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In-Office Estimation of Blood Glucose by Gingival Crevicular Blood and Finger-Prick Capillary Blood Using Glucometer

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Abstract:
Periodontitis is a common chronic inflammatory disease characterised by destruction of supporting structures of the teeth. There is a clear relationship between the degree of hyperglycemia and severity of periodontitis.
Aim: *to estimate the reliability of gingival crevicular blood as non-invasive diagnostic aid in screening for diabetes mellitus during routine periodontal examination.*
Material and methods: *in this study, a total of 61 subjects with generalised chronic periodontitis were taken from the outpatient department of periodontology and implantology. Blood samples were drawn from the gingival crevice using a capillary tube and index finger was pricked with the help of a sterile lancet for finger- prick blood samples. Samples drawn were analysed with the help of a glucometer. Blood glucose reading were then recorded and statistically analysed for proper diagnosis.*
Statistical analysis*: the recorded data was compiled and entered in a spread sheet (microsoft excel) and then exported to data editor of spss 20.0. The recorded data was compiled and entered in a spread sheet (microsoft excel) and then exported to data editor of spss version 20.0 (spss inc., chicago, illinois, usa).*
Result: *Pearson's correlation test results showed a mild positive correlation between gingival index and gcb and fcb (r=0.2612, r=0.2422) with significant value of p<0.005.*

Key Words*: Periodontitis, Diabetes Mellitus, Glucometer*

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I. Introduction:

Diabetes mellitus is a complex metabolic disorder characterised by hyperglycemia resulting from a defect in insulin secretion, insulin action or both.[1] The chronic hyperglycemia of diabetes is associated with a wide range of complications like diabetic retinopathy, atherosclerosis, cerebrovascular, cardiovascular, peripheral vascular diseases, peripheral neuropathy, progressive renal dysfunction, delayed wound healing and periodontitis.[2] However, globally, the prevalence of diabetes is predicted to be doubled from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 79.9 million individuals in India. Currently diabetes is fast growing in India with >62 million individuals diagnosed with the disease.[3]

The two main types of diabetes are classified primarily on the basis of their underlying pathophysiology. Type 1 diabetes, which constitutes about 5 to 10 percent of all cases in the United States, results from autoimmune destruction of insulin-producing β-cells in the pancreas, leading to total loss of insulin secretion. Because a person with type 1 diabetes no longer produces endogenous insulin, glucose is unable to enter target cells and remains in the bloodstream, resulting in sustained hyperglycemia. A patient with type 1 diabetes must take exogenous insulin to remain alive hence, the former name insulin-dependent diabetes.[4]

Type 2 diabetes, constitutes about 85 to 90 percent of all cases, results from insulin resistance rather than from total absence of insulin production. Autoimmune destruction of β-cells does not occur in type 2 diabetes,

and patients retain the capacity to secrete some insulin. Insulin resistance results in a decreased capacity to transfer glucose into target cells; thus, hyperglycemia develops.[4]

Periodontal disease is considered to be the sixth complication of diabetes mellitus. The interrelationship between diabetes mellitus and periodontitis has been studied for many years. Diabetes and Periodontitis interacts in a bidirectional manner. Both these conditions can produce disability, and clinicians have long assumed that these diseases are biologically linked. The diabetic state impairs the gingival fibroblast synthesis of collagen and glycosaminoglycan, enhances the gingival crevicular fluid collagenolytic activity, resulting in the loss of periodontal fibres, alveolar supporting bone, loosening and finally exfoliation of the teeth. One plausible biologic mechanism why diabetics have more severe periodontal disease is glucose-mediated advanced glycation end products (AGE) accumulation. These advanced glycation end products would affect migration and phagocytic activity of mononuclear and polymorphonuclear phagocytic cells which facilitates the bacterial persistence in the periodontal pocket and significantly increases the periodontal destruction.[2]The maturation and gradual transformation of this subgingival microflora into an essentially gram negative flora will in turn constitute the ulcerated pocket epithelium, a chronic source of systemic challenge. This in turn triggers both an infection mediated pathway of cytokine upregulation, especially with secretion of TNF-α and IL-1, and a state of insulin resistance, affecting glucose utilizing pathway. Excessive local secretion of TNF-α and IL-1 also mediates destruction of connective tissue and alveolar bone evident in periodontal disease. It had also been reported that high glucose levels decrease migration of periodontal ligament cells which compromises wound healing in periodontitis.[2]

