

Diabetes Mellitus an Epidemic to Developing Countries: Acomparision Study on the Significance of Glycated Hemoglobin Measurement by Point of Care Testing Instruments As An Alternate To the HPLC Methods.

Vipin Joseph¹, Ramesh Kumar²

¹(Department of Biochemistry DM-WIMS Medical, College, KUHS, Kerala, India)

²(Department of Biochemistry and Immunology, Metropolis, Kerala, India)

Corresponding Author: Vipin Joseph

Abstract: Diabetes is a well-established modifiable risk factor for cardiovascular and renal diseases in western countries. In 1993, the World Health Organization(WHO) Ad Hoc Diabetes Reporting Group published standardized global estimates for the prevalence of diabetes and impaired glucose tolerance in adults, based on data from 75 communities in 32 countries. The measurement of HbA1c /glycohaemoglobin in blood has become the gold standard for the long-term control of the glycaemic state of diabetic patients as presented in the DCCT and UKPDS studies. A total of 63 patients were studied which comprised of 39 females and 24 males. HbA1c of all the samples was estimated by both HPLC method (Bio- Rad D-10) and by nephelometric method (Mispa-i).The results shows that the values from the two methods shows excellent comparison which is statistically significant ($r = 0.9987$, $p < .0001$). So the results from the present study suggests that the POCT instrument can be routinely be used for the detection of glycatedhemoglobin as it gives comparable results with the NGSP certified HPLC method.

Key Words: HPLC, HbA1c, Diabetes mellitus, Nephelometry

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I. Introduction

The measurement of HbA1c /glycohaemoglobin in blood has become the gold standard for the long-term control of the glycaemic state of diabetic patients as presented in the DCCT and UKPDS studies^{1,2}. The optimal therapy of diabetic patients requires carefully validated, method-independent therapeutic target values for the glycohaemoglobin levels of diabetic patients in order to reduce the long-term risk of the late complications such as retinopathy, nephropathy and neuropathy as well as the short-term risk of life-threatening hypoglycaemia. Hemoglobin A1C is an irreversible complex that forms when glucose binds to hemoglobin. The percentage of A1C is directly related to the average glucose level over the past 6 to 8 weeks. Blood glucose levels from the past 30 days represent 50% of the total A1C result³. The American Diabetes Association (ADA) recommends an A1C goal of <7% A1C⁵ while the American Association of Clinical Endocrinology recommends a goal of <6.5%⁴. Every 1% reduction in A1C lowers the risk of developing eye, kidney, and nerve disease by 40%⁵.

More than 15 different analytical methods are currently used by the clinical laboratories, which are based on ion exchange or affinity chromatography, electrophoresis and immunological principles⁶. There are approximately 24 million A1C tests done per year. Estimating 14.6 million people diagnosed with diabetes, there should be over 58 million tests done yearly (assuming four A1C tests per year, per patient). Diabetes is a well established modifiable risk factor for cardiovascular and renal diseases in western countries^{10,11}. In 1993, the World Health Organization(WHO) Ad Hoc Diabetes Reporting Group published standardized global estimates for the prevalence of diabetes and impaired glucose tolerance in adults, based on data from 75 communities in 32 countries.¹² Currently, it is estimated that 150 million people in the world have diabetes¹³. Since the the National Glycohemoglobin Standardization Program (NGSP), implemented in 1996, standardizes GHb results among methods and laboratories¹⁴ the use of HbA1c for diabetes diagnosis and monitoring is greatly used¹⁵. Later GHb has been confirmed as a highly specific and convenient alternative to fasting plasma glucose for diabetes screening in a large Cohort study¹⁶. The NGSP standardized methods currently used highly sophisticated a costly automated instruments which are not affordable to community hospitals of small standalone laboratories. This makes GHb assay unavailable and costly to the general public. The POC instruments introduced which are easily handled and cheap makes the assay available as a routine test even to the primary care institutions. The study aims to compare the performance characteristics of the gold standard of GHb, the HPLC assay with the POC instrument using the immunonephelometric technique.

II. Material And Methods

The study is conducted on 63 samples obtained from op clinic. Both male and female with the spectrum of glycation status from 5% to 12.8% were considered for the study.

HPLC METHOD (Using Bio-Rad D-10 analyser)

The HbA1c by HPLC method was done in the NGSP certified Bio-Rad D-10 analyser. The apparatus is updated by installing the kit floppy diskette. New elution buffers and wash/diluent solution were installed and the system is calibrated. A1c low control and high control is placed at the 1st and 2nd position in the rack respectively followed by patient samples. The rack is inserted into the system through the rack door. After finishing rack loading, the 'RUN' command for test execution is given. The sample concentration is expressed as peak on a graph and also in figures.

Sample Collection and preparation

Whole blood samples are collected in vacutainer collection tubes containing EDTA (Violet Top). Sample is stable for 7 days at 2-8° C or 3 days at room temperature. In case of abnormal tube type or insufficient sample volume ie, less than 2ml then the sample is pre re-diluted in the ratio 1:300 in a 1.5 ml vial (5 microliter of sample in 1.5ml of wash/diluent solution) prior to analysis. All samples are analysed within 3 Hrs of sample collection.

