



RESEARCH ARTICLE

ASSESSMENT OF BLOOD GLUCOSE LEVEL USING GINGIVAL CREVICULAR BLOOD IN DIABETIC PATIENTS: A CROSS SECTIONAL STUDY

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ABSTRACT

Background and Aims: A high number of patients with periodontitis may have undiagnosed diabetes. It is possible that gingival crevicular blood from routine periodontal probing may be a source of blood for glucose measurements. The purpose of the study was to evaluate whether the blood oozing from the gingival crevice during routine examination could be used for determining glucose levels.

Materials and Methods: 100 patients with periodontitis and positive bleeding on probing were chosen. Blood samples of two sites were analyzed using a glucose self-monitoring device. In 50 diabetic and 50 non-diabetic patients, after testing fasting plasma glucose (FPG), glucose levels in gingival crevicular blood (GCB), and capillary fingerstick blood (FSB) samples were analyzed using the same device.

Results: The patient's blood glucose values ranged from 69.5-235.8 mg/dl. The comparison between Gingival Crevicular blood, Fingerstick blood and Intravenous blood showed a very strong correlation with a r value of 0.99 (P < 0.001).

Interpretations and Conclusions: The data from this study has shown that GCB collected during intraoral examination is an excellent source of blood for glucometric analysis.

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INTRODUCTION

Diabetes mellitus is one of the most frequent metabolic disorders and nearly half the cases are undiagnosed (Harris *et al.*, 2000). Diabetes and periodontitis seem to interact in a bidirectional manner (Grossi and Genco, 1998). The increased prevalence and severity of periodontitis seen in patients with diabetes, especially those with poor metabolic control, has led to the designation of periodontal disease as the "sixth complication of diabetes", (Loe, 1993; Ainamo *et al.*, 1990; Nishimura *et al.*, 1998) and successful periodontal therapy in diabetic patients entails the stabilization of blood glucose to a normal range (Loe, 1993; Sastrowijoto *et al.*, 1990).

In 2005, approximately 177 million people worldwide have diabetes mellitus, and this number may double by the year 2030. India ranks first in the highest numbers of people with diabetes in 2000 with 31.7 million people and it is estimated that by 2030 the diabetic patients in the nation may go up to 79.4 million (Lt Gen *et al.*, 2009). Diabetes mellitus is one of the most frequent metabolic disorders with an estimated prevalence of 7% in industrialized countries, of which nearly half the cases are undiagnosed (Hadden and Harris, 1987; Rees, 2000). Considerable effort has been made in the past few years to develop painless and noninvasive methods to measure blood glucose (Ervasti *et al.*, 1985). Glucometers are commonly used by diabetic patients for monitoring of blood glucose levels at home. Since periodontal inflammation, with or without the complicating factor of diabetes mellitus, is known to produce ample extravasated blood during diagnostic procedures (Klonoff *et al.*, 1997), and routine probing during a periodontal

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examination is more familiar to the practitioner and less traumatic compared to a finger-puncture with a sharp lancet, these devices may actually allow for painless testing of blood oozing from the gingival crevices of patients with periodontal problem during routine periodontal examination and could be a simple and relatively inexpensive in-office screening device for any patient suspected to have diabetes. They can also be used to monitor blood glucose levels in known diabetics (Kost *et al.*, 2000).

Recently, more sensitive self-monitoring devices have been developed for testing small amounts ($< 2 \mu\text{l}$) of blood and the accuracy of these glucometers has been acceptable (Rheney and Kirk, 2000). The blood glucose monitoring system used in this study is ONE TOUCH SELECT SIMPLE, which works on the principle of electrochemical methodologies. The aim of the present study was to assess the reliability of a glucose self-monitoring device for testing gingival crevicular blood glucose, comparing crevicular and fingerstick blood glucose measurements with the standard laboratory venous blood glucose measurement in diabetic and non-diabetic patients.

