Differences of salivary urea levels in plaque induced gingivitis and periodontally healthy patients in Periodontology Clinic of The Faculty of Dentistry North Sumatera University (USU) Medan

CrossMark

Aini H. Nasution,* Saipriaya S. Babu

Abstract

Objective: This study aims to know the difference of urea levels in saliva of plaque-induced gingivitis patients and in healthy patients at the Periodontology Clinic of Faculty of Dentistry USU.

Material and Methods: This analytical study used a spectrophotometer to see urea levels in 30 salivary samples of gingivitis patients and healthy patients.

Results: After the samples were analyzed, it was found that the urea level in saliva of gingivitis patients was higher with the mean score of 52.062 g/dL whereas the urea level in saliva of healthy patients was 26.614 g/dL. The results of this study are in line with several studies conducted using various methods and samples.

Conclusion: The urea level in saliva of plaque induced gingivitis patients was significantly higher when compared with healthy patients.

Department of Periodontology, Faculty of Dentistry, North Sumatera University, Medan, Indonesia

*Corresponding to:
Aini H. Nasution, Department of Periodontology, Faculty of Dentistry, University of North Sumatra, Medan, Indonesia
ainina.dentist@gmail.com

Introduction

Periodontal disease is classified according to the severity of inflammation from mild inflammation, known as gingivitis and inflammation that extends deep into the tissues and causes loss of supporting connective tissue and alveolar bone, known as periodontitis.[1]

Dental plaque is a soft layer composed of a collection of microorganisms that reproduce and adhere closely to the surface of the untreated tooth. Over time, microorganisms in dental plaque will develop and will irritate the gingiva thus damaging its supporting tissues.[1,2]

Saliva is a biological environment important for the physiology of the mouth. It achieves its mechanical functions of cleaning and protection through various physical and biochemical mechanisms.[3] The degree of acidity and buffer capacity is due to the increased bicarbonate arrangement according to the rate of secretion. This can be interpreted that the pH and buffer capacity increases as the rate of salivary velocity increases. Other parts, such as phosphates, proteins, and urea are secondary additions to the buffer capacity.[3,4] Urea, which is present in blood and saliva, is an organic substance synthesized from amino acids and carbon dioxide.[5] Some oral microbes hydrolyze salivary and dietary urea via the enzyme urease to produce ammonia and carbon dioxide which results in an increase in plaque pH.[6,7]

There are many research articles performed on urea levels intended as a biomarker for kidney disease.[4] Taye et al.[8] found elevated levels of urea and creatinine in saliva and blood in patients with chronic kidney disease when compared with healthy patients. Similar results were found by other researchers using pediatric patients with chronic kidney disease compared with healthy children.[9]

Several studies have been conducted to determine the levels of urea in saliva that can also be used as biomarkers for periodontal disease. Based on research conducted at Columbia, high levels of urea in saliva were found in gingivitis patients with End Stage Renal Disease (ESRD) compared with healthy periodontium patients.[1]

Anne et al.[10] conducted research on the chemical compositions of saliva, one of which was urea in patients with gingivitis and periodontitis without systemic disease. This research showed that there was a significant association of urea concentration in saliva with periodontal status, where there was a high concentration of urea in saliva of gingivitis and periodontitis.[10]

Several studies have been conducted on urea levels in patients with salivary periodontal disease

and patients who have systemic diseases such as chronic kidney disease. However, in Indonesia, there is still no research to determine the level of urea in gingivitis patients without systemic disease. Therefore, it is necessary to conduct research to determine the difference in urea levels in plaque-induced gingivitis patients, where this study is an early study to find alternative ways to diagnose periodontal disease or early detection of kidney disease through saliva.

