



Study to Identify the Role of High Performance Liquid Chromatography Detecting Haemoglobinopathies in Ante-natal Patients in South Gujarat

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ABSTRACT

INTRODUCTION: Haemoglobinopathy is a major genetic public health problems in India, responsible for significant morbidity and mortality. Individuals with trait (carriers) are healthy and unaware of their carrier status unless specifically screened. If a couple carry a significant haemoglobinopathy trait there is a 1 in 4 chance with each pregnancy that their child will inherit a major haemoglobinopathy. The most effective approach to reduce the burden in the society is to reduce the incidence by implementation of a carrier screening programme. This study is done to know the prevalence of haemoglobinopathies and variant of haemoglobin using cation exchange HPLC.

AIMS AND OBJECTIVES:

- To identify the pregnant woman at risk of having homozygous or heterozygous child with a severe haemoglobinopathies in tertiary level hospital of south Gujarat.

MATERIALS AND METHODS:

- The present study was carried out in a tertiary care hospital for a period of 1 month. 100 Antenatal patients attending Obstetric-out patient department for routine check-up were included in the study.
- 2 ml EDTA venous blood from pregnant mothers after informed consent. This blood is subjected for complete haemogram, peripheral blood smear and HPLC using Biorad testing system. Descriptive analysis is done and data is presented in numbers and in %.

RESULT: Out of a total of 100 pregnant women 20% were having haemoglobinopathies, whereas 80% had normal haemoglobin. Out of 20 cases, maximum number of cases i.e. Sick cell trait cases i.e. 10 % followed by β -Thalassemia trait 7 % followed by Other haemoglobinopathies are also found i.e. sickle cell disease 2% HbE homozygous 1%.

CONCLUSION: This study shows a high prevalence of haemoglobinopathies in antenatal mothers necessitating an appropriate screening strategy for antenatal mothers. We should conclude that HPLC is a sensitive technique for studying haemoglobinopathies during pregnancy.

Key Words: Antenatal mothers, HPLC, Haemoglobinopathies.



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INTRODUCTION

- ❖ Hemoglobinopathy-Major public health problem in India.
- ❖ Responsible for significant morbidity and mortality.
- ❖ Trait (carriers) - healthy and unaware of their carrier status unless specifically screened.
- ❖ In India, many studies have shown that antenatal screening followed by prenatal diagnosis is the most feasible for the prevention of birth of homozygous children [1, 2].
- ❖ In this study, we tried to find out the prevalence of hemoglobinopathies in the population in Surat, by detecting couples at risk and offer them genetic counselling and the option of prenatal diagnosis by antenatal screening for hemoglobinopathies using cation exchange HPLC.

The inherited disorders of haemoglobin (Hb) are divided into three groups:

Couple with hemoglobinopathy trait- there is a 1 in 4 chance with each pregnancy that their child will inherit a major hemoglobinopathy.

MATERIAL AND METHOD

- **Inclusion criteria**--Already registered pregnant mothers attending the antenatal clinic for routine antenatal check-up, irrespective of their gestational age, over a period of one month.
- A written informed consent was taken from the willing participants.
- A detailed history including all personal, socioeconomic and ethnic details, clinical history, obstetric and blood transfusion history was taken.
- Two ml of venous blood was collected in EDTA (ethylene diamine tetra acetic acid).

Complete blood count using automated 5 part Haematology analyzer (Beckman Coulter-DxH 800). A peripheral blood smear was prepared and stained with Geimsa's stain.

The blood samples were stored at 4°C and subjected to high performance liquid chromatography (HPLC) in batches within a week of collection. For this study, D-10 Haemoglobin Testing system (Bio-Rad Laboratories) was used to perform HPLC. It operates on the principle of HPLC and the column cation exchange cartridge.

