Original Research Paper

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Pathology

PREVALENCE OF UNDIAGNOSED HEMOGLOBINOPATHIES AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE CLINIC AT TERTIARY CARE CENTER

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ABSTRACT **Introduction:** Hemoglobinopathies are the most common of all single gene inheritance (monogenic) autosomal recessive diseases and the most severe include sickle cell disease and alpha/beta thalassemia major. As carriers are typically asymptomatic and unaware of their carrier status, carrier couple often do not realize that with each pregnancy they face a 25% risk of child with hemoglobinopathy. Pregnant women those who are carriers of hemoglobinopathy present with anemia of varying severity.

Aim And Objective:

- The aim of the study was to explore better strategies for hemoglobinopathy prevention in the preconception and antenatal phase in general practice.
- The objective of this study was to determine the prevalence of undiagnosed hemoglobinopathy carriers in random sample of pregnant women attending antenatal care clinic at tertiary care center.

Material And Method: We carried out a retrospective study in which 436 blood samples of pregnant women were analysed between August 2023 to October 2023 (3 months) in the department of pathology at tertiary care center. Total 436 blood samples of pregnant women were analysed, genotype includes 21 case of beta thalassemia trait, 6 case of sickle cell trait, 1 case thalassemia intermedia, 1 case of delta-beta thalassemia, 1 case of HbD heterozygous and 1 case of HbE heterozygous. Each blood sample was run in BIO-RAD HEMOGLOBIN VARIANT- II HPLC machine. **Results:** In present study, Prevalence of undiagnosed hemoglobinopathy carriers was 7.11%. Out of which 4.81% were beta thalassemia trait, 1.38% sickle cell trait, 0.22% thalassmia intermedia, 0.22% delta- beta thalassemia, 0.22% HbD heterozygous and 0.22% HbE heterozygous. **Conclusion:** This study reveals the high prevalence of undiagnosed hemoglobinopathy carriers among pregnant women, indicating the need to immediately implement carrier screening and genetic counseling services across the country.

KEYWORDS : Hemoglobinopathy, Prevalence, Beta thalassemia, Screening.

INTRODUCTION:

Anemia in pregnancy is one of the cause of maternal morbidity and maternal and fetal mortality in india. Hemoglobin transport oxygen to different parts of the body. Any defect in hemoglobin structure leads to its adverse functions. Screening of pregnant women for hemoglobin opathies helps in early intervention for reducing morbidity and mortality.

Pregnancies in women with hemoglobinopathies are at risk for intrauterine growth retardation, spontaneous abortion, still birth, and preterm labor.(1-4).Early symptoms are usually non-specific e.g. fatigue, weakness, light headedness, mild dyspnea with exertion.

Prevailing high maternal morbidity and mortality have always been a source of medical concern, being antenatal and intra-partum care components of the family welfare program, which are aimed at reducing maternal and fetal morbidity and mortality in india.

Basic focus of this study is to know, whether hemoglobin disorders play any role in the pregnancy outcome or not?

Hemoglobin – the red pigment of red blood cells transports oxygen to different parts of body. Inadequate availability of oxygen to fetus also leads to abortion, miscarriage or stillbirth. The primary purpose of hemoglobinopathies screening in pregnant women is to identify the mothers and fetuses vulnerable to hemolytic anemia due to other abnormal hemoglobin variants for which early intervention has been shown to markedly reduce morbidity and mortality.

For research point of view, it is not known what is the status of hemoglobinopathies and what are the hemoglobin variants prevalent in pregnant women in tertiary care center? Keeping this state of mind, this study was designed with the aim& objective: **1**.The aim of the study was to explore better strategies for early diagnosis of hemoglobinopathy in the preconception and antenatal phase in general practice. 2.The objective of this study was to determine the prevalence of undiagnosed hemoglobinopathy carriers in random sample of pregnant women attending antenatal care clinic at tertiary care center.

MATERIALS AND METHODS:

This is a retrospective study carried out in the tertiary care center pregnant women who visited for antenatal care check up were investigated.

Each subject was requested to provide the background information such as name, age, residential address, reproductive history(abortion, miscarriage, stillbirth); if any, month of gestation, history of hospitalization, history of blood transfusion, family history, consanguine/non-consanguine marriage, or pregnancy related complications.

Intravenous 2-3 ml blood samples were collected using disposable syringe and needles in disodium salt of ethylene diamine tetra acetic acid(Na_2EDTA) coated vials from each 436 pregnant women period from august 2023 to october 2023(3 months). Each blood sample was run in BIO-RAD HEMOGLOBIN VARIANT- II HPLC machine. The machine works on the principle of reversed –phase cation exchange chromatography . The guidelines to assure acceptable results:

1. The baseline-should be properly constructed
2. Peak profile & shape – Peaks should appear sharp and symmetrical
3. Order of peaks – The peaks should follow the order F, P2, P3, Ao, A2
4. Total peak area Should be 1 to 3 million
5. Hb A2 and Hb F response - The new calibration factor should be – 0.7 to 1.3

- 6 - Hb A2 retention time – The retention time of Hb A2 for the calibrator should be 3.65±0.1 C

The graph is generated (fig 1) and depending on the time of elution of Hb peak, graph is interpreted according to elution Times model given as reference literature with the machine.

