International Journal of Scientific and Innovative Research 2020; 8(1) : 8-14
P-ISSN 2347-2189, E- ISSN 2347-4971

COMPARATIVE ANALYSIS OF GLYCOSYLATED HAEMOGLOBIN OF TYPE 2 DIABETIC MELLITUS PATIENTS BY USING ION EXCHANGE CHROMATOGRAPHY AND AFFINITY NYCOCARD READER

***Anil Kumar[1], Santosh Kr. Karn[2] and RK Singh[2]**

[1]Department of Medical Laboratory Technology (Pathology) & Medical Microbiology, [2]Department of Biochemistry and Biotechnology, Sardar Bhagwan Singh University, Balawala, Dehradun ,Uttrakhand, India

*Address for Correspondence : Dr. Anil Kumar,Department of Medical Laboratory Technology (Pathology) & Medical Microbiology, Sardar Bhagwan Singh University, Balawala, Dehradun (Uttrakhand), India; Email ID: anilpoojagupta@gmail.com

ABSTRACT

The main objective of the current study was to analyze and compare the level of glycosylated hemoglobin (HbA1c) in type 2 Diabetes Mellitus (DM) patients. This study was conducted to confirm type 2 diabetes mellitus patients (n=100) who visited out patients department (OPD) of the Sardar Bhagwan Singh University, Balawala, Dehradun, Uttrakhand, India from the period of August 2018 to April 2019, by Ion Exchange Chromatography and Affinity Binding Nycocard Reader. The maximum fasting glucose level was (179.2±36.72 mg/dl) observed between the age group of 61-70 years and the maximum random blood glucose level was (199.3±43.8 mg/dl) observed between the age group of 61-70 years of patient's blood sample. The maximum values of glycosylated hemoglobin (8.9±1.06 %) and (8.8±1.1 %) were observed by Ion Exchange Chromatography and Affinity Binding Nycocard Reader respectively in same age group (61-70 years) of patients blood sample. The mean value of fasting blood glucose (161.75±15.38 mg/dl) level and random blood glucose (185.07±17.95 mg/dl) level was observed from given patient blood samples where the mean value of glycosylated hemoglobin concentration (8.35±0.44 %) and (8.14±0.61 %) were observed by Ion Exchange Chromatography and Affinity Binding Nycocard Reader respectively. The estimated level of FBS, RBS and HbA1c was indicating the maximum cases under very poor glycemic control while comparison with normal control value. However the glycosylated hemoglobin levels estimated by both methods were showing no significant variation in same patient blood samples.

***Keywords*:** Fasting blood glucose; Random blood glucose; Glycosylated hemoglobin (HbA1c); Ion exchange chromatrography; Nycocard reader; Type 2 Diabetes

INTRODUCTION

Diabetes mellitus is characterized while the defective secretion of insulin due to rapid division of beta cells inside the pancreas leading to compensate for the high blood glucose level . [1] Type 2 diabetes mellitus is increasing prevalently in developing as well as in newly industrialized countries of the world. [1,2] It is also prevalent in elderly and associated with hyperlipidemia, obesity, hypertension and cardiovascular disease. [2-4] The glycated haemoglobin (HbA1C) is non enzymatic condensation of glucose to the valine residue of β-haemoglobin to form aldimine and then by amadori rearrangement to ketoamine. [3-5] Though the clinicians have found that average blood glucose can be estimated over the previous 10 weeks . [6] Diabetes control and complications trial was published in previous 3 decades by several researchers. [5-7] Diabetic patients have been classified into different categories i.e very good glycemic control, good glycemic control, poor and very poor glycemic control to evaluate the value of HbA1c with Ion Exchange Chromatography and Affinity Binding Nycocard Reader. High performance liquid chromatography (HPLC) is the best standard method but its main drawback is that results are not available at the time of patient's visit, delay in reporting time, communicating health feedback, delay in clinical decision making,

changes in the regimen prescription may be missed . [8-10] Many methods are available for the routine measurement of HbA1c which are based on different analytical principles such as immunoassays, ion exchange chromatography, and affinity chromatography . [11-15] The choice of diagnostic method will depend on local considerations such as cost effective and availability of equipment. The objective of this study was to compare the level of glycated haemoglobin in Type 2 diabetes mellitus patients by two different methods i.e ion exchange chromatography and affinity binding Nycocard reader.

