



Original Research Article

Screening of Thalassemia in pregnant females visiting tertiary hospital in Amritsar

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ABSTRACT

Background: Thalassemic patients live a prolonged life in the light of breakthrough of treatment options and early diagnosis, and therefore it is a matter of interest and importance to have a successful reproductive outcome in such patients. Having a child is the dream and readiness of being pregnant is the inclination of not only Thalassemia intermedia patients but also females suffering from Thalassemia major. Thalassemia is an incurable disease till now and producing thalassemia children only enhances the disease burden both for the society and family. This augments the need of successful and early screening thalassemic pregnancies and counseling them for the possible outcomes.

Materials and Methods: The present study was conducted in 460 pregnant Females attending OPD of SGRDIMSAR, Sri Amritsar. Samples were taken after informed consent and processed for Complete Blood Count and Hemoglobin Fractionation using HPLC by Borad.

Results: Most of the females had hemoglobin levels between 9-10 g % forming 41.3% of the total females (Table 1). Out of 460 patients enrolled in the study, 21 Females were found to have hemoglobin variants on screening amounting to the prevalence of carrier states to 4.53% (Table 3).

Conclusion: All pregnant females should be screened for thalassemia carriers in early antenatal period and positive patients should have screening of the partner along with relevant counseling regarding treatment options. Cost effective diagnostic options should be made available in the periphery for mass screening.

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1. Introduction

A significantly consequential genetic disorder termed thalassemias has a far reaching monetary and psychological dearth. They develop an extensive emotional and social deficit on the individuals suffering from the same. Their families, society and the country also equally bear this burden. Earlier it was found to exist in enormous density in the belt ranging through the Middle-East, Indian subcontinent, Burma, Southeast Asia, and islands of the Pacific till the mediterranean basin but are now frequent throughout the world due to extensive and flourishing movement of people and consanguineous marriages.^{1,2} In the world, almost 5.2% of the individuals and even greater than 7% of expecting females carry a significant hemoglobin variant.¹ Couples are at risk of having kids

with a some or the other hemoglobin abnormality amounts approximately to 1.1%, and 2.7 per 1000 conceptions suffer throughout.¹

The numbers of hemoglobin variants surpass 3.4% in young offspring < 5 years of age. Annually, the females influenced or pretence by hemoglobinopathies amount to greater than 4.13% only in South East Asia.² Amongst these, 90% are beta-thalassemia and 4.3% amount to alpha-thalassemia.² Per year about, 1.9 lakh offspring are conceived in couples with traits, out of which almost 30% are accounting to the risk of delivering a thalassemic neonate.² Still if precautions are undertaken, severely symptomatic disorder can be archaized by many simple steps. Country like India being present on the thalassemic belt, experiences a high rate of β thalassemia minor women very variable in different states.^{3,4} India also shows such high figures because of not having a national thalassemia

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control program till date. Moreover the Lack of knowledge regarding screening and prevention of thalassaemic child births pours oil in the Fire of the alarming issue. The main objective of this research work was to study the prevalence of β thalassaemia minor pregnant women in India by screening expecting females in their early first antenatal hospital visit.⁵

2. Materials and Methods

The present observational research study was undertaken in pregnant Females attending Gynecology OPD of a tertiary care set up in Vallah, Amritsar having a good set up for diagnosing thalassaemic traits and variants, after attaining the approval of Ethical committee of the institution. 460 patients were included in the present study after calculating the sample size by Epi Info software considering a power of 99.9% and alpha error of 5% (reference of 7.9% prevalence of Thalassaemia carrier states taken from previous study by Mehandiratta SL et al.⁶

We developed a semi structured questionnaire which was pre validated by 10 faculty members of various specialties catering thalassaemic patients. The questions having a score of less than 0.80 were discarded and rest of the articles was filled by the patients. Language barrier was addressed by having the questionnaire in English, Hindi and Punjabi (Regional language spoken by most of the residents here) language to improve the feasibility of the study. The motive for screening and the possible outcomes were detailed out to the couples at the very beginning of the study. The counseling and the discussion session covered all points including how the screening will be performed, motive for this test, how much sample will be taken, time of issue of report, detailing out the report to the couple and further management in case of positive test result. All the legal, social and ethical concerns regarding the same were also undertaken. Care was taken not to miss anything due to the sensitivity of the issue. Written consent was taken from all in concern of use for screening and further implications.

