Correlation of salivary phosphorous level to dental calculus accumulation on patients of the periodontology installation in dental hospital of USU

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Abstract. Dental calculus is a hard deposit that is formed by calcification of dental plaque primarily composed of calcium phosphate mineral salts which is deposited on natural teeth and restorations and is covered by a layer of unmineralized plaque. Dental calculus plays an instrumental role in further infuriating the periodontal disease. Saliva is closely associated with the distribution pattern of supragingival calculus. Salivary phosphorous is readily absorbed by dental plaque forming calculus. Thus, this study aims to analyze whether salivary phosphorous level is associated with the dental calculus accumulation on patients of The Periodontology Installation in the Dental Hospital of Universitas Sumatera Utara.

1. Introduction
Dental calculus is a hard deposit that is formed by calcification of dental plaque primarily composed of calcium phosphate mineral salts which is deposited on natural teeth and restorations and is covered by a layer of unmineralized plaque [1]. It is a well-known fact that dental calculus itself is not an inducing agent for pathological changes that occur in gingival tissues; instead it is covered by a layer of unmineralized plaque which is proven to be the key etiological agent involved in these pathogenic mechanical [2]. Dental calculus plays an instrumental role in further infuriating the periodontal disease [1,3,4]. These hard deposits may form coronal to or apical to the gingival margin, hence named accordingly as supragingival and subgingival calculus respectively [1, 3, 5]. Dental calculus is primarily composed of mineral as well as inorganic and organic component [3,6]. The principle inorganic components are calcium, 39%; phosphorous, 19%; carbon dioxide, 1.9%; magnesium, 0.8%; and calcium phosphate [1,6]. Early plaque of heavy calculus formers contains more calcium, three times more phosphorus, and less potassium than that of non-calculus formers, suggesting that phosphorus may be more critical than calcium in plaque mineralization [1].

Mineralization of dental plaque leads to calculus formation [3]. Mineralization can begin as early as 24-48 hours. Calculus forms in layers that are parallel to the tooth surface. The layers are separated by line that appears to be pellicle which later undergoes mineralization. These lines are called incremental lines. Calcification starts in separate foci on inner surface of plaque, these foci of mineralization gradually increases in size and coalesce to form a solid mass of calculus [1, 3].
Saliva is the most available and non-invasive biofluid of the human body, permanently bathes the oral cavity and is trying to cope with an ever-changing milieu [7]. Saliva is critical to the preservation and maintenance of oral health, and any changes in its amount or quality may alter the oral health status [8]. Saliva is closely associated with the distribution pattern of supragingival calculus [6]. The most abundant component in saliva is water (approximately 99.5%), followed by inorganic and organic ions about 0.5% [5]. Saliva includes a large number of organic compounds such as: urea, ammonia, uric acid, glucose, cholesterol, fatty acids, triglycerides, glycolipids, amino acids, steroid hormones and proteins that aid in the protection of oral cavity tissues, including mucins, amylases, agglutins, glycoproteins, lysozymes, peroxidases, lactoferrin, and secretory IgA [5,7]. The inorganic component of saliva such as calcium, phosphor, sodium, potassium, magnesium, carbon dioxide, oxygen, and nitrogen [5]. Salivary calcium and phosphorous are readily absorbed by dental plaque forming calculus [9].

Phosphorus is the second most important mineral in the human body after calcium. About 80% to 90% of phosphorus is present in the bones and teeth in the form of hydroxyapatite (Ca_{10}(PO_{4})_{6}(OH))_2 and the remainder is present in the extracellular fluid (ECF), soft tissue and erythrocytes.[10] In living tissue, phosphorus exists in the form of phosphate (PO_4^3-). Most of the phosphorus in the whole blood is in the phospholipids, or red blood cells and plasma lipoprotein, and only ~1 mmol/l is found as inorganic phosphorus, which can be in different forms, the most common being HPO_4^{2-}[11]. As the SF increases, the concentrations of total protein, sodium, calcium, chloride, and bicarbonate, as well as the pH increases to various levels, whereas the concentrations of inorganic phosphate and magnesium diminish[12]. The Volpe-Manhold Index (VMI) as a method of calculus assessment was developed by AR Volpe and JH Manhold in 1962 to assess the existence and severity of calculus formation, in particular the supragingival calculus [13]. To obtain a VMI score, an examination of three lingual surface plains of the sixth anterior the mandible is the mesial, middle, and distal surface using a periodontal prob (in millimeters). The calculus score on the mesial, middle, and distal surface are averaged by summing the third score and dividing by three to obtain an index score one tooth. The index scores on all six teeth are averaged by summing entirely and divided by six to obtain a subject index scores [14].

According to the theory of mineral precipitation on the formation of calculus, calcification will occur when the pH, calcium, and phosphate saliva concentrations are high enough to cause precipitate calcium phosphate salts [15]. The initial plaque on the heavy calculus contains less potassium, more calcium and phosphorus than the non-calculus builders, so it is estimated that phosphorus is more involved in the plaque mineralization process [1].

