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RESEARCH ARTICLE

THE POTENTIAL USE OF GINGIVAL CREVICULAR BLOOD FROM INFLAMMATORY AND NON-INFLAMMATORY SITES FOR MEASURING BLOOD GLUCOSE LEVELS: A PILOT STUDY

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ABSTRACT

Introduction: Gingival Probing to assess gingivitis during routine periodontal examination will induce bleeding from the gingival sulcus which can be used to determine the blood glucose level.
Objective: To assess gingival crevicular blood glucose levels from inflamed site and non-inflamed periodontal sites and compare it with venous blood glucose and capillary blood glucose levels to check its usefulness as a diagnostic tool.
Methodology: According to inclusion and exclusion criteria 100 subjects reporting to the outpatient section of Department Of Periodontology, School of Dental Sciences, KIMSDU, Karad, were enrolled in the study. For all the participants three blood samples were collected for glucose level estimation namely gingival crevicular blood (GCB), finger capillary blood (FCB) expressed by the finger prick method and the Venous blood (VB. For Group A patients (N=50) GCB sample was collected from inflamed gingival units and for Group B patients (N=50) GCB sample was collected from non-inf1amed gingival units. The blood glucose level of the GCB and FCB samples then was evaluated by using glucometer and VB glucose level was estimated by using calorimetric method.
Result: GCB Glucose level Values (91%) are closer to the VB Glucose level values as compared to the FCB glucose levels (74%). For group A participants, correlations between VB and GCB glucose readings were high (92%) as compared to the Group B (86%).
Conclusion: Within the limitations of this study it can be concluded that gingival crevicular blood can be used for the screening the blood glucose levels And blood glucose levels of the GCB are quite comparable to gold standard laboratory venous blood glucose levels than that of the routinely done finger capillary blood glucose levels. It can also be concluded that inflamed gingival site is preferable over non-inflamed gingival site for screening blood glucose levels during routine dental check-up.

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INTRODUCTION

Diabetes is fast gaining the status of a potential epidemic globally and according to Kumar *et al*. (2013) India is projected to have around 62 million people with diabetes. (Kumar *et al*., 2013) Diabetes mellitus is associated with a wide range of complications, such as retinopathy, nephropathy, neuropathy, micro-and macrovascular disease, altered wound healing and periodontitis. (Shiela *et al*., 2009) Early diagnosis of diabetes, help to prevent its long-term complications and reduces morbidity. Dentists frequently manage dental patients using limited information about the patient's blood glucose levels. Treating undiagnosed diabetic patients in a dental clinic can lead to complications like post-operation infection, space infections, non-healing extraction socket, etc. Dental clinic can be considered as a venue of opportunity for screening and diagnosing diabetes. Currently blood glucose levels are estimated by referring the patient to a pathological laboratory or it can also be performed with the use of glucometer at chair side. Sending patient to pathological laboratory seems to be time consuming, whereas glucometer method involve finger prick which is invasive. Hence, examination of Gingival Crevicular Blood (GCB) for estimation of blood glucose levels was introduced in which gingival probing during routine periodontal examination induces bleeding from the gingival sulcus. This method can be considered less traumatic than routine finger puncture with a sharp lancet. Development of an intra oral blood sampling technique as opposed to the typically used finger site could make such tests even more suitable for use by dental practitioners. According to the study done by Shiela M. Strauss *et al*. (Gadsden, 1988) the blood glucose

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level of the blood collected from inflamed gingival units is more close to the standard laboratory glucose level values than blood glucose levels of the blood collected from the non-inflamed gingival units. To the best of our knowledge no other authors have probed the effect of inflammatory status of gingival unit on gingival crevicular blood glucose levels. So our study aimed at assessing the impact of inflammatory status of the gingival unit on blood glucose levels.

