Original Research Article

Comparative Evaluation of the Recent Diagnostic Criteria of HbA$_1$c with Fasting Blood Glucose

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Abstract

Background: Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production [1]. *Aims and Objective:* To evaluate the performance of Enzymatic and HPLC assay systems for determination of HbA1c. *Material and Methods:* This study was conducted from November, 2016 to December, 2017. The subjects under the study were enrolled from outpatient department of Medicine after obtaining written informed consent in bilingual languages from all the 100 subjects after describing all pros and cons. Fasting Plasma glucose and HbA1c were estimated in Department of Biochemistry, SRMS IMS. *Result and Discussion:* Maximum percentages of patients were in age 51 to 60 years (32%) while the least percentage of patients was from the age group 21 to 30 years (2%). Present study comprising 38% females and 62% males. HbA1c distribution in subjects by Enzymatic method in Mean $\pm$ SD (8.598 $\pm$ 2.29) with minimum of 6.2% and maximum 20.4%. The FPG distribution in Subjects 154.37 $\pm$ 40.13 (Mean $\pm$ SD) with minimum FPG of 96 mg/dl and maximum 289 mg/dl. *Conclusion:* HbA1c has come to play an integral role in the management of diabetes, one of the world's most prevalent non-communicable diseases. HbA1c defines an end point as the fuel of diabetic therapy and provides a powerful stimulus to the patients to improve their compliance. HbA1c remains the gold standard in the assessment of glycemic control with availability of standardize methods.
Keywords: Glycated Hemoglobin (HbA1c), Fasting Blood Sugar (FBS).

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production [1].

Diabetes mellitus is one of the most common non-communicable diseases globally. 425 million people have diabetes mellitus in the world and 82 million people in the South East Asian (SEA) Regions; by 2045 this will rise to 151 million. There were over 72 million cases of diabetes mellitus in India in 2017 [2].

Evidence suggests that early diagnosis and proper treatment of type 2 diabetes mellitus confer health benefits, whereas aggressive control of plasma glucose, blood pressure and cholesterol after diagnosis of type 2 diabetes mellitus may be less important than early screening [3].

In the year 1922, Banting FG *et al.*, [4] observed that initially the diagnosis of DM was mainly based on glycosuria. Thereafter, diagnosis and treatment of Diabetes mellitus have largely been based around measurement of plasma glucose concentrations which can be fasting plasma glucose (FPG), Post prandial plasma glucose (PPPG) or Random plasma glucose.

HbA1c is an important test recommended by the American Diabetes Association (ADA) and other diabetes mellitus organizations worldwide, for management of patients with diabetes mellitus [5].

HbA1c reflects average plasma glucose over the previous 8-12 weeks, providing a precise and consistent indicator of circulatory glucose levels,

allowing for accurate assessment of glycemic control as well as the potential to act as a diagnostic marker of type-2 diabetes mellitus. Based on the evidence that HbA1c level correlates with adverse disease outcomes and the fact that HbA1c targets for patient treatment, use of HbA1c as a diagnostic tool seemed a logical progression [6].

The most commonly used assay to measure chronic hyperglycemia is HbA1c. However it is not available in poor setting due to high cost. There are many reports showing the acceptable correlation between HbA1c and FBS.

Hence the aim of this study was to find the correlation between HbA1c with FBS.

MATERIAL AND METHODS
This study was done in the Department of Biochemistry in collaboration with Department of Medicine at Shri Ram Murti Smarak Institute of Medical Sciences (SRMS IMS), Bareilly, U. P. India. This study was an analytical correlation prospective study.

This study was conducted from November, 2016 to December, 2017 after obtaining due permission from Institutional Ethical Committee. The subjects under the study were enrolled from outpatient department of Medicine after obtaining written informed consent in bilingual languages from all the 100 subjects after describing all pros and cons. Fasting Plasma glucose and HbA1c were estimated in Department of Biochemistry, SRMS IMS.

