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DIFFERENCE IN HbA1C LEVEL BETWEEN BORONATE AFFINITY AND ION EXCHANGE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD IN DIABETIC PATIENT

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ABSTRACT

Glycated Hb (HbA1c) test is needed to control glycemic in high prevalence type 2 Diabetes Mellitus (DM) patients. Hemoglobin fraction separated and chemical reaction is two main concepts in the HbA1c test. Ion exchange-high performance liquid chromatography (HPLC) and boronate affinity use the first concept. Ion exchange-HPLC is a reference method in most of the clinical laboratory. Point of care testing (POCT) with boronate affinity method that has been standardized by the international institution is available. This study aimed to compare the boronate affinity POCT method and ion exchange-HPLC method. This cross-sectional study was conducted to 22 types 2 DM patients those fulfilled inclusion and exclusion criteria in January 2017 to February 2018. Level of HbA1c was assayed with boronate affinity POCT and ion exchange-HPLC method. A t-test was used to analyze data and no significant difference if p>0.005. Subjects of this study are females (59.1%) more than males (40.9%) with age mean 59.23 years old (8.1). Uncontrolled type 2 DM (77.3%) more than controlled type 2 DM (22.7%). Mean of HbA1 level was 8.0% (1.7) in boronate affinity POCT and 8.3% (1.8) in ion exchange-HPLC. T-test showed no significant difference between those two HbA1C assay methods (p>0.005). There was no difference HbA1c level between boronate affinity POCT method and ion exchange-HPLC method.

Key words: Boronate affinity, type 2 diabetes mellitus, HbA1c, ion exchange-HPLC

INTRODUCTION

Type 2 Diabetes Mellitus (type 2 DM) is a group of metabolic diseases characterized by hyperglycemia that occur due to abnormalities in insulin secretion, insulin action or both. The World Health Organization (WHO) predicts an increase in the number of people with type 2 DM in Indonesia from 8.4 million on 2004 to about 21.3 million on 2030 and an increase of 2-3 times on 2035. International Diabetes Federation (IDF) predicts an increase in the number of people with type 2 DM in Indonesia from 9.1 million in 2014 to 14.1 million on 2035.[1] The current increase in the prevalence of type 2 DM causes an increasing need for glycated hemoglobin (HbA1c) measurement for glycemic control in clinical practice especially primary care. The American Diabetes Association (ADA), the WHO and the IDF have endorsed the use of HbA1c for the diagnosis of diabetes and recommend that only NGSP certified HbA1c methods performed in clinical laboratories be used for diagnosis.[2]

Glycated hemoglobin (HbA1c) is defined as hemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chains.[3,4] Glycation is a spontaneous nonenzymatic reaction in which glucose binds covalently with hemoglobin at the amino terminus of the beta globin chain.[5,6] In the red blood cells, HbA1c formed in two basic steps: glucose binds reversibly to Hb as an aldimine or Schiff base, which in turn undergoes an Amadori rearrangement to form a stable and irreversible ketoamine.[2,6] The glycation of hemoglobin occurs at a variable over time, during the lifespan of the red blood cell, which is of 120 days.[6] Glycated hemoglobin reflects average glycemia over approximately three months and has strong predictive value for diabetes complication. Thus, HbA1c testing should be performed routinely in all patients with diabetes, at initial assessment and as part of continuing care. Measurement approximately every three months determines whether patients glycemic target has been reached and maintained. The frequency of HbA1c testing should depend on the clinical situation, the treatment regiment, and the clinician's judgment. The glycated hemoglobin test is unreliable in a condition that affects red blood cell turnover (hemolysis, blood loss) and hemoglobin variants must be considered, particularly when the HbA1c result does not correlate with the patients

Self-Monitoring Blood Glucose (SMBG) levels.[7]

There are two major analytical concepts of HbA1c measurement, based on the separation of Hb fractions and the other, and based on chemical reaction. The separation method is applied in ion exchange HPLC, boronate affinity and capillary electrophoresis. Chemical method is applied in immunochemical and enzymatic assays.[8] Ion-exchange HPLC is a reference method for HbA1c measurement of DCCT and the anchor method of the NGSP.[8,9] Ion exchange HPLC assays are currently the second most common type of HbA1c method used in the clinical laboratory according to statistics from the CAP proficiency testing program.[2]

