Med & Health Dec 2023; 18(2): 373-385 https://doi.org/10.17576/MH.2023.1802.04

ORIGINAL ARTICLE

Salivary Protein Quantification among Periodontitis Patients with Different Disease Severity: A Pilot Study

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Received: 27 December 2022 / Accepted: 24 July 2023

ABSTRAK

Kuantifikasi protein air liur dalam mencerminkan patofisiologi periodontal di kalangan pesakit dengan keparahan periodontitis yang berbeza masih kurang diketahui. Kajian ini adalah penting untuk mengenal pasti perubahan kuantiti protein dalam keparahan periodontitis yang berbeza dan seterusnya mencerminkan perubahan kompleks periodontal. Kajian awal ini bertujuan untuk mengukur dan membandingkan protein air liur melalui pengumpulan air liur yang tidak dirangsang daripada pesakit periodontitis dan individu sihat. Protokol asai radioimunopresipitasi (RIPA) digunakan untuk mengekstrak protein daripada air liur pesakit dan pengukuran dilakukan menggunakan Nanodrop Spectrophotometer 2000/2000c (A280) yang ditetapkan pada panjang gelombang 280 nm. Kepekatan protein air liur ditunjukkan dalam kumpulan sihat adalah 26.48 ± 2.95 mg/mL, keparahan 1 periodontitis adalah 22.00 ± 0.38 mg/mL, peringkat 2 periodontitis adalah 27.34 ± 1.61 mg/mL, keparahan 3 periodontitis adalah 27.11 ± 0.66 mg/mL dan keparahan 4 periodontitis adalah 24.89 ± 1.91 mg/mL. Walau bagaimanapun, perbezaan min antara semua kumpulan adalah tidak signifikan secara statistik. Tertakluk kepada limitasi kajian ini, tiada perbezaan dalam kuantiti protein air liur di kalangan pesakit dengan peringkat periodontitis yang berbeza.

Kata kunci: *Air liur; kuantifikasi; nanodrop; penyerapan; periodontitis; protein*

ABSTRACT

The salivary protein quantification reflecting the periodontal pathophysiology

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among patients with different periodontitis severity has not been well explored. This quantification is fundamental as the changes in the quantity of proteins in different severities of periodontitis can reflect the periodontal complex alterations. This pilot study aimed to quantify and compare the salivary proteins by collecting unstimulated saliva from periodontitis patients and healthy individuals. The radioimmunoprecipitation assay (RIPA) protocol was used to extract protein from patients' saliva and quantified using the Nanodrop Spectrophotometer 2000/2000c (A280) set at 280 nm wavelength. The mean concentration of salivary proteins was shown to be 26.48 ± 2.95 mg/mL in healthy patients, 22.00 ± 0.38 mg/mL in Stage 1 periodontitis patients, 27.34 ± 1.61 mg/mL in Stage 2 periodontitis patients, 27.11 ± 0.66 mg/mL in Stage 3 periodontitis patients and 24.89 ± 1.91 mg/mL in Stage 4 periodontitis patients. However, the mean difference between all groups was not statistically significant. Within the limitations of this study, there was no difference in the salivary protein quantity among patients with different stages of periodontitis.

Keywords: Absorbance; nanodrop; periodontitis; protein; quantification; saliva

INTRODUCTION

Periodontal disease is characterised by the destruction of the hard and soft connective tissue components of the periodontium in the oral cavity (Tsuchida et al. 2013). Over the last 30 years, periodontitis classification has constantly changed to reflect new and evolving scientific knowledge. Based on the new 2017 workshop, staging and grading of periodontitis have been introduced. Staging comprises the disease severity at presentation and the complexity of disease management that involve four categories (Stages 1 to 4). It is determined by the degree of clinical attachment loss, the amount and percentage of bone loss, the depth of the probing, the presence and extent of angular bony defects, tooth mobility and tooth loss as a result of periodontitis. Meanwhile, grading offers additional details on the biological characteristics of the disease, including a history-based analysis of the disease progression rate, risk assessment for further disease progression, anticipated poor treatment outcomes, and risk assessment that the disease may have an impact on the general health of the individual succumbed to the disease. Grading consists of three levels: Grade A, which is minimal risk; Grade B, which is moderate risk; and Grade C, which is high risk for progression. Grading comprises general health status and other exposures such as smoking, or the degree of metabolic control in diabetes.

