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Original Article

A Study on the Use of Gingival Crevicular Blood for Measuring Glucose to Screen for Diabetes and Comparison of Measurements of Glycated Hemoglobin in Periodontitis Cases and Gingivitis Subjects

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ABSTRACT:

Aim- Diabetes mellitus and chronic periodontitis are the most common chronic diseases in the adult world population. Many people suffering from diabetes around the globe do not know that they have diabetes. A dental / periodontal check up or treatment provides an ideal opportunity to screen for the patient particularly those who are at risk for diabetes mellitus, hypertension etc. Hence, this study aims to use gingival crevicular blood to obtain glucose level reading and compare it with glucose reading from capillary finger blood. Simultaneously chair side glycosylated hemoglobin measurement will be noted to correlate with the above two methods of blood glucose readings and to determine if gingival crevicular blood could be used as screening method for diabetes in a patient with gingivitis and periodontitis. **Methods**-60 subjects, 20 with chronic periodontitis (Group I), 20 with aggressive periodontitis (GroupII), 20 with chronic gingivitis (GroupIII) were included in this study, gingival crevicular blood, Capillary finger blood reading with glucometer and Glycosylated hemoglobin were compared. **Results**- Of all the 60 patients who participated in study; 2 patients were found to a have higher random blood glucose level, one in Group II and one in Group I, those patients were referred to physician for further detailed investigation. **Conclusion**: Gingival crevicular blood glucose and glycated hemoglobin can be effectively measured in dental settings by using chairside kits to screen for undiagnosed diabetes patients as well as to check glycemic control in diabetic patients. These tests can be performed effectively in community surveys as these tests gives readings which are rapid and accurate.

Key words: Chronic Periodontitis, Diabetes mellitus, Glycated Hemoglobin.

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INTRODUCTION

Diabetes mellitus and chronic periodontitis are the most common chronic diseases in the adult world population. Diabetes mellitus is a clinically and genetically heterogeneous group of metabolic disorder manifested by abnormally high level of glucose in blood. Whereas, Periodontitis[1] is an inflammatory disease of supporting tissues of teeth caused by specific microorganism or group of specific microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone with pocket formation, recession or both. It is multifactorial disease occurring as a result of complex interrelationship between infectious agents and host related factors.

Hyperglycemia is a result of deficiency of insulin secretion caused by pancreatic β cell dysfunction (Type 1 diabetes) or of resistance to the action of insulin in liver and muscle (Type 2 diabetes) or combination of both.[2]

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world".

It is estimated that millions of undiagnosed cases of diabetes exist and therefore there is a definite risk for an increase in number of diabetics in future. So, there is a need to increase the awareness and screen for detection of diabetes especially among those who may be at a higher risk of developing the disease. Diabetes, in its initial stages, because of its asymptomatic nature remains undiagnosed for

many years and by the time it becomes evident irreversible damage would have already occurred.[3]

Many people suffering from diabetes around the globe do not know that they have diabetes. 30 percent of the diabetics in urban India and 60 percent of those suffering from diabetes in rural India are undiagnosed. On the whole, out of a total of 3.5 crores diabetics in India, 1.33 crores go undiagnosed.[4] The issue of undiagnosed diabetes is extremely critical especially in Non Insulin dependent diabetes mellitus (Type II). This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Such patients are at an increased risk of developing macrovascular and microvascular complications. Thus there is a critical need to increase opportunities for diabetes screening and early diabetes detection, among the population.[5]

A dental / periodontal check up or treatment provides an ideal opportunity to screen for the patient particularly those who are at risk for diabetes mellitus, hypertension etc. Periodontal inflammation is known to produce ample extavasated blood during diagnostic procedure such as periodontal probing. Routine probing during a periodontal examination is more familiar both to practitioner and the patients. Thus, the ability to collect gingival crevicular blood seems to offer a more practical alternative. Because periodontal diseases may predispose individual to incident diabetes, the dental visit offers a unique opportunity to screen an especially at risk population.[3]

Hence, this study aims to use gingival crevicular blood to obtain glucose level reading and compare it with glucose reading from capillary finger blood. Simultaneously chair side glycosylated hemoglobin measurement will be noted to correlate with the above two methods of blood glucose readings and to determine if gingival crevicular blood could be used as screening method for diabetes in a patient with gingivitis and periodontitis.

