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RESEARCH ARTICLE
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SCREENING FOR β -THALASSEMIA TRAIT OR OTHER HEMOGLOBINOPATHIES IN FAMILIES OF THALASSEMIA MAJOR PATIENTS

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Abstract:
Objective:
Screening of extended families including first degree relative of Thalassemia major children for the presence of β Thalassemia minor or other hemoglobinopathies
Study Design: Cross-sectional descriptive study.
Setting: Punjab Thalassemia Prevention Program, Nishtar Hospital Multan.
Period: Six months,1st October 2023 to 1st April 2024.
Material and Methods:
CBC's and peripheral smears of 11310 first degree relatives from 138 families with index case of thalassemia major, were done. Those (7299) with MCV and MCH less than 75fl and 25pg respectively and had microcytic hypochromic blood picture on peripheral smear, were included in the study. Their 2cc blood sample was taken for Hb electrophoresis. Those whose Hb A2 levels came out to be $\geq$ 3.5% were labelled as beta thalassemia carriers, those with HbA2 levels 3.0-3.4% were considered inconclusive/ borderline and their serum ferritin levels were done to screen IDA. If they were iron deficient, they were given iron supplements for six month and Hb electrophoresis was repeated after 6 months.
Results:
Out of 7299 extended family members of beta thalassemia major patients.26.67% were beta thalassemia carriers (beta thalassemia trait), 68.78% were non carrier for beta thalassemia trait, 4.13% were repeat or borderline, while 0.39% have other hemoglobinopathies.
Conclusions: In third world countries like us cascade screening seems to be one of the most cost-effective approaches to identify carriers of beta thalassemia.

Introduction:

Genetic hemoglobin disorders are broadly divided into abnormal hemoglobin and thalassemia

syndromes. The abnormal hemoglobin is produced in normal amount but are structurally abnormal (structural variant) and hence lack the usual qualities of hemoglobin. Examples are HbD, HbS, HbC etc. Some hemoglobin like Hb-E in addition to being structurally abnormal are also produced in reduced amount (1)

The thalassemia syndromes are a diverse group of inborn disorders of hemoglobin synthesis in which one or more globin chains are either not produced at all or are produced in reduced amount. If α-globin chains are involved it is known as α thalassemia and if β-globin chains are involved it is known as β- thalassemia (2). The reduced synthesis of one globin chain not only results in hemoglobin deficiency but also in relative excess of the other globin chain, which is precipitated in erythroblasts and mature red blood cells and results in ineffective hematopoiesis and hemolysis (3).

β -thalassemia major in an untreated child causes very severe anemia which ultimately leads to death. However, with the advent of allogenic bone marrow transplantation and regular blood transfusions and iron-chelation, quality of life and life expectancy of thalassemic children has tremendously increased (4, 5)

Alpha thalassemia major not only results in hydrops fetalis and perinatal death but often results in life-threatening obstetric complications for the mother (6). Some patients have been saved recently by intrauterine transfusion, but there is always a high risk of severe physical and mental handicap (7).

Thalassemia carriers are asymptomatic and are mostly unaware of their abnormality and usually get detected will getting routine blood tests for some other medical condition or when they give birth to a child with thalassemia major after getting married to a person who is also a thalassemia carrier.

Thalassemia trait is characterized by microcytic hypochromic blood picture, (MCV < 75 fl and MCH < 25 pg) but high RBC count (>$5.5x10^{12}$/L). Hemoglobin either is within normal range, or slightly reduced (10-12g/dl). The diagnosis can be confirmed by hemoglobin electrophoresis that typically shows HbA2 ≥3.5% (2). HbA2 levels 3.0 to 3.4% are considered borderline and may occur as a result of faulty technique, coexisting α-thalassemia or iron deficiency (8, 9)

Less than 3% of thalassemia carriers are silent, having normal red cell indices and HbA2 levels. Routine screening methods cannot detect such carriers and PCR is required for its diagnosis (9).

