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Effect of Non-Surgical Periodontal Therapy on Salivary Albumin Concentration in Patients with Stage II Localized Periodontitis

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ABSTRACT

Objective: To evaluate the effect of non-surgical periodontal therapy on salivary albumin concentration in patients with stage II localized periodontitis.

Methods: Saliva samples and periodontal indices were collected from 40 individuals: 20 with stage II localized periodontitis and 20 healthy control subjects at baseline; then at 1- and 3-months following non-surgical periodontal therapy of those with stage II localized periodontitis. The saliva specimens were analyzed for albumin using the bromocresol green method. All data were analyzed using the statistical package for social sciences, version 23.0(SPSS 23.0). Independent t- test, Pearson's correlation test, and Repeated Measures of ANOVA were used for statistical analysis at a significance level of 0.05 (5 %).

Results: From baseline, through 1- to 3-months post therapy, the mean (SD) salivary albumin concentration of the test subjects reduced from 0.70 (0.13) g/dl to 0.58 (0.14) g/dl and 0.35 (0.09) g/dl respectively, while that of control subjects remained relatively stable: 0.27 (0.03) g/dl, 0.25 (0.04) g/dl and 0.24 (0.04) g/dl respectively. There was a statistically significant difference in the mean concentration of salivary albumin between the test and control groups at baseline, 1- and 3-months post therapy ($p<0.001$). Also, the reduction in mean salivary albumin concentration occurred alongside improving periodontal parameters.

Conclusion: This study showed that there was a reduction in salivary albumin concentration following non-surgical periodontal therapy among patients with stage II localized periodontitis.

Keywords: Non-Surgical Periodontal Therapy, Salivary albumin, Periodontitis

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CLINICAL IMPLICATIONS

Salivary albumin concentration could serve as a biomarker to monitor the healing of periodontal tissues after non-surgical periodontal treatment. Such a biomarker could provide the periodontist with an indirect method of assessing the healing of the periodontal tissues after non-surgical periodontal treatment. Albumin kits are readily available and affordable, and saliva collection as specimen is non-invasive. Also, affordable self-monitoring kits, which patients can use at home can be made.

INTRODUCTION

Periodontitis is a destructive inflammatory condition of the structures that support the teeth.[1] A related condition - gingivitis - is an inflammatory condition of the gingiva, without loss of attachment i.e. no periodontal pocket formed[1]. Periodontitis results in loss of connective tissue attachment, with alveolar bone destruction, leading to mobility of teeth, pain, impaired mastication and ultimately, tooth loss.[1] It is considered a multifactorial disease, caused by disruption of normal homeostatic processes by numerous bacterial species found in subgingival dental plaque.[2] Its progression may become more aggressive in the presence of systemic or environmental factors that alter the host response to plaque bacteria. These factors include systemic conditions such as uncontrolled diabetes mellitus; habits like smoking; stress; age and genetic susceptibility.[1,3]

The prevalence of periodontitis is reportedly in the range of 5 - 15% amongst adults, though it varies globally.[4] Among Nigerians, a study of the prevalence of periodontal diseases and associated risk factors in children, showed a gingivitis prevalence of 82.9%, though none had periodontitis.[5] Another study among Nigerian pregnant women, demonstrated that 48.4% of these women required complex periodontal treatment during pregnancy.[6] Among the Nigerian elderly population, 94.8% were reported to have one stage of periodontal disease or another.[7]

The management of periodontitis is usually done in phases, according to the presentation.[8] The phases include: a preliminary/emergency phase, an aetio-tropic phase/initial periodontal therapy (also known as non-surgical periodontal therapy), other adjunctive dental treatments, a maintenance or supportive periodontal therapy, and possible surgical therapy.[8] Each phase is followed by a re-evaluation in which decision for the next step of treatment is taken. After the initial periodontal therapy, it is important to effectively assess the healing progress of the tissues, in order to establish the grade of the individual case[9] and also decide whether there is a need for advanced treatment, e.g. periodontal surgery.

However, current objective methods for re-evaluation between phases of periodontal treatment are based on direct intra-oral examination and measurements around the teeth.[1] Some of these methods, (e.g. periodontal probing) may disturb the delicate healing tissues, if not carefully done. They may not even reveal detectable changes like clinical re-attachment or radiographic evidence of bone re-deposition till about 3 months post therapy. Also, though these methods can show the level of plaque biofilm deposit, they cannot show whether or not the deposits are actively causing disease or tissue response; thus, they cannot predict disease susceptibility.[10] There is therefore a need for other methods, which may be offered by biomarkers[11] such as salivary albumin.[12 13 14]