Ion exchange HPLC separates Hb species based on charge differences between HbA1c and other hemoglobins. Unlike other glycated hemoglobin fractions, HbA1c can be separated easily based on a difference in net charge. Ion exchange HPLC can identify and visualize an analytic interference that exists in a patient sample so that inaccurate HbA1c results will not be reported. The boronate affinity methods, m-aminophenyl boronic acid reacts specifically with the cis-diol groups of glucose bound to Hb.[4] The boronate affinity has been standardized by the National Glycohemoglobin Standardization Program (NGSP). This method is also included in the HbA1c checklist received by the Food and Drug Administration.[2] According to research by Razi *et al.*, sensitivity and specificity of the boronate affinity method for cut-off HbA1c 6.5% was 82.9% and 100% respectively.[10]

The various ways used to measure HbA1c are available in the form of laboratory instruments and Point of Care Test (POCT) instrument. The analytic performance of laboratory instruments is better than the performance of POCT instrument, but POCT instruments have the advantages of producing results during the patient's visits to the physician (thus meeting the clinical requirement of convenience), so that it is more efficient in terms of time, effort and cost.[6] Another advantage of using POCT HbA1c uses capillary blood so it needs a little sample, using simple techniques that can be used by untrained personnel. The National Academy of Clinical Laboratory Practice Guidelines recommends the use of POCT HbA1c for glycemic control of DM patients but not for the diagnosis of DM.[11] The American Diabetes Association recommends that the use of POCT HbA1c must be standardized and certified by NGSP.[12]

The development of POCT instrument is a recent trend.[6] Over the past several decades the availability and use of POCT have continued to increase worldwide.[13] The ultimate challenge is to find an analytic device with good specificity and clinically relevant imprecision.[6] The POCT instrument must have performance comparable to laboratory instrument, so before using POCT instrument, practitioners should review the feasibility and make a comparison of the accuracy of POCT instrument results with laboratory instruments.[8,14] The imprecision between methods accepted by the International Federation of Clinical Chemistry (IFCC) was 2.8% and <2.0% by NGSP.[15]

Several previous studies have been conducted to compare the HbA1c level between ion exchange HPLC method (laboratory instrument) with a boronate affinity method (POCT instrument). Research by Grant *et al.*, about the comparison between the boronate affinity methods with the ion exchange HPLC method concluded that POCT performance is proportional to the performance of laboratory instruments to measure HbA1c level. Another research by Sanchez-Mora *et al.*, about the evaluation of two methods of POCT HbA1c (boronate affinity and immunoassay methods) to ion exchange HPLC, obtained a coefficient of variation of 1.95% (HbA1c high level) and 2.66% (HbA1c low level) for the affinity boronate method (POCT), and the coefficient variation 3.1% (HbA1c high level) and 2.97% (HbA1c low level) for the immunoassay method (POCT). Both POCT methods have good correlation to ion exchange-HPLC (r = 0.991 for boronate affinity method and r = 0.973 for immunoassay method). Research about the comparison of HbA1c level between boronate affinity method (POCT instrument) with ion exchange HPLC method (laboratory instrument) has never been done in Dr. M. Djamil Padang Hospital. Based on the above background, researchers were interested to know the comparison between HbA1c level using POCT instrument of boronate affinity method with ion exchange HPLC method in type 2 DM patients.

METHODS

This study was an analytical study with a cross-sectional design. The study was conducted in 2018. The study was approved by the Committee of the Research Ethics of the Faculty of Medicine, Andalas University. The population studied included type 2 diabetes mellitus patient who came to the central laboratory of Dr. M. Djamil Padang. The inclusion criteria were aged over 18 years, normal hemoglobin level and filled a consent form. The exclusion criteria were hemolytic anemia, anemia

due to blood loss, variant hemoglobin and hemoglobinopathy.

The central laboratory determined analyzed HbA1c using ion exchange HPLC with Bio-rad D-10TM Hemoglobin A1C (Bio-Rad Laboratories). Bio-rad obtained NGSP certified, anchored to the IFCC reference method and traceable to the Diabetes Control and Complications Trial (DCCT) reference study. The Alere Afinion TM HbA1c Dx (Abbot) as a POCT system is based on a boronate affinity method. The POCT systems were designed to operate with ready-to-use cartridges and certified by the NGSP.

The sample was collected in two ways for two instruments. A total of 22 DM type 2 patients were selected. Blood samples were collected by venipuncture into EDTA tubes and analyzed using ion exchange-HPLC method. The sample collected by finger prick analyzed using boronate affinity method. Both samples were directly examined at the same time. Monitoring the accuracy of HbA1c examination of ion exchange-HPLC and boronate affinity methods were performed with the controlling material of the reagent device.

