

## Salivary Total Protein Level in Elderly Patients with Periodontitis Related to Type 2 Diabetes Mellitus: A Community Based Study

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### Abstract

Epidemiological study show that the prevalence of Diabetes Mellitus (DM) increases with age. Poor glycemic control exacerbates the process of periodontitis. Periodontitis reduces the flowrate of saliva and causes an increase in the production of protein levels. Salivary total Protein (STP) are strongly influenced by type 2 DM. Increased total protein levels are associated with the severity of periodontitis.

The aim of this study was to investigate the association between periodontitis related to type 2 DM and salivary total protein (STP) levels in aged people. The type of research is cross sectional design, with 114 elderly people at least 60 years old who suffered from DM and periodontitis. The diagnosis DM is established by examining HbA1c levels, while periodontitis is based on European Federation of Periodontology (EFP) and The American Association of Periodontology (AAP), The Centers Disease Control (CDC), using clinical parameters namely Clinical Attachment Loss (CAL) dan Pocket Depth (PD).

The salivary total protein (STP) levels was measured using the BCA method. Data analysis used was a linier regression. The influenced factors of STP levels are gender and severity of periodontitis, patients with severe periodontitis have the highest average salivary total protein levels.

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### Introduction

Among the elderly, the risk of periodontitis rises by 44%. Age-related immune system dysregulation, reduced type 1 collagen, and diminished wound healing response all contribute to an increased risk of periodontitis<sup>1</sup>. Periodontitis is one of the most prevalent chronic inflammatory disease in older populations. Age affects the prevalence and severity of chronic periodontitis<sup>2</sup>. At the age of 30, the rate of periodontal tissue damage is practically the same in DM patients and non-DM patients, however at the age of

more than 30 in DM patients, the severity of periodontal tissue damage is greater<sup>3</sup>. Patients with uncontrolled DM who are over 45 had a 2.9 time increased risk of developing severe periodontitis. Diabetes mellitus patients have more severe and accelerated periodontitis<sup>4</sup>. Inflammatory processes can be triggered and accelerated by hyperglycemia<sup>5</sup>. Patients with diabetes mellitus who have uncontrolled blood sugar levels may be more prone to oral infections<sup>6</sup>.

Periodontal disease is one of the most common chronic inflammatory and infectious diseases that can be diagnosed clinically, radiographically, and by several biomarkers<sup>7</sup>. With a stage II–IV diagnosis, tooth loss seems to be inversely correlated with periodontitis severity<sup>8</sup>.

Saliva contains local biomarkers that can assist in the diagnosis of<sup>6</sup> periodontal disease. Elevated salivary total protein levels are

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associated with the severity of periodontal disease and may serve as biomarker in periodontium inflammation<sup>7</sup>. Oral disease, either local or systemic causes changes in the content and composition of saliva. Salivary total protein concentration increased significantly in patients with DM. The use of antidiabetic and antihypertensive drugs affects the salivary flow rate, mucin, amylase and total protein levels in saliva. Salivary flow rate, mucin level, amylase and total protein can be used as biomarkers for the diagnosis and treatment of type 2 DM and hypertension patients with oral disease manifestation<sup>9</sup>. Salivary total protein levels in patients with gingivitis and periodontitis have increased, and are positively correlated with the severity of periodontitis<sup>7</sup>.

**Materials and methods**

Respondents were taken from primary health centers and clinics in Yogyakarta Indonesia, using a purposive sampling technique. Respondents were 114 elderly, aged at least 60 years old, suffering from type 2 DM, still having at least 6 teeth and experiencing periodontitis. The ethical feasibility of the research was approved by the Ethics Committee of The Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. The diagnosis of DM in this study was carried out by CITO laboratorium Yogyakarta.

All subjects received a complete periodontal examination by trained and calibrated 4 general dentists. Interexaminer correlation was 0.76 (p=0.01). The periodontal examination included recording full mouth PD and CAL at six sites of each tooth excluding third molars. Using a manual periodontal probe, PD was determined by measuring the distance between the gingival margin and the base of the gingival sulcus, and CAL was determined by measuring the distance between the cemento-enamel junction and the base of the sulcus.