MATERIALS AND METHODS

After obtaining due approval from the Ethical committee, a total of 100 subjects reporting to the outpatient section of Department Of Periodontology, School of Dental Sciences, KIMSDU, Karad, using purposive sampling were enrolled in the study in accordance with the inclusion and exclusion criteria from August 2015 to June 2016. After explaining the study protocol the patients who agreed to participate in the study were enrolled in the study after obtaining due informed consent. Diabetic and non-diabetic patients with age group of 35-65 years were included in the study. Patients with any systemic conditions that require prophylactic antibiotics, abnormally low or high hematocrit Eg: Polycythemia Vera, anemia, history of intake of substances that interfere with the coagulation system Eg: Coumarin derivatives, Non-steroidal anti-inflammatory drugs, heparin, history of severe cardiovascular diseases, hepatic diseases, immunological disorders, renal diseases, hematological disorders were excluded. Also patients on excessive supplemental ascorbic acid that may interfere with the glucose strip oxidation reaction were not included in the study. Periodontal examination of all the subjects was carried out by single calibrated examiner using William's graduated periodontal probe (HU Friedy USA). Only maxillary anterior sextant teeth were taken into consideration. The inflammatory status of gingiva of these teeth was assessed using the "Gingival index" (Loe and Silness, 1963). According to the gingival health the study Subjects were divided into Group A and Group B. Group A comprised of 50 individuals with at least one buccal gingival unit in maxillary anterior sextant with gingival index score ≥2 from which GCB sample was collected. Group B comprised of 50 individuals with at least one buccal gingival unit in maxillary anterior sextant with gingival index score ≤1 from which GCB sample was collected. Maxillary anterior teeth were selected for both the groups to reduce chances of sample contamination from saliva and for ease of the blood collection procedure. The selected site was isolated using cotton roll to avoid contamination of blood with saliva. William's graduated periodontal probe (HU Friedy USA) was introduced in the gingival sulcus and swept across the sulcus and wait for 30 to 60 seconds so that sufficient blood gets pooled in the sulcus. Then it was collected by using small plastic pipette. Collected blood was then transferred to glucometer test strip which was then used for estimating blood glucose level using glucometer (Accu Chek Go Glucometer manufactured by Roche Diagnostics GmbH, Germany). For Group A and Group B, this same procedure was used to induce bleeding from both inflamed and noninflamed gingival sites.

Thereafter for every patient Capillary blood was collected by initially scrubbing the subject's finger with spirit (70% alcohol) and allowed it to evaporate. Bleeding was induced using sharp sterile lancet. Blood glucose level was then measured using same glucometer (Accu chek Glucometer) as that used for measuring GCB. Venous blood sample from same patient was then drawn from patient's antecubital fossa for measurement of serum (plasma) glucose. 6 ml of blood was collected which was equally distributed in two different vacutainer containing anticoagulant. One vacutainer was for serum glucose value and the other was for was hematocrit value. Hematocrit (Packed cell volume, PCV) is the percentage of blood volume occupied by RBC. This measurement is important because the glucose meter measures whole blood glucose whereas reference laboratory instrument measures glucose in remaining plasma after separation by using calorimetric methode. Hematocrit is used to convert the reference laboratory measurement (plasma glucose) to whole blood glucose value. This corrected laboratory value is now considered the true value of blood glucose and allows for direct comparison of GCB with true laboratory value of blood glucose.

Plasma measurements can be converted to whole blood measurements by the following formula: (National Center for Health Statistics, 2010)

Hematocrit corrected venous glucose (mg/dl) =Lab (mg/dl) × (1.0 – (0.0024 × Hct))

Statistical analysis

Values of GCB glucose, corrected venous blood (VB) glucose and capillary blood glucose for both the group A and group B were statistically analysed, where the mean was calculated. The calculated mean was used to calculate the correlation of corrected hematocrit VB glucose values with GCB glucose values and capillary blood glucose values.