Results were divided according to the value of HbA1c into 3 groups i.e. good control (<7%), fair

control (7-10%) and poor control (>10%). HbA1c was estimated by enzymatic assay in Mindray autoanalyzer BS 380. FPG was estimated by enzymatic method, using Glucose oxidase and Peroxidase (GOD-POD) method. Before collecting venous blood sample using standard venipuncture subjects were asked to fast for minimum 8 hrs. For the standardization blood was drawn in sitting position from antecubital vein under aseptic conditions. 4 ml of blood was collected which was equally divided in two different vacutainers, one Ethylene Diamine Tetra Acetic acid (EDTA) vacutainers for measuring the HbA1c, and one sodium fluoride vacutainer for measuring fasting plasma glucose. Blood was allowed to sediment and centrifuged at 5000 rpm for 10 minutes. All the samples were analyzed immediately.

STATISTICAL METHODS
The data was processed and appropriate statistical analysis was done by using licensed Microsoft Excel 2007 and SPSS (Statistical Package for the Social Sciences) version 20.0. Software (SPSS Inc. Chicago, IL, USA) to compare HbA1c levels with FBS. All data were expressed as "mean ±SD". Paired student 't' test was applied to see the statistical significance of variables. A value of $p \leq 0.05$ was considered to be statistically significant. Co-efficient of correlation 'r' was determined between different biochemical indices by using Pearson product moment correlation.

RESULTS
Maximum percentages of patients were in age 51 to 60 years (32%) while the least percentage of patients was from the age group 21 to 30 years (2%). Present study comprising 38% females and 62% males.
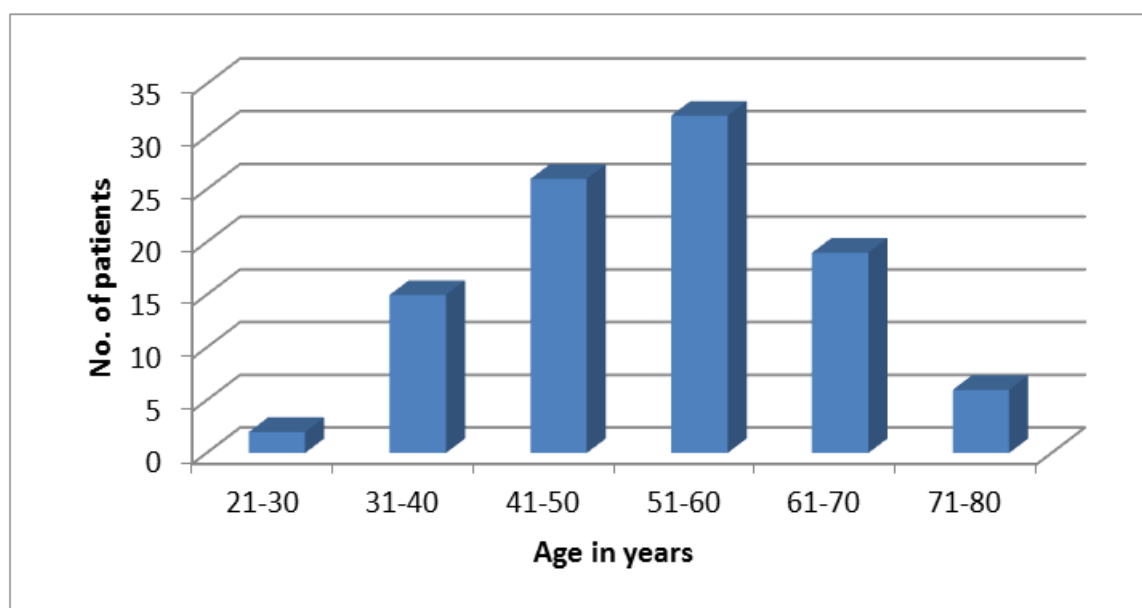
HbA1c distribution in subjects by Enzymatic method in Mean ± SD (8.598 ± 2.29) with minimum of 6.2% and maximum 20.4%.