MATERIALS AND METHODOLOGY

The study was carried out in the Department of periodontics and oral implantology Govt. Dental College and Hospital, Srinagar. Ethical clearance was obtained for the study and a written informed consent was obtained from every patient before performing the test. A total of 100 patients with generalized moderate to severe chronic periodontitis of age group between 30 and 70 years were taken for the study. They were divided into two groups, group I and group II. Group I comprised of diabetic patients and group II comprised of Non-Diabetic patient's age and sex matched controls. OPG was taken for both the groups to check the status of generalized moderate to severe chronic Periodontitis. Patients were selected based on the following criteria:

- Patients with known history of diabetes and Non Diabetic patients with generalized moderate to severe Periodontitis diagnosed clinically with presence of periodontal pockets and radiographically with bone loss were selected.
- Patients with the history of bleeding disorders, polycythemia, Patients on anticoagulant therapy, NSAIDs, severe cardiovascular, hepatic, immunologic, renal, hematological, or other organ impairment ascorbic acid medications and pregnant patients were excluded from the study.

GLUCOSE MEASUREMENT

Gingival Crevicular Blood (GCB)

As maxillary anterior teeth offer an ideal access for the collection of gingival crevicular blood, the gingival crevicular blood from either of the maxillary central incisors were taken for estimation of blood glucose levels. For each measurement, only one site with bleeding on probing was selected. Sites with suppuration were excluded from the study. After selecting the bleeding site, the site was isolated with cotton rolls. The interdental papilla between the central incisors was probed

with UNC - 15 probe. As soon as the probe was removed, the gingival crevice was observed for bleeding. At this stage, the test end of the strip (mounted on the glucose monitoring device already) was kept on to the bleeding site to obtain the blood sample on the test strip without contacting the gingival or palatal tissues. The test strip was held until the instrument beeped giving the blood glucose measurements in mg/dl (Fig. 1).



Fig 1. Estimation of blood glucose using Gingival Crevicular Blood (GCB)

Fingerstick Blood

The pulp of the finger was wiped with the surgical spirit and spirit was allowed to evaporate. The finger was then punctured with a sterile lancet. The first drop of blood was discarded and the second drop of blood was touched to the test end of the strip. It was held until the instrument gave a beep displaying the blood glucose measurements on the screen in mg/dl (Fig. 2).

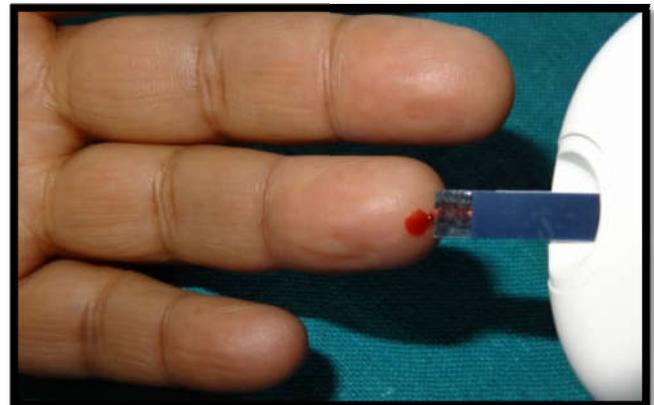


Fig 2. Estimation of blood glucose using fingerstick Blood (FSB)

Estimation of Blood Glucose Level using Venous Blood (VB)

Immediately after these two tests, a venous blood assessment was carried out. Blood was obtained by venipuncture from the anterior cubital vein, using sterile syringe and needle. 2ml of blood was collected in a plain bulb. It was centrifuged to separate the plasma from the cellular components of the blood. The reagent glucose oxidase was added to it, and this sample was immediately subjected to an Automated Chemistry analyze. The reading obtained on its screen was recorded as the level of venous blood glucose (Fig. 3)



Fig. 3. Estimation of Blood Glucose Level using Venous Blood (CVB)

Semi-automated analyzer

To analyze samples of venous blood from each patient, a reference laboratory glucose analyzer was used. This instrument was the standard type used for blood glucose determination. After the separation of cells, a glucose oxidase electrode was placed in the serum and the glucose concentration directly determined. The measurements were considered to be the true glucose concentration in this study. It is estimated that glycolysis by living cells in a vacuum tube of venous blood would decrease the glucose concentration by 7-10mg/dl per hour. To eliminate this factor, cellular metabolism of glucose was prevented by using vacuum tubes containing metabolic inhibitor fluoride.

