

COMPARISON OF COLORIMETRIC AND HPLC METHODS FOR EVALUATION OF HbA_{1c} IN DIABETIC PATIENTS

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ABSTRACT

Objectives: To measure the HbA_{1c} by colorimetric and HPLC methods and to evaluate the analytic sensitivity of both the methods in diabetic patients.

Material and Methods: It was a cross sectional analytical study conducted in Khyber Teaching Hospital and Khyber Medical College Peshawar from September 2014 to November 2014. A total of 90 individuals were included in the study and divided in two groups, one consisting of 60 known diabetics already on treatment and one consisting of 30 non diabetic individuals. The HbA1c level of both the groups was measured by both the colorimetric and HPLC methods.

Results: Mean \pm SD by colorimetric method in non diabetic individuals was 5.79 ± 0.55 percent while that by HPLC method was 5.3 ± 0.80 percent. On other hand

in known diabetic patients category (Group 2), it was seen that the Mean \pm SD by colorimetric method was 7.11 ± 1.54 percent while that by HPLC was 8.68 ± 2.93 percent. Correlation is highly significant at the 0.01 level (2-tailed)

Conclusion: This study indicated that HbA1c should be detected by HPLC method in order to get the actual picture of glycemic control of the patient and to manage the patient accordingly.

Key words: HbA_{1c}, Colorimetric method, High-performance liquid chromatography.

INTRODUCTION

The main goal of management of diabetes is to maintain the blood glucose level within or near the normal range and to avoid number of fluctuations in these levels. As the complications of diabetes mellitus have a direct relationship to glycemic control, achieving normal or near normal glycemic level is the ultimate goal for most of the patients^{2,3,4}. Diabetes mellitus is associated with pathological changes due to hyperglycemia in various systems in the body.^{1,2,3,4}

Measurement of glycosylated hemoglobin (HbA1c) is a gold standard to monitor long-term glycemic control in patients with diabetes mellitus, therapy adjustment, assessment of the quality of diabetes care and to predict the risk for the development of complications^{5,6,7}.

There are different methods for the measurement of HbA_{1c}^{1,3,8}.

In general methods for measuring glycated hemoglobin are.

1. Methods Based On Structural Differences

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2. Immunoassays (Colorimetric methods)

Affinity chromatography

1. Methods Based On Charge Differences
2. Ion exchange chromatography
3. Electroporesis
4. Isoelectric focusing
5. HPLC

Amongst these High-performance liquid chromatography (HPLC) is a standard and reference method.

MATERIAL AND METHODS

The study was conducted in KTH and KMC Peshawar. The study duration was from September 2014-November 2014. It was a cross sectional analytical study. Selection of participants was based on non probability convenient sampling method. A total of 90 individuals were included in the study. They were divided in two groups. Group 1 consisted of 60 Known diabetic patients already on treatment while Group 2 consisted of 30 non diabetic normal healthy individuals. The inclusion criterion was diabetic patients on treatment. The exclusion criteria were all secondary causes of diabetes. A semi structured questionnaire was prepared and used for recording respondent's interviews. Informed consent was obtained after briefing them on the objectives of study. The HbA1C levels of both the groups were measured using colorimetric and HPLC methods.

Statistical analysis

The results were analyzed by using SPSS version 20. Mean standard deviation of both the groups by both methods were evaluated. A "P" value of < 0.5 was supposed to be statistically significant.

RESULTS

Measurement of HbA1C in both the groups by both colorimetric and HPLC methods showed that Mean + SD by colorimetric method in group 1 was $5.79 + 0.55$ percent while that by HPLC method was $5.3 + 0.80$ percent (as shown in table 1). It was seen that in non diabetic individuals (Group 1) colorimetric method

Table 1: Mean + SD by Colorimetric method and HPLC in Controls and Patients

	Controls (Mean + SD) (n = 30)	Patients (Mean + SD) (n = 60)
Colorimetric Method	$5.79 + 0.55$	$7.11 + 1.54$
HPLC	$5.3 + 0.80$	$8.68 + 2.93$

Table 2 : Correlations in Controls

		Colorimetric	HPLC
Colorimetric	Pearson Correlation	1	.148
	Sig. (2-tailed)		.434
	N	30	30
HPLC	Pearson Correlation	.148	1
	Sig. (2-tailed)	.434	
	N	30	30

