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Original Article

Diagnostic accuracy of salivary biomarkers of bone turnover in identifying patients with periodontitis in a Saudi Arabian population

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Probing pocket depth (PPD)

Abstract *Background/purpose:* Salivary markers of bone turnover are useful biomarkers for screening patients advanced periodontal diseases with alveolar bone loss. Hence, the purpose of this study was to determine the diagnostic accuracy of deoxypyridinoline-containing degradation fragment of the C-terminal telopeptide region of type I collagen (CTX), Osteocalcin (OC) and Osteonectin (ON) in identifying patients with alveolar bone loss (BL) due to periodontitis.
Materials and method: Salivary levels of CTX, OC and ON were evaluated in ninety patients (group I, II and III with healthy, periodontitis without Type2 diabetes mellitus-T2DM and periodontitis with T2DM respectively). Group III was included since T2DM is very common among patients attending our clinics. Bleeding on probing (BOP), probing pocket depth (PPD) and BL were recorded for these patients.
Results: The concentrations of salivary CTX, OC, and ON were higher in subjects with periodontitis than in controls. Significant correlations were found between these biomarkers and periodontal parameters. CTX, OC, and ON could discriminate between healthy (group I) and diseased (group II & III) regarding BL with excellent sensitivity (90.2–100%) and good specificity (62.1–96.6%). ROC curve gave excellent discrimination regarding BL (AUC: 0.926–0.958) and PPD (AUC: 0.904–0.915). However, none of the cut-off values gave good discrimination regarding BOP.

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Conclusion: It can be concluded that CTX, OC, and ON correlated well with BL and PPD. Among the three biomarkers, ON at 81.80 ng/ml gave the best discrimination for presence or absence of bone loss.

Introduction

One of the major causes of tooth loss among adults in advanced periodontitis. It is a multifactorial immune-inflammatory disease that results in bleeding on probing (BOP), probing pocket depth (PPD) and alveolar bone loss (BL). In routine clinical practice, periodontal probing and intraoral radiographs are used to assess the presence and level of PPD and alveolar bone destruction. This traditional investigation method of BOP and PPD is invasive, painful and less acceptable by patients. Intraoral radiographs are neither sensitive nor precise enough to detect early BL.[1] Moreover, these methods can only detect past disease activity, not present periodontal disease activity. The diagnostic accuracy is limited before a certain amount of damage has already occurred in the periodontium. Hence, there is a need to develop a non-invasive, highly sensitive rapid diagnostic tool for early detection of periodontal breakdown. Currently, various components of saliva such as immunoglobulins, protein, proinflammatory cytokines and enzymes are being studied as biomarkers for screening of cases with soft tissue and hard tissue changes due to periodontitis.

Usually, in healthy patients, there is a balance between bone formation and resorption. The process of bone remodeling is controlled by various factors such as hormones, growth factors, and cytokines. This process involves osteocytes, osteoblasts, and osteoclasts. Biomarkers of bone remodeling are mainly categorized into a) those related to bone formation (connected with osteoblast activity) and bone resorption (connected with osteoclast activity); b) bone matrix proteins and resorption products of organic skeletal matrix, and c) inorganic skeletal matrix markers such as calcium and phosphorus.[2] Among these, salivary biomarkers of bone turnover such as CTX, OC and ON have been found to potentially provide an indirect estimate of bone resorption.[3–6]

CTX is formed during the process of bone degradation when insoluble type I collagen is cleaved into several fragments in the resorption compartment of osteoclast.[7] While some studies show that CTX levels in oral fluids are a potential diagnostic marker of periodontal disease activity with high sensitivity and specificity for the detection of increased bone destruction, others have reported CTX to be below the level of detection in most subjects.[8,9] Osteocalcin is the most important non-collagenous protein in bone matrix and accounts for approximately 1% of the total protein in human bone. It has been postulated as a marker of inhibition of bone formation. In diabetic patients, CTX and OC appear to be correlated probably since glucose effects of CTX are mediated through OC.[10] Similarly, ON which is also known as secreted protein acidic and rich in cysteine (SPARC) is also frequently associated with tissues with high rates of collagen turnover, such as bone[11,12] Increased levels of SPARC/osteonectin have been identified as a marker correlated with lower levels of bone loss in patients with periodontal disease.[13] While some report high levels of salivary ON, CTX, and OC[4,5] in chronic periodontitis, others report contradictorily.[6] These biomarkers have also been shown to vary following periodontal disease and its most proven systemic risk factor, type 2 diabetes mellitus (T2DM) but there are inconclusive results regarding the predictive nature of these salivary bone biomarkers.