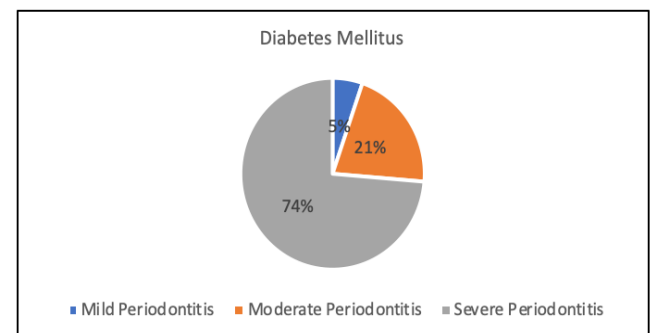
Periodontitis case definitions were established by consensus the EFP, AAP and CDC. This case definition has been widely accepted and used in both clinical and epidemiological studies<sup>10</sup>. Respondents were declared periodontitis if there was interdental CAL 2 in teeth that were not adjacent or there was CAL ≥ 3 mm with PD > 3 mm in ≥ 2 teeth. The severity of periodontitis is defined based on interdental CAL. Interdental CAL 1-2 mm (mild),

2-4 mm (moderate) and CAL ≥ 5 mm (severe).

Fasting participants with DM and periodontitis had their unstimulated entire saliva collected until approximately 2 ml was obtained. The participants were instructed not to brush their teeth, eat, drink, and smoking for 2 hours before saliva collection, and then instructed to spit saliva into a sterile flask after thoroughly cleaning their mouths with filtered water<sup>11</sup>. Following sample collection, the flasks were sealed right away and transported to the Molecular and Medicine therapy laboratory Universitas Muhammadiyah Yogyakarta. While being kept chilled at 4 °C. The salivary total protein examination technique used is BCA method. The advantages of BCA method are it's simple operation and high sensitivity are it's simple operation and high sensitivity not affected by other chemical, high accuracy and linearity. Data analysis used was a linear regression to determine the factors influenced to STP levels.

Variable	Total n (%)	Mean Salivary Total Protein (µg)
Gender		
1. Women	74 (69,4)	1,396
2. Men	40 (35,1)	1,021
Age		
1. 60-65 years old	46 (40,4)	1,289
2. 66-69 years old	18 (15,8)	1,123
3. 70-75 years old	38 (33,3)	1,103
4. 76-79 years old	12 (10,5)	1,892
Smoking		
1. No	108 (94,7)	1,277
2. Yes	6 (5,3)	1,040
DM medication		
1. No	20 (17,5)	1,120
2. Yes	94 (82,5)	1,295
Severity Periodontitis		
1. Mild	6 (5,3)	1,418
2. Moderate	25 (21,9)	1,587
3. Severe	83 (72,8)	1,602

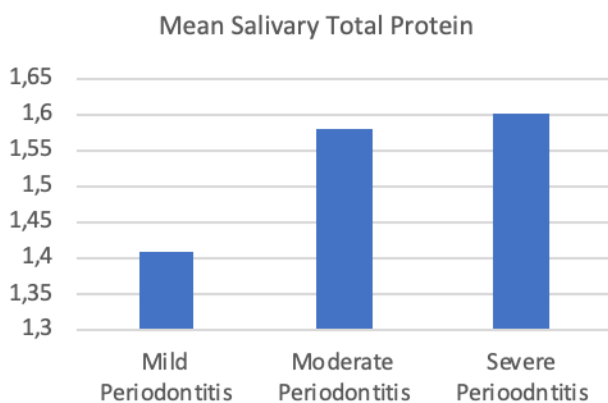
**Table 1.** The Mean Salivary Total Protein Levels.



**Figure 1.** Diabetes Mellitus and The Severity of Periodontitis.

## Results

This study involved 114 elderly aged 60 years old and over, who suffer from DM and experienced periodontitis. Respondents with DM who suffered from mild periodontitis were 6(5%), moderate were 24(21%) and severe were 84(74%) (Fig.1). Women’s mean of STP (1.396) is higher than men (1.021), and the group of participants aged 70 to 79 had the highest mean STP level. The respondents who smooking had smaller mean STP (1.040) than non smooking (1.277), and receiving DM medication had smaller mean STP (1.295) than not receiving medication (1.120) (table 1). The average amount of total salivary protein in cases of mild, moderate, and severe periodontitis was 1.418 g, 1.587 g, and 1.602 g, respectively (Fig.2). Table 2 shows that the factors associated with total salivary protein levels were gender ( $\alpha=0.037$ ) and the severity of periodontitis ( $\alpha=0.013$ ).