From the Malaysia's National Oral Health Survey for Adults in 2010, almost half (48.5%) of the participants had periodontitis (Mohd Dom et al. 2016). There are well-established

connections between periodontitis and non-communicable diseases, including diabetes and cardiovascular diseases. With the increasing number of periodontitis cases, analysing the quantity and concentration of proteins in periodontitis can provide an objective method for the early detection, prevention, monitoring and treatment of the disease (Koregol et al. 2015).

Human saliva consists of fluids and particles from a variety of oral and respiratory derivatives, and furthermore can be easily collected. Saliva secretion from the major salivary glands such as the parotid, submandibular, and sublingual, secrete a significant amount of proteins and peptides that are essential for preserving the health of the oral cavity. Saliva is sometimes referred to as the body's mirror since it can be utilised to assess both overall health and the emergence of specific diseases. A previous study by Gabelle et al. (2017) showed that plasma biomarkers can be quantified in the saliva of periodontitis patients. Therefore, there is a continuing interest in the use of saliva for protein analysis and clinical applications, particularly for early detection of periodontal disease, disease progression, and therapeutic monitoring.

Alterations in the genes, proteins or cellular pathways can be identified by genomics and proteomics approaches which can concurrently access and study a large number of potential markers. Several methods have been developed in recent years for the quantification of proteins namely, western blotting, liquid chromatography, native and denaturing one-dimensional (1-D) and two-dimensional (2-D) gel electrophoresis, and absorbance colorimetry. A quantitative proteomic workflow utilising mass spectrometry (MS) was recently developed for the screening of biomarkers in periodontitis (Rizal et al. 2020). Furthermore, a quantitative methodology based on multiple-reaction monitoring (MRM) mass spectrometry (MS) has also been developed for the screening of plasma biomarkers in saliva (Gabelle et al. 2017).

With the current studies available, there is a lack of knowledge or insufficient information on the protein quantification based on the severity of periodontitis according to the staging of the disease. Despite numerous studies had been conducted in regard to the techniques of protein quantification (Silva-Boghossian et al. 2013; Bostanci et al. 2018), studies on the quantification of salivary protein in different stages of periodontitis is very limited and is yet to be researched on. Hence, this study aimed to analyse the feasability of protein quantification of saliva in different stages of periodontitis and in comparison to healthy control groups as well as to test the research protocols for future studies.

MATERIALS AND METHODS

Ethics Statement

An investigatory study was conducted involving clinical examination and saliva collection from patients attending the Periodontics Clinic in the Faculty

of Dentistry, Universiti Kebangsaan Malaysia (UKM). The ethics approval was obtained from the UKM Medical Research and Innovation Secretariat and our study (registered as UKM PPI/111/8/JEP-2021-828). The risks and benefits of participating in this study were explained to all patients prior to signing the informed consent form.

Patient Recruitment and Data Collection

Patient was selected according to the inclusion and exclusion criteria. Criteria for selecting the subjects were patients aged at least 18 years old (≥18 years old) (Hartenbach et al. 2020) and patients must have at least 16 natural teeth. The exclusion criteria for patient selection were periodontitis patients who are diagnosed with inflammatory systemic diseases, autoimmune diseases, patients who are pregnant and nursing, tobacco smokers and taking anti-inflammatory or antibiotics within 6 months (Bostanci et al. 2018). Patients that met the inclusion criteria were recruited and written informed consent was acquired. Sample size calculation was carried out using G*power 3.1.9.4 software with power= 0.8, $\alpha = 0.05$, f=0.4 with 5 groups of subjects. The sample size calculated was 80. The sample size for the pilot study was estimated at 12-14 subjects which was 15% of the calculated sample size.

Clinical Examination

A medical history review and periodontal tissue examination were carried out to assess the patient's periodontal health. According to the periodontal and implant disease classification scheme (i.e., healthy periodontium or periodontitis with grading and staging), patients were classified into five groups based on their periodontal health conditions, as indicated in Table 1 (Caton et al. 2018; Tonetti et al. 2018).