The burden of T2DM is found to be affecting oral and general health worldwide. In Saudi Arabia alone, it is known to be around 23.9% which is moderately high.[14] With an alarming increase of about 2.7 times in the last two decades,[15] there is a need to detect and prevent periodontitis which is the sixth complication of diabetes. The worsening of alveolar bone destruction found in diabetic patients with periodontitis is found to be due to the bidirectional relationship between these diseases. Although salivary biomarkers for bone turnover appear to be an attractive method for screening and discriminating patients with alveolar bone loss who require advanced periodontal care, there is no conclusive evidence regarding its association or diagnostic accuracy. Hence, the primary objective of this study was to determine the diagnostic accuracy of deoxypyridinoline-containing degradation fragment of the C-terminal telopeptide region of type I collagen (CTX), Osteocalcin (OC) and Osteonectin (ON) in identifying alveolar bone loss (BL). Correlation and diagnostic accuracy of these biomarkers with BOP and PPD were also evaluated.

Materials and methods

Subjects

In this cross-sectional study, one hundred eighty-seven consecutive patients who attended clinics of Department of Periodontics at College of Dentistry of King Khalid University (May to December 2018) were evaluated for eligibility for participation. Patients between the age group of 25–75 years were examined for eligibility. Those who agreed to the protocol signed informed consent and were included in the study as shown in Fig. 1. The study protocol was approved by Institutional review board and ethical approval was sought from the Scientific research committee, College of Dentistry of King Khalid University, Abha, Saudi Arabia (Approval no. SRC/ETH/2017-18/061). The

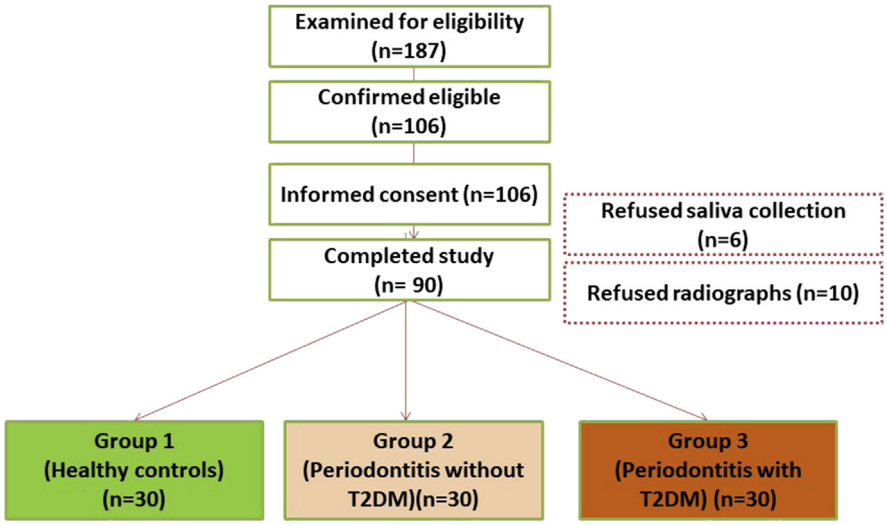

Figure 1 Flow chart of patient recruitment.

study was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008). In accordance with the classification by American Academy of Periodontology (2018),[16] patients were grouped as healthy (n = 30 in Group I) and periodontally diseased (n = 30 in Group II and III each). Periodontitis without Type2 Diabetes mellitus (T2DM) was included in Group II while periodontitis with T2DM was in Group III. Following the American Diabetes Association (ADA) criteria, group III patients had T2DM with (glycated hemoglobin) Hb A1c level $\geq$7% and were on oral anti-hyperglycemic drugs. Patients with any other systemic diseases, any medication other than oral anti-hyperglycemic drugs, the habit of smoking, artificial joints, periodontal therapy within three months or systemic antibiotics within last three months, current use of corticosteroids or non-steroidal anti-inflammatory drugs and lactating or and pregnant ladies were excluded from the study. Patients needing complex rehabilitation (stage 4) according to the new classification of periodontitis[16] and less than 20 remaining teeth were also not included due to the vast extent of periodontal destruction.

