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Original Research Article

Screening of thalassemia minor in the first and second trimesters of pregnancy

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ABSTRACT

Introduction: Hemoglobinopathy is defined as a blood disorder caused by a genetically determined change in the molecular structure of hemoglobin. Abnormalities of hemoglobin (Hb) synthesis are among the most common inherited disorders of man and can be quantitative (thalassemia syndrome) or qualitative (variant Hemoglobins). Screening is a process used to identify individuals who are at high risk of having or of developing, a specific disease when compared with the general population. In India, they are responsible for the largest number of genetic disorders and thalassemia.

Aim and Objective: To screen pregnant females in the first and second trimesters for thalassemia minor

Materials and Methods: This study was conducted at Department of Biochemistry in collaboration with Department of Obstetrics and Gynecology, at a tertiary care hospital.

The study was conducted over a period of 18 months, from January 2013 to June 2014. A complete medical history and informed consent were obtained from all participants. Levels of Hb A, Hb A₂, Hb F, and Hb variants were measured by cation exchange high-performance liquid chromatography (HPLC).

Result: Of the 1,350 pregnant females, 48 had thalassemia minor positive on screening.

Conclusion: From the obstetrician's point of view, prevention and control in pregnant women is one of the best ways to reduce the birth of severe thalassemia in major infants.

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1. Introduction

Hemoglobin (Hb) is a complex protein consisting of iron-containing heme group and the protein moiety globin.

A dynamic interaction between heme and globin gives hemoglobin its unique properties in the reversible transport of oxygen.¹⁻³ A hemoglobin molecule is a tetramer made up of two pairs of polypeptide chains; HbA is made up of one pair of alpha (α) and one pair of beta (β) polypeptide chains, represented as ' $\alpha_2\beta_2$ '. The major hemoglobin in the fetus, HbF, is made up of two alpha and two gamma (γ) chains and is represented as ' $\alpha_2\gamma_2$ '. HbA₂ is made up of two alpha and

two delta (δ) globin chains represented as ' $\alpha_2\delta_2$ '. Genes for the α chains are located on human chromosome 16. The genes for γ , β , δ chains are located on chromosome 11.

The cumulative gene frequency of hemoglobinopathies in India is 4.2%.⁴

In the world, there are 100,000 living patients with homozygous beta thalassemia, and of that, 3% are born in India.

In the most common type of beta thalassemia trait, the level of HbA₂ ($\alpha_2\delta_2$) is usually elevated. Some, but not all, of the excessive α chains, are used to form HbA₂ with δ chains while the remaining α chains precipitate in the cells, reacting with cell m.

If the mother and father both have beta thalassemia minor, then there is a one in four chance that the fetus

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can have beta thalassemia major. 50% of the children can have beta thalassemia minor, and 25% can be normal. It has autosomal recessive transmission.

Thalassemia minor is the most common form of beta thalassemia and is also known as the ‘thalassemia trait’, in which affected individuals are asymptomatic.

Asymptomatic patients are usually detected through routine hematologic testing..

The common mutation in beta thalassemia in Asian Indians are:⁵

1. IVS 1-5 (G-C)
2. 619 bp del
3. FS 8/9 (+G)
4. IVS 1-1 (G-T)
5. FS 41/42 (-CTTT)
6. CD 15 (G-A)
7. CD 30(G-C)
8. CD 16 (-C)
9. Cap site +1 (A-C)
10. Others

Pregnancy lasts about 40 weeks, counting from the first day of the last normal period. The weeks are grouped into three trimesters. First trimester (week 1- week 12), Second trimester (week 13-week 28), and third trimester (week 29-week 42).^{6,7} From the obstetrician’s point of view, prevention and control in pregnant women is one of the best ways to reduce the birth of severe thalassemia in major infants.

2. Materials and Methods

This study was conducted at the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynecology at a tertiary care hospital. The study was conducted over a period of 18 months, from January 2013 to June 2014. A complete medical history and informed consent were obtained. The tests were performed on BIORAD ‘VARIANT’ using the beta thalassemia Short program (BioRad Laboratories, California, USA). A total of 1,350 cases were studied for variants of Hemoglobinopathies.

The instrument utilizes the principle of HPLC.⁸

The simplicity of the automated system with internal sample preparation, superior resolution, rapid assay time, and accurate quantification of hemoglobin fractions make it more reliable.^{9,10}

The integrated peaks are assigned to manufacturer-defined windows derived from the retention time, i.e., the time in minutes from sample injection to the maximum point of the elution peak of normal hemoglobin fraction. The analysis time is short (6.5 min), and there is a good separation between the HbA2 values of beta thalassemia carriers from normals, with no overlap between these groups.

