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Gingival Crevicular Blood for Assessment of Blood Glucose Levels

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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 aim of this study was to compare gingival crevicular blood and fingerstick blood glucose measurements using a self-monitoring device with the standard laboratory plasma glucose measurement.

Materials and methods. 30 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 (GCBG), and capillary fingerstick blood (CFBG) samples were analyzed using the same device.

Results. In non-diabetics, the analysis of agreement failed to prove sufficient agreement between the paired methods (FPG & CFBG, FPG & GCBG, and CFBG & GCBG). In diabetics, this analysis revealed sufficient agreement only between FPG & CFBG, and between FPG & GCBG measurements.

Conclusion. Gingival crevicular blood can be used for testing blood glucose during periodontal examination in diabetic periodontal patients but not in non-diabetic individuals.

Key words: **Bleeding on probing, diabetes mellitus, gingival crevicular blood.**

Introduction

Diabetes mellitus is one of the most frequent metabolic disorders and nearly half the cases are undiagnosed.[1] Diabetes and periodontitis seem to interact in a bidirectional manner.[2] 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",[3-5] and successful pe-

riodontal therapy in diabetic patients entails the stabilization of blood glucose to a normal range.[3,6]

Considerable effort has been made in the past few years to develop painless and noninvasive methods to measure blood glucose.[7] 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,[8] and routine probing during a periodontal 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.[9]

Recently, more sensitive self-monitoring devices have been developed for testing small amounts (< 2 μl) of blood and the accuracy of these glucometers has been acceptable.[10] 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 Methods

Patient Selection

The study population was recruited from patients attending the Department of Periodontics, Dental Branch, Islamic Azad University. 30 patients with untreated moderate to severe periodontitis were enrolled in the study. Also, 50 non-diabetic patients were randomly selected from individuals attending the Department of Periodontics, Dental Branch, Islamic Azad University. Fifty diabetic patients were, in addition, selected by convenience from a list of diabetic patients, monitored regularly at Bu-Ali Hospital, Tehran Islamic Azad University, Tehran, Iran. Exclusion criteria included the following: Any indication for antibiotic prophylaxis, any bleeding disorder, severe systemic disease such as cardiovascular, renal, hepatic, immunologic, or hematological disorders, and any medication interfering with the coagulation system. All the procedures were performed in accordance with the Helsinki protocol and all subjects agreed to and signed informed consent forms regarding the protocol, which was reviewed and approved by the ethics and research committees of the Dental Branch at Islamic Azad University.

Clinical and Laboratory Assessments

The examiner was trained through standardized procedures for making the required measurements and was also calibrated prior to the study. Data were recorded by the same examiner. Thirty patients with periodontitis were examined intraorally for visual signs of periodontal inflammation. Areas with marked signs of inflammation were probed by a Williams probe, inserted into the gingival sulcus, as is commonly done during a periodontal examination. When the probe was removed the gingival crevice was observed for bleeding.[11] Bleeding gingival sites were determined and two sites with profuse bleeding on probing and access for the glucose self-monitoring device were chosen for testing gingival crevicular blood glucose (GCBG). These areas were isolated with cotton rolls to prevent saliva contamination and dried with compressed air. Probing was repeated until sufficient amount of blood appeared in the gingival crevice. The two selected areas were analyzed using the glucose self-monitoring device (ACCU-CHEK Active, Roche Diagnostics, USA) according to the manufacturer's instructions.

Fifty diabetic and 50 non-diabetic patients underwent routine laboratory measurement of fasting plasma glucose (FPG) levels. These patients also went through a periodontal examination using the same method, and only one site with bleeding on probing was selected for testing GCBG. Immediately after measuring GCBG, capillary fingerstick blood glucose (CFBG) was assessed using the same glucose self-monitoring device, using a disposable sterile lancet to take a sample from the right index finger.

Statistical Analysis

The correlation between blood glucose measurement pairs of patients with periodontitis was determined by calculating intraclass correlation coefficient (ICC). In the other two groups of the study, Pearson's rank correlation and analysis of agreement between each two methods was used according to Bland & Altman.[12-14]

Results

Patients with periodontitis included 19 males and 11 females with a mean age of 35.5 ± 11.99 years old. Non-diabetic individuals included 25 males and 25 females with a mean age of 50 ± 15 years old. Diabetic patients included 14 males and 36 females with a mean age 51.44 ± 10.02 years old.