MATERIALS AND METHODS

This is a comparative cross sectional study carried out in type 2 diabetes mellitus patients (n=100), visiting the OPD of Sardar Bhagwan Singh University, Balawala, Dehradun, Uttrakhand, India, who are confirmed by fasting blood glucose (FBG) based on world Health organization (WHO) criteria during period of August 2018 to April 2019. EDTA blood samples stored at refrigerator temperature (4°C) used for the further evaluation of the HbA1c value by using standard protocols with given methods . [10-12]

Ion Exchange Chromatography

Procedure: A mixture solution was prepared and kept it for 5 minutes at 15-25 °C. The solution was pipette into labeled reagent i.e ion exchange resin prefilled in plastic tubes in imidazole borate buffer. Separator was inserted, a rubber sleeve is 1 cm above from surface of resin suspension and it was mixed in vortex mixer for 5 minutes for firmly packed the resin to push down the separator. Then it was taken from the separator to Microtiter well and measure in ELISA plate reader at 405 nm . [16]

Pipette 20 µl haemolysate from lyse sample was taken for total haemoglobin estimation then 5ml of distilled water was added and mixed properly. The measurement was done in ELISA plate reader at 405 nm and calculation is done by using given formula. [16]

The principle behind above mentioned is observed haemolysis in the blood when the blood is mixed with lysing reagent containing a detergent and borate ions and there is elimination of labile schiff's base. Mixing of Haemosylate is done with a weekly binding cation exchange resin for 5 minutes. During this time HbA0 binds to the resin. Resin is removed from the supernatant fluid (also contain HbA1) with a special resin separator. The glycohaemoglobin percentage of total haemoglobin is determined by measuring the absorbance of the glycohaemoglobin and of total haemoglobin fraction at 405 nm in ELISA reader in comparison with a standard glycohaemoglobin preparation carried through the test procedure. [16,17]

Affinity Binding Nycocard Reader

Procedure: Pipette 5 µl of whole blood and was added to the cup with the reagent, mixed it well and kept for incubation for 180 seconds. Then to obtain homogenous suspension it was remixed with it. Added 25 µl of washing solution in test device and allowed soaking completely into the membrane for 10 seconds. The test result was read within 5 minutes using Nycocard reader . [17]

The principle behind the above mentioned, it is a borate affinity assay. The kit includes a porous membrane filter, test tubes prefilled with reagent and washing solution. The reagent contains agents that are capable to lyse erythrocytes and precipitate haemoglobin specifically as well as a blue boronic acid conjugate that binds cis diols of glycated haemoglobin. There is lysis of erythrocytes immediately when blood is added to the reagent. All haemoglobin precipitate the boronic acid conjugate binds to the cis-diol conjugation of glycated haemoglobin. Excess of colored conjugate is removed with washing solution and evaluation of precipitate by measuring the blue (glycated haemoglobin) and the red (total haemoglobin) colour between them being proportional to the percentage of HbA1c in the sample. [17]

Statistical Analysis:

As continuous variable measured SD were presented and categories were expressed as percentages. The oneway ANOVA was used for comparison of mean values and the Paired t- test and Spearman correlation were selected for these categories. The regression analysis of individual values obtained from the two measuring methods was performed . [16-18]

RESULTS

In this study, the frequency of Type 2 Diabetes Mellitus (DM) patients were identified into different age groups where the maximum

percentage (35 %) of patients affected with this disease was found under the age group of 51-60 years followed by 20 percentages of patients affected under age group of 61-70 years whereas the minimum percentage (4 %) of patients affected under age group of 21-30 years.

The mean age of this study population was 54.82±21.23 years, the mean height of the study population was 1.64± 0.05 mt, the mean weight of selected population was 61.04±3.27 kg, the mean of body mass index of study population was 24.09±1.83 kg/m^2, the mean of systolic and diastolic blood pressure were found 146.62±3.78 mm/Hg and 98.88±4.76 mm/Hg respectively as shown in Table 1.

The age-wise distributions of fasting blood sugar (FBS), random blood sugar (RBS) and glycosylated hemoglobin (HbA1c) in Type 2 diabetes mellitus (DM) patients are shown in Table 2. The normal control value of fasting blood glucose was found 90.1± 20.0 mg/dl and random blood glucose was found 180±40 mg/dl, where the good control of glycosylated hemoglobin was observed 6.0-6.9% and 7.0-7.3 % was observed poor glycemic control.

