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Correlation analysis of HbA1c versus random, fasting, and postprandial glucose levels as predictors of glycemic control in type 2 diabetes patients

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ABSTRACT

Background: Glycated Hemoglobin (HbA1c) is used for assessing glycemic control over the past 8–12 weeks. This is critical for determining the efficacy of diabetes treatment and predicting the progression of microvascular complications. However, in health-care situations where tests for HbA1c are either unavailable or unfeasible for any reason, clinicians rely only on plasma glucose values for assessing the glycemic control of the patient. **Aims and Objectives:** The purpose of this study was to determine the relationship between HbA1c and fasting, postprandial, and random plasma glucose levels. **Materials and Methods:** Routine blood samples obtained from the laboratory to measure HbA1c and plasma glucose (fasting/postprandial/random) were used for study. A total of 207 samples were used to investigate the relationship between HbA1c and fasting and postprandial glucose (PPG/PP). The correlation of HbA1c with random glucose values was investigated using 112 samples. HbA1c was estimated by immunoturbidimetry and glucose was estimated by hexokinase method. Pearson's correlation analysis was done by SPSS version 20 software. **Results:** The Pearson correlation coefficient (r) with regard to PPG/PP and HbA1c was 0.75 (P = 0.01, 95% CI), fasting glucose and HbA1c was 0.73 (P = 0.01, 95% CI), and random glucose and HbA1c was 0.59 (P = 0.01, 95% CI). **Conclusion:** PPG/PP correlates with HbA1c better than fasting or random glucose. When it is impossible to perform HbA1c measurements, PPG measurements should be used instead.

Key words: Diabetes; Fasting glucose; Hemoglobin; Postprandial glucose; Random glucose

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INTRODUCTION

Diabetes mellitus (DM) is a chronic condition that necessitates recurring medical attention, and support to avoid acute health problems and lower the chance of protracted comorbidities. Several methods can be used to assess blood glucose control in diabetic patients. Etiologic studies on insulin-independent diabetes-mellitus (type II diabetes) have posited a link between hyperglycemia and the degree of glycemic control.

Maintenance of glycemic control is the cornerstone in the management of diabetes.[1] The degree of glycemic control in a diabetic patient can be assessed by measurement of glycated hemoglobin, fasting plasma glucose (FPG), and postprandial glucose (PPG/PP). Glycated hemoglobin is the most recommended parameter to assess glycemic control at follow-up due to its correlation with average plasma glucose for past 8–12 weeks. The ADA also recommends HbA1c measurement as the criteria for diagnosing diabetes with a cutoff value of 6.5%.[2-5]

HbA1c is used for monitoring glucose control over the past 2–3 months. The glucose presents in the blood causes glycation of blood proteins such as hemoglobin. The glycation of hemoglobin is an irreversible non-enzymatic

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reaction that occurs continuously *in vivo*. The glucose reacts with the valine and lysine amino acid residues at the N-terminal end of the beta chain of hemoglobin and forms a Schiff base, which undergoes amadori rearrangement to form stable ketamine.[6]

Normal adult hemoglobin predominantly consists of HbA ($\alpha 2\beta 2$) with two alpha and two beta chains. When hemoglobin from a normal person is run through a chromatographic column, it separates into a major fraction, hemoglobin A0 (HbA0), and several minor, fast-moving component, collectively known as Hb A1 (Hb A1a+1b+1c). HbA1c is the most abundant of these fractions and accounts for approximately 5% of the total HbA fraction.[7]

The percentage of glycated hemoglobin fraction is affected by blood glucose levels. The concentration of glycated hemoglobin in the blood rises in tandem with the average plasma glucose level. As a result, HbA1c is the preferred parameter to assess the average glucose for the past 8–12 weeks. Red blood cells (RBCs) typically last for this amount of time in healthy individuals. This includes all RBCs, from the oldest to the youngest (120-days-old), and glucose levels in the previous 30 days contribute significantly more to HbA1c levels than glucose levels in the previous 90–120 days.[8,9] The HbA1c value for a normal healthy person is 4–5.9%, but it can rise to 10–12% in poorly controlled diabetic patients.[10]

The cost of the test is one of the drawbacks of using glycated hemoglobin as a predictor of glycemic control. This test is more expensive than a plasma glucose estimation.[11] Standardized monitoring systems and research laboratories are not widely used across the country, and evaluating HbA1c is more expensive than performing FPG assessments. Furthermore, there is no agreement on an appropriate HbA1c cutoff point for the diagnosis of diabetes in this high-risk population of countries, including India.[12]

The present study investigated the relationship between HbA1c and FPG, PPG/PP, and random glucose. This study would help to validate the practice of measuring FPG, PPG/PP, or random glucose as effective tools for assessing glycemic control, especially in areas where glycated hemoglobin tests are unavailable.