Data was collected on data collection sheets. Statistical analysis was performed using SPSS software v.13.0. Data obtained with the boronate affinity method was compared to ion exchange HPLC method using Student's t-test after assessment of normality of the data using Saphiro-Wilk test. The results were considered to have no significant difference if $p > 0.05$. Data was presented in the form of frequency distribution tables and diagrams.

RESULTS AND DISCUSSION

Characteristic of study subjects shown in Table 1.

Table 1. Characteristic of the study subject

Variable	n (%)	Mean (SD)
Sex		
Male	9 (40.9)	
Female	13 (59.1)	
DM type 2		
Controlled	5 (22.7)	
Uncontrolled	17 (77.3)	
Age (year)		59.23 (8.1)
Hemoglobin level (g/dL)		13.08 (1.7)

Subjects of this study were 22 patients, consisted of 40.9% male and 59.1% female with a mean age of 59.23 years (8.1). Basic Health Research Data of 2013 (Riskesdas) stated that the prevalence of DM type 2 was more common in females (1.7%) than in males (1.4%), and age group 55-64 years suffered more DM (4.8%) than other age groups. Similar findings from other studies, such as Scavini *et al*., in the US found among the Zuni Indians, the prevalence of diabetes was 57% higher among female than male members of the population. Culture, tradition and lifestyle differences might contribute to the higher prevalence of diabetes and obesity among female Zuni Indians. In contrast, Nordstrom *et al*., in Sweden found the prevalence of type 2 diabetes was 14.6% in males and 9.1% in females ($p < 0.001$). The finding was associated with the larger amount of visceral fat in males.[16] Sex-related differences in lifestyle may lead to differences in the risk of developing diabetes mellitus and, in consequence, to differences in the prevalence of this condition in females and males. However, the relationship between a known risk factor for diabetes mellitus such as obesity may not be simple. For example, in many countries of sub-Sahara Africa, females are more likely to be obese or overweight than males and might, therefore, be expected to have higher prevalences of DM. However, females in Ghana, Nigeria, Sierra Leone and rural areas of the United Republic of Tanzania were found to have lower prevalences of DM than the males in the same study areas. Although wide variations in the distribution of DM by sex have been documented in several review articles, the possible causes of this heterogeneity have never been examined in detail.[17]

This study obtained a mean Hb level of 13.08 (1.7) g/dL. Hemoglobin levels are in the range of normal values. Normal Hb levels of males is 13.5-18.0 g/dL, and females is 12.0-15.0 g/dL.[18] This study was conducted on samples with normal Hb levels because HbA1c was unreliable in the condition that affects red blood cell turnover (hemolysis, blood loss) and hemoglobin variants had to be considered, particularly when the HbA1c result did not correlate with the patients self-monitoring blood glucose (SMBG) levels.[7] Any condition that prolongs the life of the erythrocyte or decreased red cell turn over exposes the cell to glucose for a longer period, resulting in higher A1c levels. Similarly, any condition that shortens the life of the erythrocyte or its associated with increases red cell turn over shortens the exposure of the cell to glucose, resulting in lower HbA1c levels.[19] Research by Prasertwatanakorn *et al*., in Thailand found HbE homozygote samples assayed by ion exchange HPLC comparing to boronate affinity and immunoassay showed significantly lower HbA1c level ($2.44 \pm 0.33\%$, $4.88 \pm 0.35\%$, and $4.61 \pm 0.24\%$ respectively, $p < 0.01$). While HbA1c level in HbE heterozygote samples showed slightly different values. Samples from β thalassemia/HbE

with both HbE and HbF greater than 30% showed decreased HbA1c values by all three methods. Lower HbA1c levels were also found in HbH disease and HbEA Bart's disease samples when they were assayed by boronate affinity comparing to ion exchange HPLC and immunoassay (4.5±0.47%, 5.01±0.56%, and 4.89±0.65, respectively, p<0.01).[20] Hemoglobin variants can make interpretation of A1c level quite challenging as these patients can have either falsely elevated or falsely lowered A1c. The most common variants worldwide are hemoglobin S, and hemoglobin C. Overestimation or underestimation of A1c for many hemoglobin variants will differ depending on both the type of method and the specific assay used. Therefore, for patients with hemoglobin variants, it is important for the clinician to know the laboratory's method for measuring A1c.[19]

Interferences from Hb variants using ion exchange HPLC method can usually be detected in the chromatograms. Results may be either biased high or low depending on the specific method. Boronate affinity measures the ratio of glycated to nonglycated hemoglobin regardless of species, the presence of elevated HbF results in a false lowering of the HbA1c result. This interference could be a consequence of a lower glycation rate for HbF compared with HbA, leading to a lower concentration of glycated hemoglobin for given plasma glucose.[4]

