ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at www.jgpt.co.in

RESEARCH ARTICLE

The Diagnostic Value of Hba1c Turbidimetry Immunoassay in Sanglah General Hospital Denpasar, Bali, Indonesia

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Abstract

Introduction: Diabetes mellitus (DM) is a common health problem in Indonesia. Currently, Hemoglobin A1c (HbA1c) is the gold standard to diagnose and monitor DM patient. HbA1c could be assessed using turbidimetry immunoassay and High-Performance Liquid Chromatography (HPLC). However, the comparison between the two devices is never conducted. Thus, this study aimed to determine the sensitivity, specificity, and correlation of HbA1c measurements using turbidimetry immunoassay method compared to HPLC as the gold standard. Methods: A cross sectional study was conducted from May to June 2017to measure the diagnostic values and correlation test between turbidimetry immunoassay method and HPLC. Diagnostic values were measured as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Blood samples were collected in EDTA tubes and measured with turbidimetry immunoassay and HPLC, with a cut-off value of 6.5 mg/dL. Results: Fifty samples were collected during the study. HbA1c ≥6.5mg/dL were obtained from 27 samples, and normal results were obtained from 23 samples. Sensitivity, specificity, PPV, and NPV from turbidimetry immunoassay were 92.86%, 100%, 100%, and 91.67% respectively. Pearson correlation test of HbA1c result from turbidimetry immunoassay and HPLC revealed a strong positive correlation with r = 0,988. The ROC curve analysis yield AUC value of 0.926 with P=0.000 which indicate a strong diagnostic value.Conclusion: Turbidimetry immunoassay method had high sensitivity and specificity and had strong diagnostic value according to ROC analysis. There was also a strong positive correlation between turbidimetry immunoassay and HPLC HbA1c reading.

Keywords: *HbA1c, HPLC, Turbidimetry immunoassay.*

Introduction

The diagnosis of diabetes mellitus was based on blood glucose test either determined from blood glucose level (fasting and 2 hours postprandial) or the blood glycated protein product. Glucose level tends to fluctuate in a daily manner in diabetic patients who make blood glucose level less reliable to diagnose and monitor treatment efficacy. Thus, Haemoglobin A1c (HbA1c) test stands out as the diagnostic marker because of its longer half-life and less likely to fluctuate [1, 2].

Different from blood glucose level which affected by glucose intake and insulin level, HbA1c level does not fluctuate. Because its follow the half-life of hemoglobin, HbA1c has three months half-life which could describe the patient means blood glucose level for three months. It is a useful indicator for monitoring glucose control level, diet effect, as well as the efficacy of treatment [3,4,6,10]. Currently, several HbA1c measurement methods and devices are available. According to the American Diabetes Association (ADA), the HbA1c test needs to be certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized by Diabetes Control and Complications Trial (DCCT) [1,2].

Current widely used techniques are High-Performance Liquid Chromatography (HPLC) and immunoassay [5,7]. Various HbA1c test methods among clinical laboratories may result in different clinical and patient interpretation of test results which could lead to incorrect interpretations. The mistakes could lead to many consequences such as diagnostic error and miss-management of the diabetic patient

which could hasten the development of diabetic complication and financial burden. Therefore, the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry (IFCC) sought to create a standardized HbA1c examination [5,7,8,9].

So far, no documented study compares the diagnostic accuracy of immunoassay and HPLC regarding HbA1c assessment. Thus, this study aimed to determine the sensitivity and specificity of HbA1c measurements using turbidimetry immunoassay method compared to HPLC as well as assessing the correlation between HbA1c result from turbidimetry immunoassay and HPLC.

Methods

A cross sectional study was conducted at the Clinical Pathology Laboratorium Sanglah Hospital Denpasar from May to June 2017. The sample used was the patients who came to Sanglah Hospital and had their HbA1c measured with minimum samples required were 43 samples [10]. The inclusion criteria for this study were diabetes mellitus patients with normal erythrocyte index (MCV, MCH, MCHC), patients not diagnosed with diabetes mellitus with standard erythrocyte index (MCV, MCH, MCHC), and hemoglobin level within 7.5-20mg/dL. Haemolysed samples, patients with a history of thalassemia, and patients with a history of hemolytic anemia were excluded. Samples used were the patient's vein blood that collected from the middle cubital vein and stored in EDTA tubes. The HbA1c was assessed using two devices: Cobas C 501 as the test device and CE-HPLC Adams (HA-8180V) as the gold standard.

