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RESEARCH

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COMPARISON OF HBA1C LEVEL USING TURBIDIMETRY INHIBITION IMMUNOASSAY, LATEX AGGLUTINATION

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ABSTRACT

According to the National Glycohemoglobin Standardization Program (NGSP) and The Diabetes Control and Complication Trial (DCCT) the standard method of measuring HbA1c is High-Performance Liquid Chromatography (HPLC), but HPLC requires particular instrument investments, trained staff, long and relatively expensive sample processing. This an instrument that has similar performance to HPLC but its operation process is relatively simple and not costly. The purpose of this study was to analyse the HbA1c level using Turbidimetry Inhibition Immunoassay (TII) method and the HPLC method; to analyse HbA1 level using Latex Agglutination Inhibition (LAI) method and HPLC method. This research was conducted with a cross-sectional design during the period of March-April 2017. The total sample consisted of 160 samples divided into two groups. For the first group, HbA1c level using TII method and HPLC method was measured. For the second group, HbA1c level was measured using LAI method and HPLC method.The data obtained were analyzed using the Paired T-test. There was a significant difference between HbA1c levels using TII method and HPLC method {8.12(2.53)% vs. 8.49(2.63)%, $p<0.001$}. There was no significant differences between HbA1c levels using LAI method and HPLC method {7.52(1.95%) vs. 7.47(2.00)%, $p>0.05$}. This research concluded that there was a difference between the HbA1c levels using TII method and HPLC method, but no difference between HbA1c levels using LAI method and HPLC method.

Key words: HbA1C, turbidimetry inhibition immunoassay method, latex agglutination immunoassay method, HPLC method

INTRODUCTION

Hemoglobin A1c was first isolated by Huisma et al. 1958 and in 1968 Bookchin and Gallop classified it as a glycoprotein. An increase in HbA1c levels in diabetic patients was reported by Rahbar et al. in 1969. The use of HbA1c as a biomarker to monitor glucose levels in diabetic patients was first proposed by Koenig *et al,* in 1976.[1]

Hb in normal adult consists of HbA (α2β2), HbA2 (α2δ2) and HbF (α2γ2) which is 97%, 2.5% and 0.5% of total hemoglobin total, respectively. About 6% of HbA is HbA1, which consists of HbA1a1, HbA1a2, HbA1b, and HbA1c. HbA1c is the most substantial fraction which is about 5% of total HbA.1 Glycated hemoglobin (HbA1c) is a substance formed from chemical reactions between glucose and hemoglobin, through a non-enzymatic reaction between glucose and N-terminal valine of the beta chain of hemoglobin A. Glucose forms an aldimine bond with N H2- from valine in the beta chain.[2]

HbA1c in the body will be stored in erythrocytes, and will be degraded gradually along with the erythrocyte lifespan of about 3-4 months. The level of glycated hemoglobin depends on the available blood glucose. The glucose attached to hemoglobin is very stable so that HbA1c has become one of the diabetes mellitus tests worldwide.[2] The International Diabetes Federation and the American College of Endocrinology recommend HbA1c levels below 6.5%, while the American Diabetes Association recommends HbA1c levels below 7% as a good glycemic control. HbA1c levels above 8% indicate poor glycemic control.[3,4]

There are many methods of HbA1c test which can be classified based on the test principle. The first group is based on its physical, chemical or electrical properties (ion exchange chromatography, high-performance liquid chromatography, electrophoresis, isoelectric focusing). The second group is based on its structure (affinity chromatography, immunoassay) while the third group is based on its enzymatic reactions and chemical analysis (colorimetry and spectrophotometry). Each method has its advantages and limitations. The recommended HbA1c test method is the one that gives results with high precision and accuracy and does not have many interferences.[5,6]

The standard method for the HbA1c test according to the National Glycohemoglobin Standardization Program (NGSP) and The Diabetes Control and Complication Trial (DCCT) is High-Performance Liquid Chromatography (HPLC). In the HPLC method, hemoglobin will be separated into fractions based on the ion charge. HPLC has a high accuracy and precision and is relatively unaffected by hemoglobin variants. However, HPLC requires a special instrument investments, trained staff, long and relatively expensive sample processing.[6] Based on the background, this study was conducted to assess HbA1c test with Turbidimetry

Inhibition Immunoassay (TII), and Latex Agglutination Inhibition (LAI) method compared to the HPLC method.