5 ml of patient Blood sample was collected by closed system taking all required precautions in Lavendar tube EDTA vacutainers. Analysis of parameters including hemoglobin and red cell indices including MCV, MCH and MCHC etc were measured by a 5 part hematology cell counter analyzer⁷ and hemoglobin fractionation was processed by the Gold standard method of high performance liquid chromatography (HPLC)⁸ by Biorad Limited. The storage of the sample was done for a maximum of 5 days at 2-8 degrees centigrade as per Biorad protocol to prevent the development of unwanted peaks in the graph due to sample disintegration. In case of Low hemoglobin, manual pre-dilution was carried out. Calibrations and controls were processed for each run as a routine protocol of the laboratory.

In the proforma, the details about previous pregnancies and family history of any known case of thalassaemia were investigated. All the women were accessed for the features of or any symptoms of anemia, hepatosplenomegaly, and other sign pointing towards thalassaemia. Females having Low MCV, MCH or MCHC and any abnormal picture of chromatogram, were given professional advice and the husband's blood sample was taken for HPLC. After these investigations, the couples discovered at risk by chance, were counseled about the possibility of unpleasant birth and were provided the option of prenatal diagnosis and possible consequences. All the detailed medical records were accessed and evaluated for several socio-economic and clinical variables. In cases with HbA2 > 4.0%, or with variant hemoglobin were recommended corroboration and verification by DNA analysis for the same. Those identified by the nature of illness and with any traits, were told to have mandatory antenatal visits where all RBC indices were assessed, patient examined clinically. Along with the same, ultrasonographic evaluation of fetal growth was carried out every 4 weeks usually initiating at 26–28 weeks.

3. Results

It was observed from the study that most of the females had hemoglobin levels between 9-10 g % forming 41.3% of the total females (Table 1). Out of 460 patients enrolled in the study, 21 Females were found to have hemoglobin variants on screening amounting to the prevalence of carrier states to 4.53% (Table 3). The mean age of carrier females was 24.5 years whereas that of normal females was 25.8 and the difference was not significant. There was a significant difference between Hemoglobin levels and red cell indices in Carrier and Normal females (Table 2).

Table 1: Severity of anemia in pregnant females

Hemoglobin g/dL	No. of Patients	% Percentage
< 7	36	7.8
7-8	57	12.3
8-9	76	16.5
9-10	190	41.3
10-11	59	12.8
>=11	42	9.1
Total	460	100

4. Discussion

Thalassaemia pose a major health care burden in the light of the fact that no major National Health Program is active to address the issue. Thalassaemia traits are usually unaware of their situation and future prospects and thus do not seek help. This forms the basis and major objective of antenatal screening. There should be mass screening program to find females with clinically significant trait, predisposing to produce a child with major thalassaemia in case she marries a

