ORIGINAL RESEARCH

Assessment of Salivary Hemoglobin as Noninvasive Biomarker of Chronic Periodontitis in Type II Diabetics

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Abstract

Aim: The present study aimed at determining the association between salivary hemoglobin (SH) and chronic periodontitis in type II diabetic subjects and check whether SH can function as a noninvasive biomarker to assess the level of chronic periodontitis.

Materials and methods: This cross-sectional comparative study included 40 subjects aged between 40 and 65 years with at least 20 teeth remaining. The diabetic status was confirmed with HbA1c levels and categorized into group I: healthy controls with HbA1c < 5.6% and no periodontitis with bleeding on probing (BOP) at ≤10% of sites, <3% of sites with probing pocket depth (PPD) ≥4 mm, and no sites with clinical attachment level (CAL) ≥ 2 mm and group II: type II diabetes mellitus (T2DM) cases with HbA1c > 5.7% and periodontitis with BOP at >10% of sites, with CAL ≥ 2 mm and with >5% of sites with PPD ≥ 4 mm. Unstimulated fasting whole saliva was collected from each participant and the salivary hemoglobin level analyzed using a colorimetric assay kit. Both groups were compared using the *t*-test and multiple linear regression model analysis. Relationship between different variables were compared using the Karl Pearson's correlation coefficient. Statistical significance was set at 5% level ($p < 0.05$).

Results: A significant difference was observed between the salivary hemoglobin level (t −3.7710, $p < 0.001$), PPD (t −13.9023, $p < 0.001$), and CAL (t −9.3759, $p < 0.001$) between healthy controls and type II diabetics with T2DM subjects exhibiting much higher value compared to healthy controls.

Conclusion: In conclusion, data from this study demonstrated that type II diabetic adults have high prevalence of chronic periodontitis and exhibit elevated salivary hemoglobin indicating a valuable noninvasive screening method for detecting periodontitis.

Keywords: Diabetes mellitus, Periodontitis, Salivary hemoglobin, Tooth loss.

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Introduction

The literature has underlined diabetes as a major risk factor for periodontitis.[1] With a threefold enhanced manifestation in diabetic individuals compared with nondiabetic individuals,[2] periodontal diseases have exacerbated insulin resistance affecting the glycemic control. National[3] and international studies[4] have evidenced increased severity and prevalence of periodontal disease in subjects suffering from type II DM approximating to 45.9% as compared to 37.8% in nondiabetic,[5] 2.1 times higher odds of chronic periodontitis among those with diabetes in a case-control study carried out in Bengaluru, Karnataka, India.[6] Type II diabetes is the most common form of the disease concerning denture wearers. Detected most of the time at about 40–50 years, levels of this disease are projected to rise to 5.4% of the population, globally, by 2025.[7]

Advanced glycation end products (AGEs) generated due to chronic hyperglycemia can trigger inflammatory responses, vascular modifications, and altered healing capacity contributing to pathogenesis of periodontitis in diabetic patients. This relationship between diabetes and periodontitis appears bidirectional and highlights the importance for early diagnosis[8] to surmount the associated risk of tooth loss, edentulism that has a bearing upon the quality of life concorded with huge socioeconomic impacts and healthcare costs.[9]

Lack of awareness of oral health is discerned as a major cause for the high percentage of tooth loss among diabetic patients. Studies have also underlined the need for oral health measures irrespective of the type and duration of the disease[10] among diabetic patients to obviate tooth loss as one of the visible consequences of the progression of periodontal disease. The impact of diabetes complications can thus be minimized by emphasizing the screening for undiagnosed diabetes in India and comanagement of the condition by the multidisciplinary healthcare team.[11,12]

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Diagnosis of periodontitis is usually by measurement of clinical parameters, which suggests the past disease activity and present disease severity, thereby limiting its use in early diagnosis.[13] Conventionally, the Community Periodontal Index (CPI) is performed for screening for periodontal disease by pocket probing of representative teeth, even though the pocket depth is not an index of disease activity.[14] This procedure of pocket probing is not only associated with significant amount of damage predisposing periodontitis patients to bacteremia but also dismisses the scope of diagnostic parameters to determine the current status of the disease.[15]

