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RESEARCH ARTICLE

Risk Factor Analysis of Caries Through Saliva in Non-Syndromic Cleft Lips Children With or Without Cleft Palate

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ABSTRACT

Background: *The number of cleft lips or palatal cases is still very high in Indonesia, the growth of cleft lips or palatal cases was 1: 700. This prevalence varies for each region in Indonesia. The condition and composition of saliva in children affect the condition of dental caries, including protein statherin, histatin -1, and calcium minerals. Statherin has the strongest interaction with calcium hydroxyapatite, Histatin-1 has an antimicrobe and antifungal effect and calcium is an imported mineral component.* ***Objectives:*** *The purpose of this study was to analyze the children's saliva of cleft lips with or without cleft palatal non- syndromic against the risk of caries.* ***Methods:*** *This study used an Observational Analytic method with a Post Test Only Control Group Design. Saliva samples were taken by the pipetting method, saliva samples were taken from 20 children with cleft lips with or without palate and 20 normal children, aged 3-17 years, with good general health. Analysis levels of Statherin, Histatin-1, and Calcium minerals used the ELISA Method. Statistical analysis used ANOVA.* ***Result:*** *The results showed that the average level of statherin for children with a cleft lip was 0.65 µg / ml, whereas for normal children was 1.1 µg / ml, the results of the analysis of histatin-1 salivary protein showed an average of 0.86 ± 0.50 at cleft children and 1.01 ± 0.71 for normal children, mean calcium levels in saliva for cleft children is 1.09 ± 0.09 mmol / L and normal children were 1.34 ± 0.13 mmol / L. ANOVA analysis for the three salivary components were found to be p = 0.001, which means that there were significant differences between the average levels of statherin, histatin-1, and calcium in cleft lips children with or without cleft palate and normal children.* ***Conclusion:*** *The levels of statherin, histatin-1, and salivary calcium can be early diagnostic risk dental caries in children with cleft lips/palate nonsyndromic, to optimize management of preventing dental caries on clefts cases.*

Keywords: *Statherin, Histatin-1, Calcium, Non-syndromic lips/palate cleft, Caries risk*

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INTRODUCTION

Cleft lips with or without palate (CL/P) is the most common congenital craniofacial anomaly. The clefts can occur on the lip only (CL), alveolar (CA), involve both lip and alveolar, affect the palate (CP) or involve both lips and palate (CL/P). The congenital can syndromic (CL/P S) or non-syndromic (CL/P NS), it means related with another congenital or not. Prevalence cleft lip in Indonesia reaches 7.500 people. The highest prevalence in Indonesia reach 8,6% or 6-9 people of 1.000 population in East Nusa Tenggara. The multifactorial etiology of cleft lip was divided into genetic and environmental factors. Basic Health Research Data (Riskesdas) 2018, states that the prevalence of children aged 24-59 months suffering from congenital abnormalities reaches 0.53%, of which 0.08% is in the form of cleft lip disorders.[1] The cause of cleft lip or palate is generally divided into 22% genetic factors and 78% environmental factors or a combination of both.[2] So it is necessary to further explore the hereditary history and pregnancy history including the nutrition consumed by the mother during pregnancy. *Folic acid* intake during pregnancy has been shown to prevent cleft lip /palate.[3]

Dental caries in Indonesia is still a disease of the teeth and mouth with the highest prevalence in Indonesia, about 57.6% with the highest sufferer being infant.[1] Thus in cleft lip children with or without cleft palate. A study in England on 120 cases that children with cleft lip were twice risk than normal children both in deciduous and permanent teeth.[4] While the study in Texas on 183 cases found that children with cleft lip/palate had a greater risk of experiencing enamel hypoplasia and caries.[5] The causes of dental caries consist of the host, environment and agent.

Saliva contains a lot of proteins such as *IgA, IgG, Proline Rich Protein,* and *histatin* peptides which are biomolecules that can be used as biomarkers to predict the risk of dental caries.[6] *Statherin* is the only salivary protein that inhibits the precipitation of *calcium phosphate* salts.[7] *Statherin (StN43)*, a *phosphorylated* residual salivary protein similar to *osteopontin* and casein, binds to *calcium* and *hydroxyapatite* (HA). HA inhibits enamel demineralization. Demineralization causes loss of *calcium*, *phosphate* and *fluoride*. *Histatin-1* plays a role in defense of the pellicle on the tooth surface as an inhibitor of the colonization of caries-causing bacteria. *Calcium* reduction occurs in cases of dental caries.[8] *Calcium* plays an important role in the remineralization process decreasing the amount of *calcium* in saliva.

The purpose of this study was to analyze the effect of the salivary components of *Statherin*, *Histatin* and *Calcium* on the risk of caries in children with CL/P NS.