In 1998, the World Health Organization adopted the diagnostic parameters for diabetes established by the American Diabetes Association. Measuring the fasting blood glucose is considered to be the gold standard. These conventional laboratory methods employed to detect blood glucose are time consuming, invasive and require elaborative equipment. The advent of blood glucose monitors allows the clinician to assess blood glucose at the chair side. In contrast to laboratory method, results are obtained instantaneously, which helps the clinician to decide if further confirmatory tests are required to diagnose diabetes. The glucometer device may actually allow for painless testing of blood oozing from the gingival crevices of patients with periodontitis 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 diabetic patients.[5]

Periodontal inflammation with and without complicating factor of diabetes mellitus is known to produce ample extravasated blood during diagnostic procedures. Routine periodontal probing during periodontal examination is more familiar to practitioner and is less traumatic than venous blood glucose estimation. This crevicular blood oozed during periodontal probing might be an excellent source of blood for glucometric analysis using the self-monitoring glucose device (Glucometer) instead of conventional venous blood glucose.[6] This, self-monitoring device is a simple, non- invasive and a reliable method for rapid monitoring of estimating blood glucose levels in the blood. It could also be used for screening of diabetes mellitus in suspected population. The conventional laboratory methods employed to detect blood glucose are time consuming and require elaborative equipment.

In contrast to chair-side estimation, the results are obtained instantaneously which helps the clinician to make decisions immediately.[3] Also, there has been evidence that severe oral infections increases insulin resistance and metabolically interfere with the management of diabetes mellitus. In such cases, patients seek medical consultations to which they are reluctant. So, it's preferable for a dentist to perform screening of blood glucose before starting the treatment.[7]

Therefore, the present study has been designed to estimate the applicability, reliability and efficacy of gingival crevicular blood in glucose estimation as compared to finger- prick capillary blood with the use of glucometer.

II. AIM AND OBJECTIVES

To estimate the reliability of gingival crevicular blood as non-invasive diagnostic aid in screening for diabetes mellitus during routine periodontal examination.

OBJECTIVES:
Primary Objective:

1. To compare the efficacy of glucose levels in gingival crevicular blood with respect to finger-prick capillary blood in chronic periodontitis patients.

Secondary Objective:

2. To clinically evaluate the periodontal parameters and effect of diabetes mellitus in chronic periodontitis patients.

III. METHODOLOGY:

In this study, a total of 61 subjects with generalised chronic periodontitis were taken from the outpatient Department of Periodontology and Implantology, B.R.S. Dental College and Hospital. Blood samples were drawn from the gingival crevice using a capillary tube and index finger was pricked with the help of a sterile lancet for finger- prick blood samples. Samples drawn were analysed with the help of a glucometer. Blood glucose reading were then recorded and statistically analysed for proper diagnosis. Ethical approval has been obtained from the ethical committee. An informed consent was taken and the participants were enrolled in the study.

INCLUSION CRITERIA

1. Both male and female patients having >10 teeth will be taken.
2. Patients between 25-75 years of age.
3. Patients diagnosed with moderate to severe chronic periodontitis.
4. Patients with intact upper anterior teeth

EXCLUSION CRITERIA

1. Any indication for antibiotic prophylaxis.
2. Any bleeding disorder.
3. Severe systemic disease such as cardiovascular, renal, hepatic, immunologic or haematological disorders.
4. Any medication interfering with the coagulation system.
5. Patients with abnormally low or high haematocrit E.g. polycythemiavera, anaemia were excluded.
6. Patients with use of excessive supplemental ascorbic acid that may interfere with glucose strip oxidation reaction were not included in the study.

In this study, on the first day of visit, overall assessment of the patients was done by taking the medical and dental history along with proper clinical examination. Periodontal and gingival conditions were also evaluated properly. Bleeding on probing, probing pocket depth and clinical attachment levels was measured with the help of a U N C -15 probe.