Salivary albumin is a protein from the serum known as serum albumin: a 65–70 kDa globular, water-soluble protein produced in the liver.[15 16] In health, salivary albumin contributes to the normal homeostatic antioxidant properties of saliva, with mean (SD) concentration of 0.08(0.05)g/dl.[17] Under inflammatory conditions like periodontitis, there is significant decrease in the concentration of other salivary antioxidants.[18 19] However, there is a corresponding increase in the concentration of salivary albumin in these patients[20] with a mean (SD) concentration of about 0.67(0.50)g/dl.[17] The diagnostic sensitivity and specificity of salivary albumin as a biomarker in periodontal disease amongst adults has been estimated at 68 and 80, respectively.[21] Propositions for how salivary albumin increases in periodontitis include passive infiltration/leakage from gingival sulcus,[14 22 23 24 25 26 27 28] by active secretion from the salivary glands as a salivary antioxidant,[18 19 20 29] and as a response to the presence of *Treponema denticola*.[30]

Serum albumin is regarded as a marker for plasma protein leakage which occurs as a consequence of inflammatory processes.[23 24] In periodontitis, there is inflammation, ulceration and detachment of the junctional epithelium.[23] Thus if the proposition about sulcular leakage[14 22 23 24 25 26 27 28] is true, it may be inferred that after an effective treatment, as the

inflammation subsides, and the ulceration heals, albumin leakage should reduce.[12] Similarly, as the oxidative inflammatory stress associated with periodontitis subsides, the active secretion of albumin as an antioxidant from salivary glands should reduce. The concentration of albumin in gingival crevicular fluid was found to decrease significantly in people with diabetes, after non-surgical periodontal therapy.[12] The decrease in the concentration of albumin in these patients was found alongside improved periodontal outcomes. This finding suggests that as the periodontal tissues healed and reattached to the teeth, the crevicular leakage of albumin decreased accordingly. Also, as the inflammatory condition subsided, the other salivary antioxidants returned to normal levels, so the need for compensatory function of salivary albumin decreased. Since the GCF drains into the saliva, it is possible that the albumin changes could also be detected in the saliva. Therefore, following non-surgical periodontal therapy, if the level of salivary albumin increases with time, or fails to decrease over time, it could suggest that the disease activity is not being controlled. It could also signal that the factors making the patient susceptible to the disease are overwhelming the healing process. The purpose of this study was to assess the effect of non-surgical periodontal treatment on the salivary albumin levels of patients with periodontitis, who do not have diabetes.

MATERIALS AND METHODS

Study Design and Scope: This was a quasi-experimental clinical intervention study to assess the effect of non-surgical periodontal therapy on salivary albumin concentration, in patients with stage II localized periodontitis over a period of 3 months

Study Location: The Periodontics Clinic, Dental Centre, University College Hospital, Ibadan, Nigeria. The laboratory analysis of salivary albumin was performed at the hospital's chemical pathology laboratory, which is about 3 minutes' drive from the clinic.

Study Population: a. The test group – Adult individuals (18 years and above) who attended the Periodontology Clinic, Dental Centre, University College Hospital, Ibadan, Nigeria.

b. A control group of healthy volunteers among members of staff and other individuals living or working in the University College Hospital, Ibadan.

Ethical approval: This was obtained from the University of Ibadan/University College Hospital Ethical Review Committee and the West African College of Surgeons.

Sample Size Calculation: This was calculated using the formula for clinical interventional studies when endpoint is quantitative data:[31] i.e. $N = 2SD^2(Z_{A/2} + Z_B)^2 / d^2$

Standard deviation and difference of mean values from a previous study was used.

Data Collection:

a. Saliva Collection: The participants were instructed not to eat, drink, perform any kind of oral hygiene procedures, or use any scented cosmetics before saliva collection. Between 8 a.m. and 10 a.m; unstimulated saliva was collected from the test group in 3 phases: just before periodontal treatment, 1 month after the treatment, and 3 months after treatment. Also, saliva was collected from the control group at baseline, 1 month, and 3 months later. A cup of water was given to the participant to rinse out. They were then asked to remain in a relaxed position for 5 minutes without swallowing, while saliva collects in the mouth. The saliva was then drooled into the labeled 20ml sterile plastic bottle, and it was transported in an ice cooled container to the laboratory. At the laboratory, the saliva was centrifuged at 10000rpm and room temperature for 15 minutes.[17] The supernatant was then separated from the pellet, stored under -20°C,[32] and analyzed for albumin within 1 week.

Laboratory Saliva analysis:

The frozen supernatant samples were thawed and both samples and reagents were brought to room temperature. Albumin level in the supernatant was measured using an albumin estimation kit (Dialab® Albumin BCG Single Reagent.

Albumin Estimation Procedure: At room temperature, 2000microlitres of reagent was added to 20 microlitres each, of sample and standard, mixed and left to incubate for approximately 10 minutes. The absorbance of sample and standard were measured against the reagent blank within 60 minutes at 598 nm using a spectrophotometer.

The following formula was used to calculate the concentration of albumin:

CALCULATION: Albumin [g/dl] = Absorbance of sample / Absorbance of standard x Concentration of standard

Assessment of Periodontal Parameters: All clinical periodontal measurements were recorded at

baseline for both groups; they were then repeated, at 1-month and 3-months. Oral hygiene status was assessed using the simplified Oral Hygiene Index of Greene and Vermillion (1964),[33] while the gingiva was assessed using the Löe and Silness (1963)[34] gingival index. The recording of periodontal clinical parameters: maximum probing depth [MPD] and the greatest interdental clinical attachment loss [GCAL]) was done by a single examiner using a Williams periodontal probe,[12] under bright light, on the dental chair. Though full-mouth clinical examination of Probing Pocket Depth and Clinical Attachment Loss were done, only the Maximum Probing Depth [MPD] and the Greatest Interdental Clinical Attachment Loss [GCAL]) were recorded for each participant.