NEPHLOMETRIC METHOD (Using MISPA-i)

MISPA-i (Aggappe Diagnostics) used for comparison is a Point of care Testing (POCT) instrument.. This instrument uses a method which uses antigen-antibody interaction. HbA1c particle have some non-specific adsorption rate to latex particle. When mouse anti human HbA1c monoclonal antibody is added, latex HbA1c mouse anti human HbA1c complex is formed. Agglutination occurs when goat antimouse IgG polyclonal antibody interacts with monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of the latex particle.

Sample collection

Whole blood samples are collected in vacuum collection tubes containing EDTA. Sample is stable for 7 days at 2-8 °C or 3 days at room temperature.

Reagents and constituents

R1 - Latex

Glycine Buffer

R2 - Glycine Buffer

Mouse anti human HbA1c monoclonal antibody

Goat anti mouse IgG

Stabilizers

R3 - Hemolysing Reagent

PROCEDURE

Hemolysis was done by adding 10 micro litre of whole blood to 500 micro litre of R3, kept undisturbed for 5 minutes. Dispensed 180 micro litre of R1 to disposable cuvette to which 5 micro litre of the lysate was added. Immediately placed the cuvette into the mixing cum reading well. The contents of the cuvette were mixed by automatic gentle vortexing for 5 seconds. Then the cuvette was incubated for 1 minute automatically and after incubation added 60 micro liter of R2 to the cuvette immediately mixed and the sample concentration was given after 2 minutes.

Statistical analysis

Data was analyzed using SPSS version 22. Student's *t*-test was used to ascertain the significance of differences between mean values of two continuous variables. The level $P < 0.05$ was considered as the cutoff value or significance.

III. Result

The results of the study is summarized in the table (table 1)

Variable	N	Mean	SE Mean	SD	Minimum	Q1	Median	Q2	Maximum
AGE	63	51.44	1.77	14.04	23	42	51	62	81
HPLC	63	7.689	0.255	2.02	5	6	7.2	9.1	12.8
MISPA-i	63	7.754	0.254	2.018	5.1	6	7.4	9	12.9

Table (2): General Description of the study

Number of points = 63
 Correlation Coefficient (r) = 0.9987
 95% confidence interval : 0.9978 to 0.9992
 Coefficient of determination (r^2) = 0.9974
 Test : Is r significantly different than zero ?
 The two tailed p- value is <0.0001,considered extremely significant

Linear Correlation Analysis

P value and statistical significance:

The two tailed P value equals 0.8569
 By conventional criteria, the difference is considered to be not statistically significant
 Confidence interval:
 The mean of HPLC minus MISPA-i = -0.065
 95% confidence interval of this difference:
 From -0.77701 to 0.64701
 Intermediate value used in calculations
 $t = 0.1807$
 $df = 124$
 Standard error of difference = 0.360

Table (3): General description of statistical values

Group	HPLC	Mispa-i
Mean	7.689	7.754
Standard deviation	2.02	2.018
Standard error of mean	0.2545	0.25424
N	63	63

IV. Discussion

The effective use of HbA1c test results for the management of diabetes requires that the different HbA1c assay methods (at least 3 commonly used methods in India) remain closely calibrated to the method of analysis that was used in the original studies from which the interpretive criteria and clinical decision levels have been derived. Maintaining a high degree of accuracy relative to the reference method is difficult owing, in part, to the structural complexity of the glycosylated hemoglobins and the fact that different analytic methods are based on fundamentally different method principles (eg, immunoassay, HPLC, and affinity chromatography)

¹⁷.

In this study a total of 63 samples were analysed which included males and females of both spectrum of glycation status from 5 % to 12.8% were considered. Each of the patient sample was analysed by both Bio-rad D-10 analyser which uses the HPLC technique and Mispa-i that uses the immunonephelometry technique. When analysed statistically the two sets of values showed excellent comparison which was statistically significant ($r = 0.9987$, $p < 0.0001$) and the POCT appear as a satisfactory analytical alternative to the HPLC method ($t = 0.1807$, $p = 0.8569$) and both the statistical tests showed 95% confidence interval. In the central laboratory testing instrument (Bio-rad D10) the human intervention is only limited so the chance of technical errors are null or minimum, but for the POCT instrument (Mispa-i) since the human intervention happens throughout the sample analysis the chance of error exists. Both the methods are affected by factors such as sample dilution, abnormal red cell survival and hemoglobin variants.¹⁸

V. Conclusion

POCT testing for HbA1c is of increasing interest to both clinicians and patients. It provides immediate feedback, which has been shown to increase physician intervention, resulting in lower A1C values for patients. Although factors such as cost, ease of use, speed of analysis and blood volume should be considered when choosing a suitable system, guaranteed result quality is mandatory and must not be sacrificed for patient or clinician convenience. The *Mispa-i* system using immunonephelometry evaluated meets the requirements for HbA1c testing. has good precision but shows a slight positive bias and limitation in the linearity level. The *Mispa-i* system using immunonephelometry POCT appear satisfactory analytical alternatives to both central laboratory testing and each other ($t = 0.1807$, $p = 0.8569$). When used with a comprehensive quality assurance, training and documentation programme, and this instrument can offer safe, convenient HbA1c POCT testing for diabetics to augment the services of the central laboratory. Two methods shows excellent comparison which is statistically significant. ($r = 0.9987$, $p < 0.0001$). The results suggest that the POCT instrument can routinely be used for the detection of the GHb as it gives comparable results with the NGSP certified HPLC method.

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