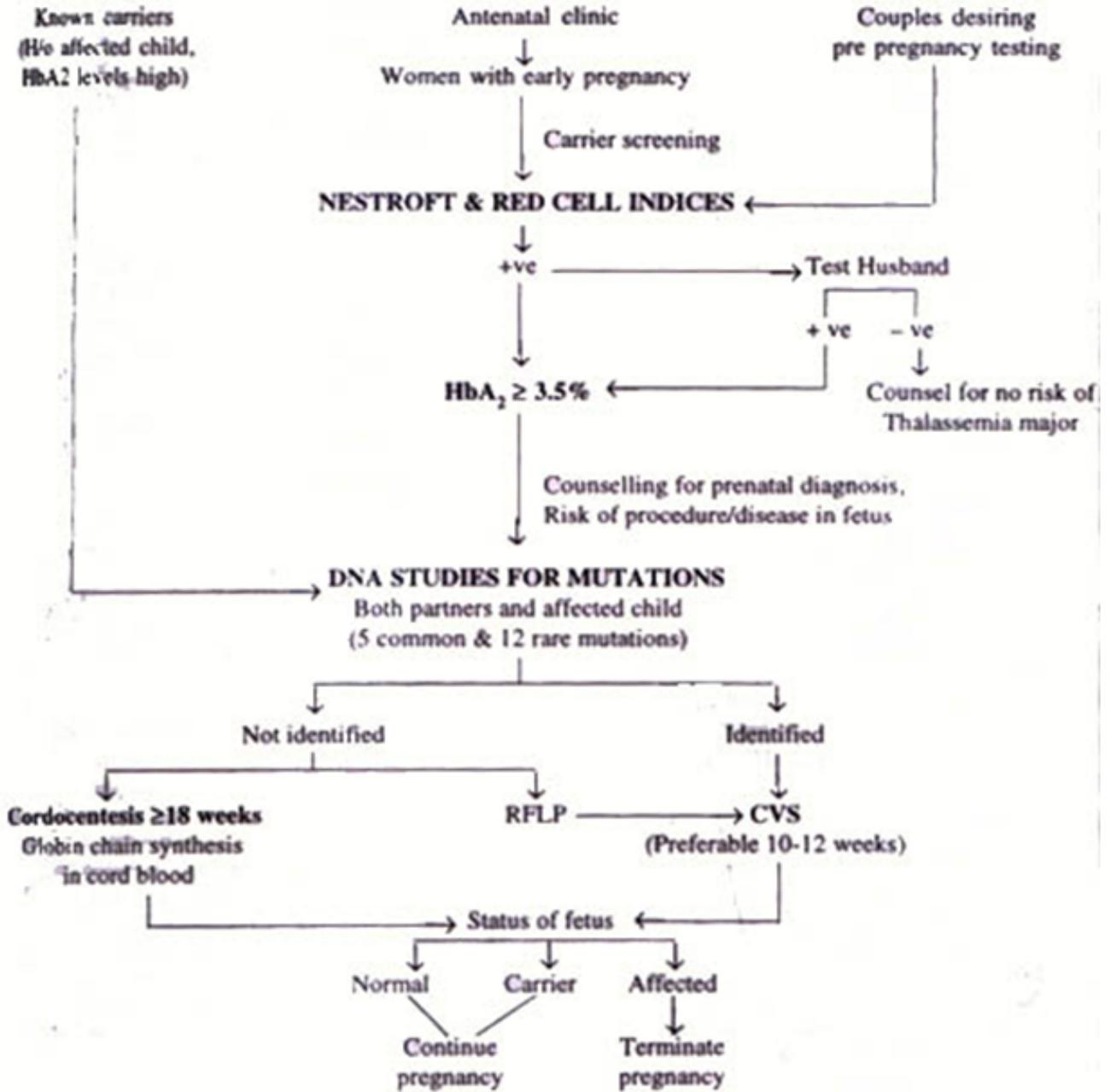


Fig. 1: Flowchart for the Protocol for screening of carriers in pregnancy

Table 2: Mean red cell indices in BTT and Non BTT cases

Red cell index	Mean value in BTT cases (Mean + SD)	Mean Value in Non BTT cases (Mean + SD)	P Value
Age (years)	24.5 + 7.2	25.8 + 4.4	0.9
Hb (g%)	8.7 + 2.1	10.2 + 1.4	<0.001
RBC x 10 ¹² /L	5.52 + 0.62	5.23 + 0.71	<0.001
MCV (fL)	63.2 + 6.5	79.5 + 4.9	<0.001
MCH (pg)	19.6 + 2.57	23.4 + 1.85	<0.001
MCHC (g/dl)	31.2 + 1.42	34.7 + 1.45	<0.001
HBA2	4.3 + 1.8	2.6 + 0.74	<0.001

Table 3: Distribution of cases according to Type of thalassemias

S.No	Type of Thalassemia variant	No of Patients	Percentage (out of 460)
1.	Beta Thalassemia Trait	14	3.00
2.	HbD Punjab	3	0.60
3.	HbE	1	0.20
4.	Delta Beta Thalassemia	2	0.43
5.	HbS	1	0.20
6.	Others	None	0.00
	Total	21	4.5

thalassemic male accidentally. This would lead to increased reproductive options to the females in terms of partner decision, prenatal diagnosis and Medical termination of pregnancy if required.⁹

The barrier in screening becomes the high prevalence of iron deficiency anemia in antenatal population, confusing the picture with similar red cell indices. Confirming the case of iron deficiency still predates the diagnosis as these two conditions still may coexist at the same time in an individual. Thus all pregnant females should have a complete blood profile during the very first antenatal visit.¹⁰

As per the protocol recommended by the evidence based guidelines prescribed by NICE, two visits of the expecting Females must be ensured in the First trimester in the time gap of which, the couple gets appropriate time for genetic screening, all required antenatal tests and decision of termination of pregnancy if required. The Screening at this time includes a complete blood count, as well as hemoglobin electrophoresis or hemoglobin high performance liquid chromatography, which is considered the gold standard the determining thalassemics. This investigation encompasses quantitation of HbA2 and HbF. Also, in case there is microcytosis (mean cellular volume < 80 fL) and/or hypochromia (mean cellular hemoglobin < 27 pg) in the presence of a normal hemoglobin electrophoresis or high performance liquid chromatography the participant must be investigated with a brilliant cresyl blue stained blood smear to find out the presence of any H bodies. Determining the levels of serum ferritin (in order to exclude iron deficiency anemia) must be undertaken at the same time.^{11–13}