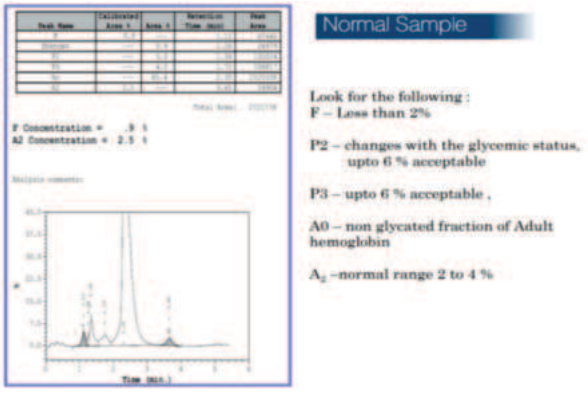

Fig -1 :Interpretation of normal sample HPLC chromatograph

OBSERVATION AND RESULT:

Table 1 : Age wise distribution of hemoglobinopathies

AGE WISE DISTRIBUTION OF HEMOGLOBINOPATHIES		
AGE	NORMAL	HEMOGLOBINOPATHIES
≤20	98	05
21-25	109	11
26-30	112	11
31-35	86	04
TOTAL	405	31

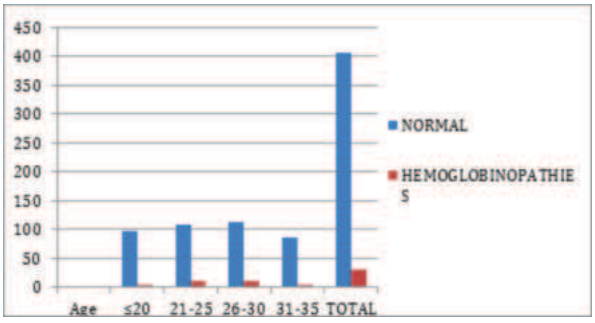

Figure-2

Table 1 & figure 2 showed maximum patients with in age group of 21-30 years of age

Table 2: Prevalence of hemoglobinopathies in pregnant women in a tertiary care hospital during AUGUST- OCTOBER 2023.

Pregnant women	No.	Percent
Normal	405	92.89
Sickle cell trait	06	1.38
β thalassemia minor	21	4.81
Thalassemia intermedia	01	0.22
□□ thalassemia	01	0.22
Hemoglobin E trait	01	0.22
Hemoglobin D trait	01	0.22
All hemoglobinopathies	436	100.00

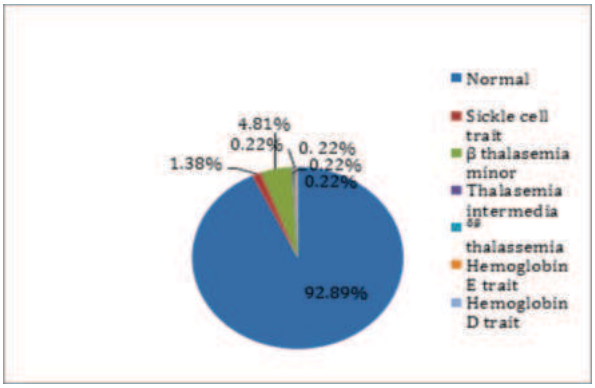

Figure-3: Percentage distribution of hemoglobinopathies

Table 2 & figure 3 : A total 436 samples were examined for HPLC . out of this samples 21(4.18%) cases were beta thalassemia trait, 06(1.38%) cases were sickle cell trait,01 case of each thalassemia intermedia, HbD trait, HbE trait, δβ thalassemia respectively.

Table: 3: Different grade of anemia in pregnant women with hemoglobinopathies.

Degree of anemia	No. of cases	Thal trait	Sickle cell trait	Thal. interm edia	HbD trait	HbE trait	δβ thalasse mia
Severe anemia (<Hb <7.0g/dl)	02	00	01	01	00	00	00
Moderate anemia (7.1-10.0g/dl)	19	18	01	00	00	00	00
Mild anemia (10.1-11.0g/dl)	06	03	02	00	01	00	00
Normal Hb (11.1g/dl)	04	00	02	00	00	01	01
	Total = 31	21	06	01	01	01	01

Table-3 shows most of carrier women with hemoglobinopathies presented with moderate degree anemia followed by mild degree anemia.