METHODS

This study was a cross-sectional study conducted in the Clinical Pathology Laboratory of the Dr. Wahidin Sudirohusodo Hospital Makassar from March to April 2017. The study population was all specimens tested for HbA1c in the Clinical Pathology Laboratory of the Dr. Wahidin Sudirohusodo Hospital Makassar. The study sample included all specimens of patients undergoing the HbA1c test at the Clinical Pathology Laboratory Installation of the Dr. Wahidin Sudirohusodo Hospital from March to April 2017. Ethical clearance was obtained from the Medical Research Ethics Commission, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital Makassar.

Statistical analysis was performed using SPSS version 22. The data was presented as the frequency distribution of sex and age, minimum, maximum, mean and standard deviation of the HbA1c level. Paired T-test was performed to assess HbA1C levels using TII and LAI compared to HPLC method. The HbA1c difference was considered statisticaly significant if $p < 0.05$.

RESULTS AND DISCUSSION

Table 1. Subject characteristics

Variable		n	%
Sex	Male	87	54.4
	Female	73	45.6
Age	20-40	13	8.1
	41-60	82	51.3
	≥ 61	65	40.6

Total subjects were 160 consisted of 87 (54.4%) males and 73 (45.6%) females. There are 13 subjects with age 20 -40 years old, 82 subjects with age 41-60 years old and 65 subjects with age ≥ 61 years old. Most subjects were male and at 41-60 years group.

Table 2. Comparison of HbA1c using TII and LAI to HPLC

Group	Methods	n	HbA1c (%) mean (SD)	p
Group I	TII	80	8.12(2.53)	<0.001
	HPLC	80	8.49(2.63)	
Group II	LAI	80	7.52(1.95)	0.454
	HPLC	80	7.47(2.00)	

This study involved 160 subjects divided into two groups. The first group was tested for HbA1c using TII and HPLC, and the second group was tested for HbA1c using LAI and HPLC. Table 2 showed that there was a significant difference between HbA1c levels using TII method and HPLC method { 8.12(2.53)% vs 8.49(2.63)%, p<0.001}. The mean of HbA1c using TII method was 0.37% lower than HPLC method (Figure 1). This result was the same as the result of Widijanti & Resti study, showing that the mean value of HbA1c by IE-HPLC was 0.16% higher than immunoturbidimetry (8.14% vs 7.98%).[7]

Although there was a statistically significant difference, it was not clinically significant. A variation of ≥ 0.5% (5.5 mmol/mol) of HbA1c values is the one considered clinically significant and is recommended to be reevaluated. In normoglycemic subjects, the expected variation in HbA1c is 0.4% (4.4 mmol/mol), so that a change in HbA1c ≥ 0.5% (5.5 mmol/mol) may happen because of other factors beside methods and individual variations.[8]

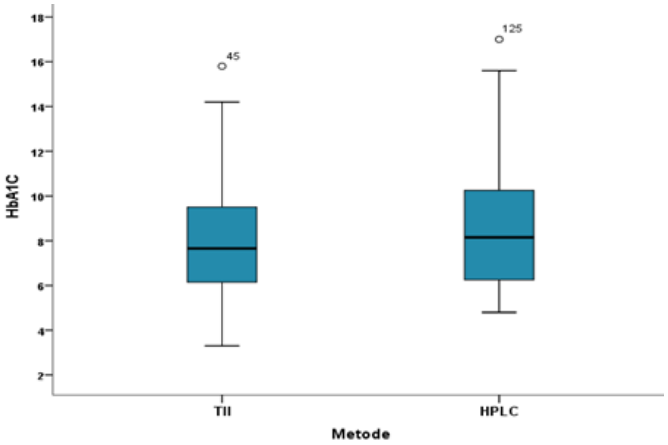

Figure 1. HbA1c level using TII and HPLC

The principle of the TII method is in the absence of HbA1c, polyhaptens (synthetic molecules containing several HbA1c epitopes) will bind free anti-HbA1c antibodies bound to microparticles forming an insoluble antibody-polyhapten complex. This complex produces significant light scattering. HbA1c in RBC lysate reacts with anti-HbA1c antibodies, forming a soluble antigen-antibody complex that reduces the light scatter. The reaction rate is measured turbidimetrically and inversely proportional to the amount of HbA1c in sample.[9,10]