Salivary biomarkers have emerged as the mainstay noninvasive screening method that reflects the activity of inflammatory reactions of an individual as a result of periodontal tissue destruction.[16] Since saliva is the first-line defense fluid of the human body, it is a desirable diagnostic fluid to analyze diseases associated with the oral cavity. Saliva is more accessible, accurate, less expensive, and present less risk of infection to the patient than current methodologies[17] and thus promising in terms of applicability.[18]

Haem is implicated to serve as a source of iron and protoporphyrin for periodontopathogens that are unable to synthesize it including porphyromonas gingivalis, thus regulating putative bacterial virulence factors.[19,20] With the focus on diagnosing periodontitis at a preliminary stage, recent advances have evaluated the levels of salivary hemoglobin as a marker for periodontitis.[21,22] These methods detect subtle bleeding from gingival tissue validating noninvasive periodontitis detection methods.[23–25] It is important to note that despite the availability of advanced biomarkers such as IL-6, prostaglandin E2 (PGE2), interleukin (IL), and tumor necrosis factor, the Pharmaceutical Affairs Law for extracorporeal diagnostic agents in Japan approves salivary hemoglobin levels for assessing periodontal conditions. With this background incorporating this test in medical check-up may be valued in promoting oral health.[21]

It is important to note that specific recommendations for oral health is not included by American Diabetes Association's Standards of Medical Care in Diabetes despite the global burden.[26] Periodontal oral point of care (POC) diagnostic devices are at apparent horizon for screening large populations and enabling access to treatment.[27] In this context salivary Hb tests that meet the criteria of simple, easy, cost-effective, with high sensitivity and specificity have become the alternative screening method to CPI.[21] Therefore, this study was undertaken to assess the relevance of salivary hemoglobin to detect chronic periodontitis in type II diabetic and nondiabetic individuals in middle-aged adults and elderly.

Materials and Methods

Study Participants

This study was conducted after approval by the Institutional Review Board (IRB) of JSS Dental College and Hospital, JSS Academy of Higher Education and Research, Mysore, Karnataka, India (JSSDCH IEC Research Protocol No. 33/2018) and in accordance with the Helsinki Declaration of 1975 (revised in the year 2000). Study population were selected from patients visiting the special clinic of JSS Hospital after obtaining an informed consent. Sample size was estimated by conducting a pilot study and estimated relationship between HbA1c and free hemoglobin was found to be 0.4953. Therefore, the sample size estimated for this study was 39 and approximated to 40 to achieve 90% power and 5% alpha error.

This comparative cross-sectional study involved subjects older than 40 years with at least 20 remaining teeth. The subjects with confirmed cases of T2DM for minimal of two years with HbA1c levels >6.4% were grouped into group II: cases (T2DM); and subjects without any history of T2DM and systemic diseases as confirmed by history, not on any systemic medication, whose HbA1c levels ≤5.6%, were grouped into group I: controls (healthy individuals).

Exclusion criteria followed for either group included subjects who demonstrated difficulties in communication, receipt of periodontal treatment within 4 weeks of saliva sampling, intraoral conditions entailing oral hemorrhage, history of salivary gland surgeries, autoimmune disease, receiving radiotherapy, systemic drug therapy for medical conditions, antibiotics, anti-inflammatory drugs except oral hypoglycemics, history of systemic illness, neurological or psychiatric disorders, alcohol abuse, smoking, pregnancy, not ambulatory and endocrinal and metabolic disorders affecting the serum/salivary glucose levels except T2DM.

Clinical Evaluation

All subjects satisfying the inclusion and exclusion criteria were subjected to a full-mouth periodontal examination. Study proforma included details of subject demographics, physical dependency, daily medication intake, oral habits, and clinical findings with receipt of past dental and periodontal treatment in specific and medical history were recorded.

Standard protocol was followed for the periodontal pocket depth (PPD) and adopted from previous studies.[15,28] The PCP-UNC 15 probe was used to measure pocket depth, BOP, clinical attachment level (CAL) at six locations per tooth (mesial-buccal, mid-buccal, distal-buccal, mesial-lingual, mid-lingual, and distal-lingual). Number of sites affected divided by the total number of sites present for each subject was considered to calculate the percentage of sites affected with BOP and PPD. Subjects with BOP at ≤10% of sites (six sites per tooth), no sites with clinical attachment loss (CAL) ≥2 mm, and <3% of sites with PPD ≥4 mm were categorized as healthy patients. The periodontitis group had BOP at >10% of sites, CAL ≥2 mm, and with >5% of sites with PPD ≥4 mm. All the clinical measurements were performed by the same periodontist.

