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Impact of P2 (Hb A1C) and P3 window in normal HPLC

S.Pandey[1]*, R.Saxena[1], R.M.Mishra[2], U.K.Chauhan[3], M.Sharma[4], Sw.Pandey[3]

[1]Deptt.of Hematology, All India Institute of medical Sciences, Ansari Nagar, New Delhi-110029, (INDIA)

[2]Dept of Environmental Biology, APS University, Rewa-486003, (INDIA)

[3]Centre for biotechnology Studies, APS University, Rewa -486003, (INDIA)

E-mail : pandeysanjaybt@rediffmail.com

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ABSTRACT

High performance liquid chromatography is an important tool for detection of Hb variants and its role has been established in prenatal diagnosis and carrier screening. We had evaluated fifteen suspected cases with normal HPLC. Patients were presenting significant clinical symptom. This study revealed few Hb variant may clinically associated with normal HPLC and produce significant pathophsiology when co inherited. So care should be taken by clinician and never ignore the unidentified peaks in HPLC.

KEYWORDS

HPLC;
Hemoglobinopathies;
Hemoglobin variants.

INTRODUCTION

Detection of various unidentified Hb variants is specified process of HPLC. These variants may be caused by mutations in globin genes. Most have no clinical impact, but a few may show altered oxygen affinity or be chemically unstable. Most unidentified Hb variants have no significant genetic implications, but a few may co-inherited with other significant hemoglobin variant and clinically significant[1]. However hemoglobin variant can be identified by electrophoretic mobility but definite identification can be achieved only by DNA analysis or amino acid sequencing[2-4]. At present time, there is limited reference laboratory capacity in India, such that the majority of unidentified hemoglobin variants identified by screening can not be definitively identified.

MATERIALS & METHODS

These investigations had been done in the department of hematology AIIMS New Delhi where patient attending the laboratory between 2 year for routine check-up. Four ml blood was taken from the patients in EDTA vials. Complete blood count and red cell indices were measured on automated analyzer (SYSMEX K-4500, Kobe Japan) Giemsa-stained peripheral blood smear were examined for red cell morphology. Quantitative assessment of hemoglobin HbF, HbA, and HbA2, were performed by HPLC (Bio-Rad-VariantTM Bio Rad, CA, USA). Mean values, standard deviation & frequency distribution was used to evaluate the hematological & clinical data.

RESULTS & DISCUSSION

Patients were sent in the laboratory with different clinical complication like jaundice, fever, pain & anemia and suspected to hemoglobinopathies. Total 15 Patients (8 male and 7 female) were identified symptomatic with normal HPLC in hemoglobin variant p2 &

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p3 windows. The mean age was 20.53±12.05.Weakness was present in 40% followed by mild fever (26.6%), anemia (20%) and jaundice (13.3 %). None of the patents were taking blood transfusion and 26.6% were asymptomatic. The patient's peripheral smears showed microcytic hypochromic red cells with few target cells. A mild degree of anisopoikilocytosis was noticed. Hematological features included HbF and HbA2 in normal range; however mean Hb (9.5 ± 2.57) serum iron (43.49± 9.57) and red cell indices were low in all patients. Hematological and Clinical features are summarized in TABLE 1 and Figure 1 respectively. Hb variants with retention times in the p2 window is 1.24 – 1.40 min. Hb A1C eluted in the P2 window. The only hemoglobin variant found to elute in this window was Hb Hope, which had a mean (SD) %Hb [45.9 (2.2)%] much greater than would be expected for Hb A1C. A hemoglobin variant with retention times in the p3 window is 1.40 –1.90 min. P3 window elution peaks had nine hemoglobin variants (four alpha and five beta variants). Hb Camden (d = 0.10 min from Hb Hope) and Hb J Oxford (d = 0.11 min from Hb Camden) can be differentiated and identified on their retention times. Retention times could not be differentiated Hb Austin, Hb N-Baltimore, and Hb Fukuyama from each other. Six hemoglobin variants with retention times of 1.68–1.78 min could be divided into two groups (group I, Hb Austin, Hb N-Baltimore, and Hb Fukuyama; group II, Hb Fannin-Lubbock, Hb J-Anatolia, and Hb J-Mexico).Retention time is not only sufficient to distinguish the two groups, the groups could be distinguished by the %Hb. Groups can be differentiated by electrophoresis. Hb Manitoba and Hb Montgomery both are α -globin variants can be differentiated by electrophoresis which have statistically identical retention times, %Hb values, and peak characteristics[5].

However all the Hb variant associated in these windows associated clinically complexity but further characterization is needed.

TABLE 1 : Hematological profile of patients

Hematological features	Mean± (SD)
HbA0 %	83.26(± 1.77)
HbA2 %	3.94± (1.0)
HbF %	0.28± (0.35)
HbC %	0.44±(0.21)
P2 %	5.24± (2.74)
P3 %	4.30± (0.35)
WBC ths/ µl	8.65 ± (2.2)
RBC millions/µl	4.40 ± (1.30)
HGB g/dl	9.5 ± (2.57)
HCT %	31.86 ± (7.37)
MCV fl	74.63± (13.61)
MCH pg	22 .98± (5.58)
MCHC g/dl	30.46± (1.74)
PLT ths/ µl	297.46± (129.83)
S.iron µg/dl	43.49± (9.57)

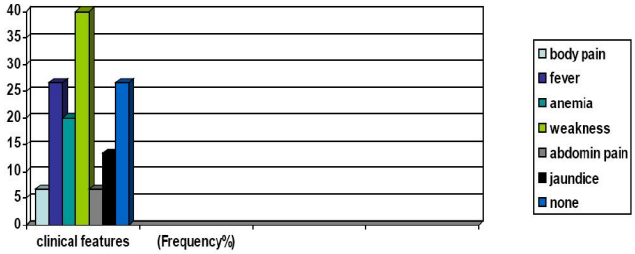

Figure 1 : Clinical profile of patients

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