# Variation of HbA1c with Hemoglobin level: A comparative study between High Performance Liquid Chromatography and Immunochromatography Analyzer

**Arabinda Mohan Bhattarai[1], Sulochana Parajuli[2], Salina Pradhananga[1]**

[1]Department of Biochemistry, Nepalese Army Institute of Health Sciences, Bhandarkhal, Syanobharyang, Kathmandu, Nepal

[2]Department of Microbiology, Manipal College of Medical Sciences, Pokhara, Nepal

**Corresponding Author**

Arabinda Mohan Bhattarai,
Assistant Professor,
Department of Biochemistry,
Nepalese Army Institute of Health Sciences (NAIHS),
Kathmandu, Nepal.
Email:
arabinda.bhattarai@gmail.com

**Keywords**

Anemia; Glycated hemoglobin; Hemoglobin variants

**Online Access**

**DOI:** 10.3126/mjsbh.v21i2.41823

*Received Date: 27 Dec, 2021*
*Accepted Date: 01 Dec, 2022*

## Abstract

**Introduction:** The measurement of glycated hemoglobin (HbA1c) is an established procedure in evaluating long-term glycemic control in diabetic patients. There are many factors like temperature, pH, lifespan of protein, substrate concentrations and hemoglobin levels that influence HbA1c estimation by any methods. The aim of the study is to estimate HbA1c by High Performance Liquid Chromatography (HPLC) and immunochromatography analyzer and find the influence of hemoglobin in HbA1c by both the methods.

**Methods:** This was a descriptive, cross-sectional study carried out in the department of Biochemistry of Shree Birendra Hospital, Chhauni, Kathmandu over a period of two months from May 2021 to June 2021. We selected patients with type 2 diabetes mellitus. HbA1c was measured in EDTA blood samples by Bio-Rad D10 (HPLC method) and Nycocard (Immunochromatography method) whereas hemoglobin was estimated using Horiba Penta XLR fully automated hematology analyzer.

**Results:** In our study of 100 known cases with type 2 diabetes mellitus, the mean hemoglobin level was 14 ± 2.09 g/dL. The mean HbA1c value obtained by Nycocard and HPLC analyzer were 7 ± 1.95 % and 7.39 ± 2.21% respectively. We found that there was a strong correlation of HbA1c values with hemoglobin levels in HPLC method p = 0.001, r = 0.6. However, we found no such correlation by Nycocard method p = 0.6, r = 0.08.

**Conclusion:** There was a positive correlation of hemoglobin with HbA1c values obtained by HPLC method however no such variation was seen with immunochromatography method. This suggests that estimating HbA1c by HPLC methods may show decreased HbA1c with decreased hemoglobin levels and increased HbA1c with increased hemoglobin levels.



## INTRODUCTION

Glycated hemoglobin (HbA1c) is the hemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chain.[1] It is formed by slow and irreversible, non-enzymatic addition of a sugar residue to the hemoglobin.[2] The main objective of our study is to find the influence of hemoglobin in estimation of HbA1c by HPLC and Immunochromatography methods.

The erythrocytes are freely permeable to glucose and the rate of production of HbA1c is directly proportional to the ambient glucose concentration, the mean concentration of glucose in the blood and the lifespan of erythrocytes which is about 120 days.[3] The HbA1c concentration gives an integrated value for glucose with wide diurnal variations over the preceding 2–3 months.[4]

Many factors are implicated in the rate of ketoamine formations which are physiological like temperature, pH, lifetime of proteins, substrate concentrations as well as the reactivity of amino groups.[5] When the changes in

HbA1c occurs by 1%, it may lead to changes of 1.4 - 1.9 mmol / L in average blood glucose concentration.[6] The medical factors like dyslipidemia, malignancy, pregnancy, liver cirrhosis and hematological factors like hemolytic anemia, iron deficiency anemia, presence of carbamylated hemoglobin in uremia, etc. may affect HbA1c levels.[7]

## METHODS

This was a descriptive, cross-sectional study done in the Department of Biochemistry of Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. This is a tertiary care referral hospital run by Nepali Army for the army personnel and their families. The study was initiated after taking approval from Institutional Research Committee (IRC/387) of Nepalese Army Institute of Health Sciences. The patients of both sexes with type 2 diabetes mellitus were included in the study. After obtaining the informed consent, blood samples of patients were collected in EDTA tubes. Known cases of type 2 diabetes were included in the study while cases with secondary diabetes like thyrotoxicosis, Cushing's, exogenous steroid use and cases with hematological disorders like thalassemia and hemolytic anemia were excluded from the study. We measured HbA1c levels using both HPLC (Bio-Rad D10) analyzer and immunochromatographic (Nycocard) analyzer.
Hemoglobin was estimated in the Horiba Penta XLR fully automated hematology analyzer. The sample size was calculated with a 95% confidence level and 10% error of margin taking the expected prevalence of type 2 diabetes in Nepal as 9.2%. [8] The formula used is n = Z2*p(1-p)/(e)2, where Z is level of confidence, p is expected prevalence and e is precision (5%). The statistical level of significance (p) is < 0.05.