Clinical examination

The primary outcome was the radiographic assessment of alveolar bone loss (BL). The amount of bone loss in the worst affect tooth in each patient was measured from cementoenamel junction to crest of alveolar bone and expressed as a proportion of its root length. The procedure was standardized by parallel technique (Kodak Ultra speed Dental Film, Eastman Kodak, Rochester, NY,USA) with a Siemens Heliodent MD model X1744 (Sirona Dental Systems, GmbH D-64625, Bensheim, Germany) and the X-ray machine was used at 70 kV and 7 mA.

BOP was measured according to gingival bleeding index.[17] PPD was measured using a periodontal probe (University of Michigan O probe with William's markings) by a double pass method by a single trained examiner for all patients. Absence or up to 15% of BL, BOP less than 10% and PPD less than 4.0 mm were considered as absence of periodontitis disease.[18,19]

Protocol for estimation of salivary bone biomarkers

Unstimulated whole saliva was collected by a clinician from each patient in the morning (9—10 am) before periodontal probing to prevent mixing of blood or GCF with saliva. Saliva was collected into a sterile 5 ml vial after they rinsed the mouth thoroughly with for 30 s and expectorated.[20] The vials with salivary samples were sealed, labeled for identification and placed in a Styrofoam box containing ice after which it was sent to the Clinical Biochemistry laboratory at the College of Medicine, King Khalid University, for storage (at $-80\,^{\circ}C$) till further analysis by an experienced senior member in Clinical Biochemistry. Antibody sandwich Enzyme-Linked Immunosorbent Assay (ELISA) was performed for the estimation of the biochemical constituents in the collected salivary samples. Commercially available ELISA kits for Human ICTP (Cross Linked C-telopeptide of Type I Collagen) (Catalog No: E-EL-H0835), Human OC/BGP (Osteocalcin) ELISA Kit (Catalog No: E-EL-H1343) were procured from Elabscience Biotechnology Inc, Houston, TX, USA and Human SPARC (Osteonectin) ELISA® Kit (Catalog No: AB220654) from Abcam, Cambridge, UK, were procured for the estimation. The levels of these constituents were estimated according to manufacturers' instructions. The analytical performance of the assay(s) was validated to confirm the manufacturer's analytical performance claims.

Statistical analysis

In descriptive statistics, all quantitative data were expressed as mean $\pm$ SD, whereas qualitative data in numbers and percentiles. Normality assumption was made. Inferential statistics includes analysis of all variables for the mean values, SD and *p* value. The statistical comparison of qualitative variables like oral hygiene practices and quantitative variables like periodontal and various salivary biomarkers between study groups was performed by chi-

square test and analysis of variance (ANOVA) along with Post Hoc (Bonferroni for multiple comparison) respectively at 95% Confidence interval (CI). Correlation analysis between various periodontal variables with salivary biomarkers was done using Pearson's correlation analysis and the result was expressed with p-value and Pearson's Coefficient. Receiver operator characteristics (ROC) curve was fitted into the data to determine the predictability of biomarkers based on binary assumption. Sensitivity (Se), Specificity (Sp), area under curve (AUC) and Youden's index[21] (YI) at 95% CI were calculated for BL, BOP, and PPD.

Results

Out of one hundred eighty-seven consecutive patients examined, hundred and six patients were considered eligible to participate in the study. Sixteen patients dropped out during the study as six of them (three males and three females) refused to give saliva for the study and ten (females) expressed inconvenience while taking radiographs after initially agreeing upon it. Hence, there were fifty-three males and twenty-seven females who completed the study. Descriptive statistics were done for the demographic data between study groups (Table 1).