RESULTS

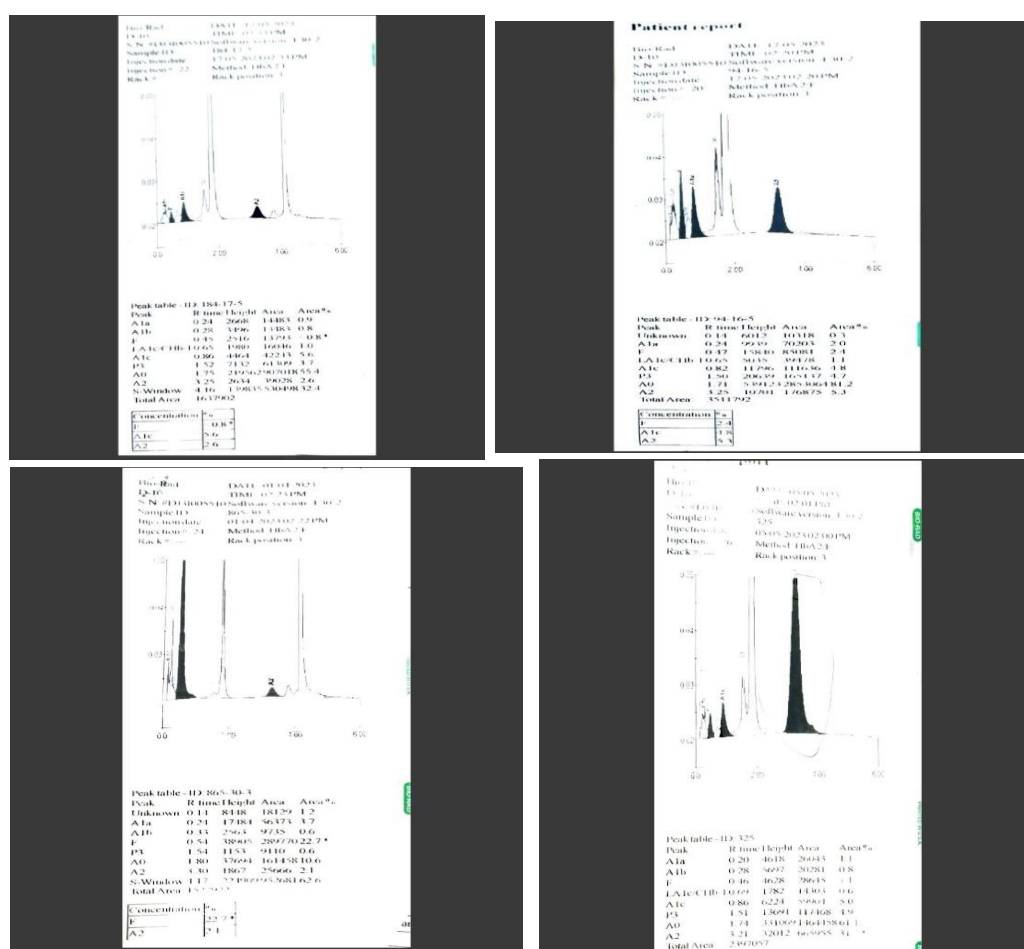


Table 1: Distribution of all case studied

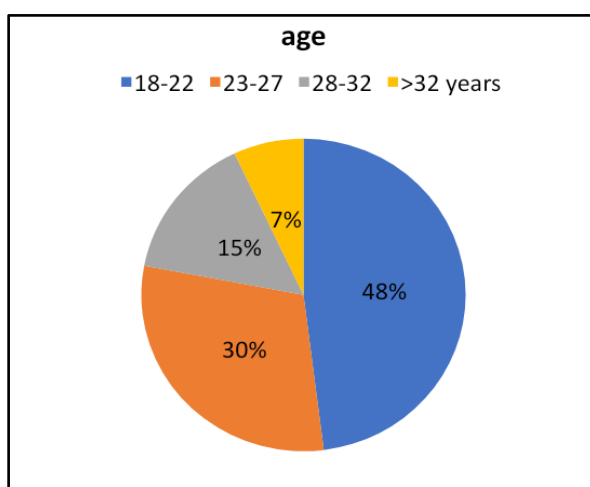
Case Distribution		No. of cases	Percentage
Normal case	NAD	80	80%
Affected case	Sickle cell trait	10	10%
	B-Thalassemia trait	7	7%
	Sickle cell disease	2	2%
	Hb E	1	1%
Total cases		100	100%