The clinical periodontal indices including full mouth periodontal probing pocket depth (PPD), clinical attachment loss (CAL), plaque index (PI), and gingival index (GI) by calibrated clinicians were obtained from the patient's case notes. Using a periodontal probe (Williams or UNC-15 probe), PPD was assessed at six locations per tooth (mesial-buccal, mid-buccal, distal-buccal, mesial-lingual, mid-lingual, and distal-lingual). Subsequently, all locations were then monitored for bleeding on probing (BoP) (Ebersole et al. 2015). The CAL was also determined at all six locations per tooth by adding the measurements of gingival recession and PPD at each site.

The percentage of sites affected with BoP and PPD were calculated by taking the number of affected sites divided by the total number of sites presented in the patient mouth. BoP classified healthy patients as being equal or less than 10% of sites (6 sites per tooth), less than 30% of sites with PPD equal or more than 4 mm, and no sites with clinical attachment loss (CAL) equal or more than 2 mm. For each patient, radiographs were used to assess the degree and severity of the alveolar bone support (Bostanci et al.

TABLE 1: Periodontitis stage

Periodontal status	Description
Healthy	No loss of clinical attachment level and no loss of bone in the radiograph
Stage I	Loss of 1-2 mm clinical attachment level and bone loss at the coronal third (15%) in the radiograph
Stage II	Loss of 3-4 mm clinical attachment level and bone loss at the coronal third (15-33%) in the radiograph
Stage III	Loss of 5 mm clinical attachment level and bone loss more than the coronal third in the radiograph
Stage Iv	Loss of 1-2 mm clinical attachment level, bone loss more than coronal third in the radiograph and 5 teeth missing due to periodontal disease

2018).

Saliva Collection and Processing

Patients were instructed to rinse their mouths with water to remove food particles before their saliva was collected. This was done approximately 5-10 minutes later to prevent sample dilution. Whole saliva was collected by passive drool method without the use of oral stimulants (unstimulated) to avoid the possibility of alternatives in saliva composition. Patients were asked to spit 5 mL of saliva sample into a cup and then the sample was transferred to a labelled 15 mL tube, centrifuged at 8000 rpm for 10 minutes at 4˚C to remove any food detritus. The sample was stored at -20˚C in a cryovial storage box in a proteomic laboratory (2010 Thermo Fisher Scientific Inc).

Protein Extraction

The radioimmunoprecipitation assay (RIPA) buffer protocol was used to process and analyse the saliva sample. To prepare the buffer, four cryovial tubes of 2 ml RIPA Buffer with Protein Inhibitor Cocktail, without SDS (x10) and SDS Solution (1% SDS). Firstly, RIPA Buffer (x10) and SDS Solution were thawed completely at 25˚C and mixed vigorously using a vortex machine (Ingenieurburo CAT, M. Zipperer Germany). The final dilution with 300 uL RIPA buffer and 300 uL SDS solution was relocated into solution into each 15 different test tubes. The stored saliva samples at 20˚C were thawed and 1000 g of each sample was mixed in each test tube with the diluted form of RIPA Buffer, respectively. The centrifuge tubes were then sealed tightly with paraffin firm and the samples were homogenised and incubated for 30 minutes to 1 hour. The lysate was then transferred to a new centrifuge tube and centrifuged at 10000 RFC for 10 minutes at 4˚C using centrifuge 5804R (Eppendorf AG Germany). After centrifugation, the supernatant with total protein extracts was transferred to a new tube for protein analysis. This method was according to the user guide RIPA extraction buffer Thermo Fisher Scientific.

Protein Quantification

The NanoDrop Spectrophotometer 2000/2000c (Protein A280) was used for protein quantification. The salivary protein quantity and concentration in periodontitis and healthy periodontium were analysed accordingly (Hughes & Chapleau 2019). The software on a PC set up for NanoDrop Spectrophotometer 2000 was set for sampling protein at absorbance of A280. Initial cleaning of both the upper and lower measurement surfaces of NanoDrop Spectrophotometer 2000 using distilled water (dH_20) was done prior to blank measurement. Pipettors (0-2 µL) with low retention were used throughout this process.