Table 2 shows the comparison of variables related to oral hygiene practices and clinical variables while Table 3 shows that BOP, PDD, BL ($p < 0.001$) and salivary CTX, OC and ON ($p < 0.001$) demonstrated a statistically significant ($p < 0.001$) gradual increasing trend from group I to group III. Details of post hoc analysis for comparative analysis of different salivary biomarkers between different study groups are given in Table 4. Following this, correlation analysis was performed between periodontal variables with salivary biomarkers (CTX, OC, and ON) using Pearson Correlation (95% CI) as shown in Table 5. It showed positive correlation of CTX with PPD ($r = 0.79$) and BL ($r = 0.82$) ($p < 0.001$) and BOP($r = 0.28$) ($p < 0.01$). OC and ON showed a similar positive correlation with BOP, PPD and BL.

It was found that CTX, OC and ON could discriminate between healthy and diseased regarding BL with excellent sensitivity (90.2—100%) and good specificity (62.1—96.6%) using the various cut off values obtained from coordinates of ROC. Similarly, regarding PPD, excellent sensitivity (94.4—100%) and fair to good specificity (63.9—83.3%). ROC curve gave excellent discrimination regarding BL (AUC: 0.926—0.958) and PPD (AUC: 0.904—0.915). Figs. 2—4 show the ROC curve for BL, PPD and BOP. Table 6 describes cut off values, sensitivity, specificity, area under curve of ROC and Youden index for CTX, OC and ON in discriminating healthy from periodontitis in terms of BOP, PPD, and BL. Among the three biomarkers, ON at 81.80 ng/ml gave the overall best discrimination which was for the presence or absence of BL ($YI = 0.91$). CTX discriminated best ($YI = 0.88$) at 29.86 ng/ml between presence or absence of BL. CTX and ON discriminated well between presence and absence of PPD too at 29.86 ng/ml and 72.85 ng/ml respectively ($YI = 0.83$). However, the none of the cut off values gave good discrimination regarding BOP.

Discussion

Our results confirm that there exists a correlation between CTX, OC and ON and alveolar bone loss. Our results also show that these biomarkers in the saliva can discriminate between presence and absence of BL and PDD but not for BOP. Similar correlation studies have been done by few other research groups to determine whether a relationship exists between clinical parameters of periodontal disease and salivary bone turnover biomarkers.[4—6,22] PPD which is the most important clinical feature of periodontal disease also showed correlation with these biomarkers. Levels of salivary CTX and OC in healthy showed greater variation

Table 1 Descriptive analysis of demographic variables between study groups.

		Group I	Group II	Group III	Total
Demographic Variables - (Expressed as N (%) — Except Age					
Gender	Male	19 (63.3)	14 (46.7)	20 (66.7)	53 (58.9)
	Female	11 (36.7)	16 (53.3)	10 (33.3)	37 (41.1)
Age	Mean ± SD	33.00 ± 9.70	41.30 ± 9.32	40.87 ± 10.88	—
Occupation	None	0	5 (16.7)	2 (6.7)	7 (7.8)
	Unskilled	6 (20.0)	7 (23.3)	8 (26.7)	21 (23.3)
	Semi-Skilled	19 (63.3)	13 (43.3)	15 (50.0)	47 (52.2)
	Professional	5 (16.7)	5 (16.7)	5 (16.7)	15 (16.7)
Education	Below 5th grade	0	3 (10)	0	3 (3.3)
	Primary School	3 (10.0)	9 (30)	10 (33.3)	22 (24.4)
	High School	20 (66.7)	11 (36.7)	15 (50.0)	46 (51.1)
	Graduate/Post graduate	7 (23.3)	7 (23.3)	3 (20)	19 (21.1)
Income	Below 4 K Saudi riyals	0	0	0	0
	4 K - 6 K Saudi riyals	2 (6.7)	5 (16.7)	0	7 (7.8)
	6 K—10 K Saudi riyals	13 (43.3)	12 (40.0)	14 (46.7)	39 (43.3)
	Above 10 K Saudi riyals	15 (50.0)	13 (43.3)	16 (53.3)	44 (48.9)
Diabetes Status	Absent (Hb A1c < 7%)	30 (100)	30 (100)	0	60
	Present (Hb A1c ≥ 7%)	0	0	30 (100)	30

Note: SD: Standard Deviation.