In this study, maxillary anterior teeth were chosen for glucose monitoring as they offer easy access for gingival crevicular blood. For each measurement, only one site with bleeding on probing was selected. After selecting the bleeding site, the site was isolated with gauze or cotton rolls and dried with compressed air to prevent saliva contamination. Prior to probing, all the subjects were made to rinse with chlorhexidine gluconate mouth wash in order to minimize microbial load in the oral cavity. The interdental papilla between the incisors or canines was probed and bleeding will be induced with a probe. Then a capillary tube is used for the collection of gingival crevicular blood and blood samples were transferred immediately to a glucometer test strip in order to avoid clotting inside the capillary tube. The test strip was held until the instrument shows beeping on meter count for 8 seconds. Later, the blood glucose measurements were recorded in mg/dl.

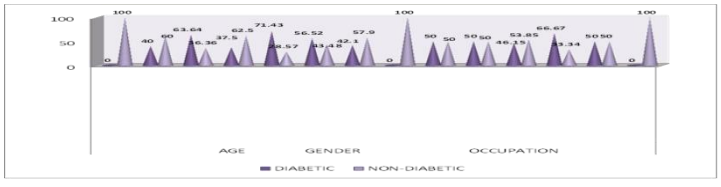
Immediately after measuring the gingival crevicular blood, finger-prick capillary blood sample was drawn from the index finger. The soft tissue surface of the finger was wiped with surgical spirit and the spirit was allowed to evaporate. The finger was punctured with a sterile disposable lancet and a drop of blood was seen on the finger. The first drop of blood was discarded and as soon as the second drop was formed, the test end of the strip was made to touch the bleeding site. This will be held until the instrument beeps showing the meter count down after 8 seconds. Later, the blood glucose measurements were recorded in mg/dl.

All the measurements were evaluated for monitoring diabetes mellitus.

IV. RESULTS

Various clinical parameters were assessed before the procedure.

1. **Questionnaire**



2. **Gingival index (Loe and Silness,1963)**

On the basis of gingival index, patients with moderate gingivitis included 10 patients in which 20% were diabetic and 80% patients were non-diabetic. Patients having severe gingivitis had maximum number of diabetic patients (52.94%) than non-diabetic patients (47.06%), indicating maximum patients with chronic periodontitis were diabetic. The mean value and standard deviation is 2.83±0.373 with a P value of <0.0001

3. **Plaque index (Silness and Loe, 1964)**

Based on the interpretation of the plaque index, patients with fair plaque index included 53 patients (86.89%) with 52.83% as non-diabetic and 47.17 as diabetic. Patients in the category of poor plaque index were 13.11% with equal percent of diabetic and non- diabetic patients having a mean and standard deviation of 3.13±0.340 with a P value of <0.0001.

4. **Periodontal index (Russell's periodontal Index, 1956)**

As per the Russell's periodontal index, patients with established destructive periodontal disease included 39.34% patients, in which 45.84% were diabetic patients and 54.16% as non-diabetic. And patients with terminal periodontal disease were 60.66% among which 48.65% were diabetic and 51.35% non-diabetic. The mean and the standard deviation as per the RPI was 4.607±0.493 with a P value of <0.0001.

5. **Clinical attachment level (Measurement Approach)**

Based on the clinical attachment level, stage 4 (more than 5mm CAL), 63.93% patients with 51.28% as diabetic and 48.725 as non-diabetic were seen having a mean and standard deviation of 3.541±0.673 and a P value of <0.0001.

6. **Bleeding on probing (Saxer and Muhlemann, 1975)**

Patients with a score of 3(bleeding immediately after probing) had 44% as diabetic patients and 56% as non-diabetic. In addition to patients with a score of 4(BOP spreading towards the marginal gingival) included equal percent of diabetic and non diabetic patients having a mean and standard deviation of 3.590±0.496 and a P value of <0.0001.

7. **Blood glucose level (Glucometer)**

   a) Gingival crevicular blood.

   b) Finger-prick capillary blood.

TABLE 1: CORRELATION BETWEEN DIABETIC/NON-DIABETIC AND FINGER CAPILLARY BLOOD

FINGER CAPILLARY BLOOD	DIABETIC	NON-DIABETIC	P-VALUE
89-198	2 (6.90%)	31 (96.88%)	<0.0001*
199-308	17 (58.62%)	1 (3.12%)	
309-418	7 (24.14%)	0 (0%)	
419-528	3 (10.34%)	0 (0%)	

