DOI Number: 10.37506/v14/i1/2020/ijfmt/192899

Polymorphic Relationship of rs7526700, rs2278651, and rs611386 Of *SLC30A1* Mothers' Gene with Mothers' High Levels of *Zinc*

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Abstract

The most common facial malformation in all populations and ethnic groups is Cleft lip and palate (CB/L). Various factors cause nonsyndromic cleft lip and palate abnormalities (CB/L NS) such as interactions between environmental and genetic factors, so that CB/L embryopathy is not clear. Deficiency of *Zn* with certain severity in pregnant women can be a risk factor for CB/L. The purpose of this study was to analyze the relationship of polymorphism of rs7526700, rs2278651, and rs611386 of the gene *SCL30A1* mother with a group of deficiency events of *Zinc* maternal. The type of this research is an observational study through cross sectional approach with the research subjects are mothers and newborns diagnosed with CB/L NS in the Perinatology Department of Dr. Hasan Sadikin Hospital Bandung and other Network Hospitals. The research was conducted in September 2016 to June 2017 with 34 groups samples. Data were analyzed using fisher's exact test and correlation test. The results showed that the majority of mothers who became the research respondents aged 25-29 years were 34% and the majority of infants who became the respondents were female as much as 60%, and most types of lip and palate disorders experienced by infants who became the respondents was Unilateral CB/L which is as much as 66%. Furthermore, the results of statistical tests indicated that there was no significant relationship between polymorphism of rs7526700, rs2278651, and *rs611386SLC30A1* maternal gene with levels of *Zinc* maternal.

Keywords*: Polymorphism, rs7526700, rs2278651, rs611386, SLC30A1, Zinc, Mother*

Introduction

Most common facial malformations in all populations and ethnic groups are the cleft lip and palate (CB/L) reported from all anomalies in the head and neck around 65%. CB/ L prevalence is quite high in Asian populations and American-Indian, of which there is around 1 in every 500 births. In Indonesia, the number of CB/L incidence is not yet known certainly. According to Kembaren L (2012), people with cleft lip disorders in Indonesia have increased by an average of 7,500 people per year.[1,2]

Failed unification of central facial skeleton part in the fourth to the tenth week of pregnancy is the cause of cleft lip and palate since the embryo experiences development and growth of facial skeleton at the end of the fourth week and the end of the eighth week of pregnancy. The process of fusion of the palate ends at the end of the tenth week of pregnancy.[3,4]

Various factors cause nonsyndromic cleft lip and palate abnormalities (CB/L NS) such as interactions between environmental and genetic factors so that CB/L of the embryopathy is not clear.[4] Both have complex

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genetic traits, environmental factors have a role in the pathogenesis of CB/L NS and genetic factors are still difficult to understand.[5-8] Some genes become candidate genes related to CB/L NS events such as the *transforming growth factor beta3 (TGFβ3)* gene, *orofacial cleft (OFC1) gene,* the *transforming growth factor α (TGFα)* gene, the *methylene tetrahydrofolate reductase (MTHFR)* gene, *drosophilamsh homebox homolog-1 (MSX1) gene*, *alpha retinoic acid receptor (RARα*) gene, *orofacial cleft 2 (OFC2)* gene, and *orofacial cleft 3 (OFC3) gene*.[5,9]

Micronutrient Deficiencies are found in developing countries, especially in Indonesia, especially deficiency of *Zinc (Zn)* and folic acid. Women in modern times like today consume a lot of food but experience less nutrient.[10] The cause of micronutrient deficiency is due to the lack of food intake and consumption of *Zn* and iron which usually present together in food sources. Oysters, beef, turkey, chicken, cereals, and processed nuts are examples of foods containing *Zn* and iron.[11]