Periodontal Treatment (Intervention): Non-surgical periodontal treatment was done for subjects in the test group by the researcher. This included supragingival scaling with ultrasonic scalers and subgingival scaling and root planing with Gracey curettes under local anesthesia. Post treatment instructions were given, tailored to each patient's peculiarities.

Care of the Control Group: This group received only oral hygiene instructions, that is, importance of oral hygiene maintenance was emphasized. They were also advised to eat healthy diets, avoid sticky, sugary, or acidic foods, and to drink enough fluids. They were given appointments for review too, and encouraged to call the Dentist' number, if they had any questions or concerns before the appointments.

Re-evaluation: Subjects were reviewed at 1 week, 1 month and 3 months from baseline. However, only on the 1 month and 3 months visits were post treatment data collected. Oral hygiene instructions were re-emphasized as required. At the end of the study period, any required follow up visits and treatments were provided.

Data analysis: All data were analyzed using the statistical package for social sciences, version 23.0 (SPSS 23.0). The Independent t- test was used to compare quantitative variables between test and control subjects. The Pearson's correlation test was used to assess the relationship between the periodontal clinical parameters/indices and the concentration of salivary albumin in the two groups. Repeated Measures of ANOVA was used to compare the means of the test and control subjects' variables at baseline, 1-month, and 3-months post intervention at 95 percent confidence level. Comparisons yielding P values of < 0.05 were considered significant.

RESULTS

Twenty test subjects and 20 matched control subjects were enrolled and completed the study. The age range of the participants was 24 to 46 years (mean (SD) = 34.70 (5.36). Twenty-six (65%) of them were within the age range of 31-40 years [Figure 1]. Male participants constituted 16(40%) while females constituted 24 (60%) (Table 1); giving a Male: Female ratio of 1:1.5. Also 26(65 %) of the subjects were civil servants, 11(27.5%) were self-employed, while 3(7.5%) were unemployed. Those who had minimum of tertiary education were 27(67.5%), while 13(32.5%) had less than tertiary education. Twenty-four (60%) of the participants were married, while 16(40%) were not married.

Table 2 shows that at baseline, 15(75%) of the test subjects, presented with fair oral hygiene, only 3(15%) had good oral hygiene, and the rest, 2(10%) presented with poor oral hygiene. All the control subjects presented with good oral hygiene at baseline. The general appearance of the gingiva was clinically healthy in 4(20%) of the test subjects at baseline, edematous in 16(80%), and none of them presented with spontaneous gingival bleeding. Among the controls, the general appearance of the gingiva was clinically healthy in all 20(100%) of the subjects at baseline. Radiographic bone loss at baseline, for all the test subjects, was within the coronal third (15% to 33%) of the involved root, and mostly horizontal.

At one-month post non-surgical periodontal therapy, 17(85%) of the test subjects had complied well with the post-operative instruction, but 3(15%) did not adequately comply with the instructions. All control subjects had complied well with the oral hygiene maintenance instructions. Also, the percentage of test subjects with good oral hygiene had increased to 12(60%), while the rest, 8(40%) only achieved fair oral hygiene. All control subjects were able to maintain good oral hygiene. The percentage of test subjects with clinically healthy gingiva (general appearance of the gingiva) increased to 15(75%), those with edematous general gingiva appearance decreased to 5(25%) and none of them developed spontaneous gingival bleeding. All the control group had maintained clinically healthy gingiva at 1-month review.

At three months post therapy, 19(95%) of the test subjects had complied well with the post-operative instructions, only 1 subject did not adequately comply with the instructions. Also, the oral hygiene of the 19(95%) subjects who complied well with instructions were good, while that of the patient who did not adequately comply was only fair.

Moreover, the general gingiva appearance of the subjects who had complied well with the post-operative instructions was clinically healthy (95%), and only the subject who did not adequately comply with the instructions returned with edematous marginal gingiva. All the subjects in the control group still reported good compliance with oral hygiene maintenance. Also, good oral hygiene with clinically healthy gingiva was recorded from all of them.

Clinical Periodontal Parameters/Indices and Salivary albumin Concentration (Quantitative variables): Normality test for the main outcome variable (salivary albumin), done using Shapiro-Wilk's test gave a value of 0.993 for the test subjects, and 0.945 for the control subjects. This showed normal distribution in both the test and the control groups. Therefore, parametric tests were used for the statistical analysis.

Comparison between test and control groups: Table 3a shows the results of the Independent Samples Test used to compare the mean test and control subjects' variables at baseline. The mean (SD) age of the test subjects was 35.15 (6.46) years, and the mean (SD) age of the control group was 34.25 (5.35). There was no statistically significant difference between the mean age of the test and control groups, (p = 0.602).