Table 2 Comparative analysis of variables related to oral hygiene practices and periodontal variables between study groups.

Variable		Group I (%)	Group II (%)	Group III (%)	Total	*p*-value
Oral Hygiene Tool	Toothbrush	9 (30.0)	8 (26.7)	12 (40)	29 (32.2)	0.91[a]
	Miswak	14 (46.7)	16 (53.3)	14 (46.7)	44 (48.9)	
	Other	7 (23.3)	6 (20.0)	4 (13.3)	17 (18.9)	
Frequency of Brushing	Once	14 (46.7)	16 (53.3)	13 (43.3)	43 (47.8)	0.82[a]
	Twice	10 (33.3)	7 (23.3)	5 (16.7)	22 (24.4)	
	Other	6 (20.0)	7 (23.3)	12 (40)	25 (27.8)	
Time taken for brushing (In minutes)	Mean ± SD	1.67 ± 1.44	1.43 ± 1.04	1.33 ± 1.17	–	0.72[b]
BOP	<10%	5 (16.7)	3 (10.0)	0	8 (8.9)	0.000¶
	≥10%	25 (83.3)	27 (90.0)	30 (100)	82 (91.1)	
PPD	<4.0 mm	30 (100)	4 (13.3)	2 (6.7)	36 (40.0)	0.000¶
	≥4.0 mm	0	26 (86.7)	28 (93.3)	54 (60.0)	
Bone Loss	<15%)	30 (100.0)	2 (6.7)	0	32 (32.2)	0.000¶
	≥15%	0	28 (93.3)	30 (100)	58 (67.8)	

¶ p value < 0.001; SD: Standard Deviation; PPD: Probing pocket depth.
[a] Chi square test.
[b] One Way ANOVA.

Table 3 Comparative analysis of periodontal variables and salivary biomarkers of bone turnover between study groups.

Variable	Periodontal Variables (Mean ± SD)			*p* value
	Group I	Group II	Group III	
BOP	9.4 ± 3.84	37.36 ± 17.90	47.07 ± 18.17	0.000¶
PPD (mm)	2.56 ± 0.63	5.13 ± 0.71	5.56 ± 0.79	0.000¶
Bone Loss (mm)	9.50 ± 4.84	22.75 ± 5.83	30.94 ± 6.10	0.000¶
	Salivary biomarkers of bone turnover (Mean ± SD)			
CTX (ng/ml)	14.45 ± 3.63	61.90 ± 11.57	70.63 ± 10.28	0.000¶
Osteocalcin (ng/ml)	8.93 ± 5.80	24.99 ± 8.97	34.40 ± 7.27	0.000¶
Osteonectin (ng/ml)	52.61 ± 8.93	109 ± 20.48	119.84 ± 16.01	0.000¶
	Percentage variation in salivary biomarkers of bone turnover with respect to healthy control			
CTX (ng/ml)	–	76.65%	79.54%	
Osteocalcin (ng/ml)	–	76.27%	74.04%	
Osteonectin (ng/ml)	–	51.73%	56.46%	
	Percentage variation in salivary biomarkers of bone turnover with respect to adjacent group			
CTX (ng/ml)	–	76.65%	14.10%	
Osteocalcin (ng/ml)	–	76.27%	27.35%	
Osteonectin (ng/ml)	–	51.73%	9.04%	

Note: ¶ p value < 0.001; PPD: Periodontal Pocket Depth; SD: Standard Deviation.

than ON. CTX had a statistically significant overall positive correlation with BL and PPD. The correlation with BL is similar to few previous studies.[8,9] The good diagnostic accuracy of CTX levels in oral fluids has suggested it as a potential diagnostic marker of increased bone destruction.[8,9] CTX has been suggested to correlate with clinical parameters of periodontal disease and to reduce following periodontal therapy, thus leading to an accurate assessment of tissue breakdown.[23,24]

In this study, the levels of ON correlated positively with alveolar bone loss and PPD. This is contrary to the negative correlation of SPARC/osteonectin in terms of bone loss in patients with periodontal disease.[4,23] Increased levels of ON in the diseased groups could be due to the potential for SPARC/osteonectin to enhance healing of degraded alveolar bone with collagen deposition.[12] It is found that lack of SPARC/osteonectin caused decreased total collagen and its production was decreased by periodontal disease. So, it appears that SPARC/osteonectin is a strong candidate for monitoring disease and improvement following treatment.