Microcytic hypochromic picture usually calls for estimation of HbA2 by HPLC. In majority of cases

of β Thal trait, there is an elevated Hb A2 > 3.5%, although variants exist. HbA2 may be normal in cases of α thalassemias and thus should be diagnosed carefully.^{14–16}

We found a prevalence of 4.5% in pregnant females screened during their first antenatal visit. In our study we detected 14 cases of Beta Thalassemia trait, 3 cases of HbD Punjab, 1 case of HbE and HbS trait and 2 cases of Delta Beta Thalassemia. Similar findings were observed in the study conducted by Most of the women belonged to low socioeconomic status and were mainly housewives. Sixty three of the 2000 women screened (3.15%) were identified as carriers of beta thalassemia trait and other hemoglobinopathies. Most of them that is 59 cases (2.95%) were beta thalassemia carriers while one each were carriers of HbE (0.05%), HbS (0.05%), HbD (0.05%), and double heterozygous for beta thalassemia and HbE. Asha Baxi et al also reported similar results in their study conducted in Indore during a period of 2 years.⁴

Lack of education and general unawareness about the disease makes husbands of carriers reluctant to get themselves screened for the disease as was also reported by Colah et al.¹⁵

HbD famous by the label of HbD Los Angeles is usually detected in North-Western India. Still some sporadic cases have been reported from Gujarat.^{13,16}

In a study conducted by Torres LDS et al, 70 cases of type D hemoglobin from South-east Brazil were diagnosed in the Hemoglobin and Hematological Genetic Diseases Laboratory of the Universidade Estadual Paulista (UNESP) a referral center for hemoglobin diagnosis. Of these, 66 (94.3%) had a profile compatible to Hb D-Punjab, eight (12.1%) were compound heterozygous Hb S/D-Punjab and 58 (87.9%) heterozygous Hb A/D-Punjab, all of which

were confirmed by molecular tests. It becomes a matter of prime importance to differentiate between HPFH and delta-beta thalassemia, as the line between the two is subtle and should be confirmed by alpha-beta-globin chain synthesis ratio and DNA analysis as the differentiation between these two conditions is not always possible from routine hematologic analyses. To reason to diagnose these two conditions especially in antenatal screening is important because HPFH is clinically asymptomatic, but interaction of $\delta\beta$ -thalassemia with β -thalassemia can result in a severe disorder.¹⁷

Until Gene therapy becomes vastly available and cost effective, the only way to treat thalassemias is preventing new births. This is only possible by screening and counseling.⁴ Cao A et al. observed a significant reduction in the birth rate of thalassemia Major cases from 1:250 to 1:4000 live births.¹⁸ This requires massive screening and robust counseling. Since India is a country with cost constraints, it will not be economical to do HPLC analysis for mass screening of β -thalassemia trait. It is thus recommended that either NESTROFT or red cell indices, or combination of the two tests be used for mass-screening program.

For those who can afford, Fetal loss of 1% attributed to early prenatal diagnosis of these disorders, future prospects ensure better scenario. In the current decade target should shift to newer molecular techniques, promising better results. PCR based techniques are currently in use for detecting globin chain mutations. ARMS (Amplification refractory mutation system), RT PCR, HRM (High resolution melting analysis), Sangers technique are some technologies coming up at a rapid pace. GAP PCR and MLPA (multiplex ligation dependent probe amplification) are innovative methods which promise detecting deletions more precisely.

The discovery of non invasive cell free DNA (cf DNA) in maternal blood has revolutionized the approach. This methods is totally free of the limitation of miscarriage. There is also no contamination risk between the mother and fetal cells.

5. Conclusion

The centers considering diagnosing and differentiating anaemia and thalassemia carriers in India are dependent on Red Blood cell indices alone due to enormous financial burden. This is not a reliable method and differentiating Iron deficiency anaemia and thalassemias is of prime importance. All centers cannot afford HPLC set up and therefore alternatives must be found for accurate diagnosis. Those affording should resort to better molecular technologies. Further policies should be made by the government to allow access of these newer technologies to the masses.

6. Source of Funding

None.

7. Conflict of Interest

None.

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