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A study on Premarital Screening for β Thalassemia Trait in Hilly Areas Around Salem

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Abstract **Original Research Article**

Hemoglobinopathies are heterogeneous group of inherited disorders of hemoglobin (Hb). Thalassemia is characterized by reduced or absent production of globin chains. This study aims at estimating the prevalence of Beta Thalassemia trait in hilly areas around salem and to validate the screening test for Beta Thalassemia trait. As a part of the ongoing school screening programme, blood samples are collected from 10th and 12th school children in 30 selected tribal blocks in Tamil Nadu. Our Medical College Hospital receives samples from three tribal blocks around Salem namely Yercaud hills, Kolli hills and Kalrayan hills. The HPLC results of samples collected from the three hilly areas around salem for period of one academic year was analysed and blockwise prevalence was worked out. Sensitivity and specificity of NESTROFT test was also calculated with the datas available in our Department. This study shows that the prevalence of beta thalassemia trait in hilly areas around salem was as high as 5.2%. Among the three hilly areas around salem, Kalrayan hills shows the highest prevalence of about 6.9%. Second highest prevalence of about 4.9% was found in Yercaud hills and Kolli hills showed a prevalence of 3.8%. NESTROFT test shows 100% sensitivity and 100% negative predictive value. Specificity was only 25%. The most cost-effective and feasible approaches are preventive genetic strategies which requires proper health education and adequate sensitization of the community.

Keywords: Thalassemia, Screening, Prevalence, NESTROFT, Sensitivity.

INTRODUCTION

Hemoglobinopathies are inherited disorders of red blood cells. Thalassemia is a heterogeneous group of inherited disorders of hemoglobin (Hb) characterized by reduced or absent production of globin chains. It is the commonest single gene disorder in the world causing a significant morbidity and mortality which was first noted in the Mediterranean population [1].

Thalassemia is one of the most prevalent disorders affecting nearly 200 million people globally and impose a heavy burden on the affected families and the health sector. Thalassemia poses a serious public health threat globally, affecting nearly 4.4/10,000 live births. India accounts for 10% of the total world incidence of thalassemia-affected children with approximately 10,000 children being born with thalassemia every year [2]. Beta- thalassemia is prevalent in a broad belt extending from Mediterranean basin to Southeast Asia. The prevalence of Beta-thalassemia trait is about 3.3% in India which varies in different parts of the country [3]. In India, there are nearly 42 million carriers of the β-thalassemia trait and its prevalence is much higher in communities like Sindhis, Punjabis, Gujaratis, Bengalis, Mahars, Kolis, Saraswats, Lohanas, and Gaurs. The only treatment available at present for thalassemia major is bone marrow transplantation, which many patients cannot afford and is limited by availability of donars. The cost of supportive care and management of a child with thalassemia major is nearly 100,000–250,000 INR/year which varies depending on the age and presence of complications [2].

As a part of the ongoing school screening programme, blood samples are collected from 10th and 12th school children in 30 selected tribal blocks in Tamil Nadu. Our Medical College Hospital receives samples from three tribal blocks around Salem namely Yercaud hills, Kolli hills and Kalrayan hills. This study aims at estimating the prevalence of Beta Thalassemia trait in these three hilly areas around salem and to validate the screening test for Beta Thalassemia trait.

MATERIALS AND METHODS

Study design: It is a cross sectional study. Ethical clearance was obtained from the institutional ethical committee. After getting parents willingness and signature in the consent form through the school principal, blood sample of 2ml each in two vacutainers (EDTA tube) are collected by the tribal Mobile Medical Unit on every mondays. Complete Blood Count, screening tests – NESTROFT (Naked Eye Single Tube Rapid Osmotic Fragility Test) and Solubility tests are carried out by Tribal Mobile Medical Unit. Second sample of screening positive cases are transported using ice carrier to Government Medical College Hospital by Tuesday.

HPLC is performed in the Biochemistry Department of Govt. Mohan Kumaramangalam Medical College, Salem to confirm screening test positive cases on every wednesdays. HPLC is performed with Bio Rad D10 Analyser available in our department. HPLC reports in excel format are sent to Tribal Medical Medical Unit, Block Medical officer, District Early Intervention Centre Medical officer and State nodal officer every week and a register is maintained in our Department. The HPLC results of samples collected from the three hilly areas around salem for period of one academic year (2018-2019) were analysed and blockwise prevalence was worked out. Sensitivity and specificity of NESTROFT test was also calculated with the datas available in our Department. Known cases of thalassemias and other hemoglobinopathies were excluded in this study.