Aims and objectives

This study was designed with the aim to evaluate the relationship of glycated hemoglobin with fasting plasma glucose, postprandial glucose and random glucose respectively. The primary objective was to estimate the correlation between HbA1c and fasting glucose, postprandial glucose and random glucose levels in plasma samples of Type 2 diabetes patients. The secondary objective was to deduce correlation of these biochemical parameters with the Estimated Average Glucose (eAG).

MATERIALS AND METHODS

This cross-sectional study was conducted in accordance with the recommended ethical guidelines. Consent for conducting requisite tests was obtained from the study participants. Diagnosis of type 2 DM was considered as the inclusion criteria for the study participants. The exclusion criteria incorporated patients with chronic inflammatory diseases, Cushing syndrome, chronic liver disease, patients on dialysis, patients taking drugs affecting glycemic state such as steroids and anti-depressants, patients with hemolytic diseases, and cases of iron deficiency anemia.

The study utilized the routine blood samples received in the laboratory for HbA1c and plasma glucose estimation (fasting, postprandial, and random). Out of all these samples received in the clinical chemistry laboratory over the course of 2 months, only the patients' samples of diagnosed type 2 diabetes cases for whom the clinician had requested both HbA1c and plasma glucose estimations were included in the study. A total of 112 samples were included to examine the relationship between HbA1c and random glucose values, while 207 samples were included to examine the relationship between HbA1c with fasting as well as PPG/PP. Therefore, no additional blood samples from the patients were obtained for this study. The blood samples drawn for the routine diagnostic workup were only subjected to the tests recommended by the clinician.

Blood samples for HbA1c were collected in EDTA vials to obtain the whole blood for analysis. Blood samples for plasma glucose estimation were collected in fluoride and oxalate-containing grey cap vials. After centrifugation, plasma was used for glucose analysis. The samples were screened for pre-analytic errors, mainly hemolysis, icterus, and lipemia. Hemolytic samples were not processed further to avoid interference in the test results. The tests were performed using a MISPA Clinia Autoanalyzer. HbA1c was estimated by immunoturbidimetry and glucose was estimated by the hexokinase method. Two levels of control were run daily for both the parameters to ensure the quality of the tests done.

Measurement of HbA1c

The HbA1c was estimated by Immunoturbidimetry method. We have used Agappe diagnostics kit for the determination of % HbA1c in human blood. This method directly determines HbA1c in whole blood by utilizing antigen-antibody interaction. The unspecific latex absorption rate for total hemoglobin and HbA1c is equal. On the addition

of mouse antihuman HbA1c monoclonal antibody (R2), a latex-HbA1c-mouse anti-human HbA1c antibody complex is developed. Later, goat anti-mouse IgG polyclonal antibodies interact with monoclonal antibodies to produce agglutination. The quantity of HbA1c absorbed onto the surface of latex particles directly correlates with the quantity of agglutination. Absorbance measures the amount of agglutination (Figure 1). A calibration curve is used to calculate the HbA1c value. The absorbance at 660 nm was measured to calculate the HbA1c% from a calibration curve.[6] The assay has an analytical sensitivity of 3%.

Measurement of glucose

Plasma glucose was estimated by the enzymatic Hexokinase Method. We have used Agappe Diagnostics kit for determination of glucose level in human blood. This method has high specificity for plasma glucose measurement. In the presence of hexokinase, glucose is converted to glucose-6-phosphate. Glucose-6-phosphate dehydrogenase, in the presence of NADP, oxidizes glucose-6-phosphate to gluconate-6-phosphate and NADPH. The rate of NADPH formation is directly proportional to the glucose concentration in the serum, measured photometrically.[13] The assay has an analytical sensitivity of 5.0 mg/dL. The following enzyme based reaction is used to determine glucose.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2}]{\text{Hexokiase}} \text{Glucose-6-Phosphate} + \text{ADP}$$

$$\text{Glucose-6-Phosphate} + NAD^{+} \xrightarrow[\text{Dehydrogenase}]{\text{Glucose-6-Phosphate}} \text{D-Gluconate-6-Phosphate} + NADH^{+} + H^{+}$$

Statistics analysis

The data were collected and documented in a Microsoft Excel spreadsheet. This included patient sex and age along with the measured HbA1c and glucose values. Estimated average glucose (eAG) was also calculated for all the patients using the formula: eAG = (28.7 × HbA1c) – 46.7 mg/dL.