OBJECTIVES OF THIS STUDY

- To assess the blood glucose level using gingival crevicular blood in periodontitis patients and gingivitis patients and compare it with capillary finger blood glucose level.
- To assess glycosylated hemoglobin level in periodontitis patients and gingivitis patients.

METHODS AND MATERIALS

This is a prospective case control study where 20 patients with chronic periodontitis, 20 patients with aggressive periodontitis, and 20 patients with gingivitis were recruited from the department of periodontics of The Oxford Dental College, Bangalore. The study was carried out for a period of one year.

The subjects were selected for the study based on the following inclusion and exclusion criteria.

Inclusion criteria

For test and control group

Routine patients presenting to clinic with no history of diabetes.

For patients with chronic periodontitis

1. Five or more teeth with probing depth greater than or equal to 5mm.
2. Bleeding on probing.
3. Clinical attachment loss more than 2mm.
4. Radiographic evidence of bone loss.

For patients with generalized aggressive periodontitis

Aggressive form of periodontal disease have been defined based on following primary features

- Rapid attachment loss and bone destruction.
- Familial aggregation of cases.

Secondary features

- Amount of microbial deposits inconsistent with severity of periodontal tissue destruction.

For patients with gingivitis

1. Probing depth not more than 3mm.
2. Bleeding on probing.
3. No signs radiographic bone loss.
4. Having received no periodontal treatment in the last 6 months.

Exclusion criteria

1. Long term antibiotic use (more than 15 days in past 6 months).
2. History of severe systemic disease that preclude regular care by dentist.

60 subjects, 20 with chronic periodontitis, 20 with aggressive periodontitis, 20 with chronic gingivitis fulfilling these criteria were selected for the study. All patients were informed of the procedure being performed and the inform consents obtained.

A detailed case history was recorded in the specially prepared Proforma (Annexure) for both the test and control groups.

Periodontal status evaluation

Clinical parameters assessed for the study were oral hygiene index, gingival index, probing pocket depth and clinical attachment level.

Radiographic examination:

Standardized intraoral periapical radiographs were taken for all the patients using long cone paralleling technique.

Procedure for collection of sample:

Maxillary sextant area was selected for collecting the gingival crevicular blood sample as this area offers best access. The area was isolated with cotton rolls to prevent saliva contamination and dried with compressed air. The probing was repeated if necessary to obtain a sufficient quantity of blood for diagnosis. For collection of GCB sample, a readily available glucometer (B Braun ^{TM}) with

compact design which requires small quantity of blood (less than 1 micro liter) and gives rapid readings was used. The paper strip was placed in the bleeding sulcus area and the blood allowed to be absorbed by paper strip. Immediately following collection of GCB sample, the pad of a finger was wiped with surgical spirit, allowed to dry and punctured with sterile lancet. Capillary finger blood sample was drawn onto the glucometer pre loaded with the test strip. Both the blood samples readings were recorded.

Glycosylated hemoglobin was measured by using commercially available chair side kit (Bayer ^{TM}). Each kit contains

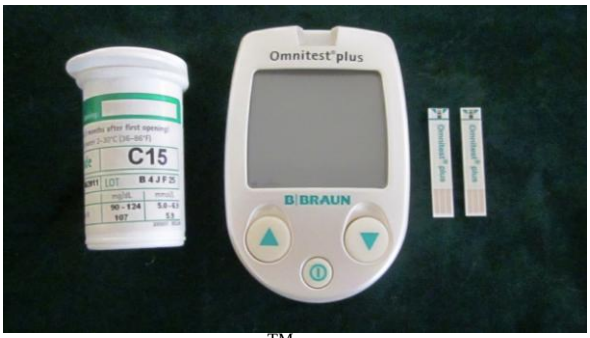
1. Reusable A1C Now^{+} monitor
2. Twenty A1C Now^{+} Test cartridges which contain antibody to HbA1c, antigen conjugates that binds to antibody, and membranes.
3. Twenty sample dilution kits each of which contains a sampler containing0.37 ml solution of buffered detergent solution with fericyanide and a blood collector.