All the three salivary biomarkers correlated least with BOP. This could be probably due to the fact that BOP is the earliest sign of periodontal disease and these salivary biomarkers are not specific to soft tissue destruction. The trends seen in this study in terms of PPD can be compared to the previous results[5,25,26] where a significant correlation

Table 4 Post hoc analysis for comparative analysis of different salivary biomarkers between study groups.

Variable	Study Group	Group I	Group II	Group III
Different salivary biomarkers between different study groups				
CTX	Group I	—	0.000¶	0.000¶
	Group II	0.000¶	—	0.001€
	Group III	0.000¶	0.001€	—
Osteocalcin	Group I	—	0.000¶	0.000¶
	Group II	0.000¶	—	0.000¶
	Group III	0.000¶	0.000¶	—
Osteonectin	Group I	—	0.000¶	0.000¶
	Group II	0.000¶	—	0.029*
	Group III	0.000¶	0.029*	—

Note: *p value < 0.05; € p value < 0.01; ¶p value < 0.001; p value (Pearson Coefficient); BOP: Bleeding on probing; PPD: Probing pocket depth.

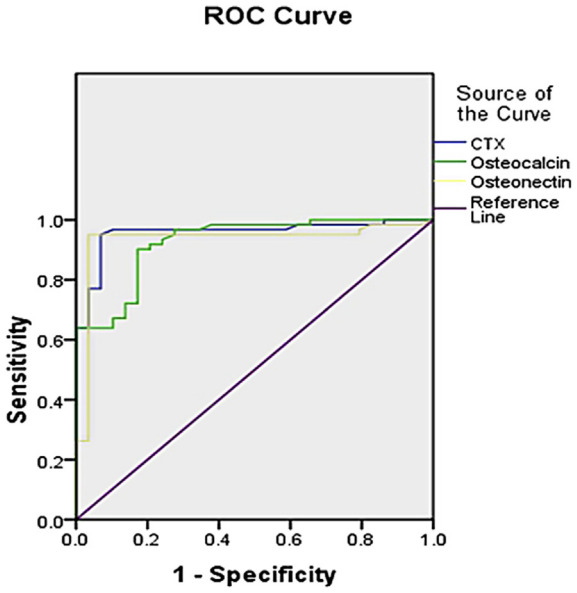

Figure 2 ROC curve of salivary biomarkers in terms of bone loss.

of OC with pocket depth, gingival index scores were observed. On the contrary, negligible or negative correlation between severity of periodontitis and OC also have been observed.[7,25,27] Increased OC levels seen in diseased patients could be due to increased periodontal disease activity. This could be explained on the fact that OC is known to act as a chemoattractant for osteoclasts[28] and essential for osteoclast differentiation. However, this is contrary to some studies that found biomineralization followed secretion of OC, which may reflect early osteoblastic differentiation. Moreover, it is suggested that OC might be an active regulator of insulin sensitivity by bone.[29] ON has also been found to predispose the body to insulin resistance, thus represent a novel and important link between obesity and diabetes mellitus.[30] This could be explained since in diabetes, periodontal membrane and the dilation of blood vessels, hemorrhage has also been a significant increase in inflammatory cells.[31]

To the best of our knowledge, this is the first report of salivary biomarkers of bone turnover such as CTX, OC and ON in an Arab population. Abha is situated in the southern region of Asir, (Saudi Arabia) at an elevation of 2270 m above sea level. Since there are no earlier studies that mention levels of salivary biomarkers in periodontitis patients in Saudi Arabia or any other Arab nation, the results of this study can be used as reference range standard (Table 7) for salivary CTX, OC and ON for these people in periodontal disease, for screening of high-risk patients with family history of periodontitis and in clinical research. Validation of this numerical scale is needed on a larger population.