Zinc is a micronutrient belongs to the category of essential minerals, in which about 3 - 10% of genes encode proteins in humans having a domain of binding *Zinc.* Zinc ions cannot pass through the cell membranes, thus the Zinc transportation requires a *Zinc Transporter.* The biological functions of *Zn* in cells are divided into 3 categories, those are catalytic, structural and regulatory systems.[7,12,13] The catalytic system of more than 300 human body enzymes requires *Zn* to function properly.[14] In structural system, *Zn* plays an important role in protein synthesis, both at the cellular and molecular stages. In the molecular level, *Zn* acts as a catalyst for DNA replication enzymes, gene transcription, RNA synthesis and protein. In cellular level, *Zn is* important to maintain cell survival, affecting the signal transduction, transcription and replication. The regulatory system of *Zn's* role is very broad, among others, as a counterweight to the levels of a number of hormones, bone metabolism, neuropsychiatry, immune system, taste and olfactory sensation systems.[12,15]

Zn deficiency with certain severity in pregnant women can be a risk factor for CB/ L.[7] Research in mice shows the role of *Zn transporters* (*ZnT*) in fetal development. Zn transporters are classified as family solute-linked carriers 30A (SLC30A / ZnTs) and SLC39A (ZiPs).[7] The *SLC30A1* gene is one of the genes in which ZnT is known to be actively expressed in developing mouse embryos and this expression depends on *the mothers' intake of Zinc*. SLC30A1 and SLC30A5 proteins have an essential function in the process of transporting *zinc* from mother to fetus.[7,13]

The purpose of this study was to analyze the relationship of polymorphism of rs7526700, rs2278651, and rs611386 of *SCL30A1* maternal gene with the incidence of *Zinc* maternal deficiency.

Research Method

This research was an observational study with a cross sectional approach in which the research subjects are mothers and newborns diagnosed with CB/L NS in the Perinatology Department of Dr. Hasan Sadikin Hospital Bandung and the Network Hospital, among others: RSUD of Bandung City of Ujung Berung, Cibabat City Hospital of Cimahi, Special Hospital for Women and Children in Bandung City, and those who came to the Foundation for Lip and Palate Disease Foundation (YPPCBL) Bandung conducted in September 2016 to June 2017. The research subjects were selected based on inclusion criteria including new mothers who gave birth to babies diagnosed with CB/L NS, newborns diagnosed with CB/L NS, mothers and babies diagnosed with CB/L NS from the Deutero Melayu tribe, as well as mothers and babies diagnosed with CB/L NS who were in good health and good general condition, selected through *consecutively Sampling.* The sample size in this study was 34 people per group.

The variables in this study were level of *zinc* maternal and infant suffered from CB/L NS as the dependent variable and polymorphism of rs7526700, rs2278651, and rs611386 of the *SLC30A1 gene for* mothers as the independent variables. Clinical examination was carried out to determine the diagnosis of cleft lip and palate (CB/L NS) and maternal blood taking from CB/L NS infants and CB/L NS infants whose Zn plasma level was examined and continued by genetic analysis (DNA isolation, PCR and RFLP and *Sanger Sequencing*). Data were analyzed through statistical test which was *fisher's exact* test. If the result did not meet the requirements but normally distributed, Pearson correlation test was performed. Meanwhile, if the data were not normally distributed, the Spearman correlation test rank was used.

Results

1. Characteristics of Research Subjects

Table 1 Characteristics of Respondents (n = 35)

No	Characteristics	Number (%)
1.	Mother	
	Age (years)	
	15 – 19	3 (8.57%)
	20 – 24	4 (11.43%)
	25 – 29	12 (34%)
	30 – 34	10 (28%)
	35 – 40	4 (11,43%)
	≥40	2 (5.71%)
2.	Infants	
	Gender	
	Men	14(40%)
	Female	21 (60%)

Table 1 shows that the majority of mothers who became respondents and aged 25-29 years old were 34% and the majority of infants who became the respondents were female, which was 60%.