After lancing the patients finger with sterile lancet, blood was drawn from patient's finger and collected in blood collector by gently touching the blood drop.

After collecting blood, the collector was inserted into sampler body and shaken 7-8 times to mix the blood with the sampler solution.

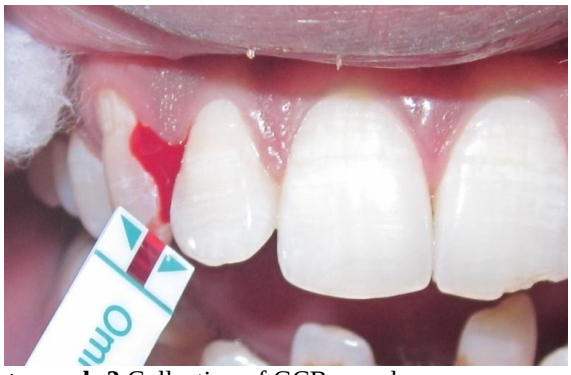
Following that, A1C Now^{+} test cartridge was opened and inserted into A1C Now^{+} monitor. Once the monitor displayed SMPL signal, this well mixed solution was placed in the cartridge. This entire procedure was completed within two minutes of opening the test cartridge, and the monitor gave reading within five minutes. All the steps were followed as recommended by manufacturer.

After collection of samples, all patients were subjected to routine periodontal therapy. While performing the study we found that chairside glycated hemoglobin kits were time and temperature sensitive. The entire procedure must be completed within 2 minutes once the test cartridge is open. The temperature of the kit should be maintained between 18^0C – 26^0C because at higher room temperature the kit shows error on the display monitor and also the kit should be kept in dry environment in order to prevent any moisture contamination.



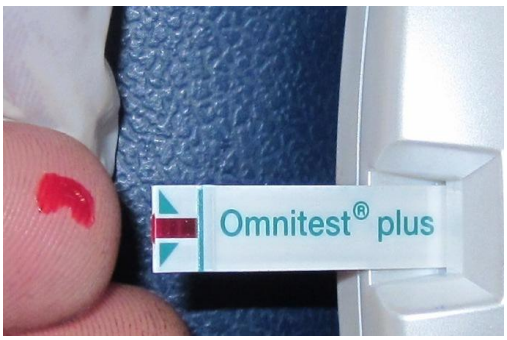
Photograph-1 B BRAUNS^{TM} glucometer along with test strips

Photograph-2 Bayers glycated hemoglobin KIT^{TM} containing test cartridge, sample dilution kit and monitor.

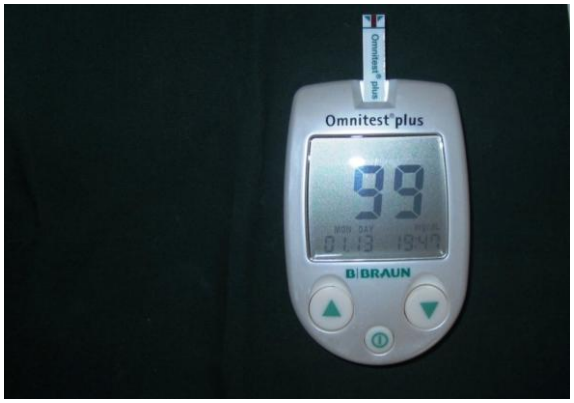


Photograph-3 Collection of GCB sample

Photograph- 4 GCB glucose reading in glucometer

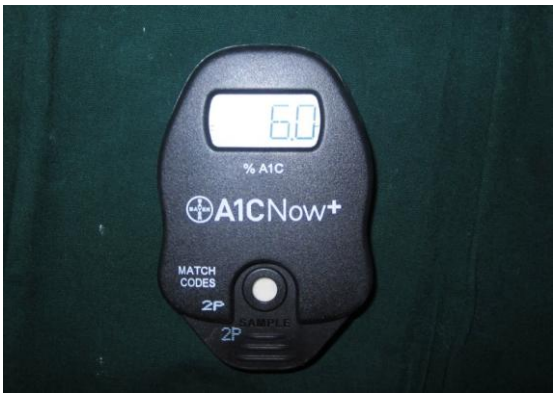


Photograph- 5 Collection of CFB sample



Photograph– 6 CFB glucose reading in glucometer

Photograph- 7 Collection of blood sample for measurement of HbA1c



Photograph- 8 HbA1c reading in chairside HbA1c kit

STATISTICAL ANALYSIS[6,7,8]

