

Comparative Evaluation of the Efficacy of Gingival Crevicular Blood with Finger Capillary Blood and Venous Capillary Blood to Assess Blood Glucose Levels for Screening of Diabetes Mellitus in Chronic Periodontitis Patients: A Cross-sectional Study

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ABSTRACT

Introduction: Diabetes mellitus is defined as a clinically and genetically diverse cluster of illnesses involving conflict in carbohydrate and protein metabolism. Periodontitis is a complex condition with several causes. The interaction between these two conditions appears to be cyclical as well as bidirectional. Gingival crevicular blood obtained through routine periodontal oral assessment could be utilised for blood glucose estimation.

Aim: To examine the efficacy of gingival crevicular blood elicited during routine periodontal probing, as a reliable source for screening of diabetes mellitus, and to compare it with finger capillary blood and venous capillary blood in chronic periodontitis patients.

Materials and Methods: This cross-sectional, in-vivo, clinical study was conducted in the Department of Periodontology at Karnavati School of Dentistry, Gandhinagar, Gujarat, India, from January 2021 to January 2022. The study included 50 patients, who were diagnosed with chronic periodontitis in the age range ≥30 years, and met the inclusion and exclusion criteria. A prior detailed history was compiled. The clinical parameters recorded were sulcus bleeding index, plaque index, gingival index, probing pocket depth, and clinical attachment level. Each patient's blood samples were collected from three different sites, Gingival Crevicular Blood (GCB) collected from gingival crevice, Finger Capillary Blood (FCB) collected from finger bed and and Venous Capillary Blood (VCB) collected from forearm for determining the

blood glucose levels. Glucose levels were compared by one-way Analysis of Variance (ANOVA). Karl Pearson's correlation was used for the comparison. Statistical Package for Social Sciences (SPSS) version 26.0 was used for statistical analysis and p-value ≤0.05 was considered as statistically significant.

Results: The mean Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) was 5.5 ± 0.61 mm and 6.76 ± 0.82 mm, respectively. The mean Plaque Index (PI) and Gingival Index (GI) score was 1.41 ± 0.25 and 1.45 ± 0.21 , respectively. The mean GCB, FCB, and VCB glucose level of the subjects were 171.58 ± 85.63 mg/dL, 179.14 ± 80.31 mg/dL and 186.96 ± 87.57 mg/dL, respectively. There was no statistical difference seen among the three methods, thus, either of the methods can be used for measuring blood glucose levels for screening of diabetes mellitus in chronic periodontitis patients (p-value=0.66). Positive correlation between FCB and VCB ($r\text{-value}=0.976$, $p\text{-value} < 0.001$). VCB and GCB, when correlated showed strong positive and highly statistically significant results ($r\text{-value}=0.934$, $p\text{-value} < 0.001$). Similarly, GCB and FCB showed a positive correlation ($r\text{-value}=0.920$, $p\text{-value} < 0.001$) which was statistically highly significant.

Conclusion: The results suggested that, the efficacy of gingival crevicular blood when compared with finger capillary blood and venous capillary blood glucose levels showed positive correlation, suggesting either can be used in dental clinics for diabetic screening purpose without any extra invasive procedures.

Keywords: Diagnostic aid, Glucometer, Glycated haemoglobin, Non invasive, Screening tool

INTRODUCTION

Diabetes Mellitus (DM) is one of the world's most commonly occurring epidemic diseases. It is heterogeneous, with the common feature of impaired glucose tolerance with altered lipid and carbohydrate metabolism [1]. Diabetes mellitus puts a significant strain on the healthcare system. Diabetes is becoming more common all across the world, particularly in India [2].

Diabetes was responsible for 10.7% of all deaths in people aged 20 to 79 in 2017, with approximately four million deaths attributed directly or indirectly to diabetes or high blood glucose [3]. It is the world's fastest growing chronic condition, on track to become the sixth leading cause of death by 2030, according to predictions [3].

Also, more than 50% of diabetic patients go undiagnosed. The classic complications of diabetes mellitus i.e. retinopathy, neuropathy, nephropathy, macrovascular disease, altered wound healing and periodontitis which has been added as a sixth complication. Severity of periodontitis and its incidence is markedly influenced by DM and also various strong evidences have shown the same [4]. A close inter relationship exist between the two signifying that they both are associated with advancing age, are asymptomatic with a bidirectional relationship.