**Figure 2.** Mean Salivary Total Protein Levels and Severity of Periodontitis.

Research Variable	Coef. $\beta$	p-value
Gender	- 0,205	0,037*
Aged	0,166	0,077
Smooking	- 0,025	0,806
Medication	0,012	0,128
Severity of Periodontitis	- 0,241	0,013*

**Table 2.** Linier Regression Test Salivary Protein Levels to Research Variable.

## Discussion

Diabetes mellitus and periodontitis have a bidirectional relationship. Advanced glycation end products (AGEs), which are extremely reactive

substances created by hyperglycemia, attach to AGE receptors on the endothelium and damage blood vessels in the periodontal tissue by causing oxidative stress. Inflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- $\alpha$ ) are rising. These inflammatory mediators have a substantial impact on how lipids and carbohydrates are digested, acting as insulin antagonists and increasing blood sugar levels. This worsens hyperglycemia in diabetics<sup>12</sup>. According to Table 1, male respondent’s average salivary protein levels are lower than those of female respondents. The group of participants aged 70 to 79 had the highest mean salivary protein level. The prevalence of periodontitis is highest in the elderly. Prevalence and severity of periodontitis is increase with age<sup>2</sup>. According to earlier studies, age, obesity, and a family history of diabetes mellitus were the main risk factors for hyperglycemia, which affected an estimated 12.4% of older people<sup>13 14</sup>. Respondents who did not smoke and those who used DM medication had higher of salivary total protein levels. With increasing periodontal severity, the mean total salivary protein tended to rise, reaching its greatest level in the group of severe periodontitis.

The aging process is associated with changes in the immune response, which causes an increased susceptibility to infection, however, there is no significant difference in total protein in the gingival sulcus fluid between elderly patients with and without periodontitis<sup>15</sup>. Result of this study showed that the mean total salivary protein tended to increase with increasing periodontal severity, peaking in the group of people with severe periodontitis. Total protein, amylase and glucose in the saliva of DM sufferers tend to be higher in males compared to females<sup>11</sup>. The increase in salivary total protein levels may be due to an inflammatory process that activates the sympathetic nervous system to increase the synthesis and secretion of several proteins thereby increasing the protective potential of saliva against disease. This increase can also be caused by increased leakage of plasma proteins into saliva due to inflammation<sup>16</sup>.

Salivary mucin, amylase and total protein are important biochemical parameters in periodontium inflammation. Salivary total protein concentration increased significantly in DM patients. Total salivary protein levels in DM and

hypertension patients can be used as a biomarker for the diagnosis and treatment of periodontitis<sup>17</sup>. Periodontitis and DM have a very strong and bidirectional relationship. Periodontitis and type 2 DM are risk factors for each other. Routine oral hygiene care and physical examination are necessary for the early prevention of type 2 DM and periodontitis<sup>18</sup>. Salivary total protein and amylase in gingivitis and chronic periodontitis respondents experienced an increase compared to healthy respondents. The salivary glands respond to periodontal disease by increasing the synthesis of several proteins<sup>16</sup>. The results of the meta-analysis stated that diabetes and healthy controls contained levels of IgA, glucose, total protein, and amylase. Measurement based on these salivary factors and how the saliva sample was taken (fasting or not fasting) can help in the diagnosis and monitoring of type 2 DM. Therefore, saliva tests may be an important tool for the diagnosis and early detection of type 2 DM<sup>19</sup>.

In comparison to healthy people, patients with gingivitis had statistically significant higher concentrations of salivary mucin, amylase, and total protein. Patients with chronic periodontitis also had statistically significant higher concentrations of salivary amylase and total protein. Total protein, salivary mucin, and amylase may all be significant biochemical indicators of periodontium inflammation<sup>17</sup>. Those with plaque-induced gingivitis have higher levels of total salivary protein than those with healthy periodontium<sup>15</sup>. Gingivitis and periodontitis patients' salivary total protein levels were 1.6 and 4.2 times greater, respectively, than in healthy individuals. Increased total protein levels may act as a biomarker in the inflammation of periodontal tissues and are related to the severity of periodontal disease<sup>7</sup>. It was also found that the severity of periodontitis in patients with DM associated with total salivary protein levels ( $\alpha=0.013$ ) (**table 2**). A few salivary parameters might be utilized to classify an adult as having diabetes. This variation in salivary composition thus indicates the use of saliva as an alternative fluid to monitor patients with diabetes mellitus, which is important given the growth in diabetes and the possibility to develop new forms of diagnosis to develop prevention methods<sup>20</sup>. The current study shows a significant association between salivary total protein levels and several

clinical markers of gingivitis and PPD in periodontitis. Salivary protein levels can also serve as a reliable predictor of gingival inflammation, periodontal pocket formation, and the severity of periodontal disease. Even if the saliva-based diagnostic method needs to be improved, it has a lot of potential for usage in the future when it comes to identifying periodontal diseases and gauging how well periodontal treatment will work<sup>7</sup>.

## Conclusions

Periodontitis and gender are significantly correlated with total protein level in saliva. With the progression of periodontitis, the mean total protein level in the saliva increased. The individuals who had severe periodontitis had the greatest average total protein in their saliva. Further research on saliva as a biomarker for early diagnosis of periodontitis linked with diabetes mellitus is necessary because it is a biomarker for many diseases.

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## Declaration of Interest

The authors report no conflict of interest.

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