Collection of Saliva

Prior to saliva collection all subjects were instructed to rinse with tap water (10 mL) for 30 seconds and expectorated. Saliva samples from subjects were collected prior to performing clinical evaluation. Modification of the method reported by Navazesh was used to collect unstimulated whole saliva.[29] Subjects expectorated 5 mL of unstimulated whole fasting saliva into a prelabeled sterile tube after following specific instructions of avoiding eating, drinking, or performing oral hygiene measures such as brushing, flossing, etc., 1 hour prior to saliva collection. Saliva samples were collected on ice and then stored at −80°C until analysis.

Evaluation and Quantification of Free Hemoglobin Levels in Saliva Sample

The salivary hemoglobin level in the saliva sample was quantitatively assessed using a commercially available Heme Assay Kit (BioVision, USA) according to the manufacturer's manual. Quantification of free hemoglobin was carried out by measuring the optical density of the saliva sample at 570 nm spectrophotometrically using Perkin Elmer Enspire Multimode Plate Reader.

Statistical Analysis

The collected data were entered in a spreadsheet application and to perform the statistical analysis using statistical program SPSS Version 20.0. Parametric tests were used to analyze data. Comparison between groups was made with the independent *t* test and correlation between HbA1c with PPD, CAL, and salivary hemoglobin with the Karl Pearson coefficient. Statistical significance was set at 5% level ($p < 0.05$).

Results

The study was initiated by first recruiting the study subjects following the inclusion and exclusion criteria mentioned in the methodology. Twenty healthy subjects were recruited in the group I (controls) and 20 subjects with type II diabetes mellitus were recruited in group II (cases). The study participants was composed of a cohort of 22 male (55%) and 18 female (45%) with a mean age of 48.95 ± 6.94 years. Whereas the mean age in the control group was 45.62 ± 6.23, the T2DM group had a mean age of 52.63 ± 5.83 as represented in Table 1 and Figure 1.

A descriptive analysis of HbA1c levels among controls (≤5.6%) and type II diabetic status HbA1c (>6.4%) was then calculated against salivary levels of Hb (in µg/mL) and chronic periodontitis criteria of PPD and CAL. The results showed that mean salivary Hb, PPD, and CAL are significantly higher in the T2DM group as compared to the healthy controls. These differences were statistically significant according to the "*t*" test. Table 2 and Figure 2 depict comparison of these two groups of HbA1c and represent a significant difference observed with the salivary hemoglobin level (t −3.7710, $p < 0.001$), PPD (t −13.9023, $p < 0.001$), and CAL (t −9.3759, $p < 0.001$) at 0.1% level of significance.

The degree and direction of the relationship between HbA1c levels with the related variables of salivary Hb conc. (in µg/mL), PPD, and CAL levels was calculated with the Karl Pearson's correlation coefficient. Although significant, a moderate correlation was found between HbA1c with the salivary hemoglobin level while there was a strong correlation between HbA1c levels with PPD and CAL. Interpreting these trends of statistical data on the scatter plot diagram in Figures 3 to 5, respectively, showed that although the points are seen somewhat scattered in a wider band positive relationship is indicated. Table 3 summarizes the correlation between HbA1c and salivary hemoglobin level, PPD, and CAL. It depicts significant positive relationship between HbA1c and salivary Hb ($r = 0.4653$, $p < 0.01$). HbA1c and PPD ($r = 0.8474$, $p < 0.001$) and HbA1c and CAL ($r = 0.7992$, $p < 0.001$) that can infer that salivary Hb, PPD, and CAL are dependent on levels of HbA1c.

