Research Paper Microbiology

A Study of β-Thalassaemia screening in antenatal mothers

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ABSTRACT

The population survey of antenatal screening for β-Thalassaemia and sickle cell heterozygotes provided an opportunity to estimate the prevalence of these traits in the antenatal mothers of Rajkot city. All antenatal mothers attending for routine checkup were screened. For each subject, venous blood was taken to determine complete blood count, red cell indices and chromatographic analysis of hemoglobin. Total of 1765 antenatal mothers were tested for Thalassaemia at Padmakuvarba Government Hospital, Rajkot. Out of these, 72 antenatal mothers were found confirm positive for Thalassaemia minor by HPLC method. 6.94% positivity was found in spouse of Thalassaemia minor antenatal mothers with 5 couple carriers for Thalassaemia. Illiteracy and unawareness found to be major reason for birth of Thalassaemia major children in the society. The aim of this article is to show the prevalence of Thalassaemia among the antenatal mothers in Rajkot city.

Keywords: β-Thalassaemia, antenatal, Red blood cell Indices, HPLC

Introduction

Thalassaemia is a heterogeneous group of inherited autosomal recessive disorders of hemoglobin synthesis characterized by absence or reduced synthesis of one or more globin chains of hemoglobin. On the basis of type of genetic abnormality, Thalassaemia is classified in two key types:

1. Alpha Thalassaemia
2. Beta Thalassaemia

β-Thalassaemia is more prevalent in Indian subcontinent. It is mainly divided into:

1. Thalassaemia Major i.e. homozygous
2. Thalassaemia Minor i.e. heterozygous

Thalassaemia Major is easily diagnosed clinically due to severity of symptoms, where as Minor is really difficult to detect. There is significant fall in the birth rate of Thalassaemia major child due to the efforts of various agencies in some countries. These efforts include extensive awareness drive, Mass screening for Thalassaemia minor in different target groups or general population, proper genetic counseling of identified minors, and prenatal diagnosis (1).

Occurrence of Thalassaemia in Our Region

In Saurashtra region of Gujarat state, Thalassaemia is a major public health problem. Our report says that the thalassaemic gene prevalence rate in Saurashtra region varies from 4% to 6%. The population of Saurashtra is approximately 15 million and thus there are nearly 6,00,000 to 9,00,000 Thalassaemia carriers (Minor) in the region.

To prevent the birth of Thalassaemia major children the screening of antenatal mothers is the prime step. The local government & Health authorities were convinced to conduct the pilot project of Thalassaemia screening of all the antenatal mothers at Padmakuwarba Government Hospital, Rajkot.

Materials and Methods

In the present study, the target group adopted is the antenatal mothers coming for routine check-ups at the Padmakuvarba Government Hospital. Blood was collected from antenatal mothers in test tube containing K3 EDTA on Every Wednesday & Saturday of the week. This K3 EDTA is recommended for the machines in which the analysis was carried out like Cell Counter (CBC) and VARIANT (HPLC). The blood samples were analyzed on Automatic Hematological Analyzer (MS4 from Melet Schloesing Company, France) within 6 hours of collection. CBC (Complete Blood Count) reports were analyzed on the basis of correlation of different red blood cell indices like Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Hemoglobin Concentration, Red Blood Cell Count, and Red Cell Distribution Width (RDW). The samples found positive were reconfirmed by HPLC on VARIANT machine(2) In pregnant females iron deficiency is very common and hence it is difficult to differentiate with Thalassaemia minors. These methods are found very reliable. The mothers found positive are counseled during next session and their spouses were called for the blood test if the antenatal mother is found positive for Thalassaemia minor. The blood collected from spouses undergoes the same procedure. In case, if both the parents are found positive they were counseled thoroughly for prenatal diagnosis.

Results

(A) Control Group

To test the reliability of decided criteria of RBCI by Cell Counter, 56 antenatal mothers were tested as a control group on both the cell counter & variant machines. 33 mothers were found negative for Thalassaemia by both Cell Counter as well as Variant, while remaining 23 were found positive for Thalassaemia by Cell counter. When further tested on Variant 13 cases were found negative and 10 were found positive for Thalassaemia trait.

Table – 1: Results of Testing of control group for Thalassaemia Minor by CBC Auto analyzer & HPLC.

No. of samples tested on CBC and HPLC	No. of Samples found Negative on CBC	Negative samples confirmed on HPLC		No. of samples Found Positive on CBC	Positive samples confirmed on Variant	
		Found Negative	Found Positive		Found Negative	Found Positive
56	33	33	0	23	13	10

The results showed that false negativity is zero and false positivity is 56% by selected methods.

(B) Test Group:
In around 7 months period, total of 62 sessions were conducted to collect the blood samples from antenatal mothers and their spouse whenever necessary. 1765 sample were collected and tested on CBC for Red Blood Cell Indices.

Table – 2: Results of testing done by RBC Indices Method by Cell Counter;

No. of sessions	No. of antenatal mothers tested	Found Negative	Found Positive
62	1765	1231	534

Here 30.26 % samples were found positive and 69.74 % samples were found Negative using CBC by Cell counter.

(C) Confirmatory Group:
534 samples found positive by RBCI methods were further tested on 'variant Machine by High Performance Liquid Chromatography (HPLC) method.