About 2 µL of dH_20 was placed on the lower measurement pedestal and the water was then wiped from both pedestal surfaces. After the initial cleaning, the sampling technique was done by using calibrated pipettes (0-2 µL) with well-fitting tips that were repetitively changed for each sample. Approximately 2 µL of the sample was measured using a pipette and placed on the lower measurement pedestal. Then, the sampling arm was lowered until a 'click' sound was heard and a spectral measurement was started with the software on the PC. The sample was removed from both pedestal surfaces directly after the measurement was completed. Each sample was measured 3 times and the average mean value was determined for each sample. Reconditioning was done after every 10 measurements. Reconditioning was done by pipetting a 2 µL dH_20 onto a lower measurement pedestal and lowering the sampling arm. The dH_20 was then removed after 30 seconds by rubbing both pedestal surfaces until no additional dark chemical residue was visible on the lab wipe.

Beer-Lambert Law

According to the Lambert-Beer law, the protein concentration in the saliva sample was detected by the absorbance measurements. In contrast, the absorbance (A) depends on the concentrations of the solution since it is affected by the intensity of the light before (Io) and after passing through the solution (I). Since mol/L is the unit used to indicate sample solution concentration, molar absorptivity was represented as L/mol· cm.

The Beer's law equation:

$$A = \log10 (Io/I) = \varepsilon * l * c$$

Where:
ε - Molar absorption coefficient or the molar absorptivity
l - Path length of the beam in the sample; and
c - Concentration of the solution

Statistical Analysis

Data analysis and differences between the stages of periodontitis and the control group were analysed using IBM SPSS statistics software version 26.0 (SPSS Inc., Chicago, IL, USA) and analysis of variance (ANOVA) was used for the numerical variables namely concentration and the absorbance of the salivary proteins.

RESULTS

Demographic Characteristics

A total of 14 dental patients were recruited in this study. Table 2 presented the demographic data of the patients engaged in this study. The age of the patients ranged from 24 to 77 years, and patients were categorised into two age groups, namely, age below 40 years (<40) to represent young-to-middle-aged adults and age 40 years and above (≥40) which represented middle-to-older-aged group. The mean age was 41.14 years. The majority of the patients were from the age group below 40 years (64.29%) and were dominated by females (57.14%) and Malay race (57.14%).

Clinical Characteristics of the Study Population

Clinical examination that included plaque score, gingival score of full dentitions, percentage of periodontal probing depth of the sites equal or more than 4 mm, and the mean of clinical attachment loss of sites was taken. Of the 14 patients, three were considered to be periodontally healthy, two were in Stage I periodontitis, three were in Stage II periodontitis, three were in Stage III periodontitis and three were in Stage IV periodontitis (Table 3). There was no significant difference in the gingival score and mean clinical attachment loss between the healthy control group and the stages of the periodontitis group. The mean plaque score was highest in Stage I periodontitis (mean=63.59 ± 23.75) and the mean gingival score was highest in Stage I periodontitis (mean=51.42 ± 25.77). The mean percentage of periodontal probing depth 4 mm (mean=32.47 ± 26.30) and the mean clinical attachment loss (mean=7.87 ± 1.66) was highest in Stage IV periodontitis. However, there were no statistically significant differences between the healthy groups and periodontitis stages and plaque score (P=0.224), the percentage of periodontal probing depth sites (P=0.129), and systemic

TABLE 2: Demographic details of patients (N=14)

Demographic	n	%
Age group		
<40 years old	9	64.29
≥40 years old	5	35.71
Gender		
Male	6	42.86
Female	8	57.14
Race		
Malay	8	57.14
Chinese	2	14.29
Indian	4	28.57
Household income		
B40 (<RM 4,849)	12	85.71
M40 (RM 4,850 - RM 10,959)	2	14.29
T20 (>RM 10,959)	0	0
Education level		
Secondary	4	14.29
Tertiary	10	85.71
Systemic Comorbidities		
Healthy	8	57.14
Diabetes Mellitus	2	14.28
Diabetes Mellitus & Hypertension	2	14.2
Hypotension	1	7.15
Hyperlipidemia	1	7.15

comorbidities (P=0.338).