Table 1: Mean and SD of age in healthy and T2DM groups of HbA1c

Groups	*n*	*Mean*	*SD*	*SE*
HbA1c (≤5.6%) Healthy (controls)	20	45.62	6.23	1.36
HbA1c (>6.4%) T2DM (cases)	20	52.63	5.83	1.34
Total	40	48.95	6.94	1.10

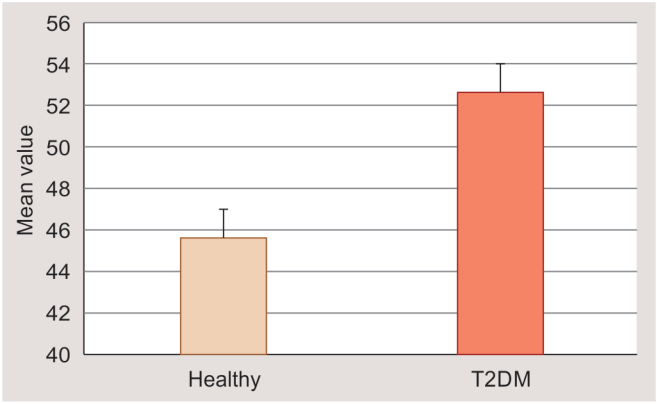

Fig. 1: Comparison of healthy and T2DM groups with mean age

Discussion

Studies have focused on the use of salivary tests as a reliable, cost-effective, specific noninvasive diagnostic tool for periodontitis to address the inherent disadvantages associated with the conventional periodontal diagnostic techniques. Current studies have indicated that increased probability of developing the disease in "high-risk" individuals can be determined by the use of inflammatory molecular biomarkers of periodontal disease.[14] Assessing the locally and systemically derived biomarkers of periodontal disease in the saliva can lead to an early detection and prevention of the progression of periodontal disease, thus maintaining systemic health.[15] Research based on salivary levels of hemoglobin as a valuable screening method for periodontitis is currently evidenced.[21] Diabetes increases the risk of prevalence and severity of periodontal disease, while the severity of periodontitis negatively affects glycemic control.[30] In this context, this study was designed to ascertain the levels of salivary hemoglobin (Hb) as a marker for periodontitis in subjects with type II diabetes mellitus.

Studies have recommended HbA1c as a standard of care (SOC) specifically in type II diabetes for testing and monitoring the disease status as an evidence of cumulative glycemic levels of the previous 3 months by the analysis of glycated hemoglobin (HbA1c) in blood.[31] Hence, HbA1c or glycosylated hemoglobin test was considered as the indicator of diabetes in this study. Based on guidelines from the American Diabetes Association,[26] individuals are considered normal if the HbA1c is ≤5.6%. Participants with HbA1c ≥ 6.5% were grouped into T2DM for further evaluation of the periodontal status. In this study, HbA1c levels were higher and statistically significant in chronic periodontitis subjects in comparison with healthy controls. This finding is in agreement with previous studies that report the positive association in adults with chronic periodontitis and elevated blood glucose and not diagnosed with diabetes and thus increasing their risk for type II diabetes.[32,33]

Studies have substantiated that challenging later stages of the periodontitis can be avoided by early detection of the process.[34] It is also evidenced that general dentists during initial screening of periodontal disease and recording practiced a simplified probing technique formulated by the American Dental Association and the American Academy of Periodontology.[35] However, to enlist drawbacks of this method, first to record the findings a competent clinician and assistant is a requisite. Second, this procedure of collection of diagnostic information involves radiographic aid, making it time- and labor-intensive, while inflicting considerable financial bearing to the consumer. Third, accuracy of readings even in the hands of experts and among the examiners is subjective. Another significant fact in order to the detect periodontal disease level by diagnostic parameters a considerable damage to tissues is needed underscoring that the current status of disease cannot be determined by clinical parameters.[15] Our hypothesis in this study

Table 2: Comparison of healthy and T2DM groups of HbA1c with Hb conc. (in µg/mL), PPD and CAL

Variable	*Groups*	*n*	*Mean*	*SD*	*SE*	*t value*	*p value*
Salivary Hb conc. (µg/mL)	HbA1c (≤5.6%) controls	20	0.00	0.00	0.00	−3.7710	<0.001
	HbA1c (>6.4%) cases	20	0.30	0.35	0.08		
PPD	HbA1c (≤5.6%) controls	20	1.78	0.67	0.15	−13.9023	<0.001
	HbA1c (>6.4%) cases	20	4.12	0.31	0.07		
CAL	HbA1c (≤5.6%) controls	20	1.37	0.62	0.14	−9.3759	<0.001
	HbA1c (>6.4%) cases		3.98	1.09	0.25		