Table-4: Various hemoglobinopathies and it's correlation with Marital status, bad obstetric and family history, gravida

CORRELATION BETWEEN MARITAL, OBSTETRIC, FAMILIAL HISTORY, GRAVIDA		
MARRIAGE	CONSANGUINE	10
	NON CONSANGUINE	21
BAD OBSTETRIC HISTORY	YES	08
	NO	23
FAMILY HISTORY	POSITIVE	01
	NEGATIVE	30
GRAVIDA	PRIMI	11
	MULTI	20

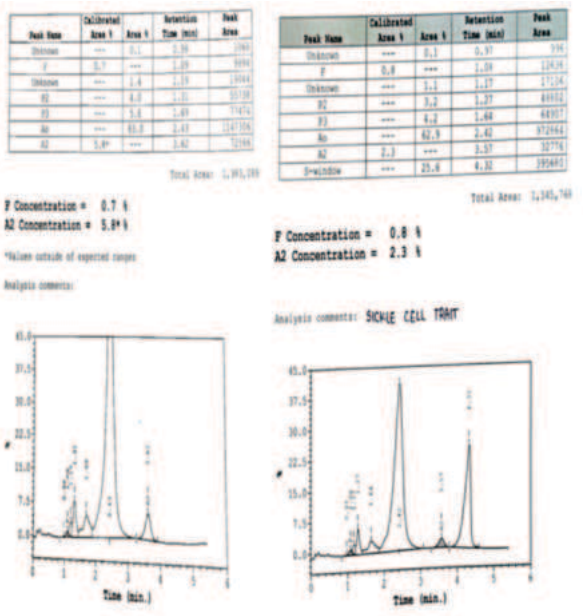

Figure-4 : HPLC chromatogram showing β-thalassemia trait And Sickle cell trait

Diagnosis of Beta thalassemia trait was made with HbA2 levels 5.8% and HbF 0.7%. In sickle cell trait HbS levels is 25.6% on HPLC chromatogram.

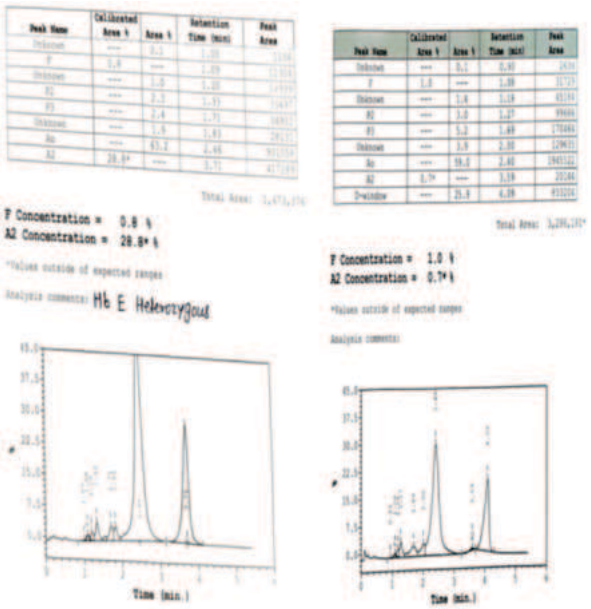

Figure – 5 : HPLC chromatogram showing HbE trait and Hb D trait

In HbE trait HbA2 levels 28.8% and HbD trait D window were seen on HPLC chromatogram.

DISCUSSION:

HPLC is emerging as one of the best methods for screening and detection of various hemoglobinopathies with rapid, reproducible and precise results(5). In our study , all the pregnant women attending at antenatal care clinic were included. Total of 06 different diagnosis of hemoglobin variants were made from 436 samples received for HPLC. Out of these, largest subgroup was of Beta thalassemia trait which was diagnosed in 21 cases(4.81%). The characterstic findings of beta thalassemia trait were mild to moderate anemia with microcytic hypochromic blood picture , target cells and basophilic stipling. HPLC value of HbA2 was >4% and HbF<2%(In some cases HbF may also be elevated).Sickle cell trait formed the second most common hemoglobinopathy , found in 06 cases(1.38%), while 4 cases had other types- one each of thalassemia intermedia, delta/beta thalassemia, HbD trait and HbE trait. The highest number of patients belonged to the age group of 21-30 years , 04 patients were in between 31-35 years of age and 05 patients were <20 years of age group. 10 couples had history of consanguine marriage. Rest all were non consanguine marriages Multigravida formed the highest percentage of cases.08 cases had history of previous abortion. The HPLC Parameters of the present study are in good correlation with the research conducted by Tejinder singh, (6)RiouJ,(7).All the pregnant women graded for anemia according to the classification of WHO. The frequency of moderate anemia was recorded to be 18 cases of beta thalassemia trait and severe anemia were found in case of sickle cell trait and thalassemia intermedia(8) (Ranbir S. Balgir study).

CONCLUSION:

HPLC is easy and convient method to rule out hemoglonopathies. As high prevalence of undiagnosed hemoglobinopathy carriers among pregnant women, indicating the need to immediately implement carrier screening and genetic traits will prevent occurrence of thalassemia major in offspring.

Detection of other variants becomes important due to complex interaction in cases with double heterozygous and homozygous states, which may lead to severe hematological abnormalities.

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