MATERIALS AND METHODS

This study used the analytical observational method with the design of the Post Test Only Control Group Design. Samples were taken by consecutive sampling method with inclusion and exclusion criteria. Saliva samples were taken by pipetting method, saliva samples were taken from 20 children with cleft lip and 20 normal children, aged 3-12 years, in good general health. Analysis levels of *statherin*, *histatin-1*, and *calcium* minerals using the ELISA method and followed by the one-way ANOVA test. Ethical Clearance conducted at FKG UNISSULA No. 018 / B.1-KEPK / SA-FKG / II / 2019.

RESULTS

From a total of 20 samples CL/P NS, the following demographic data were obtained: patients who were male 35% (n = 7) and female 65% (n = 13). Based on the clinical patient, it was found that 53% unilateral lip clefts, 26% bilateral lip clefts, 21% palate clefts were found. Control group was normal children, male 55% (n=11) and female 45% (n=9).

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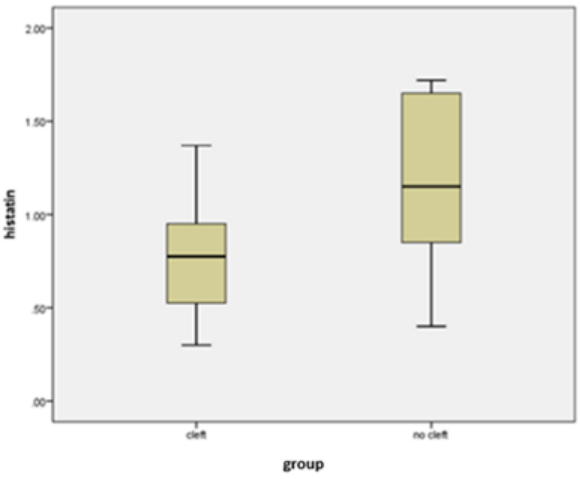
Table 1. The average value of the three salivary proteins, Statherin, Histatin and Calcium

Saliva Content	Groups	
	Cleft Lips (N : 20)	Normal (N : 20)
Statherin (µg/ml)±SD	0.65 ±0.32	1.10 ± 0.45
Histatin (µg/ml)±SD	0.86 ±0.50	1,01± 0.71
Calcium (µg/ml)±SD	1.09±0.09	1.34± 0.13

Table 2. The results of different tests with one way ANOVA, the three salivary proteins for normal children.

Saliva content	Group		Sig	Adj.
	Slit lips	Normal		
Statherin	N = 20	N = 20	0.148	Not significantly different
Histatin	N = 20	N = 20	0.002	Different meaning
Calcium	N = 20	N = 20	0.000	Different meaning

Based on the statistical test results, the statherin results were not significantly different compared to histatin and calcium.



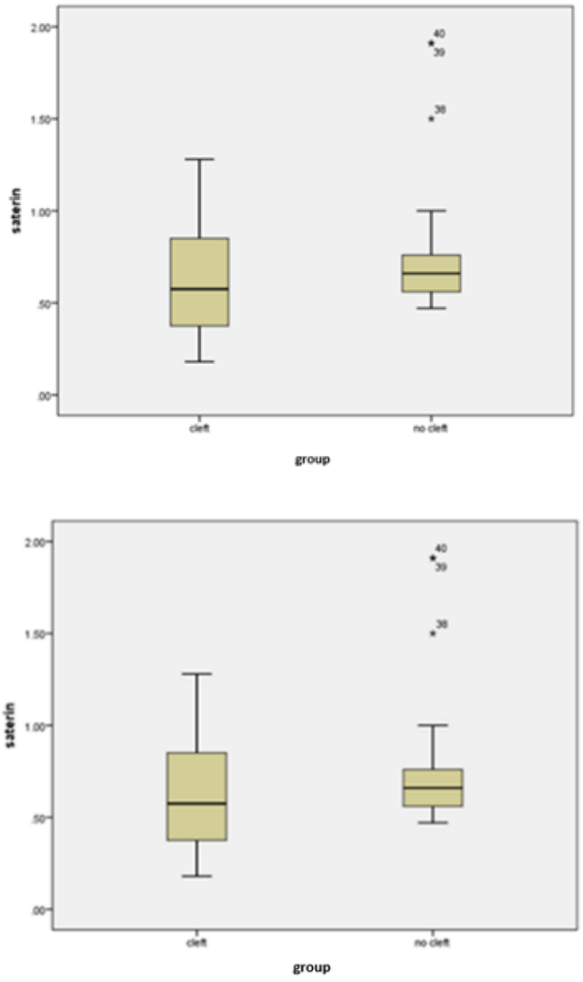


Figure 1. Diagram Anova analysis

DISCUSSION

Saliva is one of the media to diagnose an abnormality or problem in the oral cavity. Many contents in saliva can be used as a marker of the abnormal conditions. Table 2 showed that salivary *statherin* in both CL/P NS and normal children are the same, unaffected by intraoral conditions. *Statherin* is secreted by the *acinar* cells of the submandibular and parotid salivary glands where the primary structure is the residual *tyrosine rich* and vicinal *phosphoserines* with a molecular weight of 5380 Daltons and a concentration of 10 - 40 µM in human saliva. *Statherin* levels are affected by calculus degree, not by irregular tooth structure as in CL/P NS patients.[9] The CL/P NS samples did not show any significant calculus levels. Meanwhile,