HbA1c results were recorded in the table. The sensitivity, specificity, positive predictive value, and negative predictive value were calculated and the correlation was determined using Pearson correlation test. A ROC curve analysis was also conducted to assess the strength of the diagnosis of immunoassay method compared to HPLC. All of the analysis was performed using IBM SPSS Statistics 20.0

Results

50 blood samples were used during this study. Laboratory precision test was conducted and the means, standard deviations, and coefficient of variants were calculated to measure the accuracy of each device. Within-run, between days, and precision tests using level 1 (normal) and level 2 (high) Extendsure were measured fifteen times daily. The results are shown in Table 1 and Table 2.

Table 1: Within-run HbA1c precision test results of Adams (HA-8180V) with ExtendSure control

Parameter	HbA1c level (mmol/mol)	
	Level 1	*Level 2*
Mean	34	100
SD	0,0	0,4
CV(%)	0,0	0,4

Table 2: Between day HbA1c precision test results of Adams (HA-8180V) with ExtendSure control

Parameter	HbA1c level (mmol/mol)	
	Level 1	*Level 2*
Mean	33,02	97,04
SD	0,26	0,60
CV(%)	0,8	0,6

Within-run precision tests with EDTA, samples were measured twenty times, and the results were shown in Table 3.

Table 3: Within-run HbA1c precision test results of Adams (HA-8180V) with EDTA samples

Parameter	HbA1c level (mmol/mol)
Mean	37
SD	0,0
CV(%)	0,0

Cross-tabulation analysis between HbA1c reading from c501 (turbidimetry immunoassay) and Adams (HA-8180V) (HPLC) was conducted to analyze the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of immunoassay to HPLC as the gold standard. The samples were divided into two groups according to its HbA1c level with 6.5 mg/dL as the cut-off point. According to the cross-tabulation analysis, it appears that the immunoassay reading was not very different

from HPLC with only two samples in the group with < 6.5 mg/dL which were read differently by the two devices.

Table 4: HbA1c Cobas Tina Quant Haemoglobin A1c Gen 3 turbidimetry immunoassay and CE-HPLC Adams (HA-8180V) results

Groups	HbA1c Adams ≥ 6,5 mg/dL	HbA1c Adams< 6,5 mg/dL	Total
HbA1c c501 ≥ 6,5 mg/dL	26	0	26
HbA1c c501 < 6,5 mg/dL	2	22	24
Total	28	22	50

According to the cross tabulation, the diagnostic value of immunoassay can be calculated. Below is the calculation of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of immunoassay

- HbA1c Cobas Tina Quant Haemoglobin A1c Gen 3 turbidimetry immunoassay sensitivity test compared to CE-HPLC Adams (HA-8180V) as the gold standard was:

$$\text{Sensitivity} = \frac{\text{HbA1c c501 and Adams} \geq 6.5 \text{ mg/dL}}{\text{HbA1c Adams} \geq 6.5 \text{ mg/dL}} \times 100\% = \frac{26}{28} \times 100\% = 92.86\%$$

- HbA1c Cobas Tina Quant Haemoglobin A1c Gen 3 turbidimetry immunoassay specificity test compared to CE-HPLC Adams (HA-8180V) as gold standard was:

$$\text{Specificity} = \frac{\text{HbA1c c501 and Adams} < 6.5 \text{ mg/dL}}{\text{HbA1c Adams} < 6.5 \text{ mg/dL}} \times 100\% = \frac{22}{22} \times 100\% = 100\%$$

- HbA1c Cobas Tina Quant Haemoglobin A1c Gen 3 turbidimetry immunoassay positive predictive value (PPV) compared to CE-HPLC Adams (HA-8180V) as gold standard was:

$$\text{PPV} = \frac{\text{HbA1c c501 and Adams} \geq 6.5 \text{ mg/dL}}{\text{HbA1c c501} \geq 6.5 \text{ mg/dL}} \times 100\% = \frac{26}{26} \times 100\% = 100\%$$