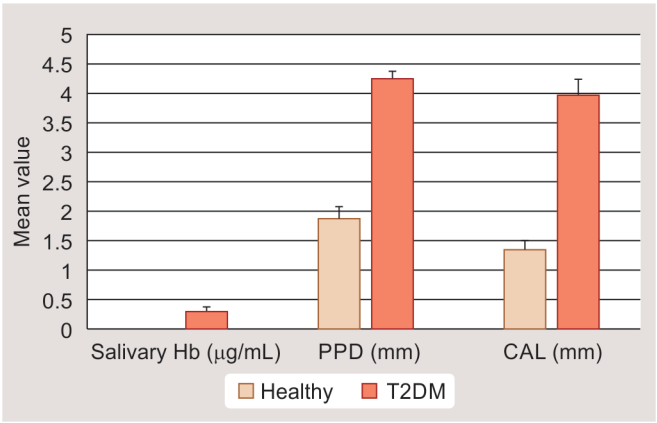

Fig. 2: Comparison of salivary hemoglobin concentration, PPD, and CAL between healthy and type II diabetic group; *p* value < 0.001

was to embark on the use of salivary molecule such as salivary hemoglobin to offer a noninvasive and valid measure in early detection of periodontal disease, thereby contributing toward assessing and monitoring periodontal disease. This can envisage the concept of individualized point-of care diagnostics by speeding up treatment decision and its implementation.

The value of evaluation of salivary hemoglobin levels to predict the severity of periodontal disease is underlined in the literature as it measures blood leaked into saliva due to destruction of the oral epithelial barrier.[36] In this study clinical parameters, such as BOP, PPD, and CAL were found to correlate strongly with salivary hemoglobin levels as evident in the previous studies.[21–25] Similarly, in this study, HbA1c levels were positively correlated with and statistically significant in chronic periodontitis subjects in comparison with healthy controls with PPD, CAL also showing positive correlation with HbA1c as illustrated by the scatter plot diagram (Figs 4 and 5, respectively).

In this study, HbA1c levels were considered while the disease extent among subjects with fasting and postprandial glucose levels was not correlated. The level of gingival inflammation and oral hygiene was however not significantly associated with glycemic control. Well-controlled diabetic patients in our study had a good periodontal status, and hence, glycemic control should be viewed as a regimen for periodontal health, which also suggests that there may be a threshold in the general population above which periodontal disease affects HbA1c values. However, larger sample size is required to confirm this finding. Studies based on the magnitude of reported HbA1c reduction following periodontal management and care, ranges from 0.27% to 0.48% at 3–4 months.[37] Studies have demonstrated a reduction of A1C by 0.6% after periodontal therapy in the absence of changes in medication and by 1.4% if changes in diabetes medications are introduced,[38] therefore emphasizing the need to consider treatment of periodontal diseases as an integral part of diabetes control.

To our knowledge, this is the first study conducted to find empirical evidence of the association between chronic periodontitis, salivary hemoglobin, and HbA1c levels. This study demonstrated that increase or decrease in HbA1c led to a significant increase or decrease in salivary hemoglobin. It is plausible that this significant association is resulting from impaired glucose levels that have raised the risk of periodontal disease with concurrent levels of increased hemoglobin potentiating the virulent effects of lipopolysaccharide from periodontopathogens, which stimulate high levels of inflammatory cytokines leading to insulin resistance and hyperglycemia.[39] Free haem present in the local periodontal environment is possibly a significant component contributing to the progression of periodontal disease.[20] However, this needs to be verified in future studies and further research in this area may ultimately lead to new strategies to prevent progression of periodontal disease and impaired glycemic control in T2DM. Although the data collected in this study were with careful quality control and the sample statistically enough to give us good power to evaluate the association, the study findings envision the limitation of its small sample representative of only one clinical setting that may be confounding the correlation between salivary Hb levels and clinical parameters.

Unstimulated whole saliva measurement is adopted by many studies to evaluate biomarkers representing various phases initiation and progression of periodontitis.[15,40,41] Many factors affect the collection of unstimulated saliva, namely the body posture, degree of hydration, and position of head during collection, circadian rhythm, etc.[42,43] To counteract this, the fasting unstimulated saliva sample was collected taking into consideration the standard method and sampling time for each subject.