At baseline, statistically significant differences (p = 0.001) were found between the OHI-s, GI, MPD and GCAL scores of the test and control groups. The mean salivary albumin concentration of the test subjects was 0.70 (0.13) g/dl, and that of the control group was 0.27 (0.03) g/dl. There was also a statistically significant difference in the mean concentration of salivary albumin between the test and control groups at baseline (p = 0.001).

At 1-month post therapy, (Table 3b), there were statistically significant differences (p = 0.001) between the OHI-s, GI, MPD and GCAL scores of the test and control groups. The mean (SD) salivary albumin concentration of the test subjects had reduced from 0.70 (0.98) to 0.58 (0.14), while the mean (SD) salivary albumin concentration of the control group only showed an insignificant change from 0.27 (0.03) to 0.25 (0.04). Between the test and control groups at 1-month post therapy, the mean concentration of salivary albumin also showed a statistically significant difference (p<0.001).

Table 3c shows that at 3-month post therapy, there were statistically significant differences (p <0.05) between the OHI-s, GI, MPD and GCAL scores of the test and control groups.

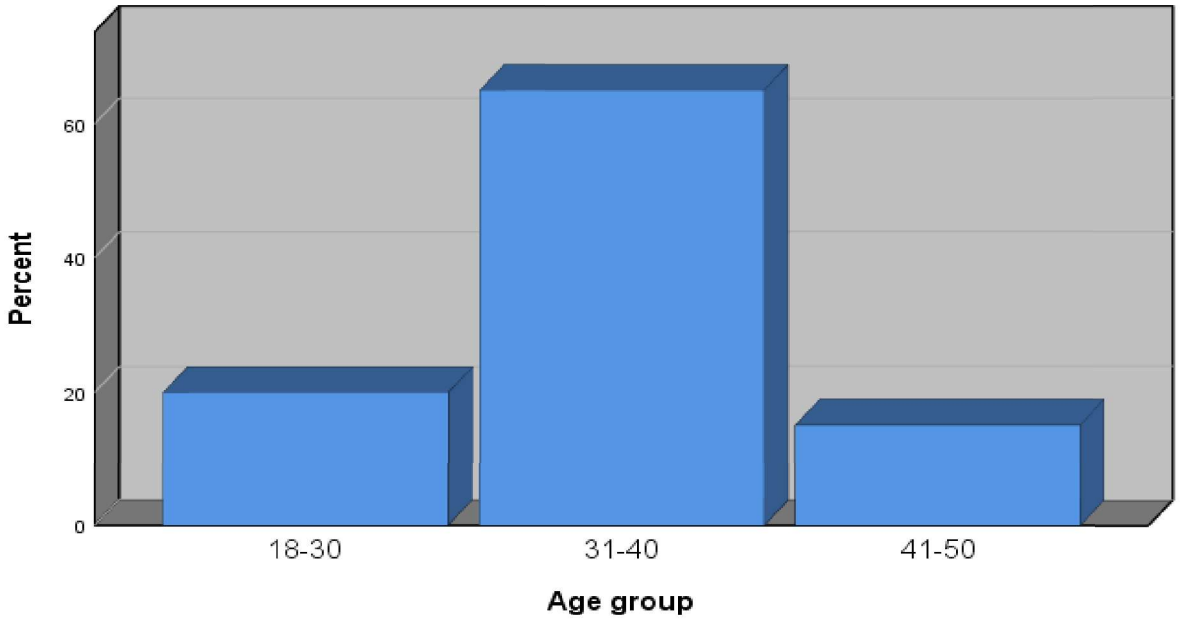

Figure 1: Age group of participants

Table 1: Socio-demographic data of the Subjects

Variables	Test n	%	Control n	%
Age group(years)				
18-30	4	20.0	4	20.0
31-40	13	65.0	13	65.0
41-50	3	15.0	3	15.0
Total	20	100.0	20	100.0
Sex				
Male	8	40.0	8	40.0
Female	12	60.0	12	60.0
Total	20	100.0	20	100.0
Occupation				
Civil servant	6	30.0	20	100.0
Self employed	11	55.0	0	0
Unemployed	3	15.0	0	0
Total	20	100.0	20	100.0
Highest Level of Education				
Primary	2	10.0	0	0.0
Secondary	11	55.0	0	0.0
Tertiary	6	30.0	14	70.0
Postgraduate	1	5.0	6	30.0
Total	20	100.0	20	100.0
Marital Status				
Married	14	70.0	10	50.0
Single	4	20.0	7	35.0
Others	2	10.0	3	15.0
Total	20	100.0	20	100.0