Microsoft Excel 2010 was used to analyze the data. Linear regressive analysis with correlation coefficient was used to compare HbA1C with Hemoglobin levels and a p-value <0.05 was considered statistically significant.

## RESULTS

Total 100 patients with type 2 diabetes mellitus were included in the study. The mean hemoglobin level was found to be 14 ± 2.09 g/dL. The mean HbA1c values obtained from Nycocard and HPLC analyzer were 7 ± 1.95 % and 7.39 ± 2.21% respectively (Table 1). There was a strong correlation of HbA1c values with hemoglobin levels in HPLC methods p=0.001, r=0.6, however, there was no such correlation in Nycocard method p=0.6, r=0.08 (Figure1and 2).

Fig. 1 Linear regressive analysis of Hemoglobin with HbA1c measured by Nycocard analyzer

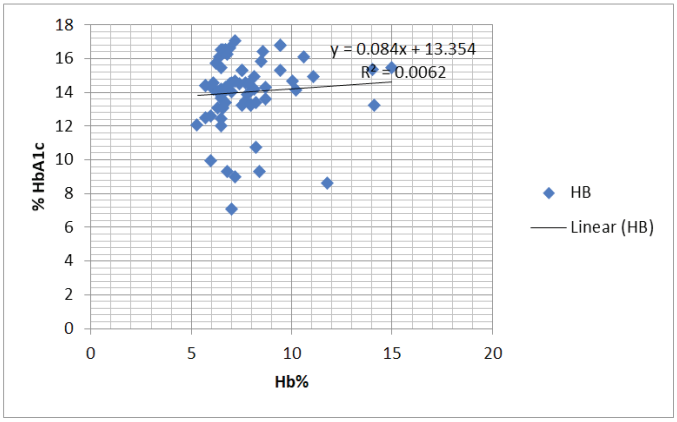


Fig. 2 Linear regressive analysis of Hemoglobin with HbA1c measured by Bio-Rad D10 analyzer

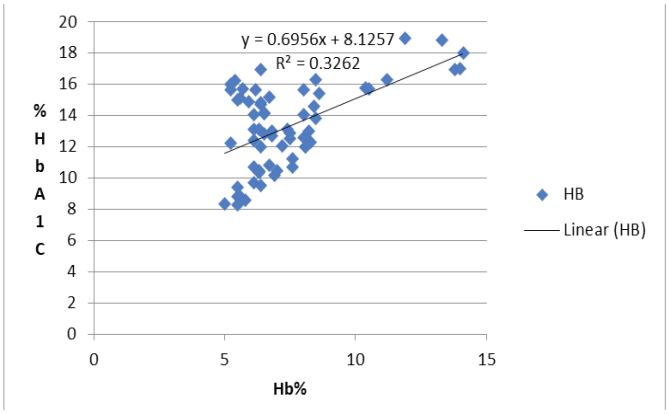


Table 1. HbA1c results obtained by HPLC and Nycocard analyzer

| | Hemoglobin Levels (g / dL) | %HbA1c (Mean ± SD) | p value | r value |
|---|---|---|---|---|
| Nycocard | 2.09 ± 14 | 1.95 ± 7.7 | 0.6 | 0.08 |
| HPLC Biorad D10 | 2.09 ± 14 | 2.21 ± 7.39 | 0.001 | 0.6 |

## DISCUSSION

In our study, we found a strong correlation of hemoglobin levels with HbA1c results obtained from the HPLC method (p = 0.001, r = 0.6), however, there was no such correlation in Nycocard method. Iron deficiency anemia is a major public health problem in developing countries like Nepal. Therefore, the estimation of HbA1c by Nycocard method has the least interference from Hb levels. The estimation of HbA1c by HPLC method is positively influenced by hemoglobin levels which must be taken into consideration in cases of iron deficiency anemia. Any condition that shortens erythrocyte survival or decreases

mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia) will falsely lower HbA1c results regardless of the assay method used.[9] The concentration of glycated hemoglobin has been reported to be increased in anemic patients because of increased production of malondialdehye and fructosamine which can be reversed by iron treatment.[9]

HbA1c as a marker of long-term glycemic control must be interpreted with caution, especially in patients with anemia, increased red cell turnover, and transfusion requirements. A study done by Roberts et al[10] who compared HbA1c results from blood samples collected in individuals with hemoglobinopathies, found differences in results in samples with hemoglobin-C trait. Similarly, study done by Diabetes Control and Complications Trial Research Group (DCCT) have found that the HPLC method can misidentify hemoglobin variants that have lost positive charges and carbamylated hemoglobin-S as HbA1c.[11]

A retrospective study done by Rekha et al[12] in Nepal has shown that sickle cell anemia is predominant in Tharu ethnic group residing in western Nepal and β-thalassemia is predominant among other ethnic groups of Nepal. In a study done by Marchand et al in Dang district of Nepal there was a high prevalence (9.3%) of sickle cell disease[13].