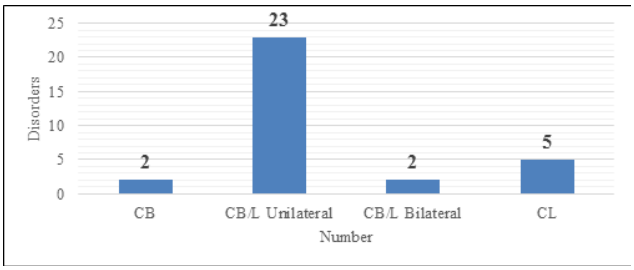

Figure 1 Distribution of Respondents by type of cleft lip and palate

Figure 1 shows that most types of lip and palate disruption experienced by infants who were respondents were Unilateral CB/L which is as much as 66% of the respondents.

2. PCR and Sequencing of rs7526700 polymorphisms

PCR products produced polymorphisms of rs7526700 for SLC30A1 gene by 352 bp. A single band of 352 bp indicated that the PCR technique has been optimized. The PCR results were then sequenced to analyze the polymorphism of rs7526700. Taq1 was primarily used in this study. From 70 samples, it was found in the mother and baby.

3. PCR - RFLPs / MboI *Digestion* polymorphism rs2278651 Digestion

Process along with enzymes *MboI carried* out for 4 hours at 37^0C produces a PCR product of 566 base pairs, rs2278651 truncatedproduce a PCR product of 566 base pairs, rs2278651 truncated *homozygothomozygot* produces base pieces 345 base pairs and 277 base pairs, while those that do notproduces base pieces 345 base pairs and 277 base pairs, while those that do not the result was 566 base pairs. RFLP results were then confirmed by using the sequencing method.

4. PCR - RFLPs / MboI *Digestion* polymorphism rs611386

The digestion process along with the enzyme *MboI* which was carried out for 4 hours at 37^0C produced a PCR product of 679 base pairs. rs611386 truncated homozygot produces base pieces of 572 base pairs and 121 base pairs, while uncollected results are 679 base pairs. Only normal (variants *wild type)* and heterozygous were found in this fragment.

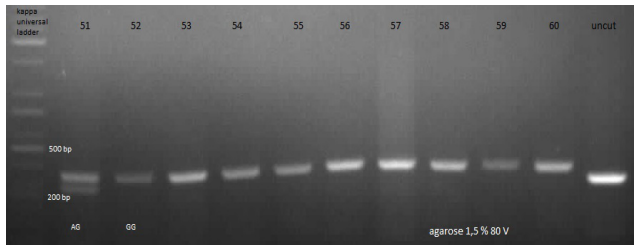

Figure 2.Electrophoresis PCR-RFLP Fragment 611386 (G> A)

5. Relationship of polymorphism rs7526700, rs2278651, and rs611386 gene *SCL30A1* mother with group eventsdeficiency *Zinc* mother

Relationspolymorphism rs7526700, rs2278651, and rs611386 gene *SCL30A1* mother with group eventsdeficiency *Zinc* mothercan be seen in Table 2

Table 2: Relationship of polymorphisms of rs7526700, rs2278651, and rs611386 of *SCL30A1* maternal gene with *zinc* maternal deficiency incidence

Mother's Gene SLC30A1	Zink maternal level		Total	p*
	<7 µg/dl	>7µg/dl		
rs7526700 Polymorphsm (GC)	4	1	5	1.00
Non Polymorphsm (GG)	24	6	30	
Total	28	7	35	
rs2278651 Polymorphsm (CT, TT)	15	3	18	0.69
Non Polymorphsm (CC)	13	4	17	
Total	28	7	35	
rs611386 Polymorphsm (GA)	0	0	0	-
Non Polymorphic (GG)	28	7	35	
Total	28	7	35	

*Fisher's Exact

Table 2 shows statistical analysis results of the rs7526700 gene polymorphism in mothers which is not significantly related to *Zinc* maternal level ($p = 1.00$ or $p > 0.05$), which means that there was no differences in levels found *Zinc* maternal between the polymorphism and non-polymorphism of rs7526700. In addition, gene polymorphisms of rs2278651 also did not have any significant relationship with *Zinc* maternal level ($p = 0.69$ or $p > 0.05$) or there were no differences in *Zinc* maternal level between the polymorphisms and non-polymorphisms of rs2278651.