Haematocrit measurement

Haematocrit (Hct) is the percentage of blood volume occupied by the red blood cells. This measurement was important because the glucose self-monitoring device measures whole blood glucose and the reference laboratory measures whole blood glucose in the remaining plasma after cell separation. To compare the measurements, haematocrit is used to correct the reference laboratory measurement for the concentrating effect on glucose due to loss of cell volume. Thus, the plasma measurements can be converted to whole blood measurements by the following formula.

Hct corrected venous glucose (mg/dl) = laboratory (mg/dl) X [1.0-(.0024 X Hct)]

As there is a natural physiological drop in the blood glucose concentration as it passes from a capillary (such as in the gingival crevice) area into a venous area due to normal cellular uptake of glucose. Thus, the measurements were corrected for direct comparison to the glucose self-monitor readings by addition of 3.5mg/dl (average drop being 2 to 5 mg/dl) to the above formula:

Corrected venous glucose = laboratory (mg/dl) X [1.0-(.0024 X Hct)] + 3.5mg/dl.

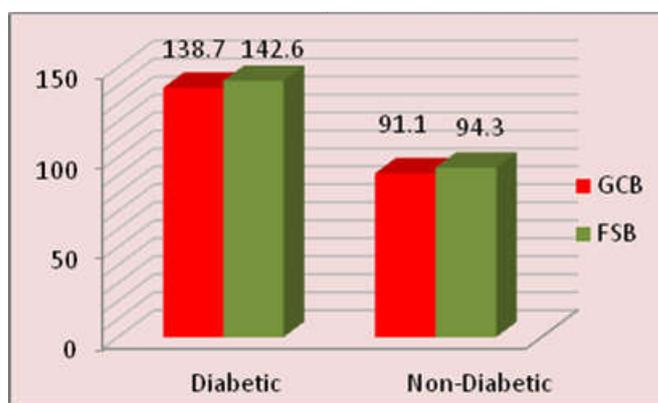
Statistical analysis

Descriptive data are presented as mean ± SD and range values. The difference between the measurements in the same individual was tested by paired 't' test. Pearson's correlation coefficient was used to assess the relationship between different measurements.

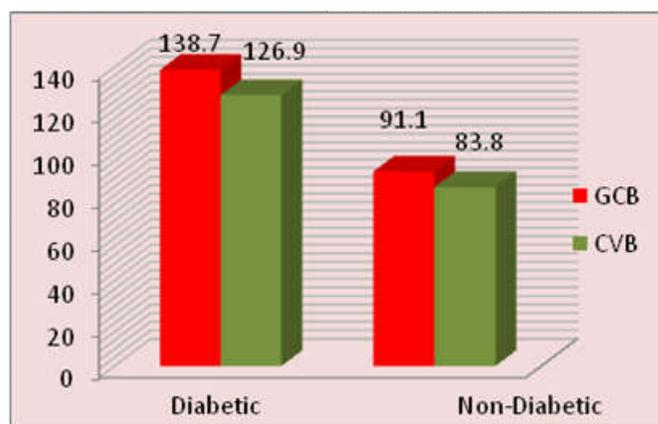
RESULTS

Comparison of Blood Glucose Levels of Gingival Crevicular Blood With Finger Stick Blood (Table - 1,Graph - 1)

On comparison of gingival crevicular blood glucose and fingerstick blood glucose measurements of Group I patients, fingerstick capillary blood glucose showed a mean value (142.6 mg/dl) than gingival crevicular blood glucose mean value(138.7 mg/dl) and showed a p-value >0.05, thus giving statistically non-significant results.