The FPG distribution in Subjects 154.37 ± 40.13 (Mean ± SD) with minimum FPG of 96 mg/dl and maximum 289 mg/dl.

	Total Subjects	Range	Mean	Standard Deviation	r- value	p value
HbA1c (%)	100	6.2-20.4	8.598	2.29	0.88	<0.0001
FBS	100	96-289	154.37	40.13		

- p value : <0.05 is considered to be statistically significant.

Positive correlation (r = + 0.882858) between FPG and HbA1c Level by Enzymatic method in subjects. p=<0.0001
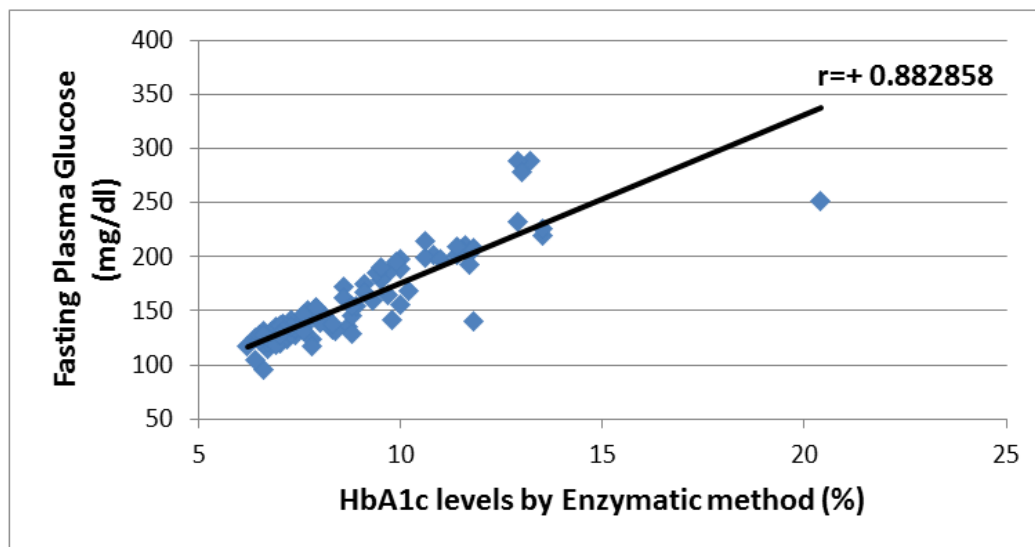
DISCUSSION

HbA1c estimation is one of the diagnostic markers of diabetes mellitus and also a useful indicator of glycemic control in diabetic patients. HbA1c is a reaction product of glucose and the N-terminal valine of the β chain of haemoglobin. It also depicts risk of diabetic complications and is a quality assurance indicator to assess the quality of diabetes care.

Routine Hemoglobin A1c (HbA1c or Glycosylated hemoglobin) testing is now widely used in clinical practices for diagnosis, monitoring and treatment of diabetic patients. Thus, measurement of HbA1c is in great demand in clinical biochemistry laboratory nowadays [7, 8]. HbA1c is a marker of patient's glycemic status in the past 2-3 months. Therefore, measurement of HbA1c is crucial for clinical decision making regarding drug dose adjustments.

HbA1c values well correlated with glycemic status in the past with only a few exceptions, provided the test was done with due care. Earlier studies have tried to correlate with values of 7– 8 point blood testing at regular interval with the HbA1c results at 1, 2, and 3 months timing and confirmed efficiency in majority of the situations. But actually 7– 8 point blood sugar in a day does not cover all the glycemic excursions throughout the period [9].

HbA1c estimation is also useful to differentiate between stress hyperglycemia and pre-existing undetected diabetes mellitus in emergency situations when insulin therapy is to be advocated in the later one [10].

HbA1c estimation is also useful to plan therapy in newly detected T2DM as per American Association of Clinical Endocrinologists (AACE) recommendation 2007 [11]. If HbA1c is above 10%, insulin should be started from the beginning, and if between 8% and 10% combinations of oral anti diabetic should be of choice. Below the value of 8%, treatment should be started with a single drug.