Diabetic screening at the dental office is usually done based on the patient's medical history and symptomatic analysis, but it has drawbacks, such as a lack of precision, objectivity, and the ability to detect diabetes early [5]. Also, diabetics on rigorous

treatment regimens or long-term dental therapy are more likely to be hypoglycemic during dental treatment. As a result, it is a dental practitioner's job to do diabetic screening and rule out undetected diabetes for specific physical issues, which could affect the treatment approach.

Dentists can diagnose and relate periodontal disease and other oral manifestation of DM that many physicians may find it less familiar and difficult to relate to [6]. Blood samples required to test blood glucose may be obtained within the mouth from the gingival crevicular area during routine periodontal examination [7]. This gives a unique opportunity for Oral Health Program (OHP) in screening for DM. Other advantages include previous experience of treating patients with medical condition that puts OHP in a bright spot for screening systemic condition like DM and Cardiovascular Disease (CVD) [8].

A field trial, utilising invasive and non invasive risk test carried out for screening DM in different dental settings in Rhodes Island, had identified little over 12% of patient diagnosed with DM and another 23% at high risk of developing the disease among the 45 year and older patients, who were unaware of the DM status at the time of presentation but later diagnosed with DM (within a year of follow-up) and prediabetes [9].

As a result, early identification of diabetes in its early stages is a top goal in healthcare. To avoid this potentially unfavorable life situation, appropriate screening devices and standardised methods are essential [10]. Despite the fact that the finger-prick method is a non invasive approach for measuring blood glucose directly and accurately. The conventional laboratory methods such as that are employed to screen for diabetes are time consuming and necessitates elaborate equipment. The advent of blood glucose monitors allows the clinicians to assess blood glucose levels at the chair side. Since, periodontal disease and diabetes are closely associated, it is likely that dentists and more so periodontists would encounter several diabetics, many of whom could be undiagnosed. Screening such patients in dental office itself could hence be beneficial [11]. Patients with undiagnosed diabetes mellitus are at greater risk for complications like coronary heart disease, stroke and peripheral vascular disease. The American Diabetes Association recommends that diabetes screening should begin at 45 years of age and be repeated every three years in and sooner and more frequently in people with risk factors such as diabetes [12]. The basic laboratory measures for screening includes fasting blood glucose, glucosuria, Haemoglobin A1c (HbA1c) and Oral Glucose Tolerance Test (OGTT) [13].

The best indicator for estimating diabetes prevalence and incidence is Fasting Plasma Glucose (FPG) which is used commonly. Fasting plasma glucose concentration of >7.0 mmol/L (>126 mg/L) is an indication for retesting. For centralised screening the analysis of HbA1c from a blood drop is recommended, though this approach is more expensive than FPG [13]. The conventional laboratory methods used for blood glucose detection are more time consuming and require elaborate equipment in contrast to the modern blood glucose monitors which enable clinicians to perform allow chair side assessment of blood glucose [11].

The present study was conducted to examine the efficacy of gingival crevicular blood elicited during routine periodontal probing as a reliable source for screening of diabetes mellitus, and to compare it with finger capillary blood and venous capillary blood in chronic periodontitis patients.

MATERIALS AND METHODS

This cross-sectional, in-vivo, clinical study was conducted in the Department of Periodontology at Karnavati School of Dentistry, Gandhinagar, Gujarat, India, from January 2021 to January 2022. The Karnavati School of Dentistry's research review board

committee gave their approval to the study (KSDEC/20-21/Apr/010). Patients were selected from the Outpatient Department of Periodontics. Total 50 patients were who were diagnosed with chronic periodontitis with age ≥30 years and who met the inclusion and exclusion criteria were included in the study. Prior to the start of the study, each subject was told about the protocol and gave their informed consent. After obtaining the informed consent, study procedures were performed.

Sample size calculation: The sample size was calculated using the following formula:

$$N = \frac{(Z\alpha)^2 (S)^2}{(d)^2}$$

Where,

Zα=1.96 (assuming the distribution was normal and confidence limit was 95%)

S=Anticipated from pilot study=35 (A pilot study was done on 10 patients before the start of the study, where standard deviation of 35 mg/dL was observed).

d=Minimum difference to be detected=10

Substituting the values in the formula, Sample size (n) was calculated to be 47.04. Hence, final sample size was 50.

To evaluate their daily routine, mood, and responsiveness, they were asked a series of routine questions. The routine questions included their daily life style regarding daily schedule, diet, habits like smoking and sleeping cycle as they directly or indirectly affects blood glucose levels. Blood samples were obtained from three different locations for each individual prior to any therapy.