Material and Methods

This study is an analytical study with cross sectional study design. The samples of this study were saliva of patients of plaque-induced gingivitis and healthy patients aged between 20-30 according to inclusion and exclusion criteria at the Dental Hospital Installation of Periodontics Faculty of Dentistry USU Medan during 2016-2017 using purposive sampling

Table 1 Distribution of demographic data based on sex and age group

Variable	Test Group		Control Group	
	n (15)	% (100)	n (15)	% (100)
Sex				
Male	10	66.7	6	40
Female	5	33.3	9	60
Age Group				
20-25	8	53.3	11	73.3
26-30	7	46.7	4	26.7

Table 2 Difference of mean urea level between test group and control group

Groups	Average x	P
Test	52.06	0.000*
Control	26.61	

Description: Mann-Whitney test p <0.05

Table 3 Mean of urea Level based on PBI score on test group

PBI Score	Mean of Urea Level on Test Group (g/dL)
31-40	32.0
41-50	65.5
>50	98.8

Table 4 Mean of urea level based on PBI score on control group

PBI Score	Mean of Urea Level on Control Group (g/dL)
0-1	11.5
2-3	15.5
4-5	31.3

method. The sample consisted of 15 plaque-induced gingivitis patients with a Papillary Bleeding Index (PBI) score of > 30 and 15 healthy patients with a PBI score < 5.

The procedure of this study includes examining the patient's gingiva using a periodontal probe. After the gingival examination, the patients involved were given sterile salivary measuring cups and told to flow their saliva into a salivary measuring cup using a passive draining method. Each sample collected as much as 3 mL was labeled and brought to the Biochemical Laboratory Falculty of Medical USU Medan and then collected in a 6 mL Falcon tube for analysis. The sample was centrifuged at 2.800 rpm for 10 minutes to obtain supernatant and then the supernatant was separated by pipette and frozen at -200C.

At the time of analysis, the sample is melted at room temperature and centrifuged at 3.000 rpm. Urea is quantified by determining the number of green indophenol reagents using a spectrophotometer at 600 nm. This technique is based on the following reaction:

$$Urea + H_2O \longrightarrow NH_4 + CO_2$$
$$NH_4\ salicylate + NaClO \xrightarrow{Nitroprusside} Indophenol$$
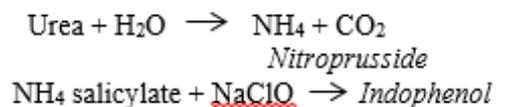
light absorbance value. Calculation of urea content is obtained using formula: Csample = Asample / Astandard × 80 mg/dL

Data analysis was performed by the Shapiro-Wilk test to see the normality of data and the Mann-Whitney test to test the difference.

Results

The results of research on demographic data is shown in table 1. The group of plaque-induced gingivitis patients were mostly male in gender (66.7%) and in the healthy group were mostly female (60%). Table 2 shows that by age group, both in plaque-induced gingivitis and healthy patients, the most were aged 20-25 years, by 63%.

The mean urea level in the test group was 52.06 g/dL. For the control group, the mean urea was 26.61 g/dL. Based on table 2, it is known that the mean urea levels in the test and control groups differed significantly at p=0.000.

Table 3 shows that based on the average PBI score, it was found that most of the study subjects in the test group had a high mean urea average on PBI> 50 scores, compared with an average PBI score between 31-40 and 41-50.

In addition, it was observed that most of the study subjects in the control group of table 4 had a low mean urea on average PBI scores between 0-1 compared with others.

Discussion

In this study, it was found that plaque-induced gingivitis patients had higher urea (52.062 g/dL) in their saliva than in healthy patients (26.614 g/dL). This result is in line with a study conducted by Castaneda et al.[10] that found an elevated urea level in saliva in patients with gingivitis and periodontitis compared with healthy patients.