Statistical analysis was done using data to find out the linear correlation (two-tailed) between HbA1c and fasting glucose, HbA1c and PPG/PP, and HbA1c and random glucose. The Pearson's correlation coefficient was calculated and the significance of this correlation was deduced. $P<0.05$ was considered significant.

RESULTS

A total of 207 samples were used to investigate the relationship between HbA1c and fasting and PPG/PP levels. The descriptive analysis showed that "HbA1c," "eAG," "Fasting," and "PP" in this study ranged from 5.20% to 14.80%, 103–378 mg/dL, 50–448 mg/dL, and 79–511 mg/dL, respectively (Table 1). In our study, we

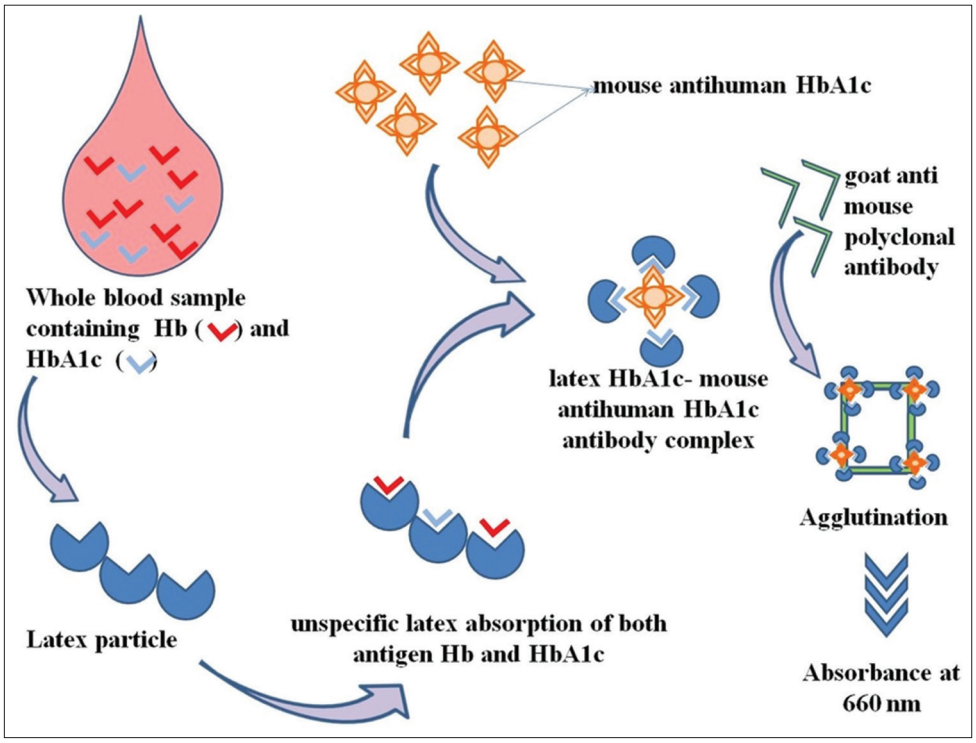

Figure 1: Immunoturbidimetry method for HbA1c estimation

Table 1: Descriptive analysis of HbA1c with fasting and postprandial glucose

Statistical parameters	"HbA1c" %	"eAG" mg/dL	"Fasting" mg/dL	"PP" mg/dL
n	207	207	207	207
Missing	0	0	0	0
Mean	8.45	196.00	164.00	225.00
SEM	0.14	4.12	5.07	6.73
Median	7.80	177.00	138.00	208.00
SD	2.07	59.30	73.00	96.80
Minimum	5.20	103.00	50.00	79.00
Maximum	14.80	378.00	448.00	511.00

eAG: Estimated average glucose, PP: Postprandial glucose

Table 2: Correlation analysis among HbA1c, eAG, fasting, and postprandial glucose

Statistical parameters	HbA1c	eAG	Fasting	PP
HbA1c				
Pearson correlation	1	1.000**	0.733**	0.747**
Sig. (two-tailed)		0	0	0
N	207	207	207	207
eAG				
Pearson correlation	1.000**	1	0.733**	0.747**
Sig. (two-tailed)	0		0	0
N	207	207	207	207
Fasting				
Pearson Correlation	0.733**	0.733**	1	0.870**
Sig. (two-tailed)	0	0		0
N	207	207	207	207
PP				
Pearson Correlation	0.747**	0.747**	0.870**	1
Sig. (two-tailed)	0	0	0	
N	207	207	207	207

