ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Prevalence of hemoglobin variants and their effect on Hba1c measurement among the indigenous population of north Bengal attending a tertiary care hospital

Soutrik Roy[1], Indrajit Nath[2],Sayantan Dasgupta[3], Arun Kumar[4], Utpal Kumar Biswas[5]

[1]Senior Resident, [2,3]Associate Professor, [5]Professor and Head, Department of Biochemistry, North Bengal Medical College and Hospital, Darjeeling, [4]Professor and Head, Department of Biochemistry, Jagannath Gupta Institute of Medical Science, Kolkata, West Bengal, India

Submission: 09-02-2022 Revision: 03-05-2022 Publication: 01-06-2022

ABSTRACT

Background: Several forms of hemoglobin variants might be present in the population being fully or partially silent. Furthermore, a high percentage of the population is already suffering from type 2 diabetes mellitus (DM) which is mainly monitored by HbA1c estimation. As the structure of hemoglobin molecule is altered by the variant status hence, this might have an impact on the HbA1c estimation. **Aims and Objectives:** The study aimed to assess the prevalence of hemoglobin variant and its effect on the HbA1c estimation. **Materials and Methods:** Samples from 439 individuals were taken and evaluated for any hemoglobin variants,andalso, the HbA1c values were measured. **Results:** About 27.4% of the study population were found to have hemoglobin variants, out of this 27.4%, 19.5% were Hb E carrier and 6.45% were Hb E diseased. About 22.3% of the population were suffering from DM. Hb variants had significant effect on HbA1C measurement in the overall study population and in the normal population with $P<0.001$. Two-way ANOVA also showed Hb variant and diabetes status significantly affected HbA1C value with $P<0.001$ in the study population. **Conclusion:** All the patients should be properly evaluated for Hb variants before interpreting the results of Hb A1C to prevent improper management.

Key words: Hemoglobin variants; Diabetes mellitus; HbA1C; High-performance liquid chromatography; Immunoturibidimetry

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v13i6.43044

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

Hemoglobin variants are highly prevalent in the sub Himalayan population of North Bengal.[1] Due to the presence of several ethnic groups in this part of the world, a significant number of individuals are associated with hemoglobinopathies. Common hemoglobin variants found in this population are mainly Hb E (both homozygous and heterozygous forms), Hb S carrier and disease and beta thalassemia carrier.[1] Other hemoglobin variants(such as Hb D carrier, Hb ES double heterozygote, and Hb C carrier) are also present though in very minor proportions.

The worldwide incidence of diabetes mellitus (DM) is increasing day by day[2] and this part of the world is no such exception. DM comprises a group of metabolic disorders that share the common feature of hyperglycemia.[3] The American Diabetes Association recommends hemoglobin A1c(A1C) as the standard laboratory assessment of glycemic control and efficacy of treatment for patients with type 1 and type 2 diabetes.[4] However, in some clinical situations, laboratory assessment using the A1C test may provide unreliable information. When an A1C result is inconsistent with a patient's clinical situation, conditions that affect red blood cell lifespan

Address for Correspondence:
Dr. Utpal Kumar Biswas, Professor and Head, Department of Biochemistry, North Bengal Medical College and Hospital, Darjeeling - 734 012, West Bengal, India. **Mobile:** +91-8777277694. **E-mail:** drutpalbiswas2010@gmail.com

and hemoglobinopathies must be considered as possible causes.[5] Hemoglobinopathies can affect the reliability of HbA1c in three ways:(1) Altering the normal process of glycation of Hb A to A1C,(2) causing an abnormal peak on chromatography, making estimation of A1C unreliable, and(3) making the red blood cell more prone to hemolysis, thereby decreasing the time for glycosylation to occur and producing a falsely low A1C result.[6] In this pretext, it is erstwhile to mention that since patients with DM are often monitored by frequent measuring of HbA1c values, all such patients also need to be examined for presence of any hemoglobin variants. Moreover, if any variants are present, then proper assessment of the patient is needed using both fasting blood glucose levels and HbA1c values simultaneously and if possible alternate methods of HbA1c measurements are to be employed.

With the above background, the present study is aimed at trying to find out the prevalence of several hemoglobin variants and their possible effect on the measurement of HbA1c.

Aims and objectives

Prevalance of Hemoglobin variants in the study population and their effect on HbA1C measurement.

MATERIALS AND METHODS

This is an observational, cross-sectional, and hospital-based study conducted in the Department of Biochemistry, North Bengal Medical College, Darjeeling, India.