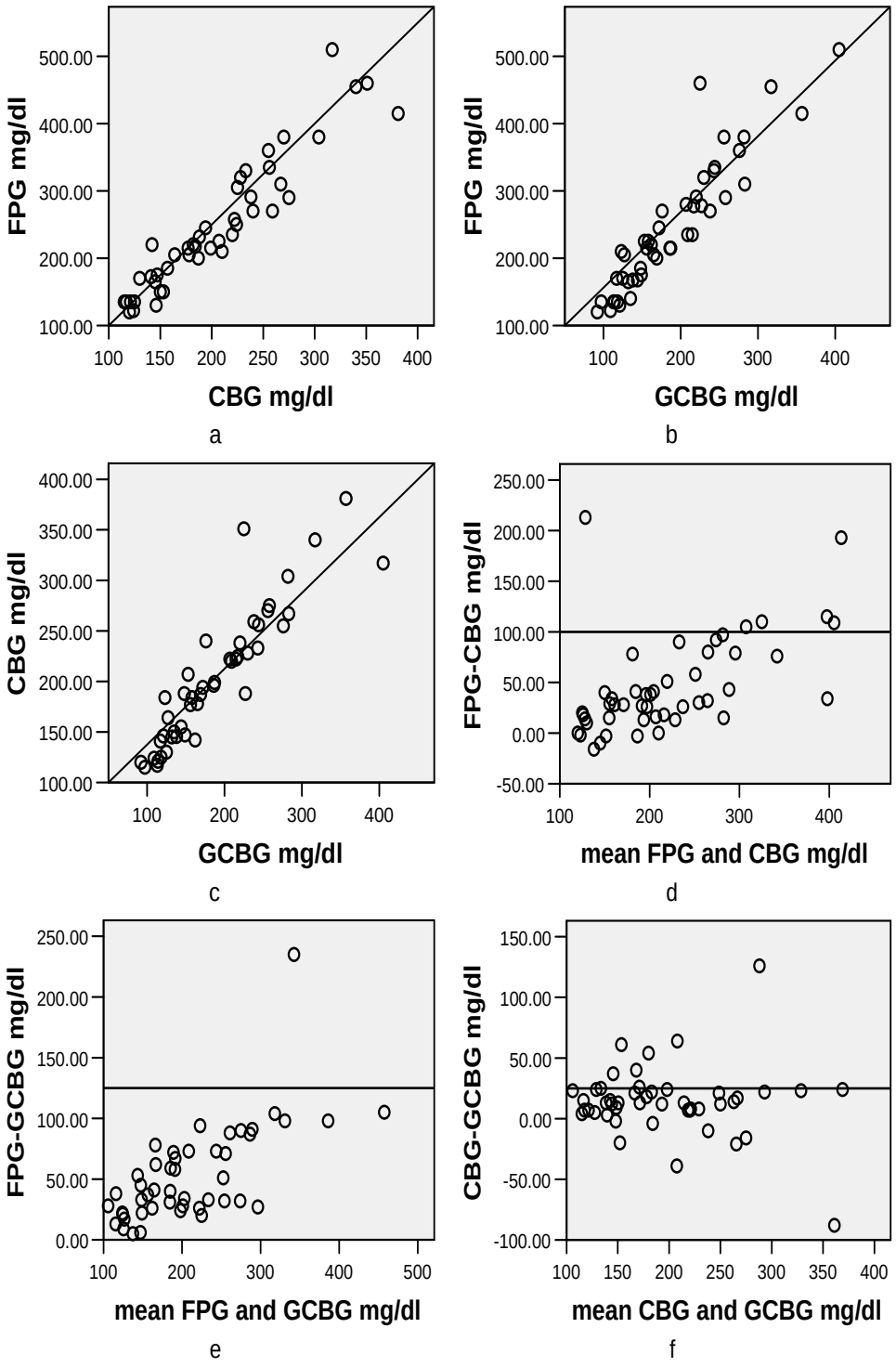

Figure 1. Mean plots of measurements in diabetic patients, with lines of equality: FPG and CBG (a), FPG and GCBG (b), and CBG and GCBG (c); and against respective differences: FPG and CBG (d), FPG and GCBG (e), CBG and GCBG (f).
FPG: fasting plasma glucose; CBG: capillary fingerstick blood glucose; GCBG: gingival crevice blood glucose.