**Correlation is significant at the 0.01 level (two-tailed). eAG: Estimated average glucose, PP: Postprandial glucose

Table 3: Descriptive analysis of HbA1c with random glucose as well as eAG

Statistical parameters	HbA1c	eAG	Random Glucose
N	112	112	112
Missing	0	0	0
Mean	8.07	185.00	153.00
Std. Error mean	0.20	5.63	7.48
Median	7.50	169.00	122.00
Standard deviation	2.08	59.60	79.10
Minimum	4.80	91.00	72.00
Maximum	14.40	367.00	541.00

eAG: Estimated average glucose

Table 4: Correlation analysis among HbA1c, eAG, and random glucose

Statistical parameters	HbA1c	eAG	Random
HbA1c			
Pearson correlation	1	1.000**	0.591**
Sig. (two-tailed)	0.000	0.000	
N	112	112	112
eAG			
Pearson correlation	1.000**	1	0.591**
Sig. (two-tailed)	0.000	0.000	
N	112	112	112
Random			
Pearson correlation	0.591**	0.591**	1
Sig. (two-tailed)	0.000	0.000	
N	112	112	112

**Correlation is significant at the 0.01 level (two-tailed). eAG: Estimated average glucose

observed a significant correlation between HbA1c and PPG/PP, as well as HbA1c and fasting glucose. The Pearson correlation coefficient (r) between PPG/PP and HbA1c was 0.747 (P=0.01, 95% CI). Pearson's correlation coefficient (r) for fasting blood glucose and HbA1c was 0.733 (P=0.01; 95% CI) (Table 2, Figure 2a and b). We have received a total of 112 samples to investigate the relationship between HbA1c and random glucose. The descriptive analysis results for "HbA1c," "eAG," and "random glucose," in this study ranged from 4.8% to 14.4%, 91 to 367 mg/dL, and 72 to 541 mg/dL, respectively (Table 3). The Pearson correlation coefficient (r) between random glucose and HbA1c was 0.591 (P=0.01, 95% CI) (Table 4 and Figure 2c).

DISCUSSION

Diabetes is a chronic condition that affects millions of individuals in low and middle-income countries, where the rate of prevalence has been increasing over the past two decades and has surpassed that of high-income countries. The epidemiology of diseases illustrates the rising disease burden due to an obesity crisis and unhealthy habits. Type 2 diabetes accounts for the majority of the total diabetes prevalence.[14] The microvascular and macrovascular complications are attributed to the morbidity and mortality during the course of the disease. The maintenance of glycemic control is the foundation of diabetes management. The degree of glycemic control in a diabetic patient can be assessed by measurement of glycated hemoglobin, FPG, and PPG/PP. Diabetes diagnostic criteria have been constantly evolving. DM is diagnosed based on plasma glucose criteria, which include FPG levels or 2-h plasma

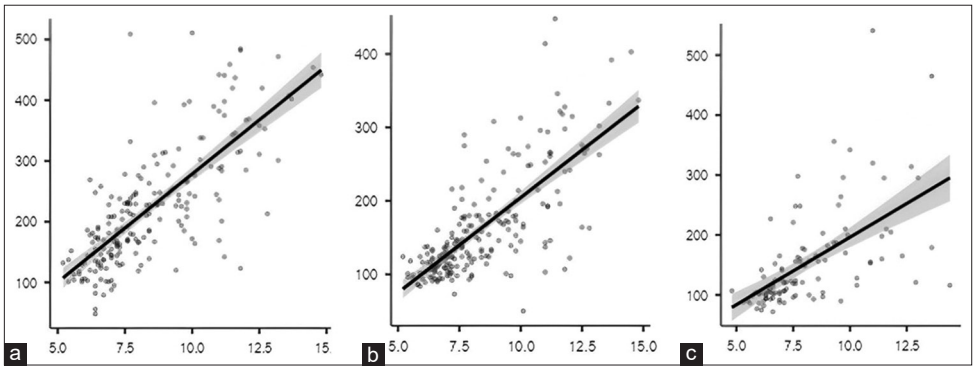

Figure 2: Correlation matrix plot of (a) HbA1c versus postprandial glucose, r: 0.747**, (b) HbA1c versus fasting glucose, r: 0.733**, and (c) HbA1c versus and random glucose, r: 0.591** (**<0.01)

PPG/PP (2h PPG) levels during a 75 gm oral glucose tolerance test, or the newer glycosylated hemoglobin (HbA1c) criteria, which reflect the average plasma glucose concentration over the previous 8–12 weeks.[15]