According to research by Patel R, et al, patients with periodontitis have higher levels of phosphorus compared with gingivitis and control group [9]. According to Prashaanthi N,et al patients with periodontitis have higher level of calcium and phosphorus saliva then healthy subjects [15].

2. Materials and Methods

This study is an observational analytic with cross sectional study design. The place of study is in Periodontology Installation in The Dental Hospital of Universitas Sumatera Utara and Integrated Laboratory in The Medical Faculty of Universitas Sumatera Utara. The samples were obtained from saliva of patients in the Periodontology Installation. Determination of the samples is done by using purposive sampling technique ie the selection of the sample by setting the subject that meets the criteria that have been determined by researchers for the purpose and objectives of the study to be achieved. In this study, the number of samples used were 40.

The inclusion criteria in this study were subjects aged 20 years and over, had six anterior mandibular teeth, not being pregnant or menstruating, systemically healthy, and willing to participate in this study. While subjects who were heavy smokers, had systemic disease that affect the periodontal tissues, such as diabetes mellitus, kidney disease, liver disease, or cancer, subjects who were undergoing hormone therapy, undergoing chemotherapy or radiation treatment, taking drugs which
may affect salivary phosphorus level, ie antihypertensive drugs, antidepressants, analgesics, and antihistamines, are excluded from this study.

The process of collecting data begins by explanation of the purpose, benefits, and research procedures that will be done to the subjects of this study. Subjects who were willing to participate in this study were asked to sign informed consent. After that, calculus was examined on study subjects based on the Volpe-Manhold Index (VMI). Then, saliva was collected without stimulation (unstimulated) and based on the spitting method, which means the subjects were instructed to sit quietly and not speak during collecting saliva procedure. Subjects were asked to drop down their head and let the saliva pool in their floor of the mouth to their maximum extent and then expectorate into saliva collecting cup about 5ml. The saliva collecting cups were then labeled and put in ice box. Saliva samples were taken to the research laboratory in less than 24 hours and stored into a deep-freezer of -80°C. Then, salivary phosphorus levels were measured using UV-Vis spectrophotometer.

3. Results
The demographic data of the subjects in this study indicated that the subjects of were mostly females, with a total of 27 subjects (67.5%). In addition, subjects < 30 years of age group were the highest number of subjects, with a total of 37 subjects (92.5%) compared with other age groups (Table 1).

| Table 1. The demographic data of study subjects |
|-----------------------------------------------|
| Variable | Number (N= 40) | Percentage (%) |
| Gender   |               |                |
| Men      | 13            | 32.5           |
| Woman    | 27            | 67.5           |
| Age group|               |                |
| < 30 years| 37            | 92.5           |
| 30 - 50 years | 2        | 5              |
| > 50 years | 1            | 2.5            |

The minimum score of the Volpe-Manhold Index (VMI) found in this study was 0, the maximum score found was 3.16 while the mean score was 0.99. It can also be seen that the minimum value of salivary phosphorus level was 0.92 nmol / μl, the maximum value was 2.56 nmol / μl, while the mean value was 1.52 nmol / μl (Table 2).

| Table 2. Score of Volpe-Manhold Index (VMI) and salivary phosphorus level |
|-----------------------------------------------|
| N | Minimum | Maximum | Mean ± SD |
|---|---------|---------|-----------|
| VMI score | 40      | 0       | 3.16      | 0.99 ± 0.95 |
| Phosphorus level | 40      | 0.92 | 2.56 | 1.52 ± 0.42 |

To find out whether there was a correlation between the salivary phosphorus level with VMI score, analytic was done using Spearman's linear correlation test. The results of Spearman's linear correlation test show that Spearman's linear correlation (r) was 0.84. This suggested that the relationship between salivary phosphorus levels with VMI score was very strong (r> 0.5). In addition, we can also note the
value of \( p = 0.00 \) (\( p < 0.05 \)). It can be concluded that based on Spearman's linear correlation test, the correlation between salivary phosphorus level and VMI score was significant (Table 3).

Table 3. Correlation between salivary phosphorus level and Volpe-Manhold Index (VMI) score

| Phosphorus level | Spearman correlation (r) | P |
|------------------|--------------------------|---|
| VMI              | 0.84                     | 0.00* |

4. Discussion

In this study, we found that the lowest score of the VMI was zero with 0.92 nmol / µl salivary phosphorus level. While the highest score of the VMI was 3.16 with 2.56 nmol / µl salivary phosphorus level. This finding suggested that subjects with a low score of VMI also had low levels of salivary phosphorus level. In contrast, subjects with a high score of VMI were followed by elevated salivary phosphorus levels. Thus, salivary phosphorus levels with VMI score have a correlation or positive relationship. The results showed a significant correlation (\( p < 0.05 \)) between the salivary phosphorus levels and VMI score based on the correlation test.