***Statistically significant p<0.05**

TABLE 2: CORRELATION BETWEEN DIABETIC/NON-DIABETIC AND GINGIVAL CREVICULAR BLOOD

GINGIVAL CREVICULAR BLOOD	DIABETIC	NON DIABETIC	P -VALUE
90-199	0 (0%)	31 (96.88%)	<0.0001*
200-309	20 (68.96%)	1 (3.12%)	
310-419	6 (20.69%)	0 (0%)	
420-529	3 (10.34%)	0 (0%)	

***Statistically significant p<0.05**

TABLE 3: CORRELATION BETWEEN GINGIVAL CREVICULAR BLOOD (GCB) AND FINGER CAPILLARY BLOOD (FCB)
T value=14.79
P=<0.00001*

The result is significant at p<0.05

In our study, the efficacy of a glucometer for the screening of diabetes using gingival crevicular blood was evaluated and the values obtained were compared using student t test which showed a positive correlation between gingival crevicular blood and capillary blood glucose level having a T value of 14.79 and P value of <0.0001 indicating a statistically significant value (Table 14).

TABLE 4: CORRELATION BETWEEN PERIODONTITIS(PI) WITH GINGIVAL CAPILLARY BLOOD (GCB) AND FINGER CAPILLARY BLOOD AND DIABETIC/NON DIABETIC				
	PERIODONTITIS		DIABETIC/NON-DIABETIC	
	r- value	p-value	r-value	p-value
GINGIVAL CREVICULAR BLOOD	0.1536	0.237	-0.826	<0.0001
FINGER CAPILLARY BLOOD	0.1217	0.350	-0.78	<0.0001

***statistically significant at p <0.05**

TABLE 5: CORRELATION BETWEEN CLINICAL PARAMETERS AND FCB AND GCB				
	GCB		FCB	
	r- value	p-value	r-value	p-value
GI	0.2612	0.042*	0.2422	0.06
PLAQUE I	-0.363	0.004*	-0.0212	0.87
BOP	0.18	0.165	0.1469	0.25
CAL	0.0786	0.547	0.0803	0.53
RPI	0.1536	0.237	0.1217	0.35

Pearson's correlation test results showed a mild positive correlation between gingival index and GCB and FCB (r=0.2612, r=0.2422) with significant value of p<0.005.
In case of plaque index, a mild negative inverse correlation was found between GCB and FCB with statistically significant value of p in case of GCB (0.004) and a non significant value of FCB (0.087).
In cases of BOP, Clinical attachment level and Russell's periodontal index, a mild positive correlation was seen (r=0.18, r=0.078, r=0.153) with a non-significant P values.

FIG:1 CHRONIC PERIODONTITIS

FIG:2 PROBING POCKET DEPTH IN UPPER ANTERIORS

FIG:3 GINGIVAL CREVICULAR BLOOD SAMPLING USING A CAPILLARY TUBE.

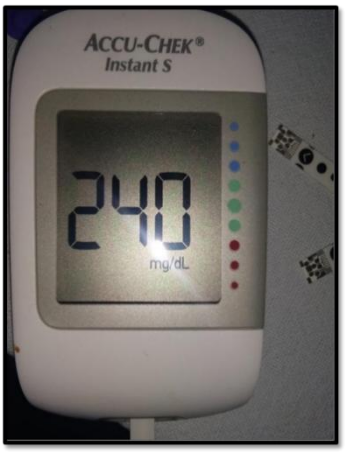


FIG:4 GLUCOMETERIC READINGS OF GINGIVAL CREVICULAR BLOOD.