Thalassemia is an autosomal recessive disorder. Both thalassemia carriers marrying each other lead to a 25% probability in each pregnancy of delivering a child with thalassemia major. Marriage of a carrier to a non-carrier will not result in thalassemia major (1,2).

Although thalassemia carriers can be identified retrospectively after the birth of the first affected child, such births can be easily avoided by premarital carrier screening. These days simple, fast, and inexpensive tests like high performance liquid chromatography (HPLC) or capillary zone electrophoresis (CE), the modern version of the classic hemoglobin electrophoresis are used for carrier screening of various hemoglobinopathies (10). These techniques can identify and quantify different hemoglobin fractions, including HbA, HbA2,HbF,HbS,HbC,HbD, and to some extent HbH **a**nd Hb Bart's. HPLC analysis should be done within seven days of sampling, as normal hemoglobin's may degrade to methemoglobins over time, leading to added peaks that prevent accurate detection of Hb types (11)

For prenatal diagnosis and genetic counseling, strategies that are PCR-based or by Southern blot can be applied to detect DNA mutations in both the parents. Modern techniques like amniocentesis performed during 16 to 20 weeks of pregnancy or chorionic villus biopsy (CVS) done during 10 to 11 weeks of pregnancy can be used to take fetal tissue samples for DNA analysis. Hemoglobin electrophoresis and DNA based analysis, can also be done on blood samples taken from fetus during the second trimester of gestation by cordocentesis (7, 1, 2).

Pakistan has one of the largest numbers of thalassemia major children in the world and providing treatment facilities to these children is far beyond the available health resources. Screening of extended families for carrier identification and genetic counseling is a practical, feasible and cost-effective approach.

The Punjab government is running a thalassemia prevention program (PTPP) which offers free of

cost prenatal diagnosis and free of cost carrier screening in the extended families of children with thalassemia major and also to the general public. It is much needed to start such programs nationwide and make screening and genetic counselling for hemoglobin disorders an intrinsic part of our health care system.

Material and Methods:

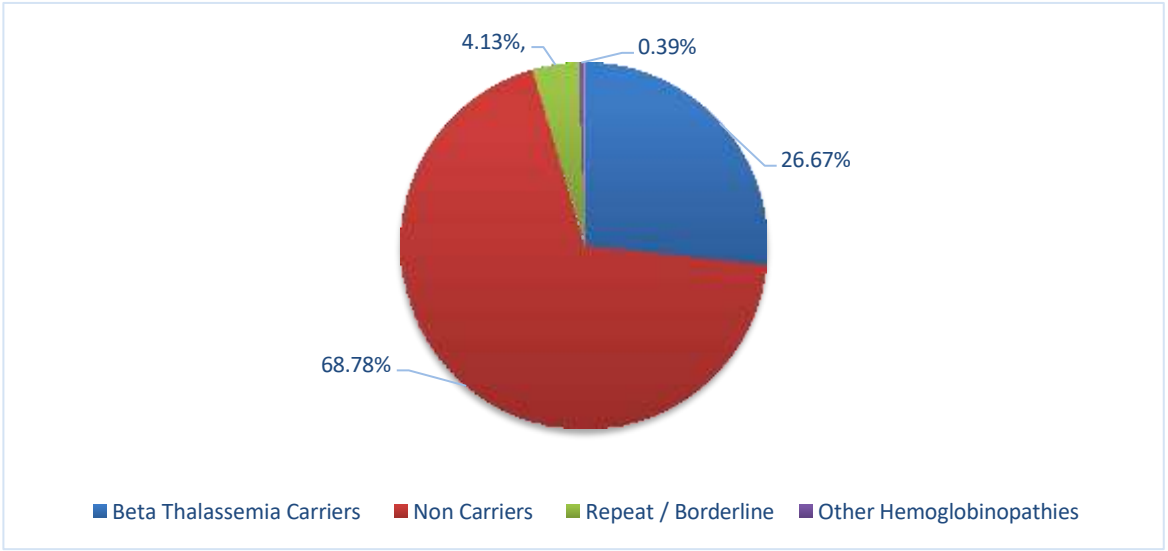
CBCs and peripheral smears of 11310