Table 2 Intra-oral findings and Compliance with instructions

Intra-oral findings	At Baseline Test n(%)	Control n(%)	1-month Test n(%)	Control n(%)	3 months Test n(%)	**Control n(%)**
Oral hygiene						
Good	3(15)	20(100)	12(60)	20(100)	19(95)	20(100)
Fair	15(75)	0(0)	8(40)	0(0)	1(5)	0(0)
Poor	2(10)	0(0)	0(0)	0(0)	0(0)	0(0)
Total	20(100)	20(100)	20(100)	20(100)	20(100)	20(100)
General appearance of the gingiva						
Clinically healthy	4(20)	20(100)	15(75)	20(100)	19(95)	20(100)
Edematous	16(80)	0(0)	5(25)	0(0)	1(5)	0(0)
Spontaneous bleeding	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total	20(100)	20(100)	20(100)	20(100)	20(100)	20(100)
Compliance with post-operative instructions						
Good	-	-	**1**7(85)	20(100)	19(95)	20(100)
Poor	-	-	3(15)	0(0)	1(5)	0(0)
Total	20(100)	20(100)	20(100)	20(100)	20(100)	20(100)

Table 3a. Comparison between test and control groups at baseline

Independent samples Test	Test Subject		Control		t-test for Equality of Means				
	Mean	SD	Mean	SD	MD	t	CI		P-value
							Lower	Upper	
Age	35.15	6.46	34.25	5.35	0.900	0.527	-2.56	4.360	0.602
OHI-s	3.04	0.98	0.23	.08	2.816	12.81	2.356	3.275	<0.001
GI	1.63	0.53	0.00	0.00	1.634	13.74	1.385	1.883	<0.001
MPD	4.70	.470	2.45	.76	2.250	11.27	1.843	2.657	<0.001
GCAL	2.70	.470	0.00	0.00	2.700	25.68	2.480	2.920	<0.001
SAC g/dl	0.70	0.13	0.27	0.03	0.436	0.436	0.372	0.5	<0.001

Simplified Oral Hygiene score--OHI-s, Gingival Index –GI, Maximum Probing Depth--MPD, Greatest Clinical Attachment Loss--GCAL, Salivary Alb Conc—SAC, Standard deviation-- SD, Mean difference – MD

Also, there was a significant reduction in the mean (SD) salivary albumin concentration of the test subjects from 0.58 (0.14) g/dl to 0.35 (±0.09) g/dl, while the mean (SD) salivary albumin concentration of the control group only minimally changed from 0.25 (0.04) g/dl to 0.24 (0.04) g/dl. The difference in the mean (SD) concentration of salivary albumin between the test and control groups was also statistically significant at 3-months post therapy ($p<0.001$).

The mean (SD) salivary albumin concentration of the test subjects reduced from 0.70 (0.98) to 0.58 (0.14), at 1-month post therapy, and 0.35 (0.09) g/dl, at 3-months post therapy. The Pearson's correlation test was used to assess for relationship between the periodontal clinical parameters and the concentration of salivary albumin in the two groups. At baseline (Table 4a), a statistically significant direct relationship was found between SAC and MPD, and between SAC and GCAL respectively, among the test group. At 1- and 3-months (Tables 4b and 4c), post therapy, no statistically significant relationship was found between SAC and any of the clinical periodontal parameters, within the period of this study. Also, among the control group, no statistically significant relationship was found between SAC and any of the clinical periodontal parameters.

Table 3b. Comparison between test and control groups at 1-month Post Therapy

Independent samples test	Test Subject		Control		t-test for Equality of Means				
	Mean	SD	Mean	SD	MD	t	CI		P-value
							Lower	Upper	
OHI-s	1.23	0.68	0.23	0.08	1.001	6.57	0.683	1.319	<0.001
GI	0.84	0.41	0.00	0.00	0.845	9.24	0.653	1.036	<0.001
MPD	4.50	0.51	2.10	0.79	2.400	11.41	1.972	2.828	<0.001
GCAL	2.30	0.47	0.00	0.00	2.300	21.88	2.080	2.520	<0.001
SAC g/dl	0.58	0.14	0.25	0.04	0.330	10.03	0.261	0.398	<0.001

Simplified Oral Hygiene score--OHI-s, Gingival Index –GI, Maximum Probing Depth--MPD, Greatest Clinical Attachment Loss--GCAL, Salivary Alb Conc—SAC, Standard deviation-- SD, Mean difference – MD

Table 3c. Comparison between test and control groups at 3-month Post Therapy

Independent samples test	Test Subject		Control		t-test for Equality of Means				
	Mean	SD	Mean	SD	MD	t	CI		P-value
							Lower	Upper	
OHI-s	0.35	0.23	0.19	0.09	0.167	3.026	0.053	0.280	0.006
GI	0.09	0.12	0.00	0.00	0.094	3.584	0.039	0.148	0.002
MPD	2.55	0.51	1.95	0.76	0.600	2.933	0.184	1.016	0.006
GCAL	0.55	0.51	0.00	0.00	0.550	4.819	0.311	0.789	<0.001
SAC g/dl	0.35	0.09	0.24	0.04	0.111	4.950	0.065	0.156	<0.001

Simplified Oral Hygiene score---OHI-s, Gingival Index —GI, Maximum Probing Depth--MPD, Greatest Clinical Attachment Loss--GCAL, Salivary Alb Conc—SAC, Standard deviation-- SD, Mean difference – MD

Table 4a. Pearson's Correlation Tests for Salivary albumin and clinical periodontal parameters among Test and Control Groups at Baseline

	At Baseline			
	Test		Control	
	Corr. Coef.	P-val	Corr. Coef.	P-val
OHI-s	0.162	0.496	0.392	0.087
GI	0.426	0.061	-	-
MPD	0.749	<0.001	0.309	0.184

Simplified Oral Hygiene score--OHI-s, Gingival Index –GI, Maximum Probing Depth--MPD, Greatest Clinical Attachment Loss--GCAL, Salivary Alb Conc—SAC, Correlation Coefficient - C.Coef.