Table 2: Prevalence of hemoglobinopathy in different religious groups

Religion	No. Screened	B-Thalassemia trait	Sickle cell trait
Hindu	56	6	3
Muslim	23	0	1
Christians	21	1	6
Total	100	7	10

Table 3: HPLC interpretation in affected cases

Disease	Hb F	Hb A2	Abnormal Hb
Normal pregnant women	0.4±2.1	3.0± 0.5	0.7±0.1
Sickle cell trait	0.9±0.7	3.3±0.4	34.6±7.3
B-Thalassemia trait	0.3 ±0.5	5.4±0.4	0.6±0.1
Sickle cell disease	12.9	2.8	67.5
Hb E	1.1	31.7	0.7±0.1



DISCUSSION

CLASSIFICATION OF β THALASSEMIA			α -thalassemia		
CLASSIFICATION	GENOTYPE	CLINICAL SEVERITY	NO. OF GENES PRESENT	GENOTYPE	CLINICAL CLASSIFICATION
β thal minor/trait	β/β^+ , β/β^0	Silent	4 genes	$\alpha\alpha/\alpha\alpha$	Normal
			3 genes	$\alpha\alpha/-\alpha$	Silent carrier
β thal intermedia	β^+/β^+ , β^+/β^0	Moderate	2 genes	$-\alpha/-\alpha$ or $\alpha\alpha/-$	α thalassemia trait
β thal major	β^0/β^0	Severe	1 gene	$-\alpha/-$	Hb H Ds
			0 genes	$-/-$	Hb Barts / Hydrops fetalis

- The approaches to prevent the birth of a thalassemia major and sickle cell disease child include carrier screening and prenatal diagnosis. Antenatal screening is the important step to identify women having the risk of producing a child affected with hemoglobinopathy. Gujarat has many high-risk communities which need antenatal screening and prenatal diagnosis for hemoglobinopathies.
- The carrier rate of β -thalassemia varies from 1 to 17 % in India with an average of 3–4 %. The overall prevalence of BTT was 7 % among antenatal women in our study. As a majority of these tribes are in the Valsad and Dang regions, antenatal women from Surat showed only a 10 % SCT prevalence in our study.
- 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:

CONCLUSION

- In the our study, 20 cases out of 100 antenatal mothers with prevalence of 20% displayed abnormal haemoglobin fraction on HPLC.
- Using HPLC method in screening for Thalassemia and sickle cell disorder in pregnant women in the region support the nation of establishing a preventive and control program of this common genetic disorder.
- Detection of carrier status during pregnancy along with couple screening provides prospective parents with the option of testing the fetus for a hemoglobinopathy. This will give parents the opportunity of planning a family without disease, also alleviating the health burden to society. A joint venture of antenatal and high risk couple screening seems to be the most fruitful strategy for control of hemoglobinopathy in India.

BIBLIOGRAPHY

1. Bhukhanvala DS, Sorathiya SM, Sawant P, Colah R, Ghosh K, Gupte SC. (2013). Antenatal screening for identification of couples for prenatal diagnosis of severe hemoglobinopathies in surat, South gujarat. *J Obstet Gynaecol India*. 63(2):123-7. doi: 10.1007/s13224-012-0271-4. Epub 2012 Nov 2. PMID: 24431619; PMCID: PMC3664685.
2. Sinha S, Kumar A, Gupta V, Kumar S, Singh VP, Raman R Sachdeva A, Kumar S, Sood SK, Gupta A. (2004). Haemoglobinopathies – thalassaemias and abnormal haemoglobins in eastern Uttar Pradesh and adjoining districts of neighbouring states. *Current Science*. 87(6):77580.