Analysis of variance (ANOVA) has been used to find the significance of study. Parameters between three or more groups of patients.

Student t test (Two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group.

RESULTS

A total of 60 subjects, 20 in Group I (Chronic generalized gingivitis), 20 in Group II (Chronic generalized periodontitis), and 20 in Group III (Generalized aggressive periodontitis) participated in the study.

The age of patients who were recruited in the study ranged between 19-55years with mean age of 24.35years±3.23 in Group I, 38.05years±8.92 in Group II and 29.10years±5.08 in group III, respectively. (Table 1)

All the patients who participated in the study were systemically healthy and were not earlier diagnosed with the diabetes.

Of all the 60 patients who participated in study; 2 patients were found to a have higher random blood glucose level, one in Group II (GCB glucose level 182 mg/dl and CFB glucose level 176 mg/dl) and one in Group I (GCB glucose level 138 mg/dl and CFB glucose level 141 mg/dl), those patients were referred to physician for further detailed investigation. The patient belonging to Group B was confirmed with diagnosis of diabetes and was started on treatment for diabetes as recommended by physician.

Out of all 60 patients who participated in the study, 1 patient in Group B had HbA1c value of 8.9% and 2 patients in Group II had HbA1c value ≥ 7% those patients were referred to physician for further detailed investigation. The patient with HbA1c value of 8.9% was confirmed with diabetes and was started on treatment for diabetes as recommended by physician.

Comparison of Hba1c levels in chronic gingivitis, chronic periodontitis and generalized aggressive periodontitis patients:

Higher HbA1c value was recorded with group II, and highest value recorded was 8.9%. The value was ranging from 5.3% to 8.9% and the mean value was 6.19%±1.08. The mean HbA1c recorded in other two groups were almost similar. HbA1c value for Group III were ranging from 5.3% to 6.9% with a mean value of 5.83%±0.42 and HbA1c value for Group I was ranging from 5.3% to 6.8% with mean value of 5.83%±0.43. Higher mean HbA1c was recorded in Group II and the mean HbA1c recorded in the other two groups were found to be almost equal. The difference in mean HbA1c between the three groups was not statistically significant (P>0.05). (Table 2)

Comparison of gingival crevicular blood glucose levels in chronic gingivitis, chronic periodontitis and generalized aggressive periodontitis patients:

Higher GCB glucose values were recorded in group II with highest value of 182mg/dl, followed by group I and Group III respectively. Values of GCB glucose level in Group II were ranging from 80mg/dl to 182mg/dl with a mean value 112.95mg/dl±22.84. Values of GCB glucose level in Group I were ranging from 85 mg/dl to 138mg/dl and mean value was 108.10mg/dl±12.76. Value of GCB glucose level in Group III were ranging from 86mg/dl to 126mg/dl and mean value was 103mg/dl±11.07. Higher mean GCB glucose level was recorded in Group II followed by Group I and Group III respectively. However, the difference in mean

GCB glucose level between the three groups was not statistically significant (P>0.05). (Table 3)

Comparison of capillary finger blood glucose levels in chronic gingivitis, chronic periodontitis and generalized aggressive periodontitis patients:

Higher CFB glucose values were recorded in group II with highest value of 176mg/dl followed by Group I and Group III respectively. Values of CFB glucose level in Group II were ranging from 80mg/dl to 176mg/dl with mean value 113.35mg/dl±20.30. Value of CFB glucose level in Group I were ranging from 84mg/dl to 141 mg/dl and mean value was 106.20mg/dl±13.50. Value of CFB glucose level in Group III were ranging from 92mg/dl to 122mg/dl and mean value was 105.85mg/dl±9.86. Higher mean CFB glucose level was recorded in Group II followed by Group I and Group III respectively. However, the difference in mean CFB glucose level between the three groups was not statistically significant (P>0.05). (Table 4)

Correlation between GCB glucose and CFB glucose levels in chronic gingivitis, chronic periodontitis and generalized aggressive periodontitis patients:

The correlation between GCB glucose and CFB glucose value was highly significant in all the three groups.