Inclusion criteria: Patients with diagnosis of chronic periodontal disease based on criteria proposed by World Workshop on Periodontology, 2017 [14], thus, periodontal disease with presence of interdental Clinical Attachment Loss (CAL) at ≥2 non adjacent teeth, or buccal or oral CAL ≥3 mm with pocketing >3 mm was detectable at ≥2 teeth. Patients having atleast 20 teeth present in upper and lower jaws and those who gave an informed consent to participate were included in the study.

Exclusion criteria: Patients diagnosed with bleeding disorders like platelet function defects such as Von Willebrand disease, purpura, haemophilia were excluded from the study. Patients taking medication interfering with the coagulation system such as aspirin and warfarin and patients undergoing treatment of anaemia, polycythaemia, gout, dialysis or any other disorder, that can cause abnormal variation in the haematocrit were also excluded from the study.

Study Procedure

A detailed case history was compiled. The clinical intraoral examination was done in a dental chair with diagnostic tools including a mouth mirror tweezers, a straight explorer, a UNC-15 probe and a William's probe by Quix™ from Hu-Friedy group single-ended, colour-coded ergonomic handle with normal illumination. The clinical parameters recorded were Sulcus Bleeding index (Mulhemann HR and Son S, 1971) [15], Plaque Index (Loe H and Silness J, 1963) [16] Gingival Index (Loe H and Silness J, 1963) [16]. Using a Williams's periodontal probe, the Periodontal Pocket Depth (PPD) from the gingival margin to the bottom of the gingival sulcus was measured. The clinical attachment level measured from Cementoenamel Junction (CEJ) to the base of the pocket was measured with the UNC-15 periodontal probe. The study participants underwent history taking, complete clinical and periodontal examination and blood investigations by single examiner only.

After history taking and periodontal examination patients with chronic periodontitis were made sit on chair side in-office blood sample estimation for blood glucose levels from:

- Gingival Crevicular Blood (GCB)
- Finger Capillary Blood (FCB)
- Venous Plasma Blood (VPB)

Patients were asked to use chlorhexidine mouthwash rinse before the screening. All of the GCB and FCB blood samples were analysed using a DrMorepen BG® glucose meter. At the same point of time, laboratory venous blood glucose estimation was done.

Gingival crevicular blood collection procedure: For estimation of blood glucose level using gingival crevicular blood, the test site was isolated with cotton roll and air dried with three-way syringe. Blood oozed out from gingival margin of the selected site was collected on to the test-strip. Site with profuse bleeding on probing was chosen. Sites which bleed easily during clinical examination were selected. Williams periodontal probe (Quix™) was used for gingival probing. When a sufficient quantity of blood was obtained, it was collected directly on the test strip of glucometer. After proper sample collection on the test strip, the glucometer unit was turned on and GCB glucose readings were recorded [Table/Fig-1]. The glucometer gives blood glucose value in about five seconds. The value was noted as mg/dL Gingival Crevicular Blood Glucose (GCBG) value.

Finger capillary blood collection procedure: The surface of the fingertip was wiped with surgical spirit to estimate blood glucose levels using finger capillary blood. A sterile lancet was used to puncture the surface of the finger, and a drop of blood was allowed to be drawn to the test strip area. The FCB glucose measurements were recorded with glucometer [Table/Fig-2]. The strip was inserted into the glucometer and the value obtained was recorded as Peripheral Finger stick Blood Glucose (PFBG) value.

Venous capillary blood collection procedure: The estimation of blood glucose level utilising venous blood was performed immediately after these two procedures. Using a sterile syringe and needle, blood was collected by puncturing the anterior cubital vein [Table/Fig-3]. A 2 mL of blood was collected in a plane bulb and analysed. Glucose readings will be noted. The glucose estimation method employed was: GOD=POD method (combined action of glucose oxidase and peroxidase) [17]. Glucoseoxidase (GOD) converts-D glucose to gluconic acid and generates hydrogen peroxide as a by product (H_2O_2). The Peroxidase (POD) enzyme produces nascent oxygen (O_2) from hydrogen peroxide, which is then combined with 4-amino antipyrine and phenol to generate red quinoneimine colour. The amount of glucose in the plasma is directly proportional to the intensity of the colour [18].

STATISTICAL ANALYSIS

Mean and standard deviation for each clinical parameter was calculated. The GCB, FCB, and VCB glucose levels were recorded as mean and standard deviation. To compare the significance of the difference between the three readings, one-way Analysis of Variance (ANOVA) was performed and Karl Pearson's correlation (r) was done to know the correlation between the three methods.