The study was pre-approved by the Institutional Ethics Committee vide Memo No. IEC/NBMC/2018-19/85 dated 07/01/2019.

The sample size (n) was calculated using the formula:
$n=z^2pq/d^2$
$n=(1.96)^2\times0.5\times0.5/(0.05)^2$
n= 384.16

where n=sample size, d= absolute precision (result expected ±5% of the true value), p=expected proportion (0.5 to yield maximum value of n), q=1–p, $z_{(1-\alpha/2)}$=1.96=value of the standard normal distribution corresponding to a significance level of a (1.96 for a two-sided test at the 0.05 level).

A total of 449 subjects were included in the study. All patients being referred to the department of biochemistry for investigations in the age group of 20–50 years of either sex were included in the study by simple random sampling along with their accompanying healthy relatives and friends. Patients suffering from chronic renal disease, moderate-to-severe anemia, suffering from cancer and receiving chemotherapy, receiving blood transfusion, and drugs affecting hemoglobin metabolism were excluded from the study.

The fasting and post-prandial blood samples were collected from the study populationunder aseptic conditions after obtaining informed consents.

Laboratory investigations

Hemoglobin variants were detected using variant II beta-thalassemia short program which utilizes the principle of high-performance liquid chromatography (HPLC).[7] HbA1c estimation was done by two methods: Particle enhanced immunoturbidometry method[8] and by HPLC method.[9] Other investigations were done using standardized reagents using our Automated Chemistry Analyzer.

Statistical analysis

The data analysis in this study is done by the help of statistical software SPSS (version 21) and Microsoft Excel 2010.All the data are expressed as Mean ± SD and p-value < 0.05 was considered statistically significant.

RESULTS

The total study subjects were grouped into four categories depending on their glycemic status(fasting blood glucose) and HbA1c percentage, as shown in Table 1.

In the study population, out of total 449 individuals, 247 (55%) were non-diabetic without Hb variants, 102 (22.7%) individuals were non-diabetic with Hb variants, 79(17.6%) individuals were diabetic without Hb variants, and 21 (4.7%) individuals were diabetic with Hb Variants.

The several types of hemoglobin variants in the study population are shown in Table 2. In the study population, 19.15% individual were HbEcarrier, 6.45% were Hb E disease, 4 were beta-Thal carrier, 2 were Hb E beta-Thal, 1 was HPFH trait, and 1 Hb D carrier.

Clinico biochemical parameters of the study subjects are shown in Table 3.

Table 1: Distribution of the study subjects in the population

Study groups	Number	Percent
Non-diabetic without Hbvariants	247	55.0
Non-diabetic with Hb variants	102	22.7
Diabetic without variants	79	17.6
Diabetic with variants	21	4.7
Total	449	100.0

When the means of the different parameters were compared among the study groups using one-way ANOVA test, it was found that there is statistically significant difference for the parameters FBS, PPBS, HbA1C (HPLC method), and HbA1C (immunoturbidometric method) with P<0.001.

From Tables 4 and 5, the non-diabetic without Hb variants group is having a mean HbA1C(HPLC) value of 4.87±0.59% with a range of 3–6.4% and a mean HbA1C (immunoturbidometry) value of 5.25±0.46% with a range of 4.1–6.8%. The non-diabetic with Hb variants group is having mean HbA1C(HPLC)value of 4.76±0.62% with a range of 2.8–5.8% and a mean HbA1C (immunoturbidometry) value of 5.36±0.26% with a range of 4.8–5.9%. The diabetic without Hb variants group is having mean HbA1C(HPLC) value 7.24±1.91% with a range of 5.1–14.2% and a mean HbA1C (immunoturbidometry) value 7.74±2.17% with a range of 5.9–16.3%. The diabetic with Hb variants group is having mean HbA1C(HPLC) value of 7.06±1.17% with a range of 5.6–9.6% and a mean HbA1C (immunoturbidometry) value of 7.41±1.27% with a range of 5.9–10.1%.The one-way ANOVA shows that there is statistically significant mean difference between study groups (P<0.001).

Comparison ofHbA1C levels by HPLC method in different study groups done using ANOVA with Bonferroni correction clearly indicates that the hemoglobin variants may have impact on the estimation of HbA1c percentage, be it in the diabetic or the non-diabetic groups.

For further evaluation, we performed independent t-test among the non-diabetic and diabetic groups with or without hemoglobin variants which have also given us statistically significant data.