RESULTS AND DISCUSSION

Datas of samples collected during the academic year 2018-19 was analysed. Total number of samples collected from 10^{th} and 12^{th} school children were 1,984. Total number of children, males and females screened blockwise is shown in Table-1. Blockwise number of beta thalassemia carriers identified is shown in Table-2. Blockwise prevalence of beta thalassemic trait is shown in Table-3 and a bar diagram, Figure-1. The overall prevalence in three hilly areas around salem is shown in a pie chart Figure-2.

NESTROFT test was used as a screening test for identifying Beta Thalassemia trait. Of the 1,984 samples collected, only 551 samples which were NESTROFT positive were subjected to HPLC for confirming the beta thalassemic trait status. Hb A2 of 4-9% was considered as Beta Thalassemic trait. It was further confirmed by parental screening. 85 ramdom samples which were NESTROFT negative was also subjected to HPLC to find out the sensitivity of the screening test. Screening test result by diagnosis is shown in Table-4. The sensitivity of NESTROFT test was found 100% and specificity was found to be only 25 %. It has 100% negative predictive value and 37% positive predictive value.

Table-1: Total number of children, males and females screened.

Tribal block	No. of Children screened	No. of males	No. of females
Yercaud hills	768	371	397
Kolli hills	504	247	257
Kalrayan hills	712	398	314
Total	1984	1016	968

Table-2: Blockwise number of beta thalassemia carriers

Tribal block	No. of beta thalassemia carriers	No. of male thalassemia carriers	No. of female thalassemia carriers
Yercaud hills	38	15	23
Kolli hills	19	12	7
Kalrayan hills	49	28	21
Total	106	55	51

Table-3: Blockwise prevalence of beta thalassemic trait

Tribal block	Prevalence of beta thalassemia carriers	Prevalence of male carriers	Prevalence of female carriers
Yercaud hills	4.9 %	4 %	5.8 %
Kolli hills	3.8 %	4.9 %	2.7 %
Kalrayan hills	6.9 %	7 %	6.7 %

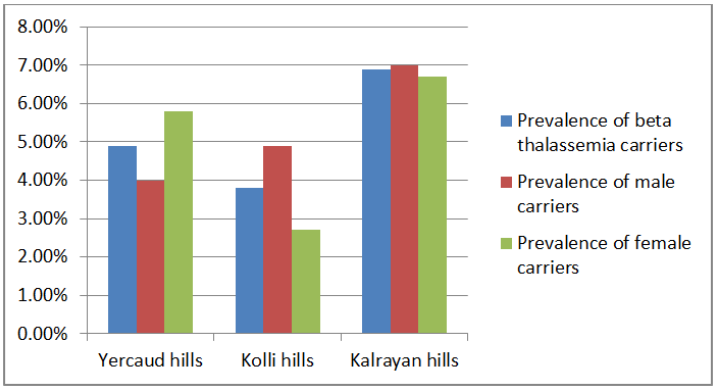

Fig-1: Bar diagram: Blockwise prevalence of beta thalassemic trait

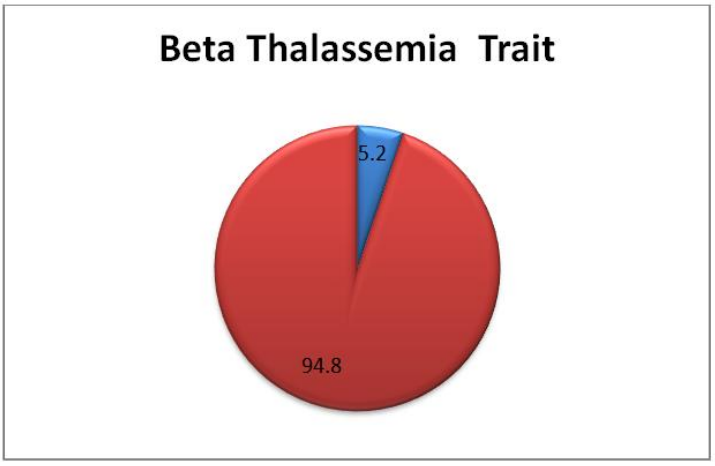

Fig-2: Pie chart: Overall prevalence in three hilly areas around salem

Table-4: Screening test- Result by Diagnosis

Screening test results	HPLC Positive	HPLC negative	total
Positive	True positive 148	False positive 257	465
Negative	False negative 0	True negative 85	85
Total	148	342	550

This study shows that the prevalence of beta thalassemia trait in hilly areas around salem was as high as 5.2%. Among the three hilly areas around salem, Kalrayan hills shows the highest prevalence of about 6.9%. Second highest prevalence of about 4.9% was found in Yercaud hills and Kolli hills showed a prevalence of 3.8%.