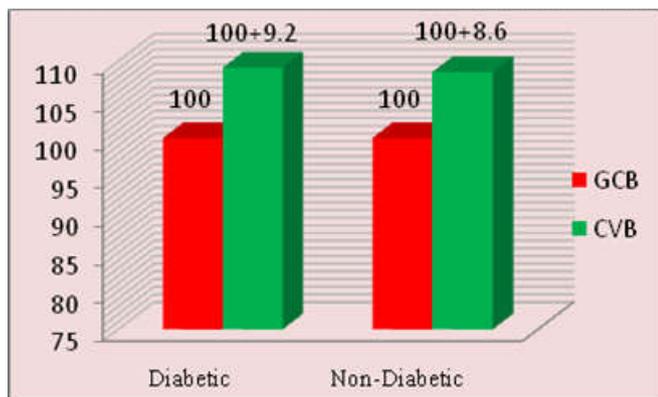


Graph 1. Comparison of Blood Glucose levels of GCB with FSB

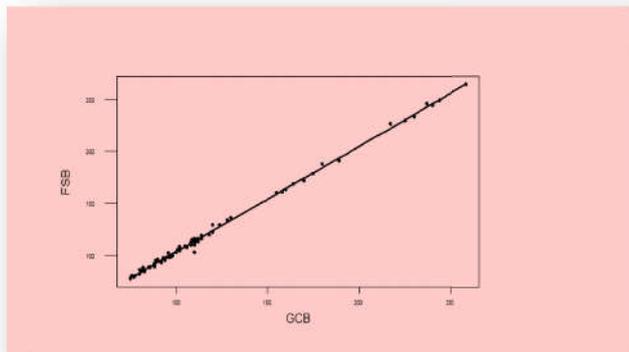


Graph 2. Comparison of Blood Glucose levels of GCB with Corrected Venous blood

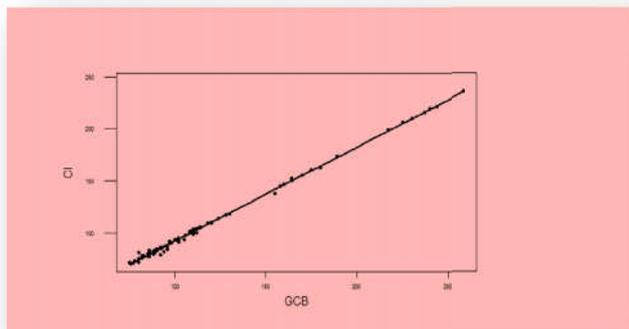
On intragroup analysis of Group II, a comparison was made between gingival crevicular blood glucose measurements and fingerstick blood glucose measurements, fingerstick blood glucose showed a mean value (94.3 mg/dl) than gingival crevicular blood glucose mean value (91.1 mg/dl), with a p-value of >0.05, thus giving statistically non-significant results.



Graph 3. Percentage Variation in Glucose Level of Gingival Crevicular Blood



Graph 4. Scatter diagram of relation between GCB and FSB



Graph 5. Scatter diagram of relation between GCB and CVB

Correlation (r) of Blood Glucose Levels of Gingival Crevicular Blood with Finger Stick Blood (Table -2, Graph - 4)

On comparison of gingival crevicular blood glucose and fingerstick blood glucose measurements of Group I patients, the Pearson’s correlation coefficient showed an r-value of 0.999 and a P-level <0.001, which shows a strong positive correlation of glucose level of gingival crevicular blood with ngerstick blood. On comparison of gingival crevicular blood glucose measurements and fingerstick blood glucose measurements of the Group II patients, the Pearson’s correlation coefficient showed an r-value of 0.982 and P-level of <0.001, which shows a strong positive correlation of

glucose level of gingival crevicular blood with ngerstick blood. Observations for the correlation of the glucose levels of gingival crevicular blood with ngerstick blood showed a signi cant positive correlation between the two measurements, in the diabetic and non-diabetic groups (p< 0.001).