HbA1C levels by HPLC method in normal population as well as entire study population showed statistically significant difference between non-variant and variant groups.

The two-way ANOVA was done taking into consideration the effect of Hb variant and diabetes status on HbA1C value. It was found that Hb variant and diabetes status were independently effecting the HbA1C value with P=0.000 each but the combined effect of Hb variant and diabetes status had no significant effect on HbA1C levels with P=0.437.

Table 2: Type of hemoglobin variants in the study population

Hb Variants	Study population				Total	
	Non-diabetic with Hb variants		Diabetic with Hb variants			
	No	%	No	%	No	%
HbE Carrier	71	15.8	15	3.34	86	19.15
HbE Disease	26	5.79	3	0.67	29	6.45
Beta-Thal Carrier	1	0.22	3	0.67	4	0.89
HbE Beta-Thal	2	0.46	0	0	2	0.46
HPFH Trait	1	0.22	0	0	1	0.22
HbD Carrier	1	0.22	0	0	1	0.22

DISCUSSION

In this study, we have found 123 subjects with hemoglobin variants out of a total of 449 subjects which are27.4%. Hence, it can be deduced that a sizeable number of study subjects are associated with hemoglobin variants in this population. Earlier reports in this direction also agrees with our observation in this North Bengal region.[1]

We also observed that out of 449 subjects, 100 number of patients is suffering from DM with poor glycemic control which is 22.3%. This also signifies the diabetic burden of the population as comparable to any other parts of the country.[2]

We have observed that majority of the population are HbE heterozygous state(carrier). Out of 123 subjects having hemoglobin variants, 86 subjects (19.15%) are diagnosed as Hb E carrier, while 29 subjects (6.45%) are Hb E

Table 3: Clinicobiochemical parameters in the study population

Parameters	Study Groups				One-way ANOVA F (3,445)
	Non-diabetic without Hb Variants (n=247)	Non-diabetic with Hb Variants (n=102)	Diabetic without Hb Variants (n=79)	Diabetic with Hb Variants (n=21)	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Age (years)	35.02±10.88	34.78.29±11.55	39.94±8.74	37.09±11.05	4.528, **P=0.003
#Sex (M/F)	96/151	31/71	46/33	11/10	*P<0.001
FBS (mg/dl)	92.15±14.60	87.96±10.32	126.72±48.38	147.48±59.53	62.64, *P<0.001
PPBS (mg/dl)	128.94±14.61	127.17±6.99	208±77.43	212.80±83.65	112.09,*P<0.001
HbA1C(%, HPLC)	4.87±0.59	4.76±0.62	7.24±1.91	7.06±1.17	95.96, *P<0.001
HbA1C(%, Immunoturb)	5.25±0.46	5.36±0.26	7.74±2.17	7.41±1.27	143.09, *P<0.001

*Significance at the level of P<0.001, **Significance at the level of P<0.05

diseased. This particular variant is hence, predominant in this population.[1]

As shownin Tables 4 and 5, the mean HbA1c values estimated by both HPLC and immunoturbidometric methods are significantly different in the different groups. Hence, it can be inferred that the presence of hemoglobin variants does affect the estimation of HbA1c levels and different methods give different values of HbA1c estimation.

Although considering unequal number of subjects in different groups, there is significant difference in the concentration of fasting and postprandial blood glucose levels as well as HbA1c percentage (measured by different methods) among the four groups, as shownin Table 3. Further, group-wise comparison is shown in Table 6 by Bonferroni's *post hoc* analysis as well as by independent t-test between the groups, as shown in Tables 7 and 8 mainly which showed statistically significant difference in HbA1C measurements between non-variant and variant group both in normal population as well as the entire study population. This observation is in accordance with the study conducted by Lorenzo-Medina et al.,[10] Furthermore, two-way ANOVA was performed to know the combined effect of the two independent variables on the outcome as the groupings were done taking into consideration two independent variables, namely, (a) status of diabetes and (b) hemoglobin variants and depicted in Table 9. Hbvariant and diabetes status were independently effecting the HbA1C value with P=0.000 each, but the combined effect of Hb variant and diabetes status had no significant effect on HbA1C levels with P=0.437.