RESULTS

In 100 study participants GCB, FCB and corrected VB glucose levels were estimated. Correlation between glucose levels of GCB and FCB with VB was assessed. The mean value was calculated with the standard deviation for all the glucose levels. The 95% confidence interval was calculated for the standard VB glucose level, considering it as a standard reading. The Coefficient of correlation (r) was calculated using the corrected venous glucose measurements as the true values for blood glucose (Table 1). The GCB glucose measurement was significantly correlated with the corrected venous glucose ($r= 0.819$, $p< 0.0001$) as compared to that of FCB glucose levels. 91(91%) of the patients GCB glucose level falls in the confidence interval of corrected hematocrit VB glucose level and only 74(74%) of the patients capillary blood glucose level falls in the confidence interval of corrected hematocrit VB glucose level. Furthermore the blood glucose level values of GCB collected from inflamed and non inflamed gingival sites were compared to that of FCB and corrected VB glucose levels. The mean value was calculated with the standard deviation for Group A study participants and Group B study participants for all the three blood glucose levels. The 95% confidence interval was calculated for the standard venous blood glucose level, considering it as a standard reading. 46(92%) of the patients GCB glucose level from inflamed gingival unit falls in the confidence interval of Corrected Hematocrit VB glucose levels (Table 2) whereas, 43(86%) of the patients GCB glucose level falls in the confidence interval of Corrected Hematocrit VB glucose level for non inflamed group (Group B) (Table 3). Coefficient of correlation (r) was calculated using the corrected VB measurements as the true

values for blood glucose. The coefficient of correlation for group A and group B is r=0.819 (p< 0.0001) and r=0.981, (p< 0.0001).

Table 1. Comparison of glucose levels of GCB, FCB with VB for all the participants

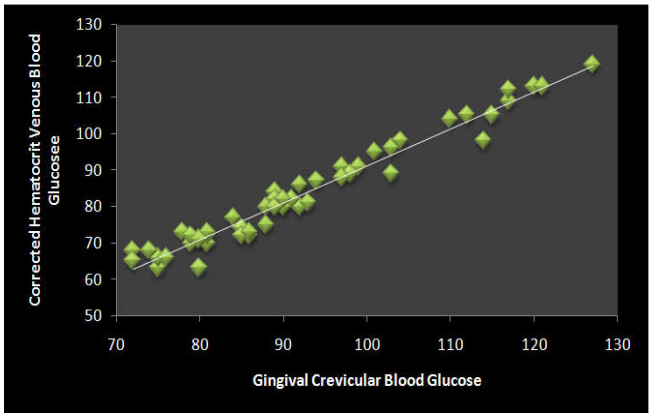
Blood Glucose Level Type	Mean	Std. Deviation	95% Confidence Interval
Gingival Crevicular	91.7	17.9	56.58 – 126.84
Finger Capillary	107.1	19.5	68.88 – 145.32
Corrected Hematocrit Venous	86.9	16.6	54.34 – 119.36

Table 2. Comparison of glucose levels of GCB, FCB with VB for Group A

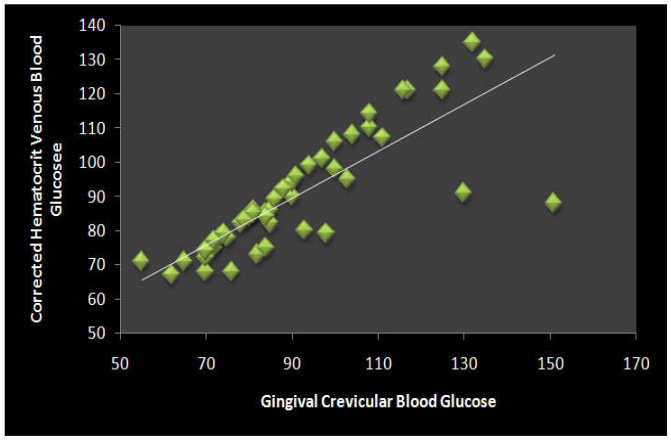
Blood Glucose Level Type	Inflamed		
	Mean	Std. Deviation	95% Confidence Interval
Gingival Crevicular	91.0	21.1	49.77 - 132.31
Capillary	111.2	21.7	68.57 – 153.79
Corrected Hematocrit Venous	90.2	17.7	55.59 – 124.85

Table 3. Comparison of glucose levels of GCB, FCB with VB for Group B

Blood Glucose Level Type	Non Inflamed		
	Mean	Std. Deviation	95 % Confidence Interval
Gingival Crevicular	92.4	14.3	64.34 – 120.42
Capillary	103	16.2	71.32 – 134.72
Corrected Hematocrit Venous	83.5	14.9	54.36 – 112.59



Graph 1:- Correlation between Gingival Crevicular Blood Glucose and Corrected Venous Glucose Level from Group A



Graph 2:- Correlation between Gingival Crevicular Blood Glucose and Corrected Venous Glucose Level from Group B

The GCB glucose measurements for both Group A and B were significantly correlated with the corrected VB glucose levels as compared to the FCB glucose levels. Correlation between GCB glucose and corrected VB level for Group A and Group B is shown in the Graph 1 and graph 2 respectively.