Comparison of Blood Glucose Levels of Gingivitis Crevicular Blood With Corrected Venous Blood (Table - 3, Graph - 2)

On comparison of gingival crevicular blood glucose and Corrected intravenous blood glucose measurements of Group I patients, gingival crevicular blood glucose measurement showed a higher mean value (138.7 mg/dl) than Corrected intravenous blood glucose mean value (126.9 mg/dl).

On comparison between the two, value of ‘t’ was found to be 1.21, which was statistically non signi cant (p >0 .05). On comparison of gingival crevicular blood measurements and Corrected intra-venous blood measurements of Group II patients, the intra-venous blood glucose showed a mean value (83.8 mg/dl) than gingival crevicular blood glucose mean value (91.1mg/dl). On comparison between the two, value of ‘t’ was found to be 1.83, which was statistically non signi cant (p >0.05).

Correlation (r) Of Blood Glucose Levels of Gingival Crevicular Blood with Corrected Venous Blood. (Table - 4, Graph – 5)

On comparison of gingival crevicular blood glucose and Corrected intravenous blood glucose measurements of Group I patients, the Pearson’s correlation coefficient showed an r-value of 0.999 and a P-level was <0.001, which shows a strong positive correlation of glucose level of gingival crevicular blood with venous blood.

On comparison of gingival crevicular blood glucose measurements and Corrected intravenous blood glucose measurements of the Group II patients, the Pearson’s correlation coefficient showed an r-value of 0.981 and P level of <0.001 was derived, which shows a strong positive correlation of glucose level of gingival crevicular blood with venous blood. Observations for the correlation of the blood glucose levels of gingival crevicular blood with venous blood have a signi cant correlation between the two, in the diabetic and non- diabetic groups (p< 0.001).

Percentage Variation in Glucose Level of Gingival Crevicular Blood (GCB) Compared To Corrected Venous Blood (VB) (Table -5, Graph 3)

Table 5, Graph 3 shows that percentage difference of blood glucose levels obtained by the gingival crevicular blood with the help of a glucometer in comparison to Corrected venous blood laboratory method was 9.2% for the diabetic group and 8.6% for the non-diabetic group. These values were found to be within the range stipulated by the American Diabetic Association (Consensus Statement on Self, 1987). This indicates strong reliability of gingival crevicular blood for blood glucose assessment using a glucometer.

Table 1. Comparison of Blood Glucose levels of GCB with FSB

Group	Variable	Mean±SD	t-value	P-value	Remarks
Diabetic	GCB	138.7±51.15	0.38	0.70	Not Sig.
	FSB	142.6±52.19			
Non-Diabetic	GCB	91.1±12.04	1.33	0.19	Not Sig.
	FSB	94.3±11.96			

Pairedt-test, Unpairedt-test, $p < 0.001$ HighlySignificant,
 $p < 0.05$ Significant, $p > 0.05$ Nonsignificant

Table 2. Correlation of Blood Glucose levels of GCB and FSB in both Diabetic and Non-Diabetic group

Group	Pearson Correlation	P-value	Remarks
Diabetic	0.999	<0.001	Sig.
Non-Diabetic	0.982	<0.001	Sig.

Carl Pearson's Correlation Coefficient: +1=positive correlation, -1= negative correlation, 0= no correlation

Table 3. Comparison of Blood Glucose levels of GCB with Corrected Venous blood

Group	Variable	Mean±SD	t-value	P-value	Remarks
Diabetic	GCB	138.7±51.15	1.21	0.23	Not Sig.
	IV	126.9±46.3			
Non-Diabetic	GCB	91.1±12.04	1.83	0.08	Not Sig.
	IV	83.8±10.8			

Pairedt-test, Unpairedt-test, $p < 0.001$ HighlySignificant,
 $p < 0.05$ Significant, $p > 0.05$ Nonsignificant

Table 4. Correlation of Blood Glucose levels of GCB and CVB in both Diabetic and non-Diabetic group

Group	Pearson Correlation	P-value	Remarks
Diabetic	0.999	<0.001	Sig.
Non-Diabetic	0.981	<0.001	Sig.