Discussion

Based on the respondents' characteristic with lower zinc level in infants compared to mothers in this study, the results of experimental studies and human epidemiological case reports indicate that severe *Zn* deficiency can cause fetal malformations including cleft lip and palate.[13] In this study, the clinical manifestations of CB/L NS are mostly unilateral CB/L. This is consistent with previous research, in which the incidence of cleft lip and palate is the largest, amounting to 45%, ceiling slit was 30%, and lip slit was only 25%.[16] However, the results of this study is different from bilateral CB/L which has more severe clinical manifestations compared to unilateral CB/L and appear less frequently.[17] Cases of CB/L and CL are considered to be two different effects and each has a different genetic etiology.

The results showed that polymorphism can disturb the balance of transporting *Zn* from mother to baby. Polymorphism in the mother directly influences the levels of *Zn* in the mother's body or in the baby's body. This assumption raises the hypothesis that gene polymorphism of *SLC30A1* in mothers can affect the levels of *Zn* in mothers and infants, but the results of research and statistical analysis showed that the three polymorphisms of *SLC30A1* genes in mothers do not affect levels of *Zn* in mothers. This happens because polymorphism in the mother influences levels of *Zn* from the beginning of embryogenesis to the second trimester, thereby increasing the occurrence of CB/L during fusion between the maxillary process and the medial process. This will not be observed in this study because the measured level is the level of *Zn* in the serum of babies who have been born (~ 9 months).

The results of genetic studies in humans showed a significant relationship between the gene expression profile for *Zn Transporter* in chronic diseases (diabetes, asthma) and carcinogenesis. Since craniofacial malformations arises from tissue misregulation which is usually coordinated during early embryogenesis. Another interesting aspect is the aspect of *Zn Transporter's* function with the possible involvement of this protein in

embryonic early nutrition.[7]

Genetic and environmental factors, including maternal nutritional status, are known to take part in the process of orofacial gaps including CB, CB/L and CL.[18,19] Some previous studies have shown that nutritional deficiencies, such as folic acid, cholesterol, multivitamins and *micronutrients* such as *Zinc* in mothers, can increase the risk of developing orofacial gaps in infants.[18-20] In contrast, fortification of food by adding folic acid to pregnant women has been shown in several studies to reduce the risk of developing orofacial gaps in infants.[18,20]

Conclusion

Most of the mothers who were respondents were aged 25-29 years and most of the babies who were respondents were female. Most types of cleft lip and palate disorders experienced by infants who were respondents were Unilateral CB/L, and there was no correlation between polymorphism of rs7526700, rs2278651, and *rs611386SLC30A1* mother gene with *Zinc* maternal levels.

Conflict of Interest: Nil

Ethical Clearance: This research has been proved by Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran Bandung Indonesia Number 837/UN6.C1.3.2/KEPK/PN/2016 approved in 26th August 2016