In this study, there were data that were not normally distributed or not homogeneous on salivary phosphorus variables. This was probably caused by researchers did not limit the subjects by periodontally healthy and suffering from periodontal disease, so that in some research subjects there were higher salivary phosphorus levels compared to salivary phosphorus levels in other subjects, where subjects with high levels of salivary phosphorus were subjects with periodontal disease.

Several studies on salivary phosphorus levels have been done before. Patel R, et al conducted a study to compare salivary phosphorus levels in three groups of subjects, namely the control group, the subjects with gingivitis, and the subjects with periodontitis. The collection of saliva as a sample was done without stimulation (unstimulated). The results showed that salivary phosphorus levels in the subjects who had periodontitis were significantly higher (14.50 ± 3.82) than in the subjects who had gingivitis (7.68 ± 1.40) and the control group (3.93 ± 1.82).[10]

The results of Patel R et al are consistent with the study of Fiyaz M, et al comparing salivary phosphate levels in subjects with periodontitis, subjects with caries, and control groups, in which phosphate is a compound of phosphorus. The study of Fiyaz M, et al used saliva without stimulation (unstimulated) as samples. The results showed that salivary phosphate levels were significantly higher in the subjects who had periodontitis (5.71 ± 0.71) than in the caries group (3.96 ± 0.55) and the control group (4.87 ± 0.35)[6]. Then Prashaanthi N, et al's study also found that salivary phosphorus levels were significantly higher in the subjects who had periodontitis (4.26 ± 0.73) than the control group (3.56 ± 0.44)[15].

The results of this study showed conformity with the research of Patel R, et al. who stated that salivary phosphorus levels were significantly higher in the subjects who experienced periodontitis compared with the subjects who had gingivitis and the control group. In addition, the results of this study were also consistent with Fiyaz M et al and Prashaanthi N, et al’s studies, which resulted in significantly higher salivary phosphorus levels in subjects with periodontitis than in the control group.

The high levels of salivary phosphorus followed by an increase in the Volpe-Manhold Index score may be due to the fact that phosphor in saliva is one of the inorganic components that play a role in the process of calculus formation[10]. Subjects with increased salivary mineralization parameters such as calcium and phosphorus, increased salivary flow rate, and poor oral hygiene have a higher risk of dental calcification of plaque that will develop calculus, and have a higher risk of developing periodontitis[7]. This is related to the role of calculus that can cause periodontal disease [1,2,3]. In conclusion, there is correlation between salivary phosphorus levels and the dental calculus accumulation as measured using the Volpe-Manhold Index score in patients of Periodontology Installation in Dental Hospital of Universitas Sumatera Utara.
References

[1] Newman M G, Takei H H and Carranza FA 2002 *Carranza’s clinical periodontology 9 th ed* (Philadelphia: WB Saunders Company) 64

[2] Aghanashini S, Bhavana P, Darshan BM, Apoorva SM, Divya B and Manjari L *JOHSR 2016* 7 42

[3] Saini R 2014 *Int J Dent Health Sciences* 1 794

[4] Kamath D G and Nayak S U 2014 *The Saudi Dent J* 26 7

[5] Eley B M and Manson J D 2004 *Periodontics 5 th ed.* (London: Elsevier Ltd) 21

[6] Jin Y and Yip H K 2002 *Crit Rev Oral Biol Med* 13 426

[7] Greabu M et al. 2009 *J of Medicine and Life* 2 124

[8] Agha F, Dizgha I M, Amirkhani S 2006 *J of Contemporary Dent Practice* 7 1

[9] Patel RM, Varma S, Suragimath G and Zope S 2016 *J of Clinical and Diagnostic Research* 10 58

[10] Raina R, Garg G, Sethi S K, Schreiber M J, Simon J F and Thomas G *J Nephrol Therapeutic* 1

[11] Kemi V 2017 Effect of dietary phosphorus and calcium-to-phosphorus ration on calcium and bone metabolism in healthy 20- to 43- year-old finish woman https://helda.helsinki.fi/handle/10138/20895 (27 Maret 2017)

[12] Almeida PD, Gregio AM, Machado MA, Lima AA, Azevedo LR. Saliva composition and functions: A comprehensive review. J of Contemporary Dental Practice 2008; 9 1

[13] Bathla S and Bathla M 2011 *Periodontics Revisited* (New Delhi: Jaypee Brothers Medical Publishers Ltd) 73

[14] Perry D A and Beemsterboer P L 2007 *Periodontology for the dental hygienist 3 rd ed* (Missouri: Saunders Elsevier) 81

[15] Prashaanthi N, Gayathri R and Vishnupriya V 2016 *J Pharmaceutical Sciences and Research* 8 623