Carl Pearson's Correlation Coefficient: +1=positive correlation, -1= negative correlation, 0= no correlation

Table 5. Percentage Variation in Glucose Level of Gingival Crevicular Blood Compared To Corrected Venous Blood

Group	GCB vs CVB
Diabetic	9.2%
Non-Diabetic	8.6%

DISCUSSION

DM is a complex disease of multiple conditions and syndromes which have glucose intolerance in common (Tsutsui *et al.*, 1985). It is the most common non-communicable disease worldwide and the fourth to fifth leading cause of death in developed countries (Amos *et al.*, 2010). DM is associated with a wide range of complications, such as retinopathy, nephropathy, micro- and macrovascular disease, altered wound healing and periodontitis (Expert Committee on Diagnosis and Classification of Diabetes Mellitus, 1997). The current classification of periodontal disease and conditions lists DM associated gingivitis under dental plaque induced gingival diseases modified by systemic factors (Armitage, 1999). Periodontitis has been proposed as a sixth complication of DM (Loe, 1993). Infact, there is a two-way relationship between DM and periodontitis.

On one hand, poorly controlled DM increases the risk for developing severe periodontitis and impairs treatment outcome. On the other, chronic inflammatory periodontal disease considerably complicates diabetic control (Grossi, Genco, 1998). So the close interrelationship between diabetes and periodontitis, it can be assumed that the dental practitioner and especially the periodontists are extremely likely to encounter an increasing number of undiagnosed diabetes patients with periodontitis. The early diagnosis of diabetes, however, might help to prevent its long-term complications that are responsible for the high morbidity and mortality of diabetic patients (Harris and Eastman 2000).

Since periodontal inflammation with or without complication factor of DM is known to produce ample extravasate of blood during diagnostic periodontal examination (Ervasti 1985), no extra procedure, e.g. finger puncture with a sharp lancet is necessary to obtain blood for glucometric analysis. Even in the case of very low gingival crevicular bleeding, a glucose measurement is possible with the use of self-monitoring device (one touch select simple), due to the low amount of blood (< 2µl) necessary to perform the analysis. Moreover, the technique described is more familiar and less traumatic to the patient than a finger puncture.¹⁹ However the method of collection of sulcular blood is critical because the resultant glucose values may be altered if there is any contamination of the collected sample by the oral tissues (tooth or gingival margin) products (saliva and plaque) (Armitage, 1999; Beikler *et al.*, 2002).

Thus, one touch select simple (second generation glucometer) offers the advantage over the first generation glucometer, which needs a larger blood sample i.e. about 10-15µl and that the blood sample had to be placed on the test strips to be wiped off later by the user after a certain time interval. Thus, giving a reading by color matching (Parker *et al.*, 1993). The present clinical study was designed to develop a rapid, simple, non-invasive, chairside test for estimation of blood glucose level of a patient using gingival crevicular blood elicited during periodontal probing, using a glucometer, by comparing it to the glucose level from nger stick blood and venous blood. The study included 100 patients, which were divided into two group's i.e.

Group I: Diabetic patients with generalized moderate to severe chronic periodontitis.

Group II: Diabetic patients with generalized moderate to severe chronic periodontitis

GCB and FSB blood glucose were measured in each patient using self-monitoring glucometric device. Since the laboratory measurement is considered to be the Gold Standard (Parker *et al.*, 1993), its comparison to GCB and FSB blood measurements allows the evaluation of accuracy and precision of each blood collecting technique and the self-monitor.

GROUP I: (Diabetic patients with generalized moderate to severe chronic periodontitis)

On comparison between GCB glucose measurements and FSB blood glucose measurements, a very strong correlation was seen with an r value of 0.999, which was statistically highly

significant (P level < 0.001). On comparison between GCB glucose measurements and VB blood glucose measurements, a very strong correlation was seen with an r value of 0.999, which was statistically highly significant (P level < 0.001). The results of this study are in agreement with the studies conducted by Parker *et al* in 1993, Fatemeh Sarlatiey *et al.* 2010 who examined diabetic patients with unknown periodontal status, and wherein a very strong correlation was observed between gingival crevicular, finger prick capillary and the intravenous blood glucose measurements, and, Beikler *et al.*, 2002, Shiela M. Strauss *et al.*, 2009, Neema Shetty *et al.*, 2013, wherein, a strong correlation was observed between GCB and finger stick capillary measured blood glucose when diabetic and non-diabetic patients with moderate to severe periodontitis were examined.