- HbA1c Cobas Tina Quant Haemoglobin A1c Gen 3 turbidimetry immunoassay negative predictive value (NPV) compared to CE-HPLC Adams (HA-8180V) as gold standard was:

$$\text{NPV} = \frac{\text{HbA1c c501 and Adams} < 6.5 \text{ mg/dL}}{\text{HbA1c c501} < 6.5 \text{ mg/dL}} \times 100\% = \frac{22}{24} \times 100\% = 91.67\%$$

According to the calculation, the immunoassay HbA1c Cobas Tina Quant Haemoglobin A1c Gen 3 appear to had a high diagnostic capability compared to the gold

standard with all of the parameters yield value above 90%. However, the diagnostic strength of the devices is needed to be calibrated and confirmed using correlation test and ROC curve analysis. The results of Pearson correlation test confirm the calculation above. The HbA1c turbidimetry immunoassay results were positively correlated with HPLC with r: 0.988 (very strong) which indicated a powerful correlation.

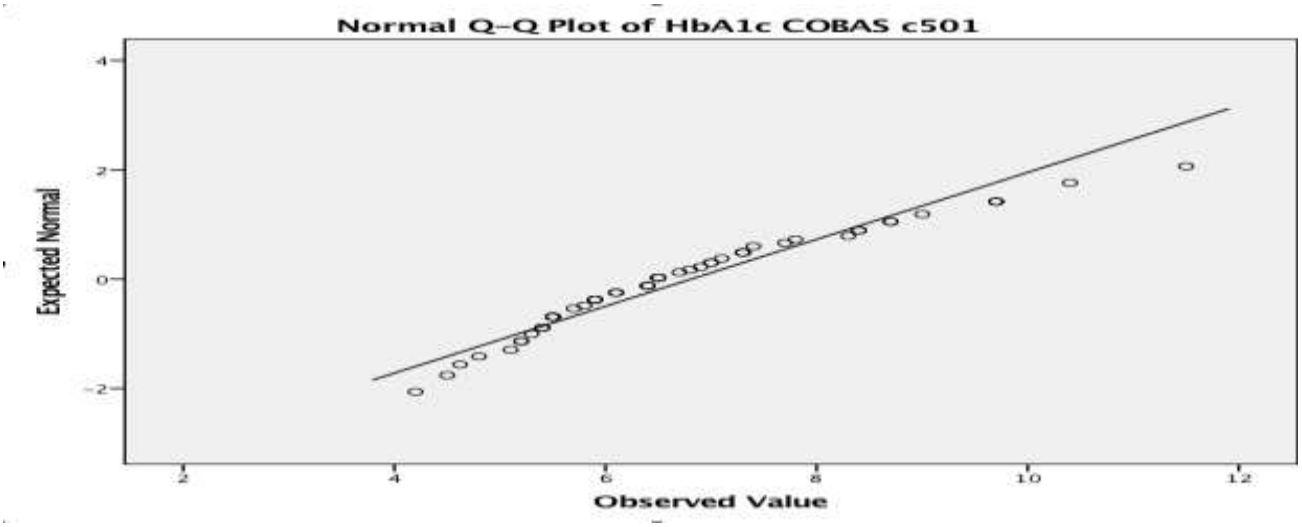

Figure 1: The correlation between HbA1c turbidimetry immunoassay and HPLC results

The result of ROC curve analysis also supports the previous study. It showed that the turbidimetry immunoassay method had Area under the Curve (AUC) value of 0.946 which indicate a strong diagnostic capability. The result of ROC is depicted in Figure 2.