For the analysis, 5 microliters of EDTA whole blood is automatically diluted with 1 mL of a hemolysing reagent. Hemolysed specimens are loaded into a 100-place sampler compartment maintained at $12 \pm 2^\circ\text{C}$. Twenty microliters of each sample are sequentially injected at 6.5-minute intervals. Built-in software controls the analysis cycle (elution gradient, column regeneration) and performs peak integration. The calibration factors for HbA2 and F are automatically calculated.¹¹

3. Results

Table 1: Distribution of thalassemia minor in pregnant females

	Patients	Percentage (%)
Normal	1302	96.5
Thalassemia minor	48	3.5
Total	1350	

Table 2: Descriptive data of parameters (Hb, MCV, MCH, RDW)

	Normal (Mean \pm SD)	Hemoglobinopathies (Mean \pm SD)
Hemoglobin (G%)	10.34 \pm 1.777	9.349 \pm 1.523
MCV (fL)	76.83 \pm 7.670	72.55 \pm 8.099
MCH (pG)	27.24 \pm 4.188	25.01 \pm 4.902
RDW	15.73 \pm 2.565	17.7 \pm 3.500

Table 3: Distribution of hemoglobinopathies in pregnant female

	Patients	Percentage (%)
Normal	1287	95.3
Hemoglobinopathies	63	4.6
Total	1350	

4. Discussion

India has a population of 1.21 billion, according to the 2011 Census. There are 4,693 endogamous communities, which includes 427 tribal groups. Although beta thalassemia and other hemoglobinopathies are seen in all the states, the prevalence is quite variable.¹²

Beta thalassemia is the most common inherited hemoglobinopathy. The prevalence of beta thalassemia trait (BTT) varies from 1.0% to 14.9% in various regions of India.

The diagnosis is made through the evaluation of positive family history or during population screening.¹³ Though a family history of thalassemia is important, a significant number of patients do not have previously affected family members.¹⁴

Given the seriousness of homozygous beta thalassemia, correct identification of BTT is important to enable family screening and genetic counseling.¹⁵

Table 4: Comparison with various studies

Parameters	Seema T et al. - 2003	Antinio et al. - 2007	RS Balgir - 2008	Shivashankara et al. - 2008	Fakher R - 2009	Present study
Hb g/dl	7.4	9.1-15.3	10.9	7.1-10.1	9.53	7.8-10.8
MCV fl	NA	<79	77	65-80	62.9	64-80
MCH pg	NA	<27	21	19-25	20.03	21-29

In this study, Hb level was found to be 7.8-10.8 g/dl, MCV was 64-80 fl and MCH was 21-29 pg. These findings are similar to those of Seema T et al., who found hemoglobin to be 74g/dl.¹⁶

Antinio et al. reported Hb levels in the range of 9.1-15.3 g/dl, MCV-<79 fl, MCH <27 pg, which is similar to our results.¹⁷ RS Balgir, in the year 2008, reported Hb levels of 10.9 g/dl, MCV-77fl, MCH-21 pg.¹⁸ Shivashankara et al. studies suggested Hb levels of 7.1-10.1 g/dl, MCV-65-80 fl, and MCH -19-25 pg.¹⁹ Fakher R studied Hb levels of 9.53 g/dl, MCV-64-80 fl, and MCH-20.3 pg, which is similar to our findings.²⁰ The alterations in the level of Hb, MCV, and MCH are due to ineffective erythropoiesis.

The maximum number of women in this study were primigravida, suggesting that women are more cautious regarding the first pregnancy as compared to multigravida.

The prevalence of beta thalassemia in our study was found to be 3.5%. The frequency of beta thalassemia carriers varies between 1 to 17 percent in different regions in India.

5. Conclusions

1,350 pregnant women were screened over a period of 18 months from January 2013 to June 2014. Of these, 63 pregnant women were found to be having hemoglobinopathies. Thalassemia major is a hemoglobinopathy that is absolutely preventable if screening of thalassemia minor is done at the right time. The awareness of prenatal diagnosis can help many families. The treatment for thalassemia major creates a financial and psychological burden. Appropriate and extensive screening, accurate detection, and counseling of at-risk couples, along with antenatal diagnosis, is a promising strategy for the reduction of morbidity due to thalassemia.

6. Additional Information

6.1. Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Grant Government Medical College issued approval: Approved. Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue. Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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None.

8. Conflict of Interest

None.

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