Statistical Package for Social Sciences (SPSS) version 26.0 was used for statistical analysis and p -value ≤ 0.05 was considered as statistically significant.

RESULTS

A total of 50 patients were included in the study with the age group 30 years and above fulfilling the inclusion criteria out of which 27 were known diabetics 23 were non diabetics. Out of 50 patients, 32 were females and 16 were males. Male to female ratio was 18:32. Diabetes showed a higher prevalence amongst females. The mean age was 51.57 ± 13.27 years. The mean PPD was 5.5 ± 0.61 mm and mean values of the CAL was 6.76 ± 0.82 mm. The mean sulcus bleeding index score was 2.55 ± 0.90 . The mean PI score was 1.41 ± 0.25 and mean gingival index score was 1.45 ± 0.21 [Table/Fig-4].

The mean FCB glucose was 179 ± 80.31 mg/dL, mean VCB glucose was 186 ± 87.57 mg/dL and GCB glucose was 171 ± 85.63 mg/dL. No significant difference between three types of readings (p -value=0.66) [Table/Fig-5]. Partial eta square was 0.006 which means that 0.6% variance was found between the different techniques which can be attributed to different reading methods, therefore, any of the methods can be used for measurement of blood glucose level. Karl Pearson's correlation was used to correlate GCB and FCB, GCB and VCB and also GCB and FCB values. Strong positive and highly significant correlation was observed between FCB glucose and VCB glucose values (r -value=0.976, p -value <0.001) [Table/Fig-6]. The VCB and GCB when correlated showed strong positive and highly statistically significant results (r -value=0.934, p -value <0.001) [Table/Fig-7]. Similarly, GCB and FCB showed a positive correlation (r -value=0.920, p -value <0.001) which was statistically highly significant [Table/Fig-8].

DISCUSSION

Diabetes increases inflammation in the periodontal tissues [19]. Over the last century, advances in science and technology have substantially increased our understanding of the pathophysiology of periodontal disease. Even though periodontal disease is an infectious illness, it can be influenced by certain systemic disorders. Diabetes mellitus is clinically and genetically a diverse illnesses involving carbohydrate and protein metabolism. There has been a lot of research done on the relationship between diabetes and inflammatory periodontal disease [20]. The interaction between these two conditions appears to be cyclical as well as bidirectional. This has been confirmed in extensive reviews by Oliver RC and Tervonen T, and by Rees TD et al., [4,21]. Appropriately periodontal disease has been recognised as the sixth complication of diabetes [22]. In fact, diabetes and periodontitis seem to interact in a bidirectional manner [23].

Periodontal inflammation, with or without a DM complication factor, is known to produce spontaneous bleeding from gingival sulcus on probing, during a diagnostic periodontal examination, it can be utilised to estimate random blood glucose levels [24].



[Table/Fig-1]: Estimation of gingival crevicular blood glucose levels with a glucometer. [Table/Fig-2]: Estimation of finger capillary blood glucose values. [Table/Fig-3]: Withdrawal of venous capillary blood for laboratory VBG estimation. (Images from left to right)

Variable	n	Maximum	Minimum	Mean±SD
Probing depth (in mm)	50	5	7	5.5±0.61
Clinical attachment level (in mm)	50	6	9	6.76±0.82
Sulcus bleeding index	50	1.10	3.92	2.55±0.90
Plaque index	50	1.10	2.40	1.41±0.25
Gingival index	50	1.07	1.92	1.45±0.21

[Table/Fig-4]: Mean probing depth, sulcus bleeding index, clinical attachment level, plaque index and gingival index of study participants.

Method of glucose estimation	Mean±SD (mg/dL)	Standard error	95% Confidence interval for mean		df	F	p-value (ANOVA)
			Lower Bound	Upper Bound			
GCBG	171.58±85.63	12.11	147.2433	195.9167	2	0.414	0.66
VCBG	186.96±87.57	12.38	162.0708	211.8492			
FCBG	179.14±80.31	11.35	156.3153	201.9647			

[Table/Fig-5]: One way ANOVA for the comparison of Finger Capillary Blood (FCB), Venous Capillary Blood (VCB) and Gingival Crevicular Blood (GCB) glucose levels.
p-value <0.05 was considered as statistically significant

FCB glucose level (mg/dL)	VCBG (186.10±87.57)		
	Mean±SD	r-value	p-value
179.02±80.31	186.10±87.57	0.976	0.001