The ion-exchange HPLC method's principle is the ion-exchange separating hemoglobin molecules based on their charge. The RBC lysate is injected into a negatively charged column. The positively charged Hb molecules move slower than the negatively charged molecules because of ionic interactions with negatively charged resin. HbA1c will be eluted first because its charge is more negative than HbA0. Other Hb fraction and several Hb variants can be identified by the ion-exchange HPLC, which will appear as an additional peak in chromatography.[9,11]

Table 2 also showed there was no significant difference between HbA1c levels using LAI method and HPLC method {7.52(1.95%) vs. 7.47(2.00)%, p>0.05}. The difference of HbA1c mean between the two methods was only 0.05 (Figure 2).

The LAI method is used to measure HbA1c, where in agglutinins (synthetic polymers containing multiple copies of immunoreactive parts of HbA1c) cause agglutination of latex particles coated with mouse monoclonal antibodies

specific to HbA1c. The agglutination causes an increase in absorbance of the suspension. The presence of HbA1c in the sample reduces the agglutination because HbA1c will compete with the Reagent Agglutinator (R2) at the antibody docking site of a microparticle.

The larger the amount of HbA1c in the sample, the lower the agglutination rate. Absorbance was measured at 550 nm wavelength, and the agglutination rate was used to calculate the concentration from a calibration curve. The percentage of HbA1c was then calculated using HbA1c and total hemoglobin values in µmol.[12]

One of the main problems in measuring HbA1c levels by various methods is the presence of hemoglobin variants. In Hb variants with high erythrocyte turnover, e.g. beta thalassemia, and Hb E disease, in which the lifespan of erythrocytes decreases, the measurement of HbA1C becomes less accurate. The immunoassay method use antibodies that recognize the amino acid glycol N-terminal. First-generation HbA1c immunoassay uses antibodies that recognize 4 to 10 amino acids in the HbA β chain, the presence of HbS and other Hb variants causes disruption in the analytical process. The HPLC method provides a chromatogram for each patient sample where the presence of variant hemoglobin can be easily detected by chromatogrphy.[12] The HPLC method can detect the presence of Hb variant because the principle is to separate HbA1c from HbA based on the difference in charge. Glycation on the N-terminal valine of the β-chain (HbA1c) will decrease the positive charge, resulting in the separation of the glycated HbA (HbA1c) and the unglycated HbA (HbA).[9,13]

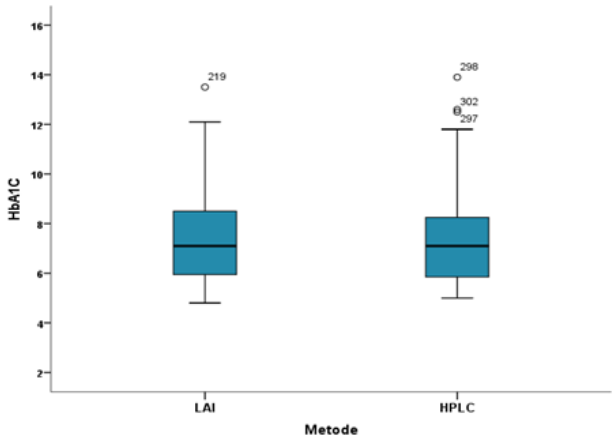

Figure 2. HbA1c level using LAI and HPLC

Limitations in this study were the presence of interferences that may affect HbA1c measurements such as anemia, hemoglobinopathy, renal failure, and hemolytic disease, which were not excluded.

CONCLUSION AND SUGGESTION

This research concluded that there was clinically significant difference between HbA1c levels using TII method, and HPLC method, no difference between HbA1c levels using LAI method and HPLC method. The researcher suggested to perform further studies that analyze hemoglobin abnormalities as an interference in HbA1c measurement.

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