Although our study population was selected among the patients and relatives, our observation has revealed that a considerable number of hemoglobin variants are present in this area. These variants are also associated with DM in a

Table 4: HbA1C levels by HPLC method in the study subjects

Category	No. of cases	HbA1Clevels by HPLC method in %					One-way ANOVA F (3,445)
		Mean	S.D.	Range	Median	S.E.M.	
Non-diabetic without Hb variants	247	4.87	±0.59	3-6.4	4.9	0.037	95.96, *P<0.001
Non-diabetic with Hb variants	102	4.29	±1.54	2.8-5.8	4.8	0.064	
Diabetic without Hb variants	79	7.24	±1.91	5.1-14.2	6.30	0.215	
Diabetic with Hb variants	21	7.06	±1.17	5.6-9.6	6.90	0.268	

*Significance at the level of P<0.001

Table 5: HbA1C levels by immunoturbidimetric method in the study subjects

Category	No. of Cases	HbA1CLevels by Immunoturbidometric method in %					One-way ANOVA F (3,445)
		Mean	S.D.	Range	Median	S.E.M.	
Non-diabetic without Hb variants	247	5.25	±0.46	4.1-6.8	5.3	0.029	143.09, *P<0.001
Non-diabetic with Hb variants	102	5.36	±0.26	4.8-5.9	5.3	0.025	
Diabetic without Hb variants	79	7.74	±2.17	5.9-16.3	6.8	0.245	
Diabetic with Hb variants	21	7.41	±1.27	5.9-10.1	7.1	0.278	

*Significance at the level of P<0.001

Table 6: Comparison of HbA1C levels by HPLC method in different study groups done using ANOVA with Bonferroni correction

(I) Status of Diabetes and Hemoglobin Variants	(J) Status of Diabetes and Hemoglobin Variants	Mean Difference (I-J)	Std. Error	Sig.
Normal non-diabetic	Non-diabetic with Hb variants	0.57563*	0.15020	0.001**
	Diabetic without variants	−2.36991*	0.16495	0.000*
	Diabetic with variants	−1.52367*	0.29008	0.000*
Non-diabetic with Hb variants	Normal non-diabetic	−0.57563*	0.15020	0.001**
	Diabetic without variants	−2.94553*	0.19126	0.000*
	Diabetic with variants	−2.09930*	0.30581	0.000*
Diabetic without variants	Normal non-diabetic	2.36991*	0.16495	0.000*
	Non-diabetic with Hb variants	2.94553*	0.19126	0.000*
	Diabetic with variants	0.84623*	0.31332	0.043**
Diabetic with variants	Normal non-diabetic	1.52367*	0.29008	0.000*
	Non-diabetic with Hb variants	2.09930*	0.30581	0.000*
	Diabetic without variants	−0.84623*	0.31332	0.043*

*Significance at the level of P<0.001, **Significance at the level of P<0.05

Table 7: HbA1C levels by HPLC method in normal population

Category	No. ofCases	HbA1C(%) by HPLC method				
		Mean	S.D.	Range	Df	P-value
Normal non-diabetic	247	4.87	±0.587	3–6.4	347	<0.001*
Non-diabetic with Hb variants	102	4.29	±1.54	2.8–5.8		

*Significance at the level of *P*<0.001

Table 8:HbA1C levels by HPLC method in the study population

Category	No. of Cases	HbA1C(%) by HPLC method				
		Mean	S.D.	Range	Df	P-value
Population without variant	326	5.44	±1.47	3–14.2	447	0.048**
Populationwith Hb variants	123	4.65	±1.88	2.8–9.6		

**Significance at the level of *P*<0.05

Table 9: Effect on HbA1C levels by two independent variables Hb variant and diabetes status using two-way ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	468.874[a]	3	156.291	95.966	0.000
Intercept	7003.443	1	7003.443	4300.258	0.000
Hbvariant	27.272	1	27.272	16.746	0.000
Diabetesstatus	269.444	1	269.444	165.444	0.000
Hbvariant and diabetesstatus	0.988	1	0.988	0.607	0.437
Error	724.731	445	1.629		
Total	13448.190	449			
Corrected Total	1193.605	448			

[a]R Squared=0.393 (Adjusted R Squared = 0.389)

sizeable number of patients. In the present study, 21 patients (21%) out of a total of 100 diabetic patients are having hemoglobin variants. As diabetic subjects are monitored frequently by HbA1c assessment, the variant hemoglobin status may interfere into the assessment of HbA1c percentage in this group of patients. We have found different methods of HbA1c measurements to also have the similar interference. Hence, patients with diabetes should be first diagnosed for presence of any hemoglobin variants and the most reliable method for assessment of glycemic status should be further evaluated besides continuous blood glucose monitoring.