Limitations and Recommendations for Future Research

This descriptive hospital-based study has many limitations. Salivary hemoglobin levels were evaluated at a single time point and in single clinical setting. It is required for a multicenter, large sample-sized prospective study with multiple measurements at different time intervals to elucidate the impact of blood sugar levels and their effect on the periodontium. As previously mentioned, in view of the multifactorial characteristic of periodontitis and T2DM monitoring factors of current and previous oral hygiene practices, change in medication, duration of the medication protocol as associated with a specific biological stage of periodontitis, and its impact on the health and number of remaining teeth are of necessity. The scope of this research was to ascertain the relevance of salivary hemoglobin levels and its association of disease and health. Sensitivity and specificity of this biomarker in comparison with other salivary

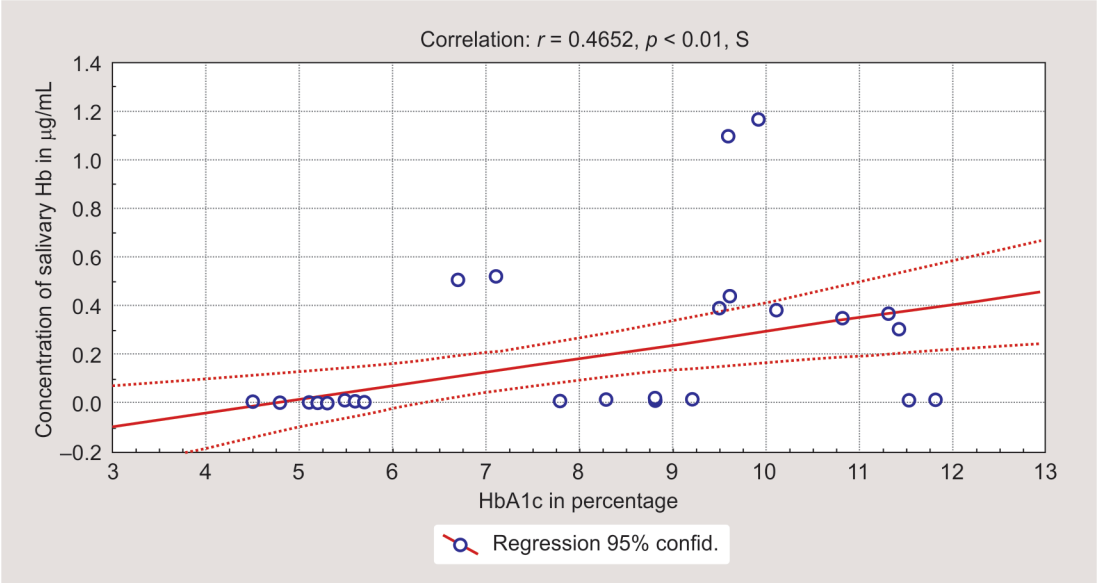

Fig. 3: Scatter diagram of the Pearson's correlation analysis showing significant positive correlation between salivary Hb and HbA1c percentage

