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Original Article

Detection of Hemoglobinopathies by HPLC in a Referral Clinical Laboratory in Nepal

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ABSTRACT

Introduction: Various hemoglobin variants are prevalent in the Nepalese population owing to the ethnic diversity of our population. Detection of asymptomatic carriers by a reliable laboratory method is the cornerstone of prevention of this serious health problem. The simplicity of the automated system with internal sample preparation, superior resolution, rapid assay time, and accurate quantification of hemoglobin fractions makes ion-exchange high-performance liquid chromatography (IEX- HPLC) an ideal methodology for the routine screening for hemoglobinopathies. We report the clinical laboratory-based prevalence of variant hemoglobin and hemoglobinopathies through IEX- HPLC analysis in a cohort of patients referred for hemoglobin electrophoresis to a referral clinical laboratory in Nepal.

Materials and Methods: The variant hemoglobin and hemoglobinopathy were diagnosed based on percentage, retention time, and peak characteristics of variant hemoglobin in a chromatogram. Peripheral blood films, reticulocyte count, serum iron profile, and sickling test were done in selected cases along with detailed family history.

Results: Hemoglobinopathy was detected in all age group but the vast majority was detected between 20 to 40 years of age. Beta thalassemia trait was the most frequently detected hemoglobinopathy in all age groups.

Conclusions: The present study conducted using IEX-HPLC reflects the magnitude of thalassemia and hemoglobinopathies in a laboratory-based population which helps to increase awareness among both health caregivers and the general population. Routine screening for hemoglobinopathy of individuals at the reproductive age group is recommended and this screening can be done through IEX-HPLC.

Keywords: Hemoglobinopathies; HPLC; Nepal; Thalassemia

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INTRODUCTION

Ion exchange high-performance liquid chromatography (IEX-HPLC) for the detection of various fractions of hemoglobin depends upon the interchange of charged groups on the ion exchange material in chromatography column with the charged groups on the hemoglobin molecule. Various hemoglobin variants can be screened using a single, highly excellent method of HPLC. HPLC is now usually used as the first-line method to diagnose hemoglobin disorders.[1] The separated hemoglobin in the HPLC system that measures $HbA1_c$, pass through photometer where changes in absorbance are measured and HbA1c is calculated

using an algorithm. When $HbA1_c$ is measured by HPLC using standard mode (including separation of individual hemoglobin fragments via ion exchange in a column of chromatography followed by its photometric quantification and calculation), variant hemoglobin can be detected if present, along with false HbA1c result in most of the cases.[2] This false estimation is due to the co-elution of hemoglobin variant with hemoglobin fraction other than HbA1c. Hemoglobin variants thus interfere with the quantification of $HbA1_c$, HbA_2, and HbF, thus generate abnormal chromatograms. Many cases with variant hemoglobin are asymptomatic and often remain undetected throughout life. Now- a- days, an increasing number of people are being detected to have hemoglobin variants especially when their blood is tested for $HbA1_c$ by the HPLC method.

Various previous studies have demonstrated HPLC to be equivalent or superior over electrophoretic methods for screening hemoglobin variants and hemoglobinopathies.[3-5] Hemoglobinopathies are an inherited problem and its timely diagnosis by population screening is needed to prevent the birth of children with clinically significant findings. Nepalese population harbors a significant number of beta-thalassemia cases.[6] The prevalence of various hemoglobinopathies in Nepal has been reported as 26.8 % for thalassemia trait and 21.6% for sickle cell disease.[6] The aim of the present study is to report the distribution of hemoglobinopathies according to age in the samples sent for variant hemoglobin detection and hemoglobin quantification by HPLC in a referral clinical laboratory.

MATERIALS AND METHODS

An observational study was conducted in a clinical laboratory of Samyak Diagnostic Pvt. Ltd. Kathmandu, from laboratory information of 1090 cases that were ordered for hemoglobin quantification and variant detection by HPLC, during the period of 56 months from April 2014 to December 2018. Bio-Rad D-10 HPLC system (Variant II Beta Thalassemia Short Program, Bio-Rad Laboratories Inc., Hercules, CA, USA) was used for the percent determination of hemoglobin A_2, F and $A1_c$ and detection of abnormal hemoglobin under the conditions specified by the manufacturer.[7] Reports and chromatograms generated by this HPLC system were studied and interpreted by observing minor component of adult hemoglobin ($A1_a$, $A1_b$), fetal hemoglobin (Hb F), a labile component of glycosylated adult hemoglobin ($LA1_c$/CHb 1), carbamylated adult hemoglobin (LA1c/ CHb 2), glycosylated adult hemoglobin ($HbA1_c$), non-glycosylated adult hemoglobin (A0), peak on HPLC (P3), S-window and hemoglobin A_2(A2). Red blood cell indices were measured on Sysmex automated hematology analyzer XN 330 (Sysmex, Milton Keynes, UK). The variant hemoglobin was identified based on their percentage, retention time, and peak characteristics. Peripheral blood films with special stains, reticulocyte count, serum iron profile, and sickling test were done in selected cases. Detailed family history was taken in the required cases. The cases diagnosed with alpha thalassemia were advised for and confirmed by a molecular diagnosis of goblin gene mutation.

Data was entered in the excel sheet and calculation was done to find out the laboratory prevalence of specific hemoglobin disorder. Ethical approval for this study was taken from the Nepal health research council (Reference number- 2033).

RESULTS

Male to female ratio was 1:1.9 in the total studied population of 1090. 123 (11.3%) different types of hemoglobinopathies and hemoglobin variants were detected on the basis of retention time, percentage of hemoglobin, and characteristics of the peak observed in the chromatogram. The normal and abnormal chromatogram is shown in figure 1A-D.