The correlation between GCB glucose level and CFB glucose level in Group I was found to be positive & very strong (r=0.871) and also statistically significant (P<0.001).

The correlation between GCB glucose level and CFB glucose level in Group II was found to be positive &very strong (r=0.863) and also statistically significant (P<0.001).

The correlation between GCB glucose level and CFB glucose level in Group III was found to be positive & strong (r=0.580) and also statistically significant (P<0.01).

(Table 5)

Table 1: Comparison of age

Group	Mean	SD	Min	Max	F	P-Value	Significant difference between
Group I	24.35	3.23	19.00	31.00	25.061	<0.001*	I vs II
Group II	38.05	8.92	29.00	56.00			II vs I, II vs III
Group III	29.10	5.08	20.00	38.00			III vs II

Differences were Statistically significant between I vs II and II vs III (P <0.001)

Table 2: Comparison of HbA1c levels in chronic gingivitis (I), chronic periodontitis (II) and generalized aggressive periodontitis (III) patients

Group	Mean	SD	Min	Max	F	P-Value
Group I	0.0583	0.0043	0.0530	0.0680	1.705	0.191
Group II	0.0619	0.0108	0.0530	0.0890		
Group III	0.0583	0.0042	0.0530	0.0690		

The difference in mean HbA1c between the three groups was not statistically significant (P>0.05).

Table 3: Comparison of GCB glucose levels in chronic gingivitis (I), chronic periodontitis (II) and generalized aggressive periodontitis (III) patients. (Values in: mg/dl)

Group	Mean	SD	Min	Max	F	P-Value
Group I	108.10	12.76	85.00	138.00	1.840	0.168
Group II	112.95	22.84	80.00	182.00		
Group III	103.00	11.07	86.00	126.00		

Table 4: Comparison of CFB glucose levels in chronic gingivitis (I) chronic periodontitis (II) and generalized aggressive periodontitis (III) patients. (values in: mg/dl)

Group	Mean	SD	Min	Max	F	P-Value
Group I	106.20	13.50	84.00	141.00	1.554	0.220
Group II	113.35	20.30	80.00	176.00		
Group II	105.85	9.86	92.00	122.00		

The difference in mean CFB glucose level between the three groups was not statistically significant (P>0.05).

Table 5: Correlation between GCB and CFB glucose levels in chronic gingivitis (I), chronic periodontitis (II) and generalized aggressive periodontitis (III) patients

Group	R	P-Value
Group I	0.871	<0.001*
Group II	0.863	<0.001*
Group III	0.580	0.007*

The correlation between GCB glucose and CFB glucose value was highly significant in all the three groups. (P<0.001).

DISCUSSION

Many people suffering from diabetes around the globe do not know that they have diabetes.[4] Diabetes especially type II diabetes go undiagnosed because hyperglycemia develops gradually. At earlier stages it is not severe enough for the patient to notice the classical symptoms; and hence leads to development of macrovascular and microvascular complications. Thus, there is a critical need to increase methods for screening diabetes in its early stages and avoid its potential complications among the population.[5]

In the view of growing number of undiagnosed diabetes and increased risk for the periodontitis patients, diabetes screening at the time of periodontal visit seems to offer a promising approach. However usefulness of this approach becomes significant only if provided values of glucose reading, obtained from GCB sample by using chair side glucometer are consistently accurate. This study supports such confidence in accuracy of GCB glucose testing in patients with periodontal disease.