We conclude from our study that estimation of HbA1c by HPLC may be influenced by hemoglobin variants, but other methods such as Nycocard may help to measure the HbA1c levels accurately but do not allow the identification of hemoglobin variants.[14] The limitation of our study are small sample size and based on participants from a single center. It would be desirable to conduct larger, multi centric study to find out the exact influence of hemoglobin and its variants in HbA1c estimation.

## CONCLUSIONS

The HbA1c estimation with HPLC method may show decrease in HbA1c by positively correlating with decreased hemoglobin levels. However, alternative self-monitoring of blood glucose levels, glycated serum albumin and serum fructosamine must be considered in such cases to find the exact HbA1c values.

## REFERENCES

1. Nasir NM, Thevarajah M, Yean CY. Hemoglobin variants detected by hemoglobin A1c (HbA1c) analysis and the effects on HbA1c measurements. Int J Diabetes Dev Ctries. 2010;30(2):86-90. DOI: 10.4103/0973-3930.62598.
2. Behan KJ, Storey NM, Lee HK. Reporting variant hemoglobins discovered during hemoglobin A1c analysis - common practices in clinical laboratories. Clin Chim Acta. 2009; 406(1-2):124-8. DOI:10.1016/j.cca.2009.06.012.
3. John WG. Glycated haemoglobin analysis. Ann Clin Biochem. 1997;34 ( Pt 1):17-31. DOI: 10.1177/000456329703400105.
4. Sacks DB. Hemoglobin variants and hemoglobin A1c analysis: problem solved? Clin Chem. 2003;49(8):1245-7. DOI: 10.1373/49.8.1245.
5. Shapiro R, McManus MJ, Zalut C, Bunn HF. Sites of nonenzymatic glycosylation of human hemoglobin A. J Biol Chem. 1980;255(7):3120-7. DOI:10.1016/0026-0495(79)90050-7
6. Gillery P, Hue G, Bordas-Fonfrede M, Chapelle JP, Drouin P, Levy-Marchal C, et al. Hemoglobin A1C determination and hemoglobinopathies: problems and strategies. Ann Biol Clin (Paris). 2000;58(4):425-9. PMID: 10932042.
7. Shrestha N, Mishra SR, Ghimire S, Gyawali B, Mehata S. Burden of diabetes and prediabetes in Nepal: A systematic review and meta-analysis. Diabetes Therapy. 2020 Jul 25:1-2. DOI: 10.1007/s13300-020-00884-0.
8. Sundaram RC, Selvaraj N, Vijayan G, Bobby Z, Hamide A, Rattina Dasse N. Increased plasma malondialdehyde and fructosamine in iron deficiency anemia: effect of treatment. Biomed Pharmacother. 2007 Dec;61(10):682-5. DOI: 10.3748/wjg.v13.i5.796
9. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM: American Diabetes Association Technical Review on Tests of Glycemia. Diabetes Care 1995; 18:896-909. DOI: 10.2337/diacare.27.7.1761
10. Roberts WL, Frank EL, Moulton L, Papadea C, Noffsinger JK, Ou CN. Effects of nine hemoglobin variants on five glycohemoglobin methods. Clin Chem. 2000;46(4):569-72. PMID: 10759485.
11. Diabetes Control and Complications Trial Research Group; Nathan DM, Genuth S, Lachin J, Cleary P, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977-86. DOI: 10.1056/NEJM199309303291401.
12. Shrestha RM, Pandit R, Yadav UK, Das R, Yadav BK, Upreti HC. Distribution of Hemoglobinopathy in Nepalese Population. J Nepal Health Res Counc. 2020;18(1):52-8. DOI: https://doi.org/10.33314/jnhrc.v18i1.2303.
13. Marchand M, Gill C, Malhotra AK, Bell C, Busto E, McKeown MD, et al. The Assessment and Sustainable Management of Sickle Cell Disease in the Indigenous Tharu Population of Nepal. Hemoglobin. 2017;41(4-6):278-82. DOI:10.1080/03630269.2017.1414058.
14. Jung CL, Kwon KJ, Hong KS, Sung YA, Lee ST, Ki CS, et al. Hemoglobin Yamagata: hemoglobin variant detected by HbA1c test. Korean J Lab Med. 2009;29(6):53640. DOI:10.3343/kjlm.2009.29.6.536.