The maximum fasting glucose level (179.2±36.72 mg/dl) was observed between the age group of 61-70 years and the maximum random blood glucose level (199.3±43.8 mg/dl) was observed between the age group of 61-70 years of patient's blood sample. The maximum values of glycosylated hemoglobin (8.9±1.06 %) and (8.8±1.1 %) were observed by Ion Exchange Chromatography and Affinity Binding Nycocard Reader respectively in same age group (61-70 years) of patients blood sample.

The minimum fasting glucose level (141.52±3.31 mg/dl) was observed between the age group of 51-60 years and the minimum random blood glucose level (163.3±55.44 mg/dl) was observed between the age group of 71-80 years of patient's blood sample. The minimum value of glycosylated hemoglobin (7.7±0.08 %) was observed by Ion Exchange Chromatography between the age group of 31- 40 years and (7.3±0.9 %) was observed by Affinity Binding Nycocard Reader between the age group of 21-30 years of patient's blood sample.

The mean value of fasting blood glucose (161.75±15.38 mg/dl) level and random blood glucose (185.07±17.95 mg/dl) level was observed from given patient blood samples where the mean value of glycosylated hemoglobin concentration (8.35±0.44 %) and (8.14±0.6 %) were observed by Ion Exchange Chromatography and Affinity Binding Nycocard Reader respectively indicating the maximum cases under very poor glycemic control while comparison with normal control value.

Table 1: Age-wise distribution of basic characteristics in Type 2 DM patients (n=100) results are expressed in (Mean ± SD)

Age-groups	No. of Patients	Age (Years)	Ht (m)	Wt (Kg)	BMI (Kg/sq.m.)	BP(Systolic) (mm/Hg)	BP(Diastolic) (mm Hg)
21-30	4	25.4±2.32	1.73 ± 0.02	56.4 ± 5.12	20.73±3.24	145.0±21.21	92.2±12.24
31-40	10	33.5±4.12	1.69 ± 0.03	58.5 ± 2.31	23.28±2.84	144.3±14.32	96.3±14.22
41-50	11	45.2±3.25	1.63 ± 0.04	63.5 ± 4.34	24.23±3.26	147.0±20.23	103.4±10.23
51-60	35	56.6±2.22	1.61 ± 0.07	65.7 ± 2.33	26.63±2.27	144.1±11.21	98.0±11.24

61-70	20	65.21±3.07	1.65 ± 0.06	63.41 ± 3.26	25.23±1.28	148.54±17.25	102.0±12.21
71-80	15	75.8±2.32	1.66 ± 0.03	60.42 ± 2.37	24.73±3.26	154.16±16.16	105.2±14.26
80-90	5	82.04±1.21	1.55 ± 0.04	59.41 ± 3.38	23.83±1.30	143.3±12.27	95.1±11.22
Total	100	54.82±21.23	1.64 ± 0.05	61.04 ± 3.27	24.09±1.83	146.62±3.78	98.88±4.76

±: Standard Deviation

Table 2: Age-wise distributions of FBS, RBS and HbA1c in Type 2 DM patients (n=100) results are expressed in (Mean ± SD)

Age-groups	FBS (mg/dl)	RBS (mg/dl)	HbA1c Ion Ex (%)	HbA1c Nyco (%)
21-30	155.23±23.31	169.22±62.46	8.7±1.2	7.3±0.9
31-40	177.4±15.22	172.3±57.32	7.7±0.08	7.7±0.8
41-50	166.31±42.47	211.2±92.24	7.9±0.14	7.5±0.9
51-60	141.52±3.31	197.43±26.43	8.7±1.07	8.5±1.7
61-70	179.2±36.72	199.3±43.8	8.9±1.06	8.8±1.1
71-80	169.3±24.35	163.3±55.44	8.2±1.03	8.6±1.2
80-90	143.3±12.22	182.3±59.21	8.4±1.14	8.6±0.9
Total	161.75±15.38	185.07±17.95	8.35±0.44	8.14±0.61

±: Standard Deviation

DISCUSSION

The patient of diabetes mellitus has a risk of hypertension, cardiovascular disease and kidney disease if there was significant rise in their systolic and diastolic blood pressure and this type of disease problem should be commonly observed in type 2 diabetes mellitus . [15-19] There are so many common causing factors of type 2 diabetes mellitus like aging, urbanization, population growth, obesity, life style with dietary aspects and physical inactivity. The population suffering with type 2 diabetes mellitus for long time also includes the complications of different diseases like retinopathy, nephropathy, autonomic neuropathy, etc. increasing the huge medical and economical burden on patients. [17-21] In current study, there was significant increased level of fasting blood sugar (FBS), random blood sugar (RBS) and glycosylated hemoglobin (HbA1c) which indicates the complication associated with diabetes mellitus.