Table 4b. Pearson's Correlation Tests for Salivary albumin and clinical periodontal parameters among Test and Control Groups at 1-month Post Therapy

	At 1-month			
	Test		Control	
	Corr. Coef.	P-val	Corr Coef.	P-val
OHI-s	0.099	0.679	0.153	0.519
GI	0.452	0.046	-	-
MPD	0.209	0.376	0.063	0.790
GCAL	0.030	0.900	-	-

Simplified Oral Hygiene score--OHI-s, Gingival Index –GI, Maximum Probing Depth--MPD, Greatest Clinical Attachment Loss--GCAL, Salivary Alb Conc—SAC, Correlation Coefficient - C.Coef.

Table 4c. Pearson's Correlation Tests for Salivary albumin and clinical periodontal parameters among Test and Control Groups at 3-month Post Therapy

	At 3-months			
	Test Group		Control Gr	
	C.Coef.	P-val	C.Coef.	P-val
OHI-s	0.416	0.068	0.037	0.876
GI	0.296	0.205	-	-
MPD	0.085	0.722	0.048	0.841
GCAL	0.085	0.722	-	-

Simplified Oral Hygiene score--OHI-s, Gingival Index –GI, Maximum Probing Depth-- MPD, Greatest Clinical Attachment Loss--GCAL, Salivary Alb Conc—SAC. Correlation Coefficient - C.Coef.

Repeated Measures of ANOVA were used to compare the means of the test subjects' variables at baseline, 1-month, and 3-months post intervention. As presented in Table 5a, for the test group, there was an overall statistically significant difference (P<0.001) in the SAC, OHI-s, GI, MPD and GCAL. The pairwise differences found between the baseline and 1-months values, as well as between baseline and 3-months values for the SAC and OHI-s were both significant. For the GI, MPD and GCAL, only between the baseline and 3-months values, as well as between 1-month and 3-months were the pairwise differences statistically significant.

Table 5b shows that among the control group, there was no overall statistically significant change (P>0.05) in any of the parameters assessed from baseline, through 1-month, to 3-months.

Table 5a. Repeated Measures of ANOVA Comparing Means of Variables at Baseline, 1- and 3- months Post Therapy Among Test Subjects

	At Baseline		At 1-month		At 3-month		F	Overall Sig.	Paired Sig.
Clinical Parameters	Mean	SD	Mean	SD	Mean	SD			
SAC	0.70	0.13	0.58	0.14	0.35	0.09	75.334	P<0.001	B-1m (P<0.001) B-3m (P<0.001) 1-3m (P<0.001)
OHI-s	3.04	0.98	1.23	0.68	0.35	0.23	133.641	P<0.001	B-1m (P<0.001) B-3m (P<0.001) 1-3m (P<0.001)
GI	1.63	0.53	0.84	0.41	0.09	0.12	80.184	P<0.001	B-1m (P<0.001) B-3m (P<0.001) 1-3m (P<0.001)
MPD	4.70	.470	4.50	0.513	2.55	0.510	130.231	P<0.001	B-1m (P=0.311) B-3m (P<0.001) 1-3m (P<0.001)
GCAL	2.70	0.470	2.30	0.470	0.55	0.510	118.769	P<0.001	B-1m (P=0.023) B-3m (P<0.001) 1-3m (P<0.001)

Simplified Oral Hygiene score--OHI-s, Gingival Index –GI, Maximum Probing Depth-- MPD, Greatest Clinical Attachment Loss--GCAL, Salivary Alb Conc—SAC.

Table 5b. Repeated Measures of ANOVA Comparing Means of Variables at Baseline, 1- and 3- months Post Therapy Among Control Subjects

Clinical Parameters	At Baseline Mean	SD	At 1-month Mean	SD	At 3-month Mean	SD	F	Overall Sig.	Paired Sig.
SAC	0.27	0.03	0.25	0.04	0.24	0.04	3.201	P=0.052	B-1m (P=0.481) B-3m (P=0.112) 1-3m (P=0.735)
OHI-s	0.23	0.08	0.23	0.08	0.19	0.09	1.918	P=0.161	B-1m (P<0.001) B-3m (P=0.041) 1-3m (P=0.041)
GI	0.00	0.00	0.00	0.00	0.00	.00	-	-	-
MPD	2.45	0.76	2.10	0.79	1.95	0.76	1.743	P=0.189	B-1m (P=0.554) B-3m (P=0.377) 1-3m (P=0.554)
GCAL	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-

Gingival Index –GI, Maximum Probing Depth-- MPD, Greatest Clinical Attachment Loss--GCAL, Salivary Alb Conc—SAC.