Glycated hemoglobin is the best variable to evaluate glycemic control, because it correlates with average plasma glucose for past 8–12 weeks. Furthermore, the test can be done at any time of day and does not require fasting. When glycated hemoglobin is used to monitor glucose control, it also overcomes variability in diet, stress, and exercise. In comparison to glucose, HbA1c has a high pre-analytical stability. The fact that there is little variation in concentration after sample collection adds to the benefits of using HbA1c as a preferred glycemic marker. The glycated hemoglobin helps in the prediction of diabetic complications and facilitates decisions. The American Diabetes Association has recommended HbA1c measurement as the criteria for diagnosing diabetes, with a cutoff value of 6.5%.[15,16]

Glycated hemoglobin is not only dependent on the level of glycemia but also on the turnover rate of RBC in the blood. The use of HbA1c values in pathological conditions such as anemia, blood loss, hemoglobinopathies, and malaria may result in an incorrect interpretation of glycemic state.[17] Moreover, due to significantly decreased erythropoietin as the stage of chronic renal disease advances, HbA1c becomes a less accurate marker for glycemic control. According to research, HbA1c should not be used alone as a glycemic control marker in hypothyroidism and unstable thyroid state. Furthermore, HbA1c does not reflect acute changes in glucose metabolism.[18]

The cost of the test is yet another barrier to using HbA1c as a glycemic control predictor. In such settings, plasma glucose estimation is preferred over HbA1c to monitor glycemic control due to the lower cost of the experiment and convenience of standardization. Plasma glucose is measured by clinicians as either fasting samples, postprandial samples, or random samples (regardless of food intake). Studies claiming the importance of using a specific sample (fasting, random, or postprandial) for evaluating chronic glycemic control in diabetic patients are contradictory.[19,20] As a result, in this study, we investigated the relationship between HbA1c levels and fasting, postprandial, and random glucose levels to identify the best sample to be used for testing in resource limited settings or where a HbA1c test facility is unavailable.

The Pearson correlation coefficient (r) between PPG/PP and HbA1c was 0.747, while the correlation coefficient (r) between fasting glucose and HbA1c was 0.733. According to these findings, postprandial plasma glucose levels correlate best with HbA1c and calculated eAG levels. The correlation coefficient between random plasma glucose and HbA1c was 0.591. PPG/PP reflects the coordinated function of carbohydrate absorption as well as the post-absorption effects of insulin and glucagon on the liver and peripheral tissues. Insulin resistance fails to adequately control the postprandial rise in glucose levels in type 2 diabetes. Several studies have been conducted to determine the relationship between PPG and FPG and HbA1c. Although there is insufficient data to accurately determine the relative contribution of FPG and PPG/PP to HbA1c.[2,19] According to some studies, PPG levels contributed most in the lower HbA1c percentile (in good or fair HbA1c values), while fasting hyperglycemia was primarily responsible for the overall hyperglycemia in patients with poorly controlled diseases (HbA1c >9%).[2,20] According to the aforementioned finding, our studies also showed that PPG is more accurate at predicting HbA1c and average glucose levels. The use of PPG values for analyzing glycemic status also allows for the inclusion of short-term fluctuations in glucose, particularly postprandial excursions. In the absence of PPG, FPG is the second best alternative to HbA1c measurements in predicting the patient's glycemic status. Another finding agreed with

ours, emphasizing the inconvenience caused to diabetic patients by overnight fasting, whereas PPG/PP caused no disruption in daily activities. Postprandial blood sugar has also been shown in studies to predict cardiovascular complications in diabetic subjects.[21]

Limitations of the study

The study had a limited sample size. More studies with large sample size are needed to establish the correlation of HbA1c with fasting, postprandial and random glucose levels in diabetic as well as healthy population. Also, since immunoturbidimetry method estimates some hemoglobin variants as well, the impact of hemoglobin variants on HbA1c could not be excluded in this study.

CONCLUSION

Our study concludes that PPG/PP estimation can be used as a standalone test for assessing long-term glycemic status in resource-constrained settings, where HbA1c testing is not available. Even though this study had a small sample size, more studies like ours are needed to strengthen our findings. It is also necessary to look into the functions of PPG and FPG in anticipating long-term complications in type 2 diabetes.

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Authors' Contributions:
SA- Conceptualized the study, collected the data and drafted the manuscript; **AKA** and **JG-** Participated in the data analysis and interpretation; **JB-** Revised the manuscript. All authors read and approved the final manuscript.

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