The long-term complications of diabetes mellitus are extensive, with retinopathy leading to vision loss, nephropathy leading to chronic kidney disease and peripheral neuropathy being the most common microvascular complications. In addition, cardiovascular disease, peripheral artery disease, and stroke are common macrovascular complications Persistent hyperglycaemia produces not only glycation of haemoglobin but also different tissue proteins and advanced glycation end products, which are one of the culprits in the development of organ complications in diabetes mellitus. Thus, persistent raised glycated Hb

values for long term (usually more than 2 years) are associated with organ damage. Klein *et al*., found 1.9 times higher risk of retinopathy with HbA1c above the highest quintile in T1DM with diabetes mellitus for more than 4 years [12]. The risk increased with age and longer duration [1].

Advantages of HbA1c over Plasma Glucose

Fasting plasma glucose is defined as testing blood sugar after no calorie intake for 8 hour. Patient preparation is also required for measurement of oral glucose tolerance test (OGTT) and Fasting plasma glucose.

In contrast the HbA1c testing is devoid of any such preparation, and bears the advantage of performing in any time of the day.

Fasting plasma glucose are altered by numerous factors like stress, acute illness, medication, venous stasis, posture, sample handling, food ingestion, prolonged fasting and exercise. These factor, are also likely affects the 2 hr OGTT. The same factors, however does not have any affects on HbA1c measurements [13].

Selvin E, Crainiceanu CM, Brancati FL, Coresh J. observed that In case of fasting blood glucose intraindividual variation in healthy persons is reported to be 5.7 – 8.5%, while interindividual variation is revealed to be upto 12.5%. Also there is high degree of intraindividual variability in the OGTT, with a CV of 16.7%, which is considerably greater than the variability for FPG [14].

Thus the reproducibility of the OGTT is poor. But compared to these two parameters used for diabetic diagnosis, HbA1c shows a relatively lower biological variation with a CV <1% Rohlfing C et al observed [15].

Glycolysis consumes glucose even in fluoride preservative for the first two hours after blood is collected, and may continue upto 4 hrs. The delay in the glucose stabilizing effect of fluoride is thought to be the result of glucose metabolism proximal to the fluoride target enolase. The rate of glycolysis is also increased in samples which contain an increased concentration of RBC, WBC and platelets [16].

This fact questions the accuracy of FPG measured and used for diagnosis of diabetes mellitus. As the same process is involved in the measurement of OGTT, like FPG its accuracy is also always questioned. In contrast HbA1c has high preanalytical stability and is stable for 1 week when stored at 4°C and for 1 year when stored at -70°C [17].

In this study, the fasting blood glucose level variation in diabetic patients was observed in between 96-289 mg/dl with 154±40.13 (Mean±SD). Positive correlation was obtained between fasting blood glucose and HbA1c level by enzymatic method which was (r) = 0.88.

CONCLUSION

HbA1c has come to play an integral role in the management of diabetes, one of the world's most prevalent non-communicable diseases. HbA1c defines an end point as the fuel of diabetic therapy and provides a powerful stimulus to the patients to improve their compliance.

Objective of this study was to compare in a prospective study the clinical efficacy glycated hemoglobin and FBS. The rapid and accurate laboratory diagnosis of HbA1c is necessary through a variety of laboratory modalities.

HbA1c remains the gold standard in the assessment of glycemic control with availability of standardize methods. The limitation of resource or cost should not be the barrier to provide the good medical care. However in resource poor settings and in conditions with limitations for using HbA1c. The results of this study also indicate that FPG can be used along with HbA1c for monitoring of diabetes as their combination enhances the diagnostic accuracy of these individual tests.

Though, HbA1c provides a better discriminate of diabetic from the non diabetic than a rapidly fluctuating variable like blood sugar. Also, the discrepancy between the results of HbA1c and results of blood sugar level of patients can give an indication to treating physician to look back into detail history and modify the therapeutic regimen accordingly.

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