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Original Research Article

Prevalence of Hemoglobinopathies in Antenatal Screening by HPLC

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ABSTRACT

We studied a total of 234 married, pregnant females coming for routine antenatal screening for presence or absence of Hemoglobinopathies over a period of one year in a tertiary level hospital. The prevalence was found to be 5.1% (12 cases). Out of these 7(2.9%) females had beta thalassemia trait and 5(2.1%) were having variant hemoglobin of which 3(1.28%) females were Hb E Heterozygous, 1(0.4%) was Hb D Punjab and 1(0.4%) was Hb Q India. Such screening for Hemoglobinopathies should be conducted as they prove to be important for the health of the mother and the child as well as spreading awareness, further screening of family members (spouses, siblings) to prevent birth of homozygous babies.

Key Words – Hemoglobinopathies, HPLC, Antenatal screening

INTRODUCTION

Hemoglobinopathy is a genetic defect that results in abnormal structure of one of the globin chains of the hemoglobin molecule. Although "pathy" means disease most of the hemoglobinopathies are not clinically apparent and very few produce serious disease. High-performance liquid chromatography is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. HPLC is a more sensitive, specific and rapid method than Hb Electrophoresis. Hemoglobin variants are mutant forms of hemoglobin caused by variations in genetics. The thalassemias and the hemoglobinopathies (the most common being sickle cell disorders) are autosomal recessive conditions affecting the quantity and quality, respectively, of hemoglobin molecules within red blood cells. These disorders are found more commonly in certain ethnic groups, lending themselves to effective ethnicity-based population screening. [2-3]

MATERIALS & METHODS

Blood samples from antenatal cases were collected in dipotassium EDTA anticoagulant vacutainers. All the specimens were analyzed on Biorad Variant HPLC (High Performance Liquid Chromatography) system. The samples were mixed by the variant II sampling station, diluted with hemolysis reagent added to each vial and injected into an assay specific analytic cartridge. The variants II dual pumps deliver a programmed buffer gradients of increasing ionic strength to the cartridge, where the hemoglobin is separated. The variant beta thalassemia short program utilizes the principles of

cation exchange HPLC. The absorbance is measured at 415 nm with secondary wavelength at 690nm and chromatogram sample report is generated.

RESULT

The prevalence of hemoglobinopathies in the cohort study was found to be 5.1%. Total number of antenatal cases studied were 234.Out of this 7(2.9%) cases were of β thalassemia trait and 5(2.1%) were having variant hemoglobin. 3(1.28%) cases were Hb E Heterozygous, 1(0.4%) was Hb D Punjab and 1(0.4%) was Hb Q India. 80% of the hemoglobin variants were found to be migrants from other states.

SR NO	HEMOGLOBIN VARIANTS	NUMBER HB VARIANTS	%
1	NORMAL	222	94.87
2	BETA THALASSEMIA TRAIT	7	2.99
3	Hb E HETEROZYGOUS	3	1.28
4	HB D PUNJAB	1	0.427
5	HB Q INDIA	1	0.427
	TOTAL	234	

Fig 1. DISTRIBUTION OF CASES

SR NO	HEMOGLOBIN VARIANTS	Hb	RBC	MCV	MCH	MCHC	HB A %	HB A2 %	HB F %	Other %
1	BETA THALASSEMIA TRAIT	10.4	5.05	61.8	20.64	33.4	93.9	4.8	1.3	-
2	Hb E HETEROZYGOUS	12.2	4.84	73.1	24.8	33.4	69.1	28.54	0.76	-
3	HB D PUNJAB	11.5	4.07	83.2	28	34.1	50.2	2.4	0.3	D-window 38.4 %
4	HB Q INDIA	11.8	3.99	86.8	30	34.2	81.9	1.4	0.6	16.10%

Fig 2. RBC Indices&hemoglobin fractions in variants.