GROUP II: (Non-diabetic patients with generalized moderate to severe chronic periodontitis)

On comparison between GCB glucose measurements and FSB blood glucose measurements, a very strong correlation was seen with an r value of 0.982, which was statistically highly significant (P level < 0.001). On comparison between GCB glucose measurements and VB blood glucose measurements, a very strong correlation was seen with an r value of 0.981, which was statistically highly significant (P level < 0.001).

The results are in agreement with the study conducted by Neema Shetty *et al* 2013 and GN Bala Raghavendra *et al.*, 2010 wherein, a strong correlation was observed between GCB, finger stick capillary and the intravenous blood glucose measurements. Beikler *et al* 2002, wherein, a strong correlation was observed between GCB and finger stick capillary measured blood glucose when diabetic and non-diabetic patients with moderate to severe periodontitis were examined. Interestingly, in our study, on comparison of glucose levels of gingival crevicular blood with finger stick blood, although the difference was statistically non-significant, still a variance was observed with a general trend of finger stick blood glucose values being slightly higher than the gingival crevicular blood glucose values. Although, both samples involved capillary blood, the reason for this could be dilution of gingival blood with gingival crevicular fluid and other contaminants (Shiela *et al.*, 2009).

When blood glucose levels of gingival crevicular blood were compared with venous blood, the difference was statistically non-significant. However, a variance was observed with a general trend of gingival crevicular blood glucose values being slightly higher than the venous values. This can be explained by the fact that gingival crevicular blood involves capillary blood. As the blood travels from capillary to venous circulation, the blood glucose values come down (by approximately 3.5 mg/dl) (Parker *et al.*, 1993) due to normal glycolysis that takes place, thus giving lower glucose levels in the venous blood. On observation of all three values, i.e. glucose levels of gingival crevicular blood, finger stick blood and venous blood, it was noted that gingival crevicular blood glucose values were lower than the finger stick blood glucose values. At the same time, these values were higher than venous blood glucose values.

Hence, gingival crevicular blood glucose levels would be closer to glucose levels of venous blood rather than the finger stick blood glucose values to that of venous blood. According to recommendations published by the American Diabetic Association in 1987 on performance guidelines for self-monitoring blood glucose devices, the glucose values should lie within $\pm 15\%$ of reference venous laboratory measurements (Consensus Statement on Self, 1987). (Consensus Statement on Self-monitoring of Blood Glucose. *Diabetes Care*, 1987; 10:95-99). For future meters, the aim of the American Diabetic Association is to reach a total variability of less than 10%. To find out whether the values of gingival crevicular blood in our study lie within this range, calculations were carried out.

The strong correlation obtained in the present study on comparison between the various blood glucose measurements indicates the feasibility of using periodontal sulcular blood as an alternative to the FP blood and IVB. The results of present study indicate that gingival crevicular blood collected during diagnostic periodontal examination may be an excellent source of blood for glucometric analysis. In addition, the technique described is safe, easy to perform and comfortable for the patient and might therefore help to increase the frequency of diabetes screening in dental offices. The sampling procedure performed in the study is much easier and less time consuming since no additional tools are necessary to collect GCB and adequate amount of blood was found to cover the strip.

Conclusion

Within the limitations of this study, it is concluded that Gingival crevicular blood (GCB) collected during examination is an excellent source of blood for glucometric analysis. The technique is safe, easy to perform, and comfortable for the patient and therefore, helps to increase the frequency of diagnosing diabetes and provides a more objective indicator for referral to physicians. Thus, the Dentist may play a vital role as a member of the health team by participating in the search for undiagnosed asymptomatic DM.

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