Source of Funding- Self

Reference

1. Lam FS-V, Bendeus M, Wong RW-K. A Multidisciplinary team approach on cleft lip and palate management. Hong Kong Dental Journal. 2007;4:38-45.
2. Kembaren L. Penderita bibir sumbing tambah 7500 per tahun. Jurnal Nasional Kuta. 2012:11.
3. Ellis E. Management of Patients with Orofacial Clefts. Dalam: Peterson LJ, Ellis E, Hupp JR, Tucker MR, editor. Contemporary Oral and Maxillofacial Surgery. Edisi ke-4. Saint Louis: Mosby; 2002. hlm. 623-45.
4. Penfold C.N. Cleft Lip and Palate and Evidance Based Care. Dalam: Booth PW, Schendel S, Hausamen J-E, editor. Maxillofacial Surgery. St.Louis Elsevier: Churchill Living Stone; 2007. hlm. 1000-25.
5. Turgut G, Ozcan A, Cakmak E, Bas L, Tatlıdede S, Ozturk O, et al. Paraoxonase-1 192 enzyme polymorphism in non-syndromic clefting: In patients and parents. Journal of Cell and Molecular Biology. 2008; 7(1):67-74.
6. Anonimous. Birth defect risk factor series: Oral clefts: Texas Department of State Health Services Birth Defects Epidemiology and Surveillance 2005.
7. Hozyasz KK, Mostowska A, Lianeri M, Offert B, Jagodzinski PP. Lack of association of polymorphic variants of genes encoding zinc transporters with the risk of orofacial cleft-affected pregnancies. FOLIA HISTOCHEMICA ET CYTOBIOLOGICA. 2010;48(4):618-23.
8. Suazo J, Santos JL, Jara L, Blanco R. Association Between Bone Morphogenetic Protein 4 Gene Polymorphisms with Nonsyndromic Cleft Lip with or without Cleft Palate in a Chilean Population. DNA and Cell Biology. 2010;29(2).
9. Lee H-K, Kim S-S, Son W-S. Characteristics of MSX1 gene in Korean nonsyndromic cleft lip and palate individuals. Korean J Orthod. 2008 4/;38(2):133-43.
10. Hozyasz KK, Kaczmarczyk M, Dudzik J, Bulska E, Dudkiewicz Z, Szymanski M. Relation between the concentration of zinc in maternal whole blood and the risk of an infant being born with an orofacial cleft. British Journal of Oral and Maxillofacial Surgery. 2009;47(6):466-9.
11. Cole CR, Grant FK, Swaby-Ellis ED, Smith JL, Jacques A, Northrop-Clewes CA, et al. Zinc and iron deficiency and their interrelations in low-income African American and Hispanic children in Atlanta. The American Journal of Clinical Nutrition. 2010;91(4):1027-34.
12. Russell R, Beard JL, Robert J. Cousins, Dunn JT, Ferland G, Hambidge KM, et al. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: The National Academies Press;2000. hlm. 442-489.
13. Helston RM, Phillips SR, McKay JA, Jackson KA, Mathers JC, Ford D. Zinc Transporters in the Mouse Placenta Show a Coordinated Regulatory Response to Changes in Dietary Zinc Intake. Placenta. 2007;28:437-44.

14. Costello RB, Grumstrup-Scott J. Zinc: What Role Might Supplements Play? Journal of the Academy of Nutrition and Dietetics. 2000;100(3):371-5.
15. Rostan EF, DeBuys HV, Madey DL, Pinnell SR. Evidence supporting zinc as an important antioxidant for skin. Int Journal of Dermatology. 2002;41(9):606-11.
16. Neville B, Damm D, Allen C, JE B. Oral and Maxillofacial Pathology. Edisi ke-2. Philadelphia: WB Saunders;2002. hlm. 2-4
17. Bender PL. Genetics of Cleft Lip and Palate Journalof Pediatric Nursing. 2000;15(4 Agustus).
18. Johnson CY, Little J. Folate intake, markers of folate status and oral clefts: is the evidence converging? International Journal of Epidemiology. 2008;37(5):1041-58.
19. Porter FD. Cholesterol precursors and facial clefting. The Journal of Clinical Investigation. 2006;116(9).
20. Munger RG, Tamura T, Johnston KE, Feldkamp M, Pfister R, Botto L, et al. Plasma Zinc Concentrations of Mothers and the Risk of Oral Clefts in their Children in Utah. Birth Defects Res A Clin Mol Teratol. 2009;85(2):151-5.