We chose to use the Youden Index along with AUC to describe the biomarker's ability for classifying disease status as YI provides maximum potential effectiveness of a biomarker. Unlike other studies, in biomarker development, levels of certain analyte may be unquantifiable below a limit of detection (LOD) and missing from the overall dataset. Disregarding these observations may negatively bias the ROC curve and thus Youden Index is also used. We have prepared a clinical interpretation based on cut-off levels because ROC areas under curve alone lack clinical interpretability. This is very significant as results of diagnostic tests would best benefit the clinician when expressed in terms of clinical gains and losses to the patients.

Within the limitations of this study, the results are novel and encouraging. Diabetic patients were included to increase the generalizability of the study as T2DM is very commonly seen in this population. One of the limitations of the study is that we included only T2DM patients who were on anti-hyperglycemic since we wanted to standardize the medications taken by the study population and during a pilot survey, a vast majority of patients attending our clinics were found to be using oral anti-hyperglycemic. To further increase its generalisability, this study should be

Table 5 Correlational analysis — Periodontal variables with salivary biomarkers of bone turnover.

Variable	BOP	PPD	Bone Loss	CTX	Osteocalcin	Osteonectin
CTX	0.007€ (0.28)	0.000¶ (0.79)	0.000¶ (0.82)	—	0.000¶ (0.87)	0.000¶ (0.85)
Osteocalcin	0.01* (0.27)	0.000¶ (0.65)	0.000¶ (0.78)	0.000¶ (0.86)	—	0.000¶ (0.81)
Osteonectin	0.005€ (0.29)	0.000¶ (0.76)	0.000¶ (0.76)	0.000¶ (0.85)	0.000¶ (0.81)	—

Note: *p value < 0.05; € p value < 0.01; ¶p value < 0.001; p value (Pearson Coefficient); BOP: Bleeding on probing; PPD: Probing pocket depth.

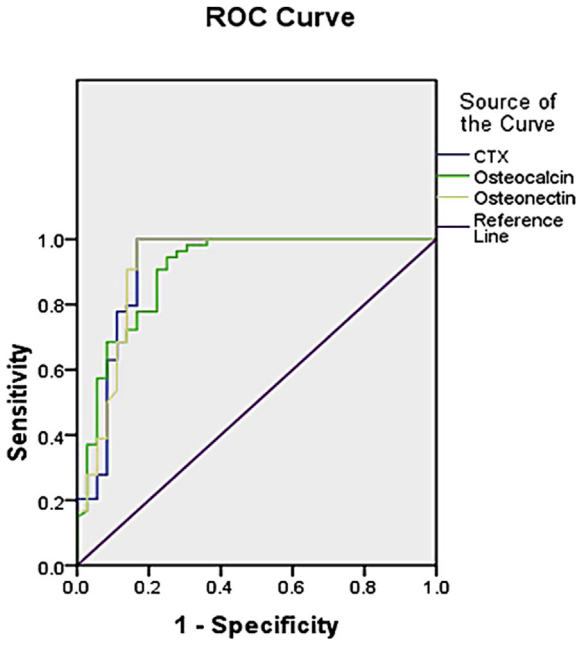

Figure 3 ROC curve of salivary biomarkers in terms of PPD.

replicated in other population and regions in a longitudinal design and in patients with prediabetic and uncontrolled diabetes mellitus. Smokers which are another major risk factor for periodontal bone destruction should also be included in the next phase of the study to have a better understanding of the association of salivary biomarkers to periodontitis.

In conclusion, this study showed that salivary biomarkers of bone turnover (CTX, OC and ON) have a statistically significant difference between periodontally healthy and diseased groups. These correlated positively with PPD and BL with a statistically significant difference between

Table- 6 Cut off values, area under curve of ROC and Youden index of CTX, OC and ON in discriminating healthy from periodontitis based on BL, BOP and PPD.