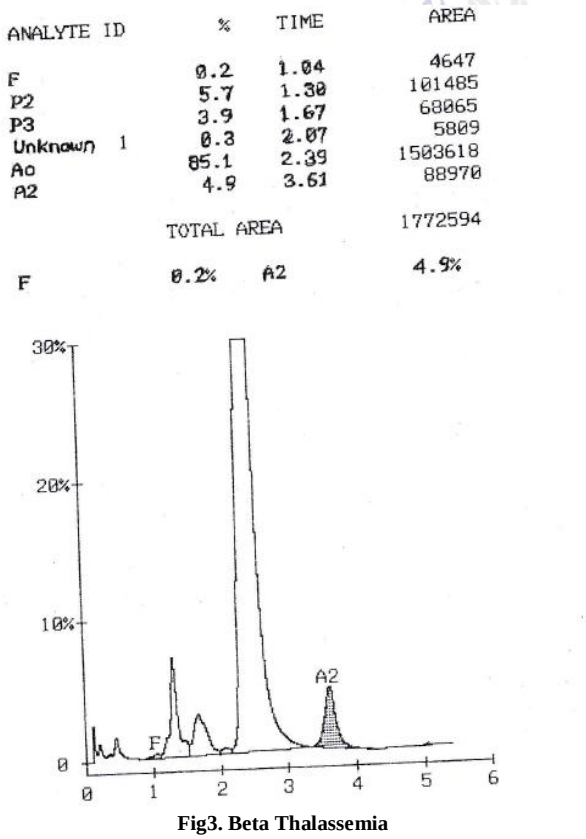

Fig3. Beta Thalassemia

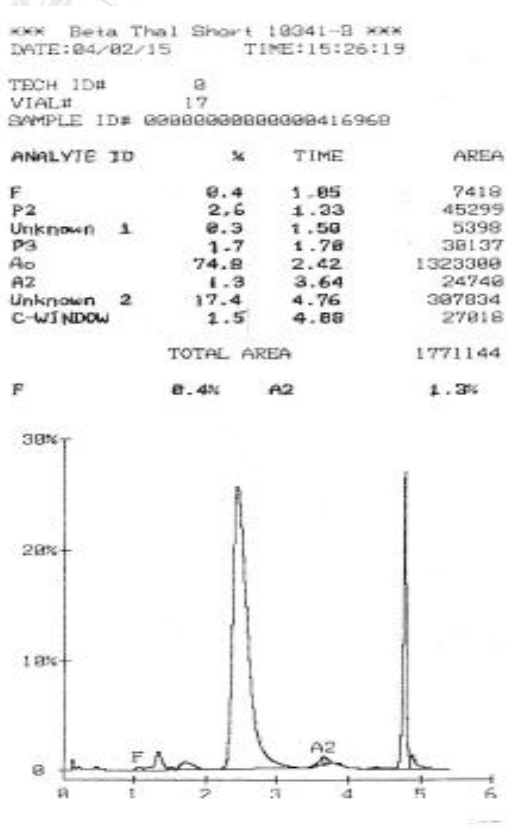

Fig 4. HbQ India

ANALYTE ID	%	TIME	AREA
F	0.3	1.11	4486
Unknown 1	0.7	1.26	11551
P2	2.7	1.36	42278
P3	3.1	1.75	49361
Unknown 2	2.4	2.11	37671
Ao	58.2	2.58	797414
A2	2.4	3.66	39048
D-WINDOW	30.4	4.09	609484
	TOTAL AREA		1591215
F	0.3%	A2	2.4%

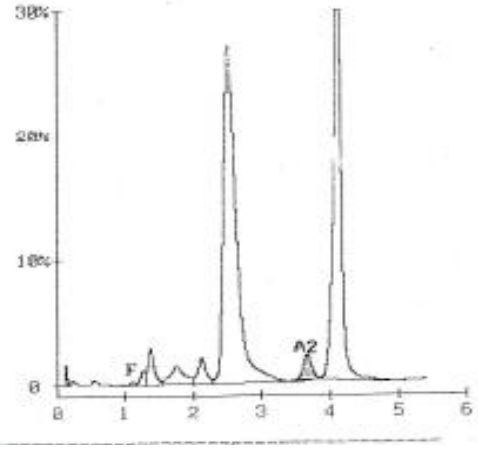

Fig 5. Hb D Punjab

ANALYTE ID	%	TIME	AREA
F	1.8	1.10	28737
P2	3.1	1.35	65076
P3	4.6	1.81	96972
Ao	62.8	2.44	1337853
A2	29.5	3.78	655543
	TOTAL AREA		2175381
F	1.8%	A2	29.5%

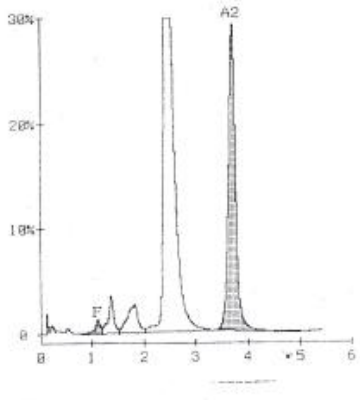

Fig 6. Hb E HETEROZYGOUS

DISCUSSION

Thalassemias are the result of genetic defects that limit the production of specific globin chains of the Hb molecule. Thalassemias are named by reference to the affected globin chain: α-thalassemia involves the α chain, β-thalassemia the β chain. The major adult Hb, HbA, consists of four globin chains (two alpha [α] and two beta [β] chains, represented as α2β2), each linked to a heme molecule. Other minor hemoglobins in adults include HbF (fetalhemoglobin, α2γ2) and HbA2 (α2δ2). Hemoglobin D is the 4th most common hemoglobin variant. It developed as a response to the selective pressures of malaria in the regions of Asia.Hb D differs structurally from normal Hemoglobin A at 121 position on beta chain, where glutamine replaces glutamic acid. [3] Hb D occurs in four forms: heterozygous Hb D trait, Hb D-thalassemia, Hb S-D disease and the rare homozygous Hb D disease, which is usually associated with mild hemolyticanemia and mild to moderate splenomegaly. Hemoglobin E or haemoglobin E (HbE) is an abnormal hemoglobin with a single point mutation in the β chain. At position 26 there is a change in the amino acid, from glutamic acid to lysine. [4] Heterozygous AE occurs when the gene for hemoglobin E is inherited from one parent and the gene for hemoglobin A from the other. This is called hemoglobin E trait, and it is not a disease. People who have hemoglobin E trait (heterozygous) are asymptomatic and their state does not usually result in health problems. They may have a low mean corpuscular volume (MCV) and very abnormal red blood cells (target cells). Its clinical relevance is exclusively due to the potential for transmitting E or β-thalassemia. [1-3]

CONCLUSION

The projected life span and quality of life of patients with hemoglobin disorders can be significantly improved. The high prevalence of carriers of structural hemoglobinopathies justifies the initiation of antenatal screening programs for hemoglobinopathies to prevent the emergence of homozygous cases of beta thalassemia major in the neonatal population. However, screening should also be promoted at pre-marital stage to avoid major hemoglobinopathies

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