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Original Article

Biochemistry Section

Comparison of NGSP Certified Methods and their Cost Analysis for Estimation of HbA1c- A Cross-sectional Study

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is a major non communicable disease that adversely impacts the health of the global community, contributing significantly to mortality. Estimation of glycated haemoglobin (HbA1c) is an integral component of diagnosis, treatment and follow-up of cases of DM as the various protocols have included it for therapy. In order to regularise the estimation of HbA1c, it is mandated by American Diabetes Association (ADA) to perform the test on NGSP certified methods only.

Aim: To compare the results of HbA1c analysis by SIEMENS DIMENSION EXL 200 and BIORAD D-10 (NGSP certified methods).

Materials and Methods: This was a cross-sectional observational study done in Department of Biochemistry, Armed Forces Medical College, Pune, Maharashtra, a tertiary care centre. One hundred and twenty blood samples in Ethylene Diaminetetraacetic Acid (EDTA) tubes for haemoglobin variant analysis, received from patients with unexplained anaemia or screening for antenatal care were included in the study. The tests for HbA1c were performed by HPLC based BIORAD D10 and enzymatic based method, SIEMENS DIMENSION EXL 200 analyser. Cost analysis of both the methods was also performed. Students paired t-test was done to look for any significant statistical difference. Kappa coefficient was also calculated to analyse for agreement between values.

Results: HbA1c estimation by SIEMENS DIMENSION EXL200 and BIORAD D-10 (both NGSP certified) showed marginally different results, but it was not significant enough to make a clinical difference (5.308% on BIORAD D10 and 5.211% on SIEMENS DIMENSION EXL 200). On performing the cost analysis, there was a significant difference observed with a figure of Rs 476.05/- per test on the SIEMENS DIMENSION EXL200, while it was significantly cheaper at Rs 266/- per test on the BIORAD D10.

Conclusion: Estimation of HbA1c on SIEMENS DIMENSION EXL200 and BIORAD D10 was not clinically significant. As per cost analysis, BIORAD D10 is a cheaper alternative and suitable for tertiary care or large volume centres.

Keywords: Cost estimation, Diabetes mellitus, Glycated haemoglobin, National glycohaemoglobin standardisation programme

INTRODUCTION

Diabetes Mellitus has a predominant position in the list of non communicable disorders that have a significant impact on health at a global level, contributing significantly to mortality while having a prevalence of 8.5% of the adult population [1]. The significant mortality associated with various non communicable diseases e.g., DM has been identified as a goal for reduction by a third, as a part of the Sustainable Development Goals of United Nations by 2030 [2]. The National Health Policy of 2017 aims to reduce the number of premature deaths till 2025 by upto 25% [3]. The prevalence of DM in India has increased significantly from 26 million cases in 1990 to 65 million cases in 2016 and the prevalence of DM in adults older than 20 years was 7.7% in 2016 [4]. In a systematic review and meta-analysis in 2021, from 27 studies of different tribes from various parts of India, the prevalence rate of DM in men, women and children were found to be as 6.04%, 6.48% and 4.94%, respectively [5].

Glycation is the process by which glucose or its derivatives are non enzymatically added to the amino acids of haemoglobin molecule in a permanent manner. The *N*-terminal valine, of the β chain of the globin molecule is the usual site of addition of glucose, and then it is termed as HbA1c [6]. This is the commonest site for addition of the glucose, though it may attach to other amino acids at other sites on both α and β chains [7]. The significance of HbA1c in the diagnosis and management of DM and Gestational Diabetes Mellitus (GDM) is understood by its inclusion in the diagnostic criteria for DM [8]. Due to the diagnostic and therapeutic significance of HbA1c, the method of its estimation becomes extremely significant. There are multiple known methods for the estimation of HbA1c; while one set of methods physically separate the different fractions of haemoglobin from each other by using various techniques, while in the next set of methods, it is estimated by chemical reactions [8]. The analytical methods for glycated haemoglobin are based on charge differences on glycated and non glycated haemoglobin: cation exchange chromatography, electrophoresis and iso electro focusing. The analytical methods for analysis based on the differences in structures of the glycated haemoglobin include boronate affinity chromatography and various immunoassays [8]. The boronate affinity chromatography method is capable of detecting glycation in α and β chains N-terminal valine, as well as on lysine residues [8]. There is a slight difference in the products measured by boronate affinity chromatography as compared to other methods of analysis, as this method estimates the total glycated haemoglobin [9]. The reference method for estimation of glycated haemoglobin in the DCCT trial was cation exchange chromatography. The National Glycohaemoglobin Standardisation Programme (NGSP) was established in 1996, in order to standardise the values of glycated haemoglobin among the various laboratories analysing HbA1c to the DCCT method [6]. It is recommended by American Diabetes Association (ADA) that only NGSP certified methods be used for the analysis of HbA1c [8]. For any method of analysis to be NGSP certified, it has to have a total imprecision (CV%) of <4% and the 95% CI with a reference laboratory should fall within the clinically significant limits of ±1% HbA1c [10]. Various factors are known to influence the levels of glycated haemoglobin, including various haemoglobinopathies that reduce RBC survival, Vit C and Vit E use, Iron deficiency anaemia, hypertriglyceridaemia, hyperbilirubinaemia, uraemia, chronic alcoholism, chronic ingestion of salicylates and opiate addiction [11-15]. The interpretation of

HbA1c in some cases varies with the race and ethnicity, G6PD deficiency (Glucose 6 Phosphate Dehydrogenase), sickle cell anaemia, pregnancy (second and third trimesters), recent blood loss, haemodialysis, postpartum period, blood transfusion or erythropoietin therapy [16-24]. The presence of various different chains in haemoglobin alters the rate of glycation of haemoglobin hence the interpretation of HbA1c becomes complicated in case of haemoglobin variants [9].