Limitations of the study

A more elaborate multi centric study is further required for proper translation of study results in the general population. Furthermore, further study should be conducted in a larger number of diabetic population with Hb variants to find out the effect of Hb variants in HbA1C measurement precisely.

CONCLUSION

- Among the overall study subjects, 123 out of 449 individuals (27.4%) were found to have some kind of hemoglobin variants which area sizeable number. Among the variants, Hb E carrier is 19.15% and Hb E disease is 6.45%
- Within the diabetic patients (100 numbers), 21numbers (21%) of patients were associated with some kind of hemoglobin variants. Hence, a significant number of patients suffering from Type 2 DM are associated with hemoglobin variants in this population
- HbA1c values measured by HPLC method and immunoturbidimetric method in the overall study population revealed significant difference
- Hence, all the patients including diabetes patients should be carefully evaluated, whether they have associated hemoglobin variants. If they have variants, they should be properly monitored by adequate methods of HbA1c measurement and blood glucose levels. They should be given more attention to prevent any untoward complications.

ACKNOWLEDGMENT

The Authors like to thank Department of Biochemistry, NBMC, Darjeeling for their hole hearted support for the study.

REFERENCES

1. Goswami BK, Pramanik R, Chakrabarty S, Pal PP, Banerjee S and Bandyopadhyay A. Spectrum of hemoglobin variants in the population of Northern region of West Bengal: An ethno genetic proposition. J Family Med Prim Care. 2014;3(3):219-223.

https://doi.org/10.4103/2249-4863.141614

2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843.
   https://doi.org/10.1016/j.diabres.2019.107843
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2013;36(1):S67-S74.
   https://doi.org/10.2337/dc13-s067
4. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37(1):S81-S90.
   https://doi.org/10.2337/dc14-s081
5. Radin MS. Pitfalls in hemoglobin A1c measurement: When results may be misleading. J Gen Intern Med. 2014;29(2):388-394.
   https://doi.org/10.1007/s11606-013-2595-x
6. Dam AR, Ahuja AJ, Singh M and Singh R. Limitation of HPLC methodology for HBA1c estimation. Indian J Pathol Microbiol. 2013;56(4):483-484.
   https://doi.org/10.4103/0377-4929.125413
7. Kutlar A, Kutlar F and Wilson JB. Quantitative of hemoglobin components by high-performance cation-exchange liquid chromatography: Its use in diagnosis and in the assessment of cellular distribution of hemoglobin variants. Am J Hematol. 1984;17(1):39-53.
   https://doi.org/10.1002/ajh.2830170106
8. Metus P, Ruzzante N, Bonvicini P, Meneghetti M, Zaninotto M and Plebani M. Immunoturbidimetric assay of glycated hemoglobin. J Clin Lab Anal. 1999;13(1):5-8.
   https://doi.org/10.1002/(sici)1098-2825(1999)13:1<5:aid-jcla2>3.0.co;2-s
9. Davis JE, McDonald JM and Jarett L. A high-performance liquid chromatography method for hemoglobin A1c. Diabetes. 1978;27(2):102-107.
   https://doi.org/10.2337/diabetes.27.2.102
10. Lorenzo-Medina M, De-La-Iglesia S, Ropero P, Nogueira-Salgueiro P and Santana-Benitez J. Effects of hemoglobin variants on hemoglobin A1c values measured using a high-performance liquid chromatography method. J Diabetes Sci Technol. 2014;8(6):1168-1176.
   https://doi.org/10.1177/1932296814538774

Authors Contribution:
SR, UKB, IN-Concept and Design of the study, prepared first draft of manuscript; **UKB, IN**- Interpretation of results, literature review; **IN, SD**-Coordination, review of literature, final manuscript preparation, **UKB, AK**-Statistical analysis and interpretation, final revision of manuscript.

Work attributed to:
North Bengal Medical College & Hospital, Darjeeling, West Bengal, India.

Orcid ID:
Dr. Soutrik Roy - https://orcid.org/0000-0001-9437-4414
Dr. IndrajitNath - https://orcid.org/0000-0002-6807-6736
Dr. SayantanDasguptav - https://orcid.org/0000-0002-8483-7072
Prof. Dr. Arun Kumar - https://orcid.org/0000-0002-8800-0296
Prof. Dr. Utpal Kumar Biswas - https://orcid.org/0000-0002-4714-0065

Source of Support: Nil, **Conflict of Interest:** None declared.