DISCUSSION

The American Diabetes Association recommends that screening for diabetes should start at age 45 years and be repeated every 3 years in persons without risk factors, and earlier and more often in those with risk factors for diabetes (Shiela *et al*., 2009). Early detection of an undiagnosed systemic condition significantly improves the prognosis of the condition, improves the patient's quality of life and lessens the complications associated with the condition. (Waghmare *et al*., 2011) In view of certain difficulties encountered during blood glucose level estimation using VB and FCB some authors tried using GCB as an alternative and found mixed results. Study conducted by Waghmare *et al*. (Parker *et al*., 1993) suggested that GCB samples show false glucose readings when compared with FCB. Similar results were obtained by Beikler *et al.* (2002) Parker *et al.* (Yamaguchi *et al*., 2004) and Yamaguchi *et al*. (Muller and Behbehani, 2004) In a study conducted by Muller, *et al*. in 2005 (Narula *et al*., 2014) on diabetic and non-diabetic patients with gingivitis and moderate-to-advanced periodontitis, the results failed to provide any evidence for the usefulness of GCB for testing blood sugar during routine periodontal examination. Contrary to earlier reports present study has found out the usefulness of the GCB for determining the Blood glucose levels. This could be attributed to two reasons; firstly previous studies collected GCB through strips by directly placing them near the gingival crevice (Stein and Nebbia, 1969). This may lead to contamination of the sample. Secondly, hematocrit value was not used to correct the VB glucose levels to whole blood glucose levels. Both of these errors are corrected in this study.

In the current study small plastic pipettes were used for collection of GCB. The small plastic pipette transferred the blood onto a test strip using chip blower for glucose measurement in glucometer. Gingival site was selected and isolated with gauze to avoid contamination of blood with saliva. Gingival Blood samples during probing (which induces bleeding) was collected from the sulcus using small plastic pipette. This is an improved modification done over other methods used in the past intraoral blood glucose studies by Stein Nebbia (Tsutsui *et al*., 1985) and Tsutsui *et al*. (Caraway, 1976) They transferred the blood glucose onto the test strip either by wiping blood directly from hemorrhagic gingival tissue with the test strip itself or by rubbing blood onto the test strip from blood ladened dental curette. Hence manual timing of the test strip reaction and the wiping of the test strip have been identified as the significant sources of error when using glucose self monitors. These sources of errors are eliminated in our study by using the plastic pipette, which is a quick means of transferring blood to the glucometer. (Gadsden, 1988) Thus according to the results of present study GCB value could be predictably used for the blood glucose level identification. In fact the GCB blood glucose values are quite close with corrected VB glucose level values than Blood glucose level of finger prick method. Furthermore one more important aspect highlighted in this study is, the blood glucose level value of the GCB from inflamed gingival sites are closer to the gold standard VB glucose level values than GCB blood glucose

level values of non inflamed sites. This difference was studied by only single author as per our knowledge (Gadsden, 1988). This difference could be because of the inflammation there will be increase in vascular permeability. So there will be frank oozing of the fresh blood with less cellular uptake of the serum products like glucose by peripheral cells. In this study preferably buccal aspect of maxillary anterior teeth was selected as a site of GCB collection as this area provides best access with least chances of contamination during sample collection. (Gadsden, 1988) A natural physiological drop in the blood glucose concentration is observed as it passes from a capillary (such as in the gingival crevice) area into a venous area. This is due to normal cellular uptake of glucose. The average drop for a fasting individual is 2 to 5 mg/dl or an average of 3.5 mg/dl (Caraway, 1976). Because of this the GCB blood glucose level value is always bit higher than the VB glucose level values.

Conclusion

Within the limitations of this study it can be concluded that GCB can be used for screening the blood glucose levels which are quite comparable to gold standard laboratory VB glucose levels than that of the routinely done FCB glucose levels. It can also be concluded that inflamed gingival site is preferable over non-inflamed gingival site for screening blood glucose levels during routine dental check-up.

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