The urea level in normal saliva was 17-41 g/dL.[10] The results of this study obtained a urea level of 52.062 g/dL in the plaque induced gingivitis group which showed that this value exceeded normal urea levels in saliva. This increase may be due to the salivary alkalization process due to the formation of ammonia and carbon dioxide which indicates high levels of urea in saliva.[10]

The results of this study are also consistent with research conducted by Tanaka et al.[11] in Japan where it was found that there was an increase in urea levels in saliva of gingivitis patients and periodontitis with different severity compared with patients without periodontal disease.[11]

In addition, a study conducted by Junior et al.[5] in Brazil found that there was an increase in urea levels in patients with gingivitis and chronic periodontitis compared with healthy patients. The results showed similar results with this study which showed elevated urea levels in gingivitis patients. In the study, it was explained that this increase in urea can be caused by bacterial activity, especially anaerobic gram-negative bacteria. Among several components of saliva, urea is an organic compound that is the final product of protein catabolism. Hydrolysis of urea by bacterial urease enzymes results in the continuous formation of ammonia which causes the periodontal tissue to be cytotoxic.[5]

On top of that, Mattioli et al.[8] research results are also in accordance with the results of this study that there is an increase in urea levels in gingivitis patients. According to these researchers, urea is an organic compound contained in saliva in the oral cavity. Increased levels of urea can be caused by the formation of ammonia resulting from the activity of bacterial ureolysis plaque.[8]

In this study, it can also be seen that the higher the bleeding score, the higher the level of urea in saliva. This is in accordance with a study conducted by Arnaud et al.[12] who explained that the urea levels evaluated in the study may be correlated with inflammatory agents such as interleukins and growth factors.[12]

Based on this research it can be concluded that there is significant difference of urea level in saliva of plaque-induced gingivitis patients compared with healthy patients. In addition, there was an increase in mean urea levels in saliva of plaque induced gingivitis patients based on an increase in PBI scores.

Conclusion

There is a significant difference of salivary urea levels in plaque-induced gingivitis compared to healthy patients. This was proved by obtaining the results of this study that showed that the salivary urea level of patients with plaque-induced gingivitis was significantly higher than patients with healthy periodontium. In addition, there was an increase in mean urea levels in saliva of plaque-induced gingivitis patients based on an increase in PBI scores.

Acknowledgment

Conflict of Interest

The authors report no conflict of interest.

References

1. Ariyamuthu VK, Nolph KD, Ringdahl BE. Periodontal disease in chronic kidney disease and end stage renal disease patients. NCBI 2013;3: 2.
2. Panagakos FS, Davies RM. Gingival diseases their aetiology, prevention and treatment. Croatia: Alenka Urbancic; 2011. p. 33-36.
3. Gupta M, Abhishek. Oral conditions in renal disorders and treatment considerations. J Saudi 2015;27: 113-119.
4. Ahmed N, Mehmood A, Dawani N, et al. Salivary urea: a marker for chronic renal disease. J Med Dent 2015;4: 1-2.
5. Junior AB. Evaluation of organic and inorganic compounds of saliva of patients with chronic periodontal disease. Rev Odonto Cienc 2010;25: 243-238.
6. Djordjevic S, Ivanovic D, Ivanovic T. Effect of 10% urea solution on epithelialization of thermally wounded gingiva. J Serbian 2013;60: 147-148.
7. Patricia-Del VDA. Saliva composition and functions: a comprehensive review. J Contem Dent Pract 2008;9: 2-3.
8. Mattioli TM, Koubik CA, Ribas M. Salivary flow rate, calcium, urea, total protein and amylase levels in fanconi anemia. J PHO 2010;32: 46-48.
9. Reddy SM, Abdelmagyd HAE, Shetty SR, et al. Minimal invasive periodontal surgery: a Review. J Dentomaxillofac Sci 2017;2: 81-85.
10. Castaneda HA, Moya CA. Chemical salivary composition and its relationship with periodontal disease and dental calculus. Braz J Oral SCI 2015;14: 159.
11. Tanaka T, Arakawa H, Tsurumoto A, et al. Screening of periodontitis with salivary enzyme tests. J Oral Sci 2006;48: 177-183.
12. Arnaud, Nakamura M, Slots J. Salivary enzymes origin and relationship to periodontal disease. J Periodontal Res 1983;18: 559-569.