The paired GCBG samples revealed an intraclass correlation coefficient of 0.96 ($P < 0.001$).

Table 1 shows the results of the analysis of agreement comparing blood glucose measurements in non-diabetic subjects. The mean ± SD difference measurements in FPG and CFBG samples was 16.3 ± 10.11 mg/dl and the 95% coefficient of agreement was 19.82 mg/dl (1.96 times the standard deviation of the differences). If the differences were normally distributed 95% of the differences were expected to lie within the

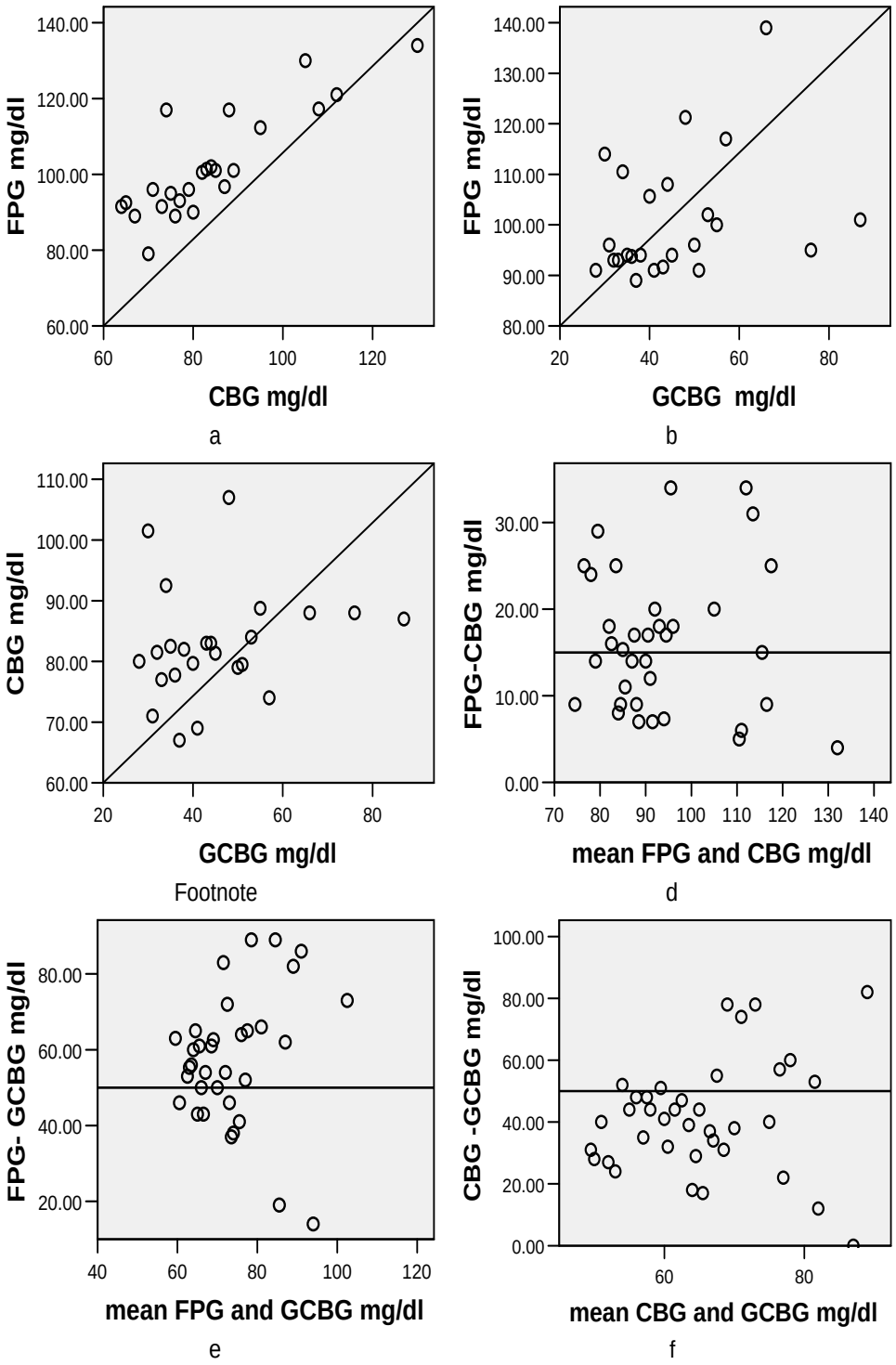

Figure 2. Mean plots of measurements in non-diabetic patients, with lines of equality: FPG and CBG (a), FPG and GCBG (b), and CBG and GCBG (c); and against respective differences: FPG and CBG (d), FPG and GCBG (e), CBG and GCBG (f).
FPG: fasting plasma glucose; CBG: capillary fingerstick blood glucose; GCBG: gingival crevice blood glucose.