Table 3 : Correlations in Patients

		Colorimetric	HPLC
Colorimetric	Pearson Correlation	1	.832**
	Sig. (2-tailed)		.000
	N	60	60
HPLC	Pearson Correlation	.832**	1
	Sig. (2-tailed)	.000	
	N	60	60

**.Correlation is significant at the 0.01 level (2-tailed).
[P value= 0.01]

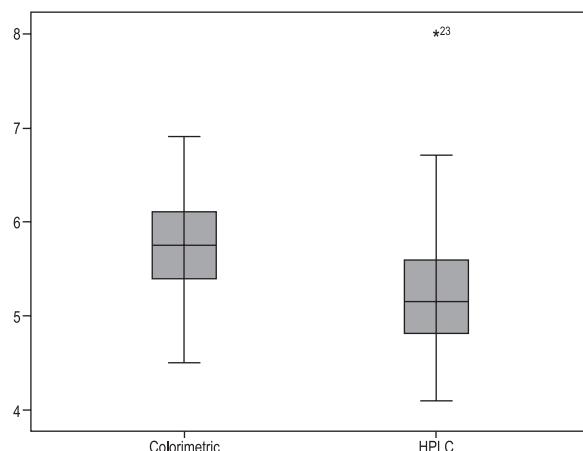


Figure 1: Box plot of Controls by Colorimetric and HPLC method

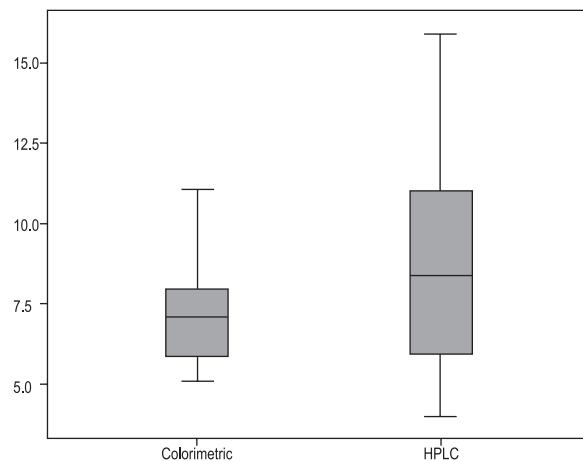


Figure 2: Box plot of Patients by Colorimetric and HPLC method

gave a higher value of HbA1c then the HPLC method. Figure 1 shows a boxplot of values of HbA1c in Group 1 individuals by colorimetric and HPLC method. While in the known diabetic patients category (Group 2), it was seen that the Mean + SD by colorimetric method was $7.11 + 1.54$ percent while that by HPLC was $8.68 + 2.93$ percent as shown in table 1. In Group 1 individuals the colorimetric method gave a lower value of HbA1c when compared with HPLC method. Figure 2 shows a boxplot of values of HbA1c in patients by colorimetric and HPLC method.

DISCUSSION

Glycosylated hemoglobin is a hemoglobin compound produced when glucose reacts non enzymatically with the amino group of hemoglobin (a protein). The rate of glycosylated hemoglobin (HbA1c) formation is directly proportional to the plasma glucose concentrations. As the average red cell life span is approximately 120 days so the glycosylated hemoglobin level at any one time shows the average blood glucose level over the previous 2 to 3 months. HbA_{1c} has also been recommended and used for the diagnosis of diabetes mellitus

when HbA1c levels are above 6.5% (48 mmol/mol). Accurate HbA1c results are essential for monitoring and appropriate treatment of diabetic patients.

In our country the routine methods used for the measurement of HbA1c are colorimetric methods based on immunoassays. High-performance liquid chromatography (HPLC) is a standard and reference method used for standardization of other methods performed for routine estimation of HbA_{1c}.^{6,9,10,11} Studies regarding comparison of colorimetric methods and HPLC methods have been done. Such a study was done in Iraq by A.A. Mansour in 2013 and it concluded that HbA1c estimation by HPLC in patients with sickle cell trait should be the preferred method and its estimation by colorimetric method should be banned¹⁵.

CONCLUSION

This study indicates that we should perform HbA1c only by HPLC method especially in diabetics, because it shows the actual glycemic control of the patient and helps to properly manage the patient in time and moreover this is a gold standard method used worldwide. Colorimetric method can be used in rare instances preferably for screening purposes only.

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