GCB glucose testing is one of the method by which the patients can be screened for diabetes in the dental clinic. The dentist can also collect CFB blood at the time of dental visit as done in various studies.[3,9-11] In this study we have simultaneously recorded CFB glucose values and compared it with GCB glucose values and found high correlation between GCB glucose and CFB glucose values in all three groups.

Screening using CFB glucose samples are generally performed without regard to the specific finger from which blood is collected and glucose measured. So, in the same manner GCB glucose reading was obtained from the site with adequate bleeding on probing. In this study bleeding on probing was more pronounced in periodontitis groups as compared to gingivitis group, but as the chairside kits are highly sensitive, it gives accurate reading with a very small fraction of blood (1µl).[12]

The use of GCB to measure glucose is likely to be more acceptable to the dental professional and the patients because the patients anticipate dental intervention in the dental office. The procedure involving probing and GCB sample collection takes very little time and does not increase patient's discomfort during the probing and while samples are drawn. Unlike screening for diabetes using CFB sample, diabetes screening using GCB sample can occur while the dentist clinically probes to gather the necessary data for diagnosis of periodontal disease. Such screening makes use of blood sample that would generally be swabbed away. Thus, measurement of glucose through GCB involves quick and simple intraoral procedure with minimal cost, and so dental professionals may be motivated to implement diabetes screening using GCB sample.

In this study we did not collect venous blood samples to use as a gold standard with which to measure glucose in the laboratory, as in other studies[9,13] nor did we collect duplicate GCB and CFB samples, as was done in a study[9] because it has been proven through these studies that there is high degree of correlation between chairside glucose measurement kit and routine laboratory tests performed for diagnosis of diabetes.[9,13]

Participants in this study were not fasting, nor were the results adjusted based on the time since the participant last ate, as was the case with few studies.[10,14] Various studies [15,16] failed to correlate that the length of time since last food intake could affect blood glucose concentration. This is because blood glucose level is maintained by negative homeostasis i.e. when blood glucose level rises above the normal, the liver converts the excess glucose into glycogen and similarly when glucose falls below normal level, the liver converts glycogen back into glucose in order to maintain constant blood glucose level. In the present study there was no statistically significant difference between group I, II and III with regard to length of time since participants had their last food intake, as it could not have affected one group more than the other in terms of agreement between CFB and GCB glucose readings.

In this study the correlation value between GCB and CFB glucose readings were 0.871 in Group I, 0.863 in Group II and 0.580 in Group III, these correlation values were highly significant and similar as values obtained in previous studies. [9,10,11]

Participants in the present study were divided into three groups, Group I (Chronic gingivitis), Group II (Chronic periodontitis) and Group III (Generalized aggressive periodontitis). Previous studies included only chronic periodontitis and a healthy control group.[3,17] Gingivitis group was included to find out a possible role of low grade chronic inflammation on glycemic control. Aggressive periodontitis patients were recruited in this study in order to see if rapid progression of this disease could have an effect on glycemic control. Peridontally healthy patients were not recruited because obtaining blood sample from healthy gingiva is difficult and therefore ethically unacceptable.

It was found that, higher GCB glucose values were recorded in group II with highest value of 182mg/dl, followed by group I and Group III respectively. Of all the 60 patients who participated in study; 2 patients were found to a have higher random blood glucose level, one in Group II (GCB glucose level 182mg/dl and CFB glucose level 176 mg/dl) and one in Group I (GCB glucose level 138mg/dl and CFB glucose level 141mg/dl). Those patients were referred to physician for further detailed investigations. The patient belonging to Group B was confirmed with diagnosis of diabetes and was started on treatment for diabetes as recommended by the physician. This elevated level of blood glucose in chronic periodontitis and chronic gingivitis patients can be attributed to long term inflammatory status in patients[18,19] as compared to aggressive periodontitis patients where disease is rapidly progressive.[20] In the present study, chronic periodontitis patients had poor oral hygiene index compared to the other two groups, results also showed increase level of GCB glucose and glycated hemoglobin levels in chronic periodontitis.