Figures 1 and 2 demonstrated the mean concentration of salivary protein (mg/mL), and the salivary protein absorbance (280 nm) among periodontitis patients (N=14). There were no significant difference among the healthy control group and four different severity of periodontitis with concentration of protein, F(4,9)=3.28, p<0.06, n2p=0.59, and with quality of light absorbed by solution, F(4,9)=3.28, p<0.06, n2p=0.5. Post hoc testing revealed no significant difference between the healthy control group and four differetce severity of periodontitis with concentration of protein, healthy control group (mean=26.48 ± 2.95), Stage 2 (mean=27.34 ± 1.61), Stage 3 (mean=27.11 ± 0.66), Stage 4 (mean=24.89 ± 1.91) having a higher concentration of protein than Stage 1 (mean=22.00 ± 0.38). The results were not statistically significant due to the *P*-value for that effect was higher than 0.05.

DISCUSSION

The purpose of this pilot study is to analyse the demographics of patients with periodontitis, to determine salivary protein absorbance at 280 nm and to examine the concentration of salivary protein in periodontitis patients. Of the 14 patients included in the current study, 11 (78.6%) had periodontitis, with two patients in Stage I, three in Stage II, three in Stage III, and three in Stage IV. The majority of patients were female (57.1%). The participant distribution among age groups was 24-77 years. Out of the five parameters, only the age factor was determined to be a statistically significant factor of periodontitis (P= 0.043). According to Bhadbhade (2015), the prevalence and severity of periodontal disease tends to increase as patients age. This could be due to the negligence of treatment and oral hygiene practice which can result in disease progression (Bansal et al. 2015). In this study, the mean age for Stage III was the highest compared to the rest of the severity of periodontitis.

Males (54.54%) had a higher frequency of periodontitis compared to females (45.45%). This can be due to a difference in the immune-regulatory dimension as well as the socio-behavioural differences between different genders (Ioannidou

TABLE 3: Clinical characteristics of patients

Characteristics	Healthy n=3	Periodontitis				P-value
		Stage I, n=2	Stage II, n=3	Stage III n=3	Stage IV n=3	
Full mouth plaque score (%)	32.80 ± 10.62	63.59 ± 23.75	39.03 ± 7.18	54.80 ± 19.23	54.93 ± 16.76	>0.05
Full mouth gingival score (%)	9.40 ± 6.11	51.42 ± 25.77	35.43 ± 5.14	32.32 ± 5.63	46.35 ± 4.86	>0.05
% Probing depth sites ≥4mm	0	10.90 ± 9.48	12.20 ± 3.50	11.50 ± 5.85	32.47 ± 26.30	>0.05
Mean CAL (mm)	0	5.12 ± 0.02	6.09 ± 0.58	6.01 ± 1.23	7.87 ± 1.66	> 0.05
Systemic comorbidities	0	0	2.33 ± 2.52	2.00 ± 2.65	2.67 ± 2.31	>0.05

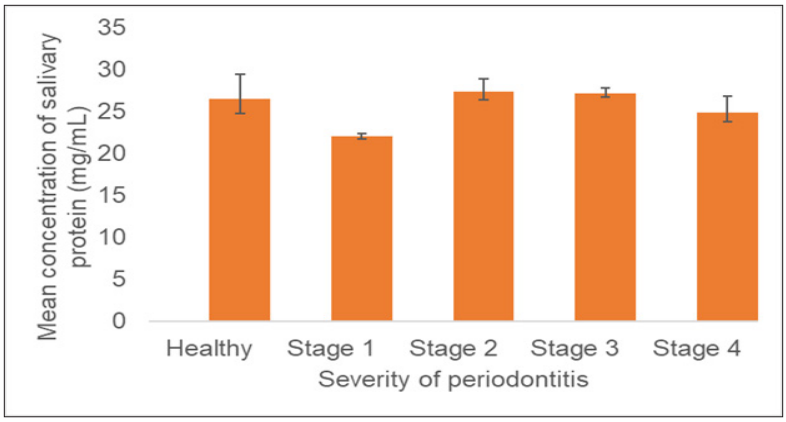

FIGURE 1: Mean concentration of salivary protein (mg/mL) among periodontitis patients (N=14)

2017). The term gender relates to social roles, behaviours, attitudes and gender predisposition can also depend on hormones, genetics, behaviour, and stress. In our study, males had a higher prevalence of periodontitis although the female participants were higher in number. This result coincides with similar results reported in the literature which indicated that males have a higher risk of developing periodontitis (Natto et al. 2019).