Complete blood count of patients with beta thalassemia trait shows low normal hemoglobin, decreased MCV (< 80 fl) and MCH (<25 pg) [4].

NESTROFT test is used as the screening test since HPLC is expensive and cannot be done for all school children. This test is based on the principle of decreased osmotic fragility of red cells in Beta Thalassemic trait. NESTROFT reagent stock solution of 10% saline is prepared by dissolving Nacl-90g, Na2HPo4-13.655g and NaH2PO42H2O-2.4g in distilled water and made upto 1 litre by adding distilled water. Working solution of 0.36%saline is prepared ideally at the time of procedure. 2 ML of buffered saline and 2ml of distilled water are taken in two test tubes. One drop of Sample is added in both test tubes and left undisturbed for half an hour and tubes are read against a white paper on which thin black lines are drawn. If the lines are visible through the contents of the test tube containing the reagent and Distilled water, the test is negative. If the lines are not visible, then the test is positive [5].

NESTROFT test fulfills the criteria of a good screening test. A good screening test must have high sensitivity, repeatability and high negative predictive value. It must be simple, safe and cost effective [6]. NESTROFT test shows 100% sensitivity and 100% negative predictive value. Specificity is only 25% since it gives false positive results in iron deficiency anaemia.

Hb A2 value of 2 – 3.4% is considered as normal. A value of 4- 9 % is considered as beta thalassemic trait while a value of 3.5 to 3.9 % is considered as unequivocal and reported as borderline beta thalassemic trait. Such cases need further evaluation by parental screening and DNA analysis. HbA2 is lower in the presence of coexistent iron deficiency anaemia or α thalassemia trait. They are at a risk of false negative diagnosis of BTT. Hence information about iron status is vital and most critical [7].

DNA analysis for beta thalassemia is done by mutation analysis for common mutations or HBB gene sequencing. The five β-globin mutations, viz. IVS1-5 (G→C), 619 bp del, IVS1-1(G→T), CD41/42 (-TCCT) and CD8/9 (+G), tend to account for more than 85 % of β-thalassaemics, which facilitates the use of a cocktail of primers for these sites as a diagnostic test for BTT by amplification-refractory mutation system (ARMS) test [8].

Our study population is at high risk of having a beta thalassemic major child who are dependent on life long frequent transfusion. Though Bone marrow transplantation is curative but it is very expensive and there are limited availability of donors. So the best way is to prevent the birth of thalassemic major child by genetic counseling. This is an autosomal recessive disorder and the disease occurs only if both the parents are carriers. This screening programme targets the premarital population so as to prevent marriages between the carriers. This can be achieved only by creating awareness about the disease burden and the way of preventing it. Though Premarital screening is the simplest and best approach for thalassemia prevention, it may not be feasible in India because of its social and cultural taboos [9].

Antenatal screening appears to be the most effective way in the present scenario. This programme also aims at screening all antenatal mothers by HPLC for carrier state. Pregnant women must be screened before 10-12 weeks of pregnancy. If mother is a carrier the father must also be screened. If both parents are carriers, prenatal diagnosis is resorted to for preventing the birth of a child affected with beta-thalassaemia or other haemoglobinopathies. This requires proper health education and adequate sensitization of the individual and community to accept these remedial measures [10].

Premarital thalassaemia screening was first carried out in 1975 by Silvestroni and colleagues in Latium, Italy, as part of a school prevention programme. Screening for sickle cell anaemia began before this, in Virginia in 1970. Nationwide screening programmes also began in Canada, Cyprus, Greece, Italy and the UK during the 1970s, with proven success [11].

Sicily is the largest island in the Mediterranean Sea and one of the 20 regions of Italy. In the 1980s, about 50 newborns per year with beta thalassemia major were observed. From 1983, in Sicily prenatal diagnosis programmes for the prevention of the most important haemoglobin disorders were developed. An article published in 2015 reviews the results of the ongoing programme for prevention of haemoglobinopathies in Sicily, through a retrospective analysis of data collected over 30 years of activities in their centre. These programmes were found to be very effective and has created a greater public awareness about thalassemia and its prevention in the target population. There was decline in the incidence of thalassaemia major and sickle cell anaemia from 1 in 245 live births in the absence of prevention to 1 in 2000, a reduction of about 85% [12].

CONCLUSION

The most cost-effective and feasible approaches are preventive genetic strategies. Major efforts need to be directed towards control by genetic counselling and prenatal diagnosis. A prerequisite for a successful prevention programme is health education, public awareness and sensitization, and screening for identification of heterozygotes or carriers. By the ongoing programme, in near future we will be able to reduce the prevalence of beta thalassemia in Tamil Nadu.

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