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histatin and *calcium* levels differed significantly between cases and controls. This shows that anatomical conditions affect the levels of *histatin* and *calcium* in saliva, while *statherin* has no effect. *Histatin* plays a very important role in the *hydroxyapatite* bonding process, so that *histatin* is often used as a material to prevent abrasion and enamel lysis.[10] *Histatin* can also be used as prevention for infection due to implant failure because it has an antimicrobial effect as well as the main function of *histatin* in the peptidomic sphere. *Histatin* can also prevent gingivitis because of the effect of *histatin* which can reduce the entry of microorganisms.[11] *Histatin-1* is part of the salivary protein which has an antibacterial function to inhibit the occurrence of dental caries.[12] *Histatin-1* protein interacts with *mucin-7*; *mucin-5B*; and *cystatin-S* which will form the biological structure. The interactions between these salivary proteins provide antimicrobial functions and also form pellicles in the enamel to reduce microbial adhesion and reduce the demineralization process.[13] The malfunction of salivary glands can lead the cariogenic and bacterial colonization which can increase the risk of caries. This situation will be exacerbated by abnormal anatomical conditions in the case of CL/P NS causing irregular tooth arrangement so that the risk of caries becomes greater.

In this study, it was found that calcium levels in children with CL/P NS were lower than in controls. This was consistent with *Whelton's* research that irregular tooth arrangement had an effect on salivary *calcium* levels, which is lower than that of regular (normal) teeth.[14] Irregular tooth arrangement causes an imbalance of the demineralization and remineralization processes. Re mineralization causes the loss of *calcium*, *phosphate,* and *fluoride* ions and is replaced by *fluorapatite* crystals which are more resistant to acid dissolution and substantially larger than the original crystal, thereby providing a better (smaller) surface to volume ratio. Therefore the larger apatite crystals in remineralized enamel are more resistant to enamel demineralization by organic acids. Otherwise, plaque concentrations and levels of *Ca* and P ions in saliva can affect the balance of demineralization and remineralization of enamel.[15]

The enamel crystals contain apatite carbonate which is demineralized by organic acids (lactic acid) which are produced by the cellular activity of plaque bacteria from the carbohydrate diet. Increasing the level of *Ca* in the remineralization solution can increase the rate of mineral deposition on tooth.[16] Progress of dental caries is about the relationship between demineralization and remineralization is replaced by apatite crystals which are more resistant to acid solution and larger natural crystals. This proceses caused the larger apatite crystals to be more resistant to enamel damage with residual organic acids and inhibit demineralized processes that induces caries. Crystalline enamel containing carbonate apatite that can be demineralized by organic acids produced by cellular plaque bacteria through consumption of carbohydrates.[17]

Besides, the composition of saliva also affects gingivitis and vice versa. In cases of gingivitis, it is usually accompanied by an increase in *sthaterin* levels and inhibits *calcium binding*. Gingivitis can prevent *statherin* from binding to *calcium* in saliva. All *statherin* molecule chains have a tendency to inhibit calcium and *phosphate* deposition.[18] *Statherin* molecules bind strongly to HA in the form of *statherin-N terminals* which can inhibit secondary *calcium phosphate* deposition. *Stahterin* inhibits the calcium deposition process with calcium-binding saliva so that it cannot survive in the plaque solution. Statherin also prevents the absorption of high molecular weight glycoproteins (HMWGPs) on the tooth surface, then inhibits the attachment of HMWGP which binds to bacteria, including Streptococcus mutans. Statherin-rich layer on the tooth surface showed that high calcium zone facilitates mineralization of the tooth enamel, without further precipitation that actually resulted in the mineralization of tooth plaque.[19] The interaction between *statherin* and *calcium* cause the

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influence each other function. The condition low levels of *statherin* will be followed by abnormal conditions in salivary calcium levels which can affect the risk of caries which is supported by severe gingivitis conditions.[20]

CONCLUSION

The levels of *statherin, histatin-1*, and salivary *calcium* could be early diagnostic risk dental caries on children with CB/P NS to optimize management of prevention dental caries on clefts cases.

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