interval between 13.42 and 19.17. Moreover, differences (D: FPG, CFBG) were regressed on the means (M: FPG, CFBG) of measurements. Both the constant and predictor regression parameters were not significant (P = 0.177 and P = 0.885 respectively). Finally, absolute values of the residuals from the model were regressed on M (P = 0.102).

There was also a significant correlation between FPG and CFBG measurements (r = 0.72, P < 0.001). Regression of FPG on CFBG showed an intercept of

38.57 ± 8.62mg/dl (P < 0.001) and a regression coefficient of 0.74 ± 0.1 (P < 0.001).

The Mean ± SD difference measurements in FPG and GCBG samples was 57.54 ± 16.38 mg/dl and the 95% coefficient of agreement was 32.11mg/dl (1.96 times the standard deviation of the differences). If the differences were normally distributed 95% of the differences were expected to lie within the interval between 52.88 and 62.19. Moreover, differences (D: FPG, GCBG) were regressed on the means (M: FPG, GCBG) of measurements. The constant regression parameter was significant (P = 0.041) but the predictor regression parameter was not significant (P = 0.23). Finally absolute values of the residuals from the model were regressed on M (P = 0.001).

FPG and GCBG were not correlated (r = 0.17, P = 0.23). Regression of FPG on GCBG showed an intercept of 92 ± 7.57mg/dl (P < 0.001) and a regression coefficient of 0.2 ± 0.169 (P = 0.23).

The mean ± SD difference measurements in CFBG and GCBG samples was 41.24 ± 16.91 mg/dl and the 95% coefficient of agreement was 33.14 mg/dl (1.96 times the standard deviation of the differences). If the differences were normally distributed 95% of the differences were expected to lie within the interval between 36.43 and 46.05. Moreover, differences (D: CFBG, GCBG) were regressed on the means (M: CFBG, GCBG) of measurements. Both the constant and predictor regression parameters were not significant (P = 0.17 and P = 0.27 respectively). Finally, absolute values of the residuals from the model were regressed on M (P = 0.001).

CFBG and GCBG were not correlated (r = 0.1, P < 0.48). Regression of CFBG on GCBG showed an intercept of 79.4 ± 7.54 mg/dl (P < 0.001) and a regression coefficient of 0.12 ± 0.17 (P = 0.48). Correlation between glucose levels of different methods are shown in Figure 1.

Table 2 shows the results of the analysis of agreement comparing blood glucose measurements in diabetic patients. The mean ± SD difference measurements in FPG and CFBG samples was 40.96 ± 40.81 mg/dl and the 95% coefficient of agreement was 79.99 mg/dl (1.96 times the standard deviation of the differences). If the differences were normally distributed 95% of the differences were expected to lie within the interval between 29.36 and 52.56. Moreover differences (D:FPG, CFBG) were regressed on the means (M: FPG,CFBG) of measurements: D = −45.02 ± 0.39 M mg/dl. Both the constant and predictor regression parameters were significant (P < 0.001). Finally, absolute values of the residuals from the model were regressed on M (P = 0.91).

There was also a significant high correlation between FPG and CFBG measurements (r = 0.94, P < 0.001). Regression of FPG on CFBG showed an intercept of −33.86 ± 15.52 mg/dl (P < 0.034) and a regression coefficient of 1.37 ± 0.073 (P < 0.001).