This study obtained an uncontrolled group of DM type 2 (77.3%) more than the controlled type 2 diabetes group (22.7%). This result was similar to Alcala *et al*. study in Mexico on the comparison of HbA1c content levels of immunoassay method with HPLC, from 178 total samples obtained 96 cases with HbA1c ≥7% (uncontrolled) and 82 cases with HbA1c <7% (controlled).[21]

Data obtained from the study on both method found to have a normal distribution using the Shapiro-Wilk test. The Mean of HbA1c level for boronate affinity method 8.0% (1.7) and ion exchange HPLC method 8.3% (1.8) are shown in Figure 1.

This study found the mean HbA1c level of ion exchange-HPLC method was higher than boronate affinity method. The student's t-test was calculated to assess significant differences between these two methods, and no significant difference was found (p>0.05) (Table 2).

Pearson's correlation coefficient between the boronate affinity method (POCT) and ion exchange HPLC method was used to assess the performance of POCT. As shown in Figure 2, the correlation for POCT vs. HPLC was r = 0.974 (p =0.000) indicate a strong correlation and statistically significant.

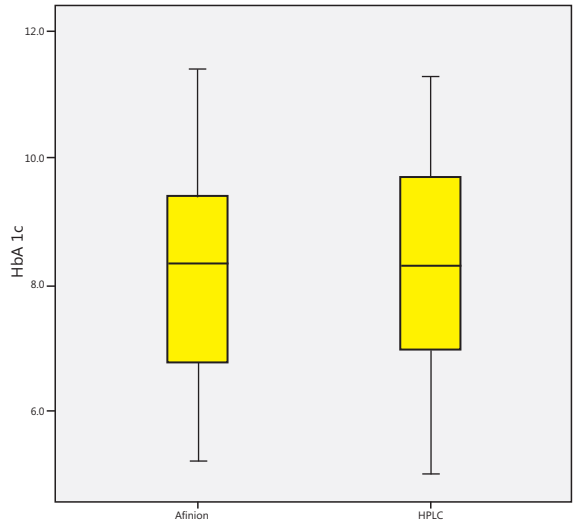

Figure 1. The mean of HbA1c level for boronate affinity method and ion exchange HPLC

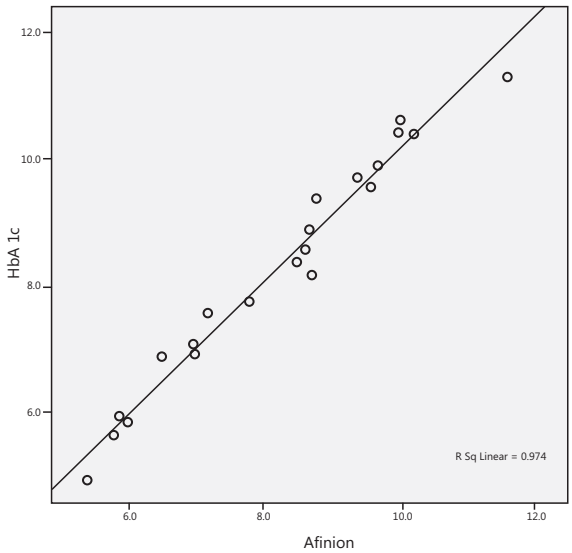

Figure 2. Correlation of boronate affinity method and ion exchange HPLC method for measurement of HbA1c level

Table 2. The difference means of HbA1c level between boronate affinity methods with ion exchange-HPLC method

Variable	Mean (SD) (%)	p
HbA1c level boronate affinity method	8.0 (1.7)	0.58*
HbA1c level ion exchange-HPLC method	8.3 (1.8)	

* t-test (p>0.05), the difference was not statistically significant

Diabetes mellitus, especially type 2 diabetes (DM2) represents a significant public health issue, not only due to its high prevalence and incidence, but also because it is associated with high morbidity and mortality.[22] Microvascular complications of diabetes, including nephropathy, neuropathy, and retinopathy, impose a high cost on the patients and health system. The incidence of these complications is associated with a patient's long-term glycemia. HbA1c measurement is a standard method to investigate the long-term glycemic control of the patients. Thus, its precise analysis by laboratory methods to follow-up the patients and treat them is essential. Because employing a reference method (HPLC) is not affordable for all laboratories, the necessity for replaceable and strongly correlated to those of HPLC is clarified.[23]

