.03 - 6.14) and in CL group of women of 0.72 (0.23 - 6.16). The Mann-Whitney statistical test obtained a p-value of 0.798 (p> 0.05), which indicates that there was no significant difference in DNA methylation. This test proved that the DNA methylation in the CL group and CLP mothers were relatively the same.

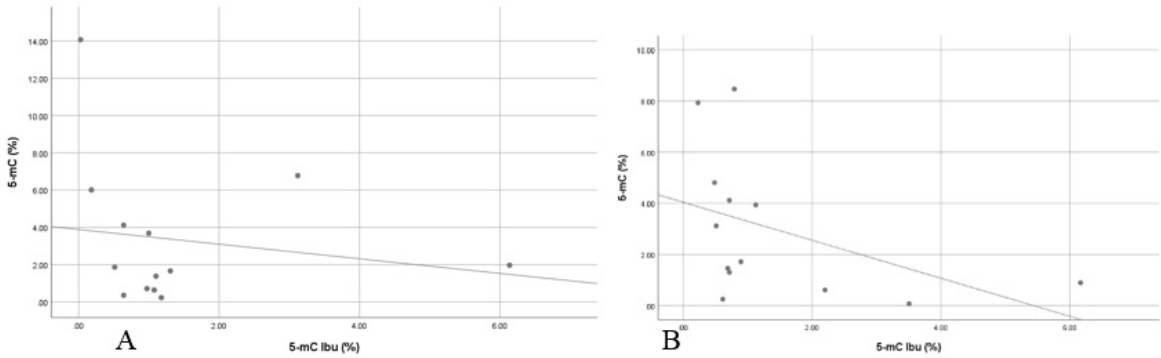

Figure 2. (A) Scatter plot of the correlation between DNA methylation of CLP patients and CLP mothers. (B) Scatter plot of correlation between DNA methylation of CL patients and CL mothers.

The Spearman Rank correlation test showed correlation between DNA methylation of CLP patients and CLP mothers with r = -0.259 and p = 0.394. This test proved that there was no significant correlation (p> 0.05) between DNA methylation of CLP patients and CLP mothers. Correlation test for DNA methylation of CL patients with CL mothers showed r = -0.492 and p = 0.087, which proved that there was no significant correlation (p> 0.05) between DNA methylation of CL patients and CL mothers.

Discussion

Epigenetic influences on a person's traits involve changes in how DNA is packaged, expressed, and converted into proteins without involving variations in DNA sequence. There are chemical modifications that change the level of gene activity, even though the underlying DNA remains the same. These modifications can be attached to the DNA itself or to the histone proteins that are wrapped around the DNA. In DNA methylation, which is the most studied epigenetic mechanism, methyl group binding occurs in the DNA, which affects the expression of nearby genes, often rendering them inactive.[6] Methylation of DNA occurs by covalent addition of methyl group to 5-carbon of cytosine ring by DNA methyltransferases, yielding 5-methylcytosine (5-mC).

Research by Alvizi (2017) found positive correlation between DNA methylation from lip tissue and blood of patients with oral cleft, which suggests that blood is a suitable material for methylation testing in CL/P.[3] This fact underlies the method of collecting blood data in this study.

The results of this study found that there was no difference in methylation between CL and CLP. Similar results were obtained in a study by Khan et al., 2018 regarding the methylation of long interspersed nucleotide element-1 (LINE-1) in CL lip tissue which was examined by bisulfite conversion and pyrosequencing. The study found no significant difference between CL and CLP methylation levels, and no significant differences between methylation levels according to sex. The difference between the medial and lateral sides of the cleft was apparent in male infants but not female infants, and in infants whose mothers did not take folic acid supplements during the periconception period but not in the offspring of mothers who took the supplements.[9]

This study resulted in a median value of DNA methylation in CLP higher than in CL. A study by Sharp et al. (2017) found similar results that the DNA methylation profiles of CL and CLP are more similar to each other than DNA methylation profiles of cleft palate (CP). The methylated regions differed more between CP and CL, than between CP and CLP, and more differed methylated regions between CP and CPP than between CL and CLP. This suggests that the three subtypes have different DNA methylation profiles, but the DNA methylation profiles of CL and CLP are more similar to each other than the DNA methylation profiles of CP. The implication of this oral cleft study is to remind that CL, CLP and CP should be analysed separately and not combined into a single entity or CL/P for analysis.[10]

This study found that DNA methylation between CL mothers and CLP mothers was relatively the same. This is in accordance with a study conducted in Saudi Arabia by Al Faishal et al., (2018) who indicated the transmission of epimutation BRCA1 from mother to child. Verification of methylation in positive mother-infant pairs in the promoter region, analyzed by pyrosequencing in three pairs, found that maternal and neonatal leukocyte DNA showed similar methylation patterns and rates across CpG sites analyzed.[11] A study by Iacobazzi et al., (2014) obtained different results. Folic acid and hyperhomocysteine metabolism disorders are thought to play a role in the incidence of CL/P. A case-control study showed that mothers of children with CL/P NS had higher concentrations of total plasma homocysteine during fasting as well as after a methionine loading test, compared with control mothers. Research on the use of periconceptual folate has been shown to prevent CL/P.[12]

This study also found no correlation between DNA methylation of CL and CL mothers, and between CLP and CLP mothers. A slightly different finding was obtained by Jin et al. (2015) who suspected a possible correlation between hyperhomocysteine and CL/P by the involvement of abnormal DNA methylation, cell proliferation, apoptosis, and migration during embryogenesis. High plasma homocysteine levels have been observed in mothers after the birth of babies with CL/P.[13]

Conclusion

This study found no difference in methylation between CL and CLP, with the median value of DNA methylation in CLP higher than that in CL. This study found that DNA methylation between CL mothers and CLP mothers was relatively the same. This study also found no correlation between DNA methylation of CL and CL mothers, and between CLP and CLP mothers. Further studies are needed to examine blood homocysteine levels in CL and CLP and in their respective mothers.

Ethical Clearence: This observational study has passed the ethical test with clearance letter no E.5.a/037/KEPK-UMM/III/2020.

Conflict of Interest: The authors declare that

there is no conflict of interest in this work.

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