The mean ± SD difference measurements in FPG and GCBG samples was 55.22 ± 41.03 mg/dl and the 95% coefficient of agreement was 80.42 mg/dl (1.96 times the standard deviation of the differences). If the differences were normally distributed 95% of the differences were expected to lie within the interval between 43.56 and 66.88. Moreover, differences (D: FPG, GCBG) were regressed on the means (M: FPG, GCBG) of measurements: D = 0.337 M mg/dl. The constant regression parameter was not significant (P = 0.18) but

Table 1. Agreement of fasting plasma glucose (FPG), capillary fingerstick blood glucose (CFBG) and gingival crevice blood glucose (GCBG) measurements in non-diabetic subjects

	FPG,CFBG (N = 50)	FPG,GCBG (N = 50)	CFBG,GCBG (N = 50)
Minimum difference	-2	14	0
Maximum difference	51	89	82
Mean difference ± SD*	16.3 ± 10.11	57.54 ± 16.38	41.24 ± 16.91
95% CI of lower limit**	96.99,80.746	52.88,40.07	80.746,40.07
95% CI of upper limit	104.81,88.45	62.19,46.65	88.45,46.65
Coefficient 95% of agreement	19.82	32.11	33.14
Limits95% of agreement	13.42,19.17	52.88,62.19	36.43,46.05

*: Standard Deviation; **: Confidence interval

Table 2. Agreement of fasting plasma glucose (FPG), capillary fingerstick blood glucose (CFBG) and gingival crevice blood glucose (GCBG) measurements in diabetic subjects

	FPG,CFBG (N = 50)	FPG,GCBG (N = 50)	CFBG,GCBG (N = 50)
Minimum difference	-16	5	-88
Maximum difference	213	235	126
Mean difference ± SD*	40.96 ± 40.81	55.22 ± 41. 03	14.26 ± 28.45
95% CI of lower limit**	215.84,183.4	215.84,168.16	183.4,168.16
95% CI of upper limit	269.79,220.31	269.79,207.04	220.31,207.04
Coefficient 95% of agreement	79.99	8.427	55.762
Limits95% of agreement	29.36,52.56	43.56,66.88	6.17,22.34

*: Standard Deviation; **: Confidence interval

the predictor regression parameter was significant ($P < 0.001$). Finally, absolute values of the residuals from the model were regressed on M ($P = 0.751$).

There was also a significant high correlation between FPG and GCBG measurements ($r = 0.92$, $P < 0.001$). Regression of FPG on GCBG showed an intercept of 2.14 ± 15.23 mg/dl ($P = 0.889$) and a regression coefficient of 1.28 ± 0.76 ($P < 0.001$).

The mean ± SD difference measurements in CFBG and GCBG samples was 14.26 ± 28.45 mg/dl and the 95% coefficient of agreement was 55.76 mg/dl (1.96 times the standard deviation of the differences). If the differences were normally distributed 95% of the differences were expected to lie within the interval between 6.17 and 22.34. Moreover, differences (D: CFBG, GCBG) were regressed on the means (M: CFBG, GCBG) of measurements. Both the constant and predictor regression parameters were not significant ($P = 0.059$ and $P = 0.389$, respectively). Finally absolute values of the residuals from the model were regressed on M ($P = 0.006$, significant).

There was also a significant high correlation between CFBG and GCBG measurements ($r = 0.91$, $P < 0.001$). Regression of CFBG on GCBG showed an intercept of 39.74 ± 11.32 mg/dl ($P < 0.001$) and a regression coefficient of 0.86 ± 0.05 ($P < 0.001$). Correlation between glucose levels of different methods in diabetic and non-diabetic subjects of the study as well as the mean plots measurements against their references are shown in Figures 1 & 2.

Discussion

The results this study showed that there was a highly significant correlation (ICC: 0.96) between subsequent measurements of glucose concentrations in gingival crevicular blood samples of two sites with profuse bleeding on probing, and therefore, the device is reliable.

Muller et al[15] compared the conclusions drawn following the approach of correlation/regression analyses of comparative data of diagnostic methods and measures of calculating limits of agreement. They mentioned that correlation analysis is inappropriate when assessing agreement between two methods of measurements. They also state that correlation measures association and not agreement. Therefore, any overall summary measure, such as the correlation coefficient does not help a clinician interpret a measurement. Regression analysis attempts to predict an observed measurement by another observed measurement. It suggests that one measurement can be modeled by another, which is not the case in measurement comparison and the key to method comparison is quantification of disagreement of the measurements,[12] not of residuals.[15] In order to eliminate any discrepancy, we used both methods in our study.