The high performance liquid chromatography (HPLC) is the best standardized method for estimation of glycosylated hemoglobin and also used in clinical haematology laboratory for the determination of HbA1c from patients blood samples but a drawback of this method is that the patients result are not available at the time of patient visit in the outpatient department (OPD) . [22-24]

The present study was started by thinking the above problem of the patient results, in this study we were involved the two methods like Ion exchange chromatography and Affinity binding Nycocard reader for determination of HbA1c concentration and we found the results of the test within 5- 15 minutes. Affinity Nycocard reader will give results within 5 minutes and ion exchange chromatography will give results within 15 minutes and there was showing no significant difference between the results of these methods.

In this study, the maximum fasting glucose level (179.2±36.72 mg/dl) was observed between the age group of 61-70 years and the maximum random blood glucose level (199.3±43.8 mg/dl) was observed between the age group of 61-70 years patient's blood sample. The maximum values of glycosylated hemoglobin (8.9±1.06 %) and (8.8±1.1 %) were observed by Ion Exchange Chromatography and Affinity Binding Nycocard Reader respectively in same age group (61-70 years) of patients blood sample. The highest frequency of glycosylated hemoglobin > 8.0 % was observed by Gautam *et al*., 2014 [17] with both method, which was similar finding with this study and there were showing no major differences between the results of HbA1c from type 2 diabetes mellitus blood sample by both method namely Ion Exchange Chromatography and Affinity Binding Nycocard Reader. The above data indicate the maximum cases under very poor glycemic control while comparison with normal control value in the population of patients near the Balawala, Dehradun, Uttrakhand, India. It also reflects the poor management of diabetes mellitus patients in this region because of poverty, poor health education, ignorance, inactive in physical activity and unawareness about the disease, etc.

The comparable finding was observed with positive correlation between fasting blood glucose level, random blood glucose level and glycosylated hemoglobin concentration by Baral et al. [24]; Giacco et al. [22] and Pokharel et al. [23] .

This study was mainly focused on preventing the delay time for reporting of patients HbA1c test result with accuracy. The mentioned techniques applied for the consume of reporting time, it gives reading of the patient samples within 15 minutes at the time of patient visit outpatient department of the Sardar Bhagwan Sigh University, balawala, Dehradun, India without any statistically significant differences between the test report of the same patient blood sample. The conventional methods which are easy to monitor by technician, the reference ranges of the method, diagnostic protocol and risk assessment procedure are updated regularly for increasing reports quality, reliability for the diagnosis and management of patients with diabetes mellitus by International Diabetes Federation (IDF) or World Health Organization (WHO).

CONCLUSION

The estimated level of fasting blood sugar (FBS), random blood sugar (RBS) and glycosylated hemoglobin (HbA1c) was indicating the maximum cases under very poor glycemic control while comparison with normal control value. However the glycosylated hemoglobin levels estimated by both methods were showing no significant

variation in same patient blood samples. So the both methods are preferred and can be used in clinical hematology laboratory to run the patient blood sample for HbA1c tests and results should also be provided at the time of patient visit, which should be made easy to doctor for taking decision in changing the treatment.

ACKNOWLEDGEMENT

Authors are thankful to Dr. G.D. Singh, Managing Secretary, Sardar Bhagwan Singh University, Dehradun, Uttrakhand, India for providing laboratory facility and resources for this research work.

Financial Support: The study does not have any financial support.

Conflict of interest: The authors of the article have no conflict of interest.