Among the three races, Malays were documented to have a higher prevalence of periodontitis (54.54%) compared to Chinese (18.18%) and Indians (27.27%). The largest groups of Malaysians consist of three main races which are the Malays, Chinese, and Indians. The Bumiputera composition is 69.8%, Chinese composition is 22.4% while Indian is 6.8% as of statistic in 2021 (Department of Statistics Malaysia 2021). In conjunction with

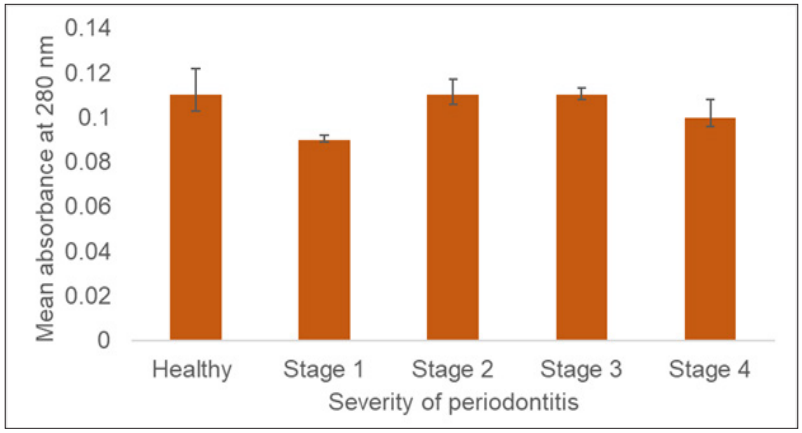

FIGURE 2: Mean absorbance at 280 nm for salivary proteins among periodontitis patients

the racial composition ratio among the three major races, there are higher numbers of Malays in Malaysia which also reflects in the sample size in this study. Cultural differences and genetics play roles in the differences of the prevalence.

Based on a study by Steele et al. (2015), socioeconomic factors such as income and educational level influence an individual's oral health. An individual's income may influence the individual's ability to access oral health care services and this can significantly affect their oral health. There is a correlation between the educational level of the adult population and the level of oral health knowledge (Márquez-Arrico et al. 2019). In this study, the highest mean income was in Stage IV periodontitis and the highest mean education level was in Stage I periodontitis.

The clinical findings reported were based on subjects prior to periodontal therapy and were used to classify the subjects into respective severity of periodontitis. Staging is the process of classifying the severity of a patient's disease. The primary determinant is the mean CAL of each periodontitis group. The classification of the severity of periodontitis provides a framework for the introduction of biomarkers in the diagnosis and prognosis of the disease. Based on the results obtained, the mean gingival score and the mean clinical attachment loss had no significant differences among the healthy group and the severity of periodontitis. For the periodontal probing depth, Stage IV periodontitis had the highest mean of PPD (in terms of percentage of sites). Furthermore, an association with plaque score or poor oral hygiene are said to increase the risk of periodontitis by two- to five-fold. In this study, the mean of plaque score was the highest in Stage I periodontitis, followed by Stage IV, Stage III, and Stage II. A study by Nazir (2017) revealed that with poor oral hygiene and increased plaque accumulation, the severity of periodontitis increases as well. Our results were slightly inconsistent due to the imbalance sample size in each group.

Numerous studies have demonstrated the association between periodontitis and comorbidities such as type 2 diabetes mellitus (DM), cardiovascular disease, hypertension (HTN), and hyperlipidemia (Holmstrup et al. 2017). In our study, the mean of clinical attachment loss sites was higher in patients with DM (M=74.50 $\pm$ 39.89), followed by hypotension (M=30.0), DM+HTN (M=28.00 $\pm$ 2.73), and lastly hyperlipidemia (M= 17.00). A study has been conducted by Sperr et al. (2018), which evaluated 1199 individuals with periodontitis, and the majority of the cases were associated with comorbidities including hypertension, hyperlipidemia and allergies. In this study, 42.86% of the periodontitis patients had one or more comorbidities.