The results of measurements in non-diabetic patients showed that although there was a highly significant correlation between the paired samples of fasting plasma glucose and capillary fingerstick blood glucose, the analysis of agreement according to Bland and Altman failed to prove sufficient agreement between the paired methods (FPG & CFBG, FPG & GCBG, and CFBG & GCBG).

The results of measurements in diabetic patients showed that there was a highly significant correlation between each two methods, but analysis of agreement revealed sufficient agreement only between FPG & CFBG and FPG & GCBG measurements.

In this study, we also compared GCBG and CFBG to the laboratory measurement of FPG at the same time. FPG measurement with the highest level of diagnostic accuracy is known as the "Gold Standard" and its comparison to the other two methods is necessary to assess the correctness of each blood collecting technique and device.[11] Because the glucose self-monitoring device and laboratory tests do not measure the same components, the reported figures are likely to vary between methods. Laboratory tests generally test plasma, but a glucose self-monitoring device uses whole blood. As a result, if both tests are taken at the exact same time, the self-monitoring device is likely to show a lower figure than the laboratory results. The new standard method has to prove sufficient agreement with the "gold standard" as mentioned earlier,[15] but the GCBG and CFBG was not compared to FPG measurements in neither of their studies.[15,16] Comparing the measurements of CFBG with GCBG is correct but not sufficient and the amount of disagreement between GCBG measurements and laboratory-measured FPG is essential for drawing a definitive conclusion, although any new method cannot be measured completely without error.[17,18] This comparison using laboratory-measured FPG was not done in previous studies except the study of Parker et al,[11] who have investigated the correlation of fingerstick and crevicular gingival blood with whole blood glucose, measured in a laboratory analyzer.

We have also examined both known diabetic and non-diabetic patients in two different procedures. The results from non-diabetic patients in our study were in agreement with the results of the studies performed previously on patients with diabetic mellitus.[11,19] Beikler et al[20] examined both diabetic and non-diabetic patients with moderate and severe periodontitis while the study population of Muller & Behbehani[16] con-

sisted mainly of non-diabetic patients. The results drawn following the approach of correlation/regression analysis and the analysis of agreement in the present study were also comparable to both studies of Muller & Behbehani,[15,16] except that they did not compare GCBG and CFBG to FPG and our positive results are mainly because of this comparison.

All of these studies aim to develop a safe, rapid and noninvasive approach to screen diabetes, which is a major problem especially in periodontal management. Successful resolution of periodontal inflammation involves the stabilization of blood glucose. Therefore, multiple measurements of a diabetic patient's blood glucose allows the periodontist to better assess the patient's diabetic control as treatment progresses.[21] All the studies started from 1969 by Stein et al[22] and Tsutsui et al[23] to the more recent studies of Beikler et al[20] and Khader et al[19] attempted to prove that extravasated blood from the gingival crevice due to inflammation can provide an acceptable source for measuring blood glucose in diabetic patients. Yamaguchi et al[24,25] have investigated a method for noninvasive blood glucose measurement using the gingival crevicular fluid, (entailing the use of a high sensitivity glucose testing tape to evaluate the possibility of using this fluid for noninvasive blood glucose measurement, and concluded that gingival crevicular fluid could be used as a method of blood glucose measurement. These studies have reported only the correlation coefficient in their results.

We chose ACCU-CHEK Active® glucometer because it is the most widely-used glucometer in Iran and requires less than 1 μlit blood. Gingival crevicular blood samples revealed glucometer readings in all the cases and we did not obtained any error message neither in diabetic nor in non-diabetic cases. The fact that bleeding on probing was sufficient in our cases might be due to the high prevalence of periodontal inflammation in our population.

Strauss et al,[26] in a recent similar study based on characteristics of the blood collection site, reported that gingival crevicular samples are suitable to screen for diabetes in individuals with sufficient bleeding on probing to obtain a sample without touching the tooth or gingival margin. It can be interpreted that since periodontal inflammation with the complicating factor of diabetes produces ample extravasated blood during routine periodontal examination, the examiner can easily and accurately test the blood glucose in uncontrolled diabetic patients.

It can be concluded that, based on the results of the present study, gingival crevicular blood for is useful for testing blood glucose during routine periodontal examination in diabetic periodontal patients but not in non-diabetic ones. Future research should be directed at comparing different glucose self-monitoring devices in larger populations.

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