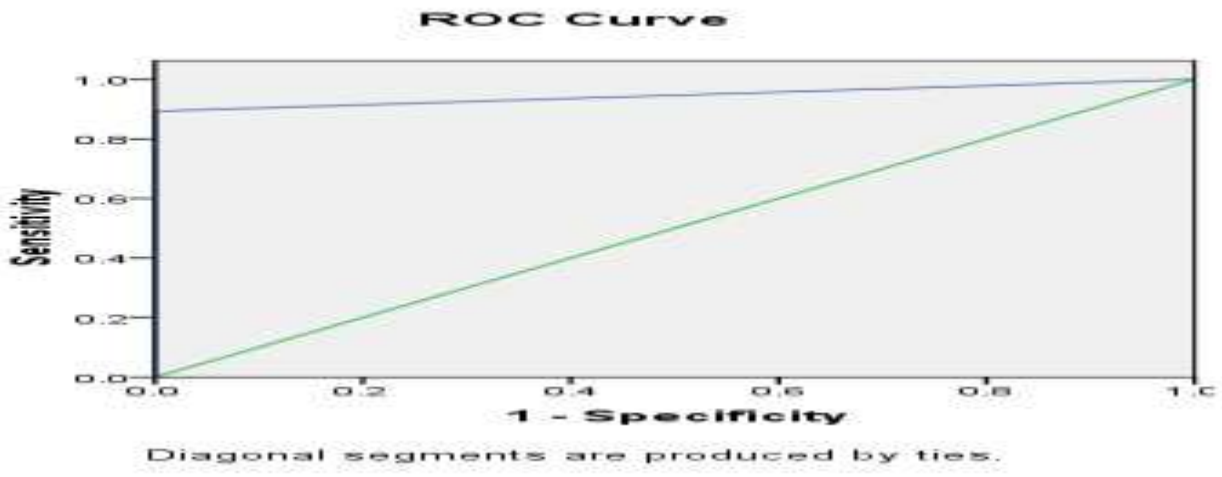

Figure 2: ROC curve analysis of immunoassay HbA1c Cobas Tina Quant Haemoglobin A1C

Discussion

The availability of the HbA1c test has enhanced diabetic care, and its measurement has become an integral part of the management of diabetes. Also, the relationship between the improved glycemic control and risk of diabetic complications has been established [11, 12]. The HbA1c levels of samples can be reliably measured by using various methods such as High-Performance Liquid Chromatography (HPLC), immunoassay, boronate affinity chromatography, and enzymatic assay.

Several studies have reported observed differences between HbA1c measurements based on different techniques since the methods were standardized using a widespread reference model and calibrated with the same calibrator. The two main routine methods used in many countries are immunoturbidimetric and enzymatic assays [13, 14]. However, in some situations these two methods tend to yield results with undesirable differences; thus it is essential to compare the results from these methods which are used by different laboratories [15].

In the enzymatic assay, the technique was based on digesting hemoglobin samples with a specific protease to generate fructosyl amino acid.

The measuring protocol was in line with the Diabetes Control and Complications Trial (DCCT) and National Glycohemoglobin Standardization Program standards (NGSP) [16]. Though some studies reported that the HPLC method could detect abnormal hemoglobin with favorable reproducibility and a CV < 1%, this technique needs a sizeable dedicated device and instead of a time-consuming procedure. Also, many trained staffs are needed to maintain the instrumentation [15,17,18].

The immunoassay can be performed by an automated analyzer. Thus this method does not take a long time for measuring a large number of samples. However, in this method, the total hemoglobin needs to be assessed by an additional measurement. On the other hand, the enzymatic assay also provides an accurate, fast and uniform reaction and the error obtained from this method has been reported to be <1% [15,19]. Enzymatic method is also a fully automated system that requires no sample preparation and has a fast running time.

As indicated in other studies a relationship and concordance between these two methods support the reliability of both approaches, if the assay protocol is appropriately standardized. Although the HbA1c measured values should be monitored periodically by Quality Control (QC) observations and each laboratory is responsible for determining the accurate reference values and correction equations for more reliable results. The American Diabetes Association (ADA) has suggested that one crucial remaining issue with the HbA1c test is the lack of available and adequate assay to manage diabetes, especially in developing countries [20].

The turbidimetric immunoassay is easy to use and more available in most developing countries especially in significant rural populations where limited access to advanced devices and laboratories performing the proper assays is still an unsolved problem [21, 22]. So far no considerable superiority between various measurement methods has been reported and thus the immunoturbidometric and enzymatic method which are both reliable and easy to perform can be used as alternative methods to HPLC measuring system with its known limitations.

Conclusion

HbA1c turbidimetry immunoassay was proved to have a high diagnostic value which was proven by its sensitivity, specificity, PPV, and NPV compared to the gold standard. The HbA1c reading of turbidimetry immunoassay also strongly correlated with HPLC and, according to ROC curve analysis, it had strong diagnostic accuracy. Furthermore, a comprehensive study is needed to validate these findings further as well as to increase the ability of the study to detect a much smaller deviation.

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