[Table/Fig-6]: Pearson's correlation coefficient between Finger Capillary Blood Glucose (FCBG) and Venous Capillary Blood Glucose (VCBG).
p-value ≤0.05 was considered as statistically significant

GCB glucose level (mg/dL)	Venous capillary blood glucose		
	Mean±SD	r-value	p-value
171.58±85.63	186.10±87.57	0.934	0.001

[Table/Fig-7]: Pearson's correlation coefficient between Gingival Crevicular Blood Glucose (GCBG) and Venous Capillary Blood Glucose (VCBG).
p-value ≤0.05 was considered as statistically significant

GCB glucose level (mg/dL)	FCB glucose level		
	N	r-value	p-value
171.58±85.63	179.02±80.31	0.920	0.001

[Table/Fig-8]: Pearson's correlation coefficient between Gingival Crevicular Blood Glucose (GCBG) and Finger Capillary Blood Glucose (FCBG).
p-value ≤0.05 was considered as statistically significant

The gold standard method of measuring blood glucose level in diabetic patients is venous plasma glucose that has the highest level of diagnostic accuracy [25], and it must be compared to the further two different non invasive methods in order to evaluate the correlation of different methods for screening for blood glucose levels. Partheeban I et al., found in their study a strong positive correlations between these three methods in both diabetic and non diabetic patients, where they have used venous blood glucose level as a gold standard, the sensitivity and specificity of fasting plasma glucose was 93% and 100%, respectively in both their groups [26]. The mean and SD of FCB was 179±80.31 mg/dL, VCB was 186±87.57 mg/dL and GCB was 171±85.63 mg/dL. The ANOVA showed no significant difference between all three types of readings (p-value=0.66). That shows no difference in any of the method for blood glucose measurement.

Earlier studies done by Stein GM and Nebbia AA [27], Beikler T et al., [28], Khader Y et al., [29] and most recent by Rapone B et al., [30] sought to demonstrate that extravasated blood from the gingival crevice as a result of bleeding on provocation during periodontal examination, can be used to measure blood glucose in diabetes patients these findings were similar to the present study. According to the current study findings, Pearson's correlation coefficients between VCB and FCB, FCB and GCB and GCB and VCB were 0.976, 0.920 and 0.934, respectively, which were statistically highly significant respectively suggesting a strong positive correlation (r). The result of the present study is similar with most of the previous

studies of Shetty S et al., [31] and Strauss SM et al., [32] which suggest that GCB sample analyses are satisfactory for screening diabetic patients with adequate bleeding on provocation, and that samples can be obtained quickly.

Results of study conducted by Jain S et al., [33] were similar to the present study where Pearson's correlation showed a strong positive correlation between the two measurements (r -value=0.893, p-value <0.001). A recent study done by Vummidi AV et al., also demonstrated, strong positive correlation between gingivitis and

periodontitis patients [34]. The present study data reveals that estimating sulcular blood glucose levels has an association with capillary and venous blood glucose levels, implying that measuring sulcular blood could be a useful tool in diagnosing diabetes patients. The ability to do the test at the chair side and the lack of a long wait time for results are two of the advantages of GCB glucose testing. In addition, a dentist can carry out the treatment [30].

According to the American Diabetic Association, blood glucose monitoring devices have a prediction error of less than 15% of the laboratory norm [35]. Also, dental practitioners prefer intraoral sampling for DM screening since the sample may be taken during normal scaling and the strip system provides a more objective indicator for physician referral than the usual medical history review and observation of symptoms [36]. In the present study, GCB was used for blood glucose estimation in all 50 subjects. Out of 50 subjects, 27 were known diabetics 11 were non diabetics and 12 were diagnosed of being diabetic at the time of chair side screening. Patients who had just been diagnosed with diabetes, were then referred to a physician, who validated the results using fasting blood sugar and postprandial blood sugar estimation. Out of 50, there were 32 females and 16 males. Prevalence of diabetes was higher in the females.

Limitation(s)

Larger sample sizes with more precise outcome measurement should be used in future investigations.

CONCLUSION(S)

Gingival crevicular blood obtained during routine periodontal examination can be used for in-office diabetic screening. When correlated with the FCB and VCB blood glucose measurements (r -values=0.976 and 0.934) which shows strong positive correlations. The GCB glucose estimation is a safe, easy to perform, non invasive, less time-consuming chair-side method for diabetic screening. It can be used for diagnosing undiagnosed asymptomatic patients with diabetes in dental office and can prove to be a reliable method for referral to physician.

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