		Cut-off (ng/ml)	Se (%)	Sp (%)	AUC	95% CI	YI
Bone loss	CTX	19.87	96.7	89.7	0.958	0.92—1.00	0.85
		29.86	95.1	93.1			0.88
		40.95	93.4	93.1			0.86
	OC	9.16	98.4	62.1	0.926	0.87—0.98	0.60
		10.38	96.7	72.4			0.68
		15.43	90.2	82.8			0.72
	ON	66.70	95.1	86.2	0.934	0.87—0.99	0.81
		72.85	95.1	93.1			0.88
		81.80	95.1	96.6			0.91
PPD	CTX	19.87	100	77.8	0.914	0.84—0.99	0.77
		29.86	100	83.3			0.83
		40.95	98.1	83.3			0.81
	OC	9.16	100	52.8	0.904	0.83—0.97	0.52
		10.38	100	63.9			0.63
		15.43	94.4	75.0			0.69
	ON	66.70	100	77.8	0.915	0.84—0.98	0.77
		72.85	100	83.3			0.83
		81.80	98.1	83.3			0.81
BOP	CTX	19.87	72.0	62.5	0.704	0.53—0.87	0.34
		29.86	69.5	62.5			0.31
		40.95	68.3	62.5			0.30
	OC	9.16	80.5	37.5	0.637	0.45—0.81	0.17
		10.38	75.6	37.5			0.17
		15.43	67.1	37.5			0.04
	ON	66.70	69.5	62.5	0.684	0.50—0.86	0.31
		72.85	69.5	62.5			0.31
		81.80	68.3	62.5			0.30

Sensitivity (Se); Specificity (Sp); Receiver operator characteristic (ROC); Area under curve (AUC); 95% Confidence interval (CI); Youden Index (YI)Se + Sp-1; Probing pocket depth (PPD); Bleeding on probing (BOP).

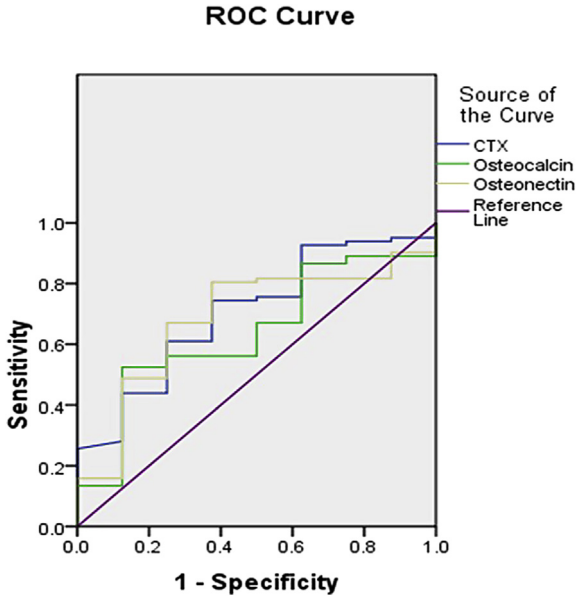

Figure 4 ROC curve of salivary biomarkers in terms of BOP.

Table 7 Clinical interpretation and reference range of salivary biomarkers based on cut off values obtained from the coordinates of ROC curve and YI for identification of patients as healthy and periodontally diseased.

Salivary Biomarker	Clinical patients	Cut off values
CTX ng/ml	BL present	29.86 ng/ml and above
	PPD present	29.86 ng/ml and above
	BOP present	—
OC ng/ml	BL present	15.43 ng/ml and above
	PPD present	15.43 ng/ml and above
	BOP present	—
ON ng/ml	BL present	81.80 ng/ml and above
	PPD present	81.80 ng/ml and above
	BOP present	—

Receiver operator characteristics curve (ROC) curve; Youden Index (YI); Alveolar bone loss (BL); Probing pocket depth (PPD); Bleeding on probing (BOP).

healthy and diseased patients. It can be concluded that CTX, OC and ON have high discriminating power to identify most prominent clinical features of periodontitis. ie, BL and PPD. The reference range presented here may be used as a screening tool for identifying high risk patients at an early stage because timely detection of periodontal disease can be crucial in the clinical management of periodontal patients.

Conflicts of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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