Table-3: Results of testing done by HPLC method using Variant Machines;

No. of samples tested on Variant (HPLC)	Found Negative on Variant	Found Positive on Variant
534	462	72

13.48 % samples were found positive and 86.52 % samples were found negative by HPLC using variant Machine.

(D) Spouse Testing:
The spouse of all 72 antenatal mothers were called for testing. Despite out best efforts only 43 spouses came for testing. All these spouses were tested by both RBCI and HPLC method.

Table – 4: Results of test of spouses of antenatal mothers found positive by variant

Test conducted by RBCI using Cell counter			Test conducted by PHLC using Variant		
No. of spouse tested	Found Negative	Found Positive	No. of spouse tested	Found Negative	Found Positive
43	38	5	43	38	5

5 spouses were found positive and 38 spouses were found negative for Thalassaemia by both RBCI and HPLC method. Hence, we found 5 at risk couple i.e. both the partner carrier for Thalassaemia(3).

To summarize, out of 1765 antenatal mothers tested by us, 72 were fond positive for Thalassaemia minor with percentage positivity of 4.08 % and out of 72 Thalassaemia minor antenatal mothers, 5 spouses were also found positive for Thalassaemia minor.

Discussion
Our aim was to prevent birth of Thalassaemia Major children. This we attempted to achieve by many means like creating awareness among general population by screening of different target groups for heterozygotes. i.e. Thalassaemia minors, proper counseling of Thalassaemia minor and prenatal diagnosis. Despite of many efforts by Government and other agencies, lots of Thalassaemia major children have been borne in our region itself.

In India, lot of attention is given to disease like Polio, AIDS, Tuberculosis, etc. under National Health Program by Government. There is no doubt about the seriousness of these diseases, but Thalassaemia must be give the status in National Health Program which has incidence of 10, 000 cases per year causing immense physical, mental and financial burden on family, society, as well as health organizations (4).

The on-going pilot project of screening the antenatal mothers as a target group has been initiated to set an example to the Government and other charity organizations that Government and the Non-Government Organizations together can work better for prevention of Thalassaemia.

In the present study, initially we wanted to involve only the antenatal mothers of first trimester so that if any couple found Thalassaemia Minor, Medical intervention can be done before the permissible gestation age for Medical Termination of Pregnancy. But then we decided to involve all antenatal mothers who came for routine check-up because in this region there are some couple who have more than one Thalassaemia major children and we didn't want to miss the chance to counsel them if not for current pregnancy, but for the future.

Major hurdles for this project
- ⊡ Illiteracy is a very big problem especially among the females in this region. They don't even know their addresses also. Mostly they belong to lower socio-economic class. At such time it is difficult to convince them about the consequences of Thalassaemia. They don't come to collect even their reports.
- ⊡ Out of total 1808 people tested (including antenatal mothers and spouses) only 1642 came to collect their reports personally. Remaining reports were dispatched by Courier and post but all were returned due to incomplete addresses.
- ⊡ All the 72 spouses of the antenatal mothers that were found positive for Thalassaemia trait were called for testing. It was shocking that despite of all the efforts, only 43 spouses turned up for the test while remaining 29 have not came for testing. It's quite possible that out of these 29, some might be Thalassaemia minor and thus the chance of Thalassaemia major birth increases.

The five couples found Thalassaemia minor, in this case both the partners were thoroughly counseled and referred to our genetic clinic for prenatal diagnosis. Only 3 antenatal mothers were found eligible for prenatal testing. The other 2 mothers were having advance pregnancy i.e. third trimester and hence they were just counseled thoroughly without any further procedures. The Chorionic Ville Samplaing for 2 pregnancies and amniocentesis for 1 pregnancy were conducted. Out of these 3 fetuses, one was found Thalassaemia major while other two were not. The couple with Thalassaemia major fetus was counseled properly and as a result the pregnancy was terminated.

Conclusion
This type of projects will create awareness up to some extent among the most unaware people like illiterate and poor. Government should work in collaboration with the Non-Government organization to conduct integrated National Health Program to prevent the birth of Thalassaemia major children. The project should be evaluated for appropriateness, cost-benefit analysis as well as outcome and hence decision can be made to take this up as an integrated National Health Program.

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REFERENCES

1. Lau YL, Chan LC, Chan AYY, Ha SY, Yeung CY, Waye JS, Chui DHK. Prevalence and genotypes of - and -thalassemia carriers in Hong Kong - implications for population screening. N Engl J Med (1997) 336: 1298 - 1301. | 2. El-Tayeb EN, Yaqoob M, Abdur-Rahim K, Gustavson KH. Prevalence of beta-Thalassaemia and sickle cell traits in premarital screening in Al-Qassim, Saudi Arabia. Genet Couns. (2008) 19(2): 211-8. | 3. Mary Petrou. Screening for beta Thalassaemia. Indian Journal of Human Genetics (2010) Jan-Apr; 16(1): 1-5. doi: 10.4103/0971-6866.64934 | 4. S. Longlais, J. Ford, D. Chatiyat. Carrier screening for Thalassaemia and haemoglobinopathies in Canada. Joint SOGC-CCMG Clinical Practice Guideline.(2008) No. 218. October 2008.