DISCUSSION

This study evaluated the effect of non-surgical periodontal therapy on salivary albumin concentration in patients with stage II localized periodontitis. The stage II of the disease was specified in order to limit the extent to which the results may be confounded by the severity, complexity of management, as well as the extent of teeth involved. The study participants were adult patients aged 18 and above, as periodontitis is reported as commoner among adults. [4 5 7] The test subjects were matched for age and sex with the control subjects in order to minimize chances of these factors affecting the results.[22 35 36 37] (Saliva flow rate has been found significantly higher in younger age groups than in the older age groups, and also significantly higher in males than in females).[22] The effectiveness of the age matching is supported by the result of the t-test which showed that there was no statistically significant difference in the mean age between the test and control groups, with a mean difference of 0.900 and a p-value of 0.602. For the sex matching, the same male: female ratio (1:1.5) among the test subjects was also represented among the control subjects.

In this study, it was observed that the ages of the patients who presented with stage II periodontitis ranged from 24 to 46 years, and more than half of them were within the age range of 31-40 years. This could have been due to the fact that periodontitis has been reported to increase with increasing age.[4 5 7 38 39] Thus, the stage II of the disease which is a relatively early stage might likely be concentrated around the young adults as observed in this study.[40 41 42 43] A mean (SD) salivary albumin concentration of 0.70 (0.13) g/dl was found among the patients with stage II periodontitis in this study. This finding is close to that reported by Henskens *et al.*[17] (mean [SD] = 0.67 [0.50] g/dl). Also, this study found a mean (SD) salivary albumin concentration of 0.27 (0.03) g/dl in the healthy control group, which is higher than that reported by Henskens *et al.*[17] (mean [SD] = 0.08 [0.50] g/dl). Such variation was commonplace from study to study. For example, Shaila *et al.*[22] found a mean (SD) salivary albumin concentration of 0.04 (0.012) g/dl among periodontitis patients in their study, and 0.009 (0.004) g/dl for healthy control subjects. This pair of results are also very much lower than that of Henskens *et al.*[17] Koduru *et al.*[44] found a mean (SD) albumin value of 0.28 (0.19) g/dl in healthy control subjects, and 0.31 (0.19) g/dl in chronic generalized periodontitis patients: also, different from the studies above.

The different studies had different criteria for selection of their test and control subjects. And different methods of salivary albumin estimation chosen and used in the different studies could have had some effect on the outcome of each study. Ahmed *et al.*[13] submitted that the reason these results differ from one study to another may be due to the diversity in selection criteria of samples, metabolic control, and wide range of age, which can limit direct comparison. Terrapon *et al.*[25] also encountered a variability (from month to month) in

some of the dentate subjects they studied. Oppenheim and Hay[45] reported that procedures as trivial as tooth brushing can induce changes in the concentration of salivary albumin. And Henskens *et al*.[17] have also noted a significant positive correlation between the salivary albumin concentration and the severity of periodontitis. However, the results of Gora *et al*.[46] and Wakde *et al*.[47]who also used unstimulated saliva and colorimetric method among 20 periodontitis patients and 20 healthy control subjects; were 0.51 (0.162) and 0.602 (0.21) among the periodontitis patients; with 0.07 (0.176) g/dl and 0.1435 (0.05) g/dl among the healthy control subjects respectively. Thus, other factors, which may include the selection criteria, socio-economic and racial factors of the study groups may contribute to the variations.[48] [49]

A statistically significant difference of 0.436 (p <0.001) was found between the mean concentration of salivary albumin of the test and control groups at baseline. This supports the submissions of Shaila *et al*.[22], Henskens *et al*.[28], Gonçalves *et al*.[27], Moore *et al*.[20], who noted that salivary albumin concentration was significantly increased in patients with periodontitis. Ahmed *et al*,[13] following their study of salivary albumin concentration in patients with periodontitis, also observed similar significant difference, and stated that "as a biochemical marker, salivary albumin may provide an objective phenotype that could lead practitioners to an early diagnosis" of periodontitis. Gora *et al*.[46] and Wakde *et al*.[47] also both found a significant rise in salivary albumin concentration in chronic periodontitis patients and concluded that salivary albumin concentration may serve as an important biochemical parameter of inflammation of the periodontium. Though a multiple logistic model revealed that disease progression was not related to the levels of any of 9 protein biomarkers which included albumin, but related to the subjects' smoking habit.[48] However, in a comparative study of salivary proteins from patients with generalized aggressive periodontitis and healthy patients, 11 proteins which included albumin were found altered in the generalized aggressive periodontitis patients.[49] The associated proteins included high abundance proteins also associated with inflammation such as α-amylase, lactoferrin, IgG2, IgA2, and albumin.[49]