Multiple studies have been done in India in order to compare the results of HbA1c by different methods but none has been done on the enzymatic method, which is routinely used by there clinical chemistry laboratory [25-27]. The methods available in present setting include cation exchange chromatography on BIORAD D10 analyser as well as enzymatic methods on SIEMENS DIMENSION EXL 200, both of which are NGSP certified. Cost analysis of any analyte is an inescapable part of administrative task of the healthcare centre as it helps the administration in determining which method of analysis is suitable at a particular scale of operations. This study was planned in order to compare the results of HbA1c by these analysers and to perform the cost analysis of both methods.

MATERIALS AND METHODS

This cross-sectional observational study was undertaken in Department of Biochemistry, Armed Forces Medical College, Pune, Maharashtra. The duration of the study was from February 2021 to July 2021. A total of 120 samples were selected following inclusion criteria. As this was a Short-term Studentship project of ICMR (STS Reference 2020-01582) and had to be completed in a short duration, the sample size was kept at 120 (as approximately 20 patients reported monthly). The duration of the study was initially planned to be the case load for three months, but due to reduced patient load during COVID-19 pandemic, the duration had to be increased.

Inclusion criteria:

- All samples received for estimation of HbA1c, irrespective of their glycaemic or haemoglobin variant status.

Exclusion criteria:

- Any recipient of blood transfusions in the previous 12 weeks.
- Samples with inadequate blood volume.

Study Procedure

Blood samples were collected in EDTA vacuum evacuated tubes (2 mL) and stored at -20°C, until analysis was done. As per protocol, the samples were collected every day and processed weekly.

The HbA1c was estimated on both the analysers BIORAD D10 analyser as well as by enzymatic methods on SIEMENS DIMENSION EXL 200.The HPLC analyser BIORAD D10 is routinely used for performing haemoglobin variant analysis in patients as it is widely used for this clinical purpose, and it performs HbA1c analysis by default in all the samples. Proper quality control was performed on both the systems, as per the protocols already laid down. The cost analysis was done by including the cost of the kits for performing the tests as per the instructions (SIEMENS DIMENSION HbA1c flex and BIORAD D-10 Recorder Pack), different consumables (SIEMENS DIMENSION A1C calibrator) and quality controls (BIORAD LYPHOCHECK Diabetes Control Level 1 and 2) , that were used for performing all these tests.

STATISTICAL ANALYSIS

The data was analysed using SPSS version 23.0 software. The qualitative data was analysed for frequencies. Mean with standard deviation of HbA1c was calculated for both methods. Kappa statistics was measured to see the degree of agreement between two methods of estimation of HbA1c. Student's paired t-test was used to check whether mean HbA1c measured by two methods was statistically different or not, for which $p<0.05$ was taken as significant.

RESULTS

Out of 120, 65% (n=78) of the subjects were predominantly female with mean age of 25.4 years [Table/Fig-1]. The mean HbA1c on BIORAD D10 was 5.308±0.8986, while by SIEMENS DIMENSION EXL200, it was 5.211±0.9022. The association between the mean by these two methods was 0.936, which was not statistically significant [Table/Fig-2]. Kappa coefficient statistics measuring the agreement between the results on nominal scale (Yes/No) by two methods was found to be 0.561 which was statistically significant. This showed that, there was moderate strength of agreement between two methods. Samples came for patients with unexplained anaemia or screening for antenatal care and it was found that Ante Natal Care (ANC) samples were 30 and there were 35 samples of anaemia. The haemoglobin variant status showed anaemia and ANC to be in highest cases [Table/Fig-3]. The highest levels of foetal haemoglobin seen in the present study was 5.2%.

S. No.	Age in years	Males	Females	Total
1	<21 years	11	4	15
2	21-30 years	11	65	76
3	31-40 years	14	07	21
4	>40 years	06	02	08
	Mean age (years)	29.3	25.4	26.8
	Total	42	78	120

[Table/Fig-1]: Age and gender distribution (N=120).

Variables	Mean	Number of samples	Std. Error mean
HbA1c by HPLC	5.308±0.8986	120	0.0820
HbA1c by siemens	5.211±0.9022	120	0.0824

[Table/Fig-2]: Comparison of HbA1c by both the methods (p value=0.936).