REFERENCES

1. Rhea JM., Molinaro R. Pathology consultation on HbA1c methods and interferences. American Journal of Clinical Pathology. 2014; 141(1):5-16.
2. Little RR., Roberts WL. A review of variant hemoglobin interfering with hemoglobin A1c measurement. Journal of Diabetes Science and Technology. 2009; 3(3):446-451.
3. Gupta S., Jain U., Chauhan N. Laboratory diagnosis of HbA1c: A review. Journal of Nanomedicine Research. 2017; 5(4):1-10.
4. Garcia AH., Ruiz AA., Cedillo CB. Effect of the method to measure levels of glycated hemoglobin on individual clinical decisions, comparison of an immunoassay with high-performance liquid chromatography. American Journal Clinical Pathology. 2009; 132(3): 332-335.
5. Reddy A., Sachan A., Rao S., Mohan A. Clinical applications of glycosylated hemoglobin. J Clin Sci Res. 2012; 2(1): 22-33.
6. Razi F., Esfahani EN., Farzani MR., Tootee A., Qorbani M., et al. Effect of the different assay of HbA1c on diabetic patients monitoring. Journal of Diabetes and Metabolic Disorders. 2015; 19(6): 1-4.
7. Grant DA., Dunseath GJ., Churm L. Comparison of a point-of-care analyzer for the determination of HbA1c with HPLC method. Practical Laboratory Medicine. 2017; 8: 26-29.
8. Genc S., Gurdol F., Kanzamar-Ozer M., Ince N., Ozcelik F., et al. The analytical performance of four different glycated hemoglobin methods. Medical Chemistry. 2014; 4(6): 501-505.
9. Michael RS. Pitfalls in hemoglobin A1c measurement: When results may be misleading. J Gen Intern Med. 2013; 29(2):388-394.
10. Prasertwatanakorn W., Vattanaviboon P., Rerksngarm T. The effect of thalassemia and abnormal hemoglobins on HbA1c assays by ion exchange HPLC, boronate affinity chromatography and immunoassay. J Med Tech Assoc Thailand. 2016; 44(3): 5843-5853.
11. Mora CS., Olivia MS., Riejos PF., Mateo J., Padillo JP., et al. Evaluation of two HbA1c point-of-care analyzers. Clin Chem Lab Med. 2011; 49(4): 1-5.
12. Subcharoan U. Comparative study of HbA1c measurement using HPLC, immunoassay and affinity chromatography techniques based on hemoglobin typing. J Med Tech Assoc Thailand. 2013; 41(1): 4448-4456.
13. Stirk H., Allen KR. Measurement of glycated hemoglobin by boronate affinity high-pressure liquid chromatography. Ann Clin Biochem. 1999; 36(1): 232-234.
14. Rukmini MS., Ashritha A., Nishmitha P., Yalla D., Christy A., Manjrekar P. Analytical calibre of high performance liquid chromatography and ion exchange chromatography resin methods in estimation of glycated hemoglobin: a comparitive study. Biomedical Research. 2017; 28 (4): 1765-1769.
15. Sekerija M., Poljicanin T., Erjavec K., Liberati-Cizmek AM., Prasek M., Metelko Z. Gender differences in the control of cardiovascular risk factors in patients with type 2 diabetes - a cross-sectional study. Intern Med. 2012; 51: 161-166.
16. Asryani T., Nasrul E., Rikarni., Prihandani T. Difference in HbA1c level between boronate affinity and ion exchange high performance liquid chromatography method in diabetic patient. Indonesian Journal of Clinical Pathology and Medical Laboratory. 2019, 25(2): 174-179.

17. Gautam N., Dubey RK., Jayan A., Nepaune Y., Padmavathi P., Chaudhary S., Jha et al. Comparative study of glycated hemoglobin by ion exchange chromatography and affinity binding nycocard reader in type 2 diabetes mellitus. Nepal Medical College Journal. 2014; 16(2-4):103-108.
18. Kautzky-Willer A., Kamyar MR., Gerhat D. Sex-specific differences in metabolic control, cardiovascular risk, and interventions in patients with type 2 diabetes mellitus. Gend Med. 2010; 7: 571-583.
19. Nitin S. HbA1c and factors other than diabetes mellitus affecting it. Singapore Med J. 2010; 51: 616-622.
20. Burtis C., Ashwood E., Bruns D. Tietz Text book of clinical chemistry and molecular diagnostics. India, Elsevier. 2006; 4: 879-881.
21. Mahajan RD., Mishra B. Using Glycated Hemoglobin HbA1c for diagnosis of Diabetes mellitus:An Indian perspective. Int J Biol Med Res. 2011; 2: 508-512.
22. Giacco F., Brownlee M. Oxidative Stress and Diabetic Complications. Cir Res. 2010; 107: 8-70.
23. Pokharel DR., Gautam N., Archana J., Nagamma T., Kumar R., Sapkota RM. Frequency of Type 2 Diabetes mellitus and Impaired Glycemia in a Teaching Hospital of South-Western Nepal. Asian Journal of Medical Sciences. 2011; 2:202-206.
24. Baral N., Koner BC., Karki P., Ramaprasad C., Lamsal M., Koirala S. Evaluation of New WHO Diagnostic Criteria for Diabetes on the Prevalence of Abnormal Glucose Tolerance in a Heterogeneous Nepali Population – The Implications of Measuring Glycated Hemoglobin. Singapore Med J. 2000; 41(6): 264-267.