This study found a statistically significant difference between the means of the OHI-s and MPD of the patients with stage II localized periodontitis and the periodontally healthy individuals. This is in keeping with previously established differences between features of a healthy periodontium and cases of periodontitis.[1] [8] [50] [51] [52] [53] At baseline, this study detected a positive relationship between SAC and MPD, and between SAC and GCAL respectively. This compares with the submission of Terrapon *et al*.[25] who found a weak correlation between the average pocket depths in each patient and the concentration of salivary albumin. Ahmed *et al*.[13] also reported positive correlation between salivary albumin and all clinical periodontal parameters assessed in their study. Thus, this study suggests that the extent of periodontal periodontal inflammation probably corresponds to the extent of plasma albumin leakage from the gingival sulcus, which then corresponds with the concentration of albumin in the saliva. The findings of Gopinath *et al*.[54] and Jabber *et al*.[55] also attest to the variations of salivary albumin concentration with the health status of the periodontium. No statistically significant relationship was found between SAC and any of the assessed clinical periodontal indices at 1- or 3-months post therapy. It is possible that more time is required for manifestation of a statistically significant correlation between the healing periodontium and the salivary albumin concentration. Kardesxler *et al*.[12] reported similar findings stating that the only consistent correlation between the clinical indices and the inflammatory parameters they assessed was between the GCF volume and the concentrations of IL-6 and tissue plasminogen activator. The healing of periodontal tissues improves with time.[56] [57] [58] [59] [60] A longer period of study may help to draw more definite conclusions on the relationship between SAC and the clinical indices .

After initial periodontal treatment, the means of the OHI-s, GI, MPD and GCAL scores showed significant decreases from baseline through 1- to 3-months. This suggests that after the bacterial plaque was removed from the periodontal tissues, and the tissues were maintained in relatively healthy conditions, the periodontal tissues began to heal, with obliteration of the periodontal pocket.[61] [62] [63] [64] [65] For instance, the related clinical parameters assessed by Kardesxler *et al*.[12] plaque index, bleeding on probing, and pocket depth levels exhibited similar significant decreases at the two points assessed. The difference in the means of OHI-s, GI, MPD and GCAL scores also reached a statistically significant level with p-value of <0.001 in each test subject, from baseline through 1- and 3-

months. Thus, the change in the index scores of these periodontal clinical parameters, detected by this study, was likely due to healing following the non-surgical treatment intervention.[61 66 67 68 69 70 71 72] Such findings were also observed by Kardesxler *et al*:[12] who noted that after initial periodontal treatment, plaque index, bleeding on probing, and pocket depth levels of their participants exhibited significant decreases at 1- and 3-months review ($p<0.05$).[12] Among the control subjects, no statistically significant difference was found in the means of OHI-s, GI, MPD and GCAL scores. This further strengthens the suggestion that the change in the index scores of the clinical parameters among the test group was likely influenced by the intervention of non-surgical treatment.

The mean salivary albumin concentration score of the test subjects reduced to a statistically significant level with p-value <0.001 from baseline, through 1 month, to 3 months post intervention. Thus, as ulceration in the periodontal tissues healed, and inflammation resolved, plasma albumin leakage via the gingival sulcus is likely to decrease, reducing the concentration of albumin in the saliva. This is in agreement with a previous study among diabetic subjects, which reported a decrease in concentration of albumin in gingival crevicular fluid.[12] From this study, it is apparent that as the clinical periodontal parameters improved, salivary albumin concentration reduced. The submissions of other studies[14 22 23 24 25 26 27 28] also suggest that the increased sulcular leakage due to inflammation and ulceration in the periodontal tissues contributed to increased albumin concentration in patients with periodontitis. Moreover, Kardesxler *et al*.[12] noted that the decrease in the concentration of albumin also occurred alongside improved periodontal indices. However, Novakovic *et al*.[73] reported significant increase in albumin ($p < 0.001$), among other salivary antioxidants, after scaling and root planing treatment. Nonetheless, the subjects who participated in the study of Novakovic *et al*.[73] could have been a mixture of different stages of the disease, which could have influenced the outcome. Among the control group in this study, the mean salivary albumin concentration showed no statistically significant change, from baseline, through 1 month, to 3 months review. Thus, the decrease in the mean salivary albumin concentration of the test group may have resulted from the intervention.

The limitation of the study may be related to the fact that saliva flow rate may have varied at the different stages of assessment, depending on variations in factors such as the subject's mood, state of hydration or even depending on whether the patient is already hungry at the point of collection. However, the participants were given the due instructions to ensure accuracy of outcome, the study had to depend on their claimed compliance.

CONCLUSION

This study found a statistically significant difference between the salivary albumin concentration in periodontally-healthy individuals and in patients with stage II localized periodontitis. Also, among patients with stage II localized periodontitis, there is a difference in salivary albumin concentration before and after non-surgical periodontal therapy.

RECOMMENDATIONS:

1. A longer period of such study may draw more definite conclusions on the role of salivary albumin as a periodontal biomarker.
2. A multicenter study may also improve on the findings.
3. Longitudinal validation to determine the predictive values of salivary albumin as a periodontal biomarker is required.

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Conflict of interest

None declared

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