S. No.	Diagnosis	(N) Number of cases	% of total
1	Sickle cell disease	03	2.5
2	Generalised weakness/giddiness	04	3.3
3	Thalassaemia	18	15
4	Ante Natal Care (ANC)	30	25
5	ANC (Ante Natal Care) with Anaemia	29	24.2
6	Anaemia	35	29.2
7	Others (sibling with haemoglobinopathy)	03	2.5

[Table/Fig-3]: Indications of Hb variant analysis in participants (N=120).

The cost analysis was performed on both the methods. For performing the cost analysis, the cost of the kit was factored, along with the cost of all consumables, including quality control, whenever it was run on the analysers [Table/Fig-4].

Variables	Siemens dimension EXL200	Biorad D 10
Cost per kit	Rs 19458.9/- per 120 tests	Rs 65000/400 tests
Basic cost per test (cost of kit/ Number of tests)	Rs 162.16/-	Rs 162.5/-
Final cost per test (including controls and calibration costs added to basic cost per test)	Rs 476.05/- per test	Rs 266/- per test

[Table/Fig-4]: Comparison of cost of kits by both methods.

The basic cost of the test of HbA1c by both methods was close to each other, as seen in [Table/Fig-4]. However, on addition of the quality control that is supposed to be run every time the samples were processed, the final figures showed that BIORAD D10 was cheaper as compared to SIEMENS DIMENSION EXL 200.

DISCUSSION

The dilemma faced by clinical laboratories regarding the use of a suitable NGSP certified method for HbA1c analysis, is due to negligible information available regarding the cost incurred in performing the tests by different methods. Additionally, whether there is any clinical difference in HbA1c results by different methods doesn't have significant data. The comparison of HbA1c by different methods has been done multiple times [28,29]. In a study of 110 samples, HPLC based method had higher mean as compared to the immunoturbidometric mean. They also found a higher variation of HbA1c by immunoturbidometric methods, though it was within the acceptable range [30]. In a similar study from India with 137 samples, the mean HbA1c was higher with HPLC based method as compared to immunoturbidometric method, though conversely HPLC had a higher variation in the results [17].

High concentrations of HbF (Foetal haemoglobin), beyond 15% may interfere with HbA1c estimation with the SIEMENS DIMENSION EXL200, but not on the BIORAD D10 [31], hence in the present study, no interference by foetal haemoglobin was seen. This study was done to compare the results of HbA1c analysis on the analysers available in present setting, i.e., SIEMENS DIMENSION EXL200 and BIORAD D10, both of which are NGSP certified methods. By virtue of being NGSP certified, both the methods are recommended for clinical use and are supposed to give comparable results. In this study, the mean HbA1c, the mean by BIORAD D10 was found to be 5.308%, while on the SIEMENS DIMENSION EXL200 was 5.211%. This difference can be accepted as both the methods are NGSP certified.

The other significant component of the study was the cost analysis of both the methods. The cost per kit is easily available as the cost is mentioned on all the kits by the actual manufacturers of the kits. The basic cost per test by SIEMENS DIMENSION EXL200 was Rs 162.16/-, while on the BIORAD D10 was Rs 162.5/-. There was hardly any cost-difference noted per kit initially. The cost of the quality control used routinely for assessing the quality of results generated by each method was subsequently added to the cost by each method. Finally, the cost seen on SIEMENS DIMENSION EXL200 was Rs 476.05/- per test while on the BIORAD D10 were Rs 266/ test. In a study of cost analysis of lab parameters by Declerck B et al., of 156 parameters, it was seen that high volume and automated tests had lower costs [32]. This was also similar to the findings of Mouseli A et al., from a comparison of rates from 34 laboratories in a city [33]. In a study by Gujral S et al., in a haematopathology laboratory, suggested that all laboratories would have separate cost per test and the cost decreased with the increase in volume of the tests [34]. Unnikrishnan R and Mohan V also suggested that the interpretation of HbA1c in India by clinicians should be done meticulously keeping in mind the history of drug intake, distribution of haemoglobinopathies in different geographical locations or anaemia in the patient [35].

For any healthcare establishment, the choice of platform to perform an analysis depends on the cost of the analyser as well as the recurring cost per test. WHO has brought out the laboratory test costing tool in order to facilitate each laboratory to calculate the cost of a specific test [36]. Since all healthcare establishments have to charge the patients for the test, it becomes extremely important that the cost analysis should be performed. In case there is a drastic difference in the sensitivity of the different methods or equipments, then a difference in the cost per test would be acceptable, otherwise a significant cost difference is not justifiable. In this case, there was a significant difference in the cost analysis of the both NGSP certified methods and it may vary in other centres, depending on the protocols of quality control being followed, as well as by the types of the quality control material being used by them.

Limitation(s)

The sample size could have been higher and other types of patients if included, may have given a wider picture.

CONCLUSION(S)

Present study has shown that HbA1c estimation by both methods (both NGSP certified) had marginally different results, but it is not significant enough to make a clinical difference. On performing the cost analysis, there was a high difference observed in both the methods as BIORAD D10 was found to be cheaper than SIEMENS DIMENSION EXL200. Hence BIORAD D-10 is a commercially viable platform for estimation of HbA1c, especially for large or tertiary care centres.

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