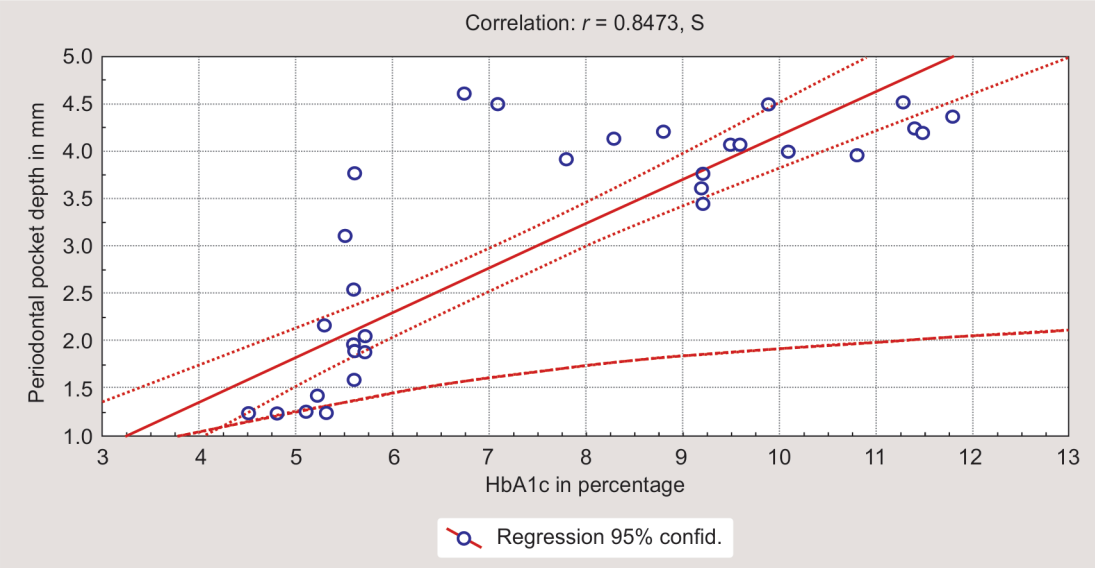

Fig. 4: Scatter diagram of the Pearson's correlation analysis showing significant positive correlation between periodontal pocket depth and HbA1c percentage

Table 3: Correlation between HbA1c with salivary Hb conc. (in µg/mL), PPD and CAL by Karl Pearson's correlation coefficient

	HbA1c		
Variables	*r value*	*t value*	*p value*
Salivary Hb	0.4653	3.2404	<0.01
PPD	0.8474	9.8364	<0.001
CAL	0.7992	8.1952	<0.001

biomarkers for identification and discriminating periodontitis in T2DM in the population need to be investigated.

Conclusion

Monitoring of salivary hemoglobin can be considered a valuable tool in clinical practice for diagnosing periodontal disease among risk groups. The results of this study evidenced that type II diabetic adults have high prevalence of chronic periodontitis and exhibit elevated salivary hemoglobin indicating a valuable noninvasive screening method for detecting periodontitis. Comparison of salivary hemoglobin in healthy and type II diabetic condition showed an increased salivary Hb concentration in the diabetic condition over healthy subjects indicating the susceptibility of the diabetic condition with chronic periodontitis.

Active efforts to include primary prevention for chronic dental diseases need to be a part of the country's efforts to control the burden of noncommunicable diseases. This is attainable with promotion of multidisciplinary management of diabetics that encompasses comprehensive periodontal and endocrinologist expert management. Early detection of periodontal diseases can lead to an effectual way of preventing tooth loss, insulin resistance, and complications.

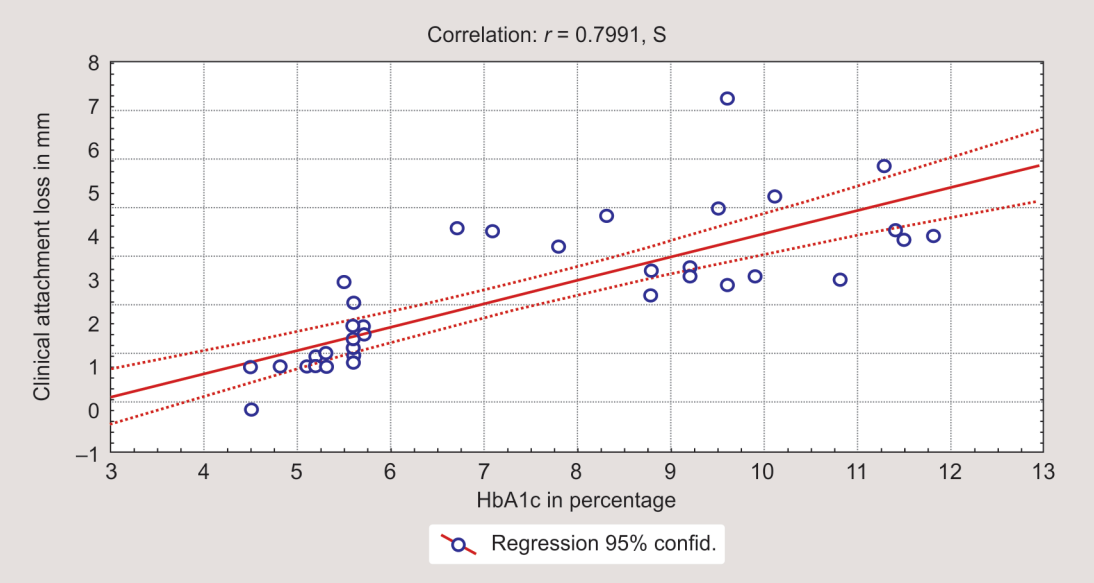

Fig. 5: Scatter diagram of the Pearson's correlation analysis showing significant positive correlation between HbA1c percentage and CAL

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