The National Academy of Clinical Biochemistry laboratory practice guidelines recommended the use of POCT HbA1c. The main advantages of HbA1c determinations using POCT are a simplification of the pre-analytical phase, including administrative procedures and hospital circuits, prompt availability of results, and finally, these devices may be handled by nursing professionals for immediate results in the ambulatory patient.[22] Point of care HbA1c testing offers potential benefits for diabetes care, especially for patients who experience barriers to traveling to laboratories for blood draws or repeated follow-up visit. Research has demonstrated that the availability of HbA1c test results provide during the same visit is associated with improvement in glycemic control.[13] This study assesses turn-around-time (TAT) for each instrument. The TAT was three minutes for the boronate affinity method (POCT), and three hours for the ion exchange HPLC. This significant TAT difference leads to faster patient treatment and improved outcomes, including enhanced physician and patient satisfaction.

In this study, the POCT device showed good correlation with the laboratory method (ion exchange HPLC). This result is in agreement with those previously reported. The research by Mora *et al.*, in Spain on the evaluation of two HbA1c POCT obtained the mean difference between POCT method of boronate affinity with HPLC (CI 95%) 0.27% (p <0.001), correlation coefficient r = 0.991, and concluded POCT of boronate affinity method shows a good correlation with ion exchange HPLC.[22] The research by Muller and Jones in Germany, also evaluate POCT of boronate affinity to the gold standard HPLC laboratory method and found good correlation (r=0.9653).[21] The research by Subcharoan, in Thailand, demonstrated a strong correlation between HPLC and boronate affinity method in measuring HbA1c level at r =0.902.[24]

This study found no significant difference on HbA1c level between boronate affinity method and ion exchange HPLC method. The research by Stirk *et al.*, in the UK found HbA1c results obtained from the Primus (boronate affinity) for the diabetic patient group compared very well with those from the Variant analyzer (ion exchange HPLC).[25] The research by Arzuhal *et al.*, in Turkey, found correlation between the difference of the two method (boronate affinity and ion exchange HPLC) and the low and the high hemoglobin concentrations was statistically non significant (r-0.149, p=0.3343; r=0.263, p=0.05494).[26] The fact that HbA1c and nonglycated Hb have different chemical properties allows for the separation of fractions and the quantification of HbA1c. This principle is applied in ion exchange chromatography, affinity chromatography, and capillary electrophoresis.[27] Ion exchange HPLC separates Hb species based on charge difference. Boronate affinity is a structurally specific method that recognizes the cis-diol groups of glucose bound to Hb. Affinity separation of glycated Hb typically uses m-aminophenyl boronic acid and depends on a particular interaction between the glucose on glycated Hb and the immobilized boronic acid.[2]

Glycated hemoglobin is hemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chains.[3] Carbohydrates (such as glucose) can bind non-enzymatically to proteins (such as hemoglobin) in a process known as glycation.[5] Hemoglobin is a protein present in the erythrocyte cytoplasm.[17] This study uses capillary blood for the boronate affinity method (POCT) and whole blood with EDTA for the ion exchange HPLC method. The use of different samples gives an almost close result because HbA1c present in the erythrocyte. Capillary blood sample use instead of conventional venipuncture is beneficial in certain groups, such as newborns or patient with a chronic disease which sample volume is an important consideration. Another advantage is collecting capillary blood can be done by untrained personnel because of the simple procedure.

Limitation of this study was the possible effects of hemoglobin products such as carbamylated Hb did not consider. Chronic renal failure develops in many diabetic patients. Almost half of all individuals with end-stage renal disease in the USA have diabetes.[4] One of the most frequent interferences in HbA1c determination is a modification of hemoglobin by carbamylation which occurs in patients with chronic renal failure.[22] Both glycation and carbamylation are post-translational modification of hemoglobin involving the free amino

groups, especially the N-terminal valine residues.[26] Urea derived isocyanate can lead the formation of carbamylated hemoglobin, which can be indistinguishable from HbA1c when using certain HbA1c assay method.[9] In this study, sample measurement was not done in duplicate for each instrument. Duplication aims to obtain more reliable results.

CONCLUSION AND SUGGESTION

In summary, HbA1c levels obtained using the boronate affinity method (POCT instrument) are not different from the HbA1c level of the HPLC method (laboratory instrument). Thus the boronate affinity method in POCT form can be considered for use by clinicians, especially in primary health care.

Further research that considers a patient with carbamylated Hb and does duplication of sample measurement is needed.

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