The RIPA Buffer protocol was used in this study for protein quantification. This method involves the separation of cellular components without ultracentrifugation into the cytoplasm, organelles, intermediate filaments, and nucleus (Rizal et al. 2020). RIPA Buffer is an optimal buffer for lysing cultured

mammalian cells without causing proteolysis or interfering with biological or immunological processes. RIPA Buffer comes with an SDS solution but is not premixed, which is ideal for immunoprecipitation. In contrast, the presence of SDS can negatively affect the antigen-antibody reaction and protein extraction, which is prevented by the presence of the preservative used in RIPA Buffer. In this study, RIPA buffer was used to lyse and extract protein from the saliva sample. It is used when carrying out the western blot technique or in immunoprecipitation assay.

NanoDrop Spectrophotometer 2000/2000c (Protein A280) was used to investigate the whole-saliva protein concentrations from periodontitis and healthy individuals. The present study was designed to compare the salivary protein quantity and concentration in periodontitis and healthy periodontium. This variability may or may not reveal a significant difference in protein absorbance at 280 nm (A280) and calculated the protein concentration (mg/mL) at different severity levels of periodontitis compared to healthy controls. The absorbance at 280 nm is primarily due to the presence of aromatic chains on the amino acids Tryptophan and Tyrosine. The usage of Protein A280 is fast and simple that requires only a small sample with no reagents or standard curves.

The total saliva sample was analysed and found that the concentration of protein in all the different severity and healthy groups ranged from 24-27 mg/mL whereas only stage 1 showed a reading of 22 mg/mL. According to previous studies, the concentration of protein should increase as the severity of periodontal disease increases. However, in this study, the hypothesis failed to achieve. This could be due to several factors like, patients might have underlying systemic diseases such as DM which will indirectly increase the amount of protein in the saliva samples (Hartenbach et al. 2020). The other reason can be due to the method of saliva collection, where the patient was forced to spit the saliva which caused the process to occur under sympathetic stimulation. There are few studies reported that more protein can be found in saliva if the sample is taken by sympathetic stimulation compared to parasympathetic stimulation. According to the Thermo Scientific NanoDrop Spectrophotometers guideline, the quality of light absorbance at 280 nm should be less as the severity of the periodontitis disease increases. However, in this study the healthy control group, stage 2, and stage 3 showed an average reading of 0.11 but, the other two severity showed lesser readings, stage 1 showed 0.09 and stage 4 showed 0.10. This error can be due to many factors such as sample heterogeneity, dirty pedestal, and multiple measurements done without cleaning and reconditioning the pedestal surfaces prior to starting the new measurement session.

In this pilot study, we only analysed the acceptable protein concentration in the saliva of subjects diagnosed with different severity of periodontitis before the proteomic analysis of the saliva. This study was based on protein

quantification of saliva using RIPA Buffer protocol whereas other studies were mainly focused on the comparison of salivary protein concentration of non-periodontal disease and periodontal disease patients. An average of 1 to 100 mg of protein can be collected and processed for proteomic analyses depending on the sample type, cell density and extraction techniques (Feist & Hummon 2015). It is recommended that further studies should be implemented to identify specific proteins and compare the different severity of periodontitis using different methods such as chromatography-based techniques, western blotting, and enzyme-linked immunosorbent assay (Aslam et al. 2017).

The main limitation of this study was its exploratory nature. This limited the ability to draw firm conclusions on the salivary protein concentration among periodontitis patients with different severity and those with healthy periodontium. The descriptive analysis can however be used as a guide in future studies and as a reference for specific protein markers in periodontitis and other comorbidity diseases. The study protocols were also deemed feasible for future large-scale studies investigating salivary protein concentration among periodontitis patients.

CONCLUSION

The range of salivary protein concentrations in patients with periodontitis was 22.00-24.89 mg/mL. Within the limitations of this study, there was no difference in the salivary protein quantity among patients with different stages of periodontitis. This suggested that analysis of salivary proteins in periodontitis patients may offer more insights into a better understanding of the disease activities, more detailed analysis of specific protein markers as well as its relationship with other clinical parameters were warranted.

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