This could be attributed to chronic inflammation of this form of periodontitis. Of three groups, PPD and CAL were higher in aggressive periodontitis cases. But the GCB glucose and HbA1c were lower in comparison to chronic periodontitis group. This could be due to rapid progression of the disease, whereas, chronic periodontitis is slow onset disease. Evidence suggest that periodontitis induced bacteremia and endotoxemia causes elevation of serum proinflammatory cytokines such as interlukin-1β (IL-1β), c reactive proteins (CRP), interlukin-6 (IL-6), tumor necrosis factor α (TNF-α), which have been demonstrated to produce alteration in lipid metabolism leading to hyperlipidemia. These cytokines initiate destruction of pancreatic β cells, lead to development of diabetes, and produce insulin resistance syndrome similar to that observed in established cases of diabetes. [21]

In this study we simultaneously measured glycated hemoglobin in dental setting using chairside glycated hemoglobin kit. Higher HbA1c value was recorded with group II. Out of all 60 patients who participated in the study, 1 patient in Group B had HbA1c value of 8.9% and 2 patients in Group B had HbA1c value ≥ 7%. Those patients were referred to physician for further investigations. The patient with HbA1c value of 8.9% was confirmed with diabetes and was started on treatment for diabetes as recommended by the physician. A study compared[22] non fasting blood glucose level between periodontally healthy patients and those with the advanced periodontal disease and the results showed that glucose levels were significantly higher in periodontitis cases than in controls. As mentioned above, the increased HbA1c in our study could be attributed to presence of low grade infection in periodontitis patients which leads to increased production of proinflammatory cytokines such as IL-1, IL-6 and prostaglandins particularly, PGE_2, which ultimately leads to development of insulin resistance. Our results are consistent with other reports[22,23] that collectively suggests that periodontitis is associated with elevated blood glucose levels in adults who have not been diagnosed with diabetes.

HbA1c was measured using a chairside test in accordance with the manufacturer's instructions. Such tests are internally reliable and their results correlate well with the laboratory value (r = 0.72-0.90).[24,25] One of the advantage of such a test is that it can be performed quickly and easily in a dental office setting. Hence this study's finding offers greater potential benefit of HbA1c screening in dental office. The dental office seems to be a reasonable setting to monitor glycemic control in patients with diabetes.[26] HbA1c is commonly used as treatment end point in clinical trials in diabetology because measurement of glycated hemoglobin accurately reflects the mean blood glucose concentration over the preceding 1-3 months. Further such a rapid chairside test also permits the clinician to determine the fitness of the diabetic patients to undergo periodontal surgery at short notice.

A number of participants in this study had elevated levels of HbA1c, GCB and CFB readings albeit normal and therefore may have the potential to develop type 2 diabetes. Twenty five subjects in this study had HbA1c values ≥ 6%, which suggest that substantial fraction of this population are at risk for diabetes. According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken.[27]

The average age group of the patients with chronic periodontitis in this study was 37.9±8.92 years as compared to 24.35±3.23 years in chronic gingivitis and 28.8±5.08 years in aggressive periodontitis patients. Although the age differences were statistically significant, and the reading of glycated hemoglobin were higher in chronic periodontitis patients as compared to other two groups, these differences were not statistically significant. The increased level of glycated hemoglobin in Group II could be attributed to the accumulation of advanced glycation end products and increased glucose concentration in tissues with time.[28]

Interestingly, results of this study show higher levels of blood glucose and glycated hemoglobin in males as compared to females. Out of 60 patients, 37 were male participants, 14 belonging to Group II, 16 belonging Group III and 6 belonging to Group I. This gender difference correlates with previous studies which state that chronic periodontitis[29] and aggressive periodontitis[29]are more prevalent in males as compared to females. Studies also have proven that diabetes is more prevalent in males as compared to females[110] and this higher prevalence of diabetes in males is attributed to their lifestyle, smoking, and increased LDL levels, hence the male population could have higher propensity for development of diabetes.

Further the clinical importance of present results suggest that gingival crevicular blood glucose and glycated hemoglobin can be effectively measured in dental settings by using chairside kits to screen for undiagnosed diabetes patients as well as to check glycemic control in diabetic patients. These tests can be performed effectively in community surveys as these tests gives readings which are rapid and accurate.

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