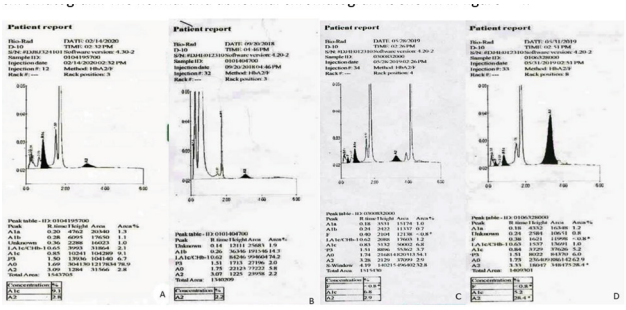

Figure 1: A) Normal Chromatogram, B) Beta-thalassemia major, C) Sickle Cell heterozygous and D)Hb-E heterozygous

The age distribution was from 6 months to 77 years with slightly female predominance of 1:1.2 (55 male and 68 female). Age wise prevalence of various haemoglobinopathies in this study is shown in table 1.

Table 1: Prevalence of hemoglobinopathies in various age groups

Diagnosis	Age Group (Years) Number of Cases														Total Cases (%)
	0-10		11-20		21-30		31-40		41-50		51-60		>60		
	19		10		30		37		19		6		7		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Beta-Thalassemia minor/Trait	5	1	3	1	6	11	15	18	2	12	1	4	2	4	85 (69.1%)
Hb E Heterozygous	1	1			1				2		1				6 (4.9%)
Compound heterozygous for Hb S and Beta-Thalassemia minor		1	1		2				1						5 (4.1%)
Hereditary Persistence of Fetal Hemoglobin Trait	1														1 (0.8%)
Beta-Thalassemia homozygous	1		1	1											3 (2.4%)

Table 1: Prevalence of hemoglobinopathies in various age groups (contd.)

Diagnosis	Age Group (Years) Number of Cases														Total Cases (%)
	0-10		11-20		21-30		31-40		41-50		51-60		>60		
	19		10		30		37		19		6		7		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Compound heterozygous of Hb E and Beta-Thalassemia Intermediate		1													1 (0.8%)
Alpha Thalassemia	1	4			1	1									7 (5.8%)
Hb H		1		1											2 (1.6%)
Beta-Thalassemia major	1														1 (0.8%)
Sickle cell homozygous with Beta-Thalassemia			1												1 (0.8%)
Sickle cell heterozygous			1			1								1	3 (2.4%)
Hb E Homozygous						1	2	1		2					6 (4.9%)
Sickle cell homozygous						1									1 (0.8%)
Hb D Punjab Heterozygous							1								1 (0.8%)
Total cases	19		10		25		37		19		6		7		123 (100%)

Abbreviation: M= Male F= Female

DISCUSSION

Hemoglobinopathy is a major health burden described in various parts of Nepal. This study provides only a glimpse of the institutional prevalence of hemoglobin disorder in various age groups, which is studied in a single centre with the use of the HPLC technique. Over a period of 56 months of observational study, the laboratory-based prevalence of hemoglobinopathy was 11.3%. β- thalassemia trait was the commonest variant found in our study, the same is reported earlier.[3,8] Alpha thalassemia with a prevalence of 5.8% was the second most common hemoglobinopathy in our study. Hb E hemoglobinopathy was found as the most common hemoglobin variant with equal homozygous and heterozygous forms. Findings from one of the recent population-based studies involving the maximum number of districts in Nepal suggest that sickling disorder was the most common hemoglobinopathy followed by β-thalassemia.[9] The higher prevalence of β- thalassemia trait in our laboratory-based study as well as the population-based study may be due to increased rate of patient and population screening for presence of hemoglobin variants and hemoglobinopathy.

The more number of female cases in our study is due to the higher number of female patients referred at our centre since we receive a biological specimen from various infertility clinics and antenatal check-up clinics in Kathmandu. The finding of our study is important for patients at their reproductive age. Antenatal screening of variant hemoglobin and hemoglobinopathies through IEX-HPLC helps in the identification of carriers. Thus, our finding advocates the importance of testing for hemoglobin abnormality of the partner if any hemoglobin abnormality is found in females in antenatal checkups.

There are other ways for hemoglobin analysis. Alkaline cellulose acetate and citrate agar electrophoresis are the most widely used methods for hemoglobin electrophoresis with limitation of poor resolution. Isoelectric focusing provides excellent resolution but is labor-intensive. The simplicity of sample preparation, superior resolution of the method, accurate quantitation of hemoglobin concentration, and complete automation makes this IEX-HPLC the ideal methodology for routine screening of hemoglobin disorders in a clinical laboratory.[4]

The algorithmic approach in the clinical laboratory through detailed family history, complete blood count with red blood cell morphology, serum iron chemistry, a sickling test, reticulocyte count, test for Hb-H inclusion, and variant analysis through IEX-HPLC helps in the screening of various thalassemia syndrome and variant hemoglobin. Molecular studies may be required in cases of alpha thalassemia.

CONCLUSIONS

This is a report on the use of HPLC in determining the presence of hemoglobin variants and hemoglobinopathies in a large number of individuals in the clinical laboratory Nepal. Hemoglobin fraction analysis by ion-exchange in HPLC has the advantage of quantifying HbF and HbA2 along with hemoglobin variant screening in a single reproducible system making it an excellent tool to screen for hemoglobin variants and hemoglobinopathy.

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