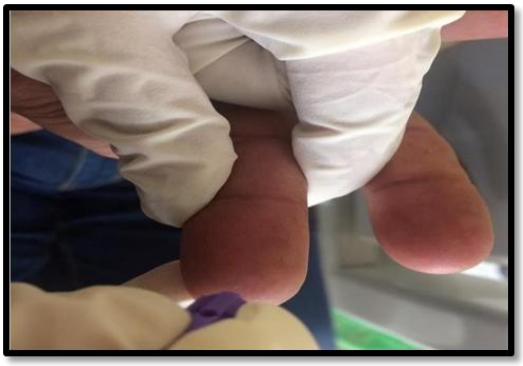


Fig.5 FINGER-PRICKING USING A STERILE LANCET

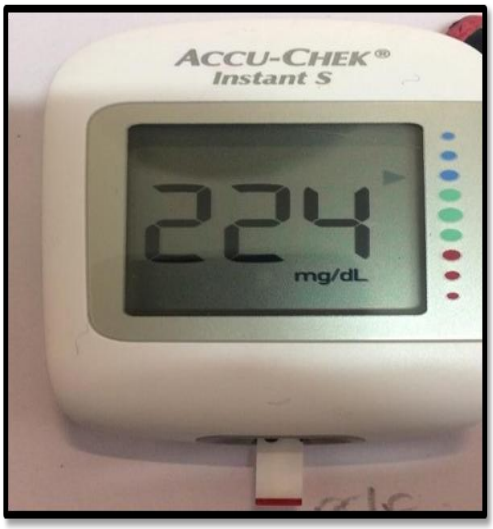


FIG:6 GLUCOMETERIC READINGS OF FINGER-PRICK CAPILLARY BLOOD.

V. DISCUSSION

(Expert committee on the Diagnosis and Classification of Diabetes Mellitus, 1997)[8]- The American Diabetes Association recommends the screening for diabetes should start at the age of 45 years and be repeated every 3 years in persons without the risk factor and earlier and more often in those with the risk factor. Moreover testing should be carried out in individuals who are obese, have 1st degree relative with diabetes, have delivered a baby weighing >4.05 kgs or have been diagnosed with gestational diabetes, or are hypertensive (>140/90) and have a HDL cholesterol level >250mg/dl or had a previous testing having an impaired glucose tolerance or an impaired fasting glucose.

Stein and Nebbia[9] were the first to describe a chairside method of diabetic screening with gingival blood. They transferred blood onto the test strip by wiping blood directly from hemorrhagic gingival tissue. **Tsutsui et al, 1985**[10] reported the rubbing of blood onto the test strip from blood laden dental curette. Rubbing or direct wiping of intraoral blood on the test strip will not produce a uniformly timed reaction and may damage the strips chemical indicator surface.[11] In this study, Reagent strips were used which showed out to be fairly reliable and accurate for clinical use.

Also significant contamination may occur from saliva and oral debris present at the wiped gingival area or from plaque and crevicular fluid on the dental curette from the entry into the gingival sulcus.

In the present study, isolating the bleeding gingival site with a gauze piece and then rapidly sampling blood with the capillary tube was an improvement over the past studies. Using the capillary tube might not eliminate contaminations; however, it does reduce saliva, plaque and debris by collecting free-flowing blood just inside the gingival crevice.

All the studies started from **1969** by **Stein et al** and **Tsutsui et al** to the more recent studies of **Beikler et al** attempted to prove that extravasated blood from gingival crevice due to inflammation can provide an acceptable source for measuring blood glucose in diabetic patients.

However, because majority of the patients are usually apprehensive whenever invasive techniques are used, we have incorporated the non-invasive method where the blood oozing out during routine periodontal examination is checked for diabetes. In this study, 2% Lignocaine jelly was used as a topical anesthetic and was applied 1 minute prior to gingival prick. This makes the gingival prick almost painless. Finger prick produces trauma to fingers, as well as subjective symptoms of pain invariably. Fingertips are full of nerve endings (receptors) which make finger prick very painful. Gingival blood sampling may be more comfortable in patients because of local anesthesia application.

American Diabetes Association[11] in their consensus statement on blood glucose monitoring (1987) said that manual timing of the test strip reaction and the wiping of the test strip are significant sources of error when using glucose self-monitors. To over-come these errors

Periodontal inflammation with and without complicating factor of diabetes mellitus is known to produce ample extravasated blood during diagnostic procedures (**Ervasti et al, 1985**).[12] Even in case of a very low, gingival crevicular bleeding a glucose measurement is possible with the help of a self-monitoring device due to the low amount of blood (2ul) necessary to perform the analysis. Moreover the technique performed is less traumatic than the gold standard technique. According to the findings and other studies, there is a strong correlation between GCB and FCB (**Parker et al, 1993**) showing a highly significant value ($r>0.975$, $p<0.0001$).

In this study, no invasive procedure such as finger puncture with a sharp lancet is necessary to obtain blood for glucometric analysis. The technique of using GCB is less traumatic and less time consuming and does not cause any discomfort to the patient motivating the dental professionals to implement diabetes screening using a GCB sample and feel comfortable and confident in doing so. Furthermore, especially among older persons (a population at greater risk for diabetes), pocket depth ≥ 4 mm and/or excess bleeding on probing is common and increases with age, even exceeding 50% in some sample.

On comparison between the GCB, FCB and intravenous blood glucose measurements, a strong correlation was seen with a p value of <0.001 in diabetic patients with chronic periodontitis showing a highly statistically significance. The results of this study are in agreement with the studies conducted by **Parker et al, 1993** who examined diabetes patients with unknown periodontal status, having a strong correlation between the GCB, FCB and intravenous blood glucose measurements reporting that 92% of the GCB measurements with non-wipe glucose self-monitor were within 15% of the true glucose concentration.

In our study, after proper isolation and drying of the site, sample was collected from the outer surface of the gingiva, thus eliminating the possibility of contamination with crevicular fluid. None of the subjects under study reported pain / discomfort and no complications have been reported after sampling by this method. The glucometer used is a self-timing, second-generation monitor and is approved by Federation Dentaire Internationale (FDI) for off-finger testing. It requires very low amount of blood (1 µl), thus allowing to perform the analysis even in cases with very mild gingival inflammation.

Tellervo Ervasti et al, (1985)[12] examined the periodontal health status of 50 adult diabetes and 53 healthy controls. The diabetic group was further subdivided into three subgroups according to the control of diabetes. Poorly controlled diabetics suffered significantly more from gingival bleeding than those with good or moderate control.

With regard to the development of painless and non-invasive, methods to measure blood glucose, considerable efforts have been made in the past few years (**Kost et al, 2000**). However, until now, none are in routine clinical practice (**Klonoff, 1997**).

Katz J, (2001)[13] analysed 10590 subjects for normal and abnormal (higher than 120mg/dl) serum glucose levels, their periodontal status reflected as CPITN was corrected with their glucose level category. The result of this study

reflected a significant association between elevated blood glucose levels and periodontal disease as measured by CPITN.

Muller et al, (2005) compared the conclusions drawn following the approach of correlation/regression analyses of comparative data of diagnostic method and measures of calculating limits of the agreement. They mentioned that the correlation analyses are inappropriate when assessing agreement between two methods of measurement. Therefore, an overall summary measured that the correlation coefficient does not help a clinician to interpret the result. Regression analyses attempts to predict an observed measurement by another observed measurements suggesting one measurement can be modelled by another which is not the case in measurement comparison leading to quantification of disagreement of measurements. So, in order to eliminate the discrepancy we have used both methods in the study. **Muller and Behbehani, 2005** failed to obtain any correlation between GCB and FCB.

Yamaguchi et al, (2005)[14] have investigated a method for the non-invasive blood glucose measurement using gingival crevicular fluid entailing the use of a high sensitive glucose testing tape to evaluate the possibility of using this fluid for the measurements reporting only the correlation coefficient in their results.

VI. SUMMARY AND CONCLUSION

The present study was undertaken to estimate the applicability, reliability and efficacy of gingival crevicular blood in glucose estimation as compared to finger-prick capillary blood with the use of glucometer. The results of the present study indicate that GCB 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. Although not a test to diagnose diabetes, such screening is an important aid in identifying those for whom follow-up tests regarding possible diabetes are warranted. Furthermore, the costs associated with the purchase of a readily available glucometer and individual test strips are extremely modest. Thus, with minimal cost and a limited investment of time for patients and clinicians, dental professionals can play a critical role in supporting their patients' overall health.

The data thus collected was compiled and subjected to statistical analysis. It was concluded that:

Gingival crevicular blood and finger-capillary blood had an inverse negative correlation with that of diabetic and non-diabetic glucose value (-0.826, -0.78). Both the correlation had a statistically significant P value (<0.0001).

Pearson's correlation test results showed a mild positive correlation between gingival index and GCB and FCB ($r=0.2612$, $r=0.2422$) with significant value of $p<0.005$.

In case of plaque index, a mild negative inverse correlation was found between GCB and FCB with statistically significant value of p in case of GCB (0.004) and a non-significant value of FCB (0.087).

In cases of BOP, a mild positive correlation was seen ($r=0.18$) with a non- significant P values.

In cases of Clinical attachment level a mild positive correlation was seen ($r=0.078$) with a non-significant P values.

In cases of Russell's periodontal index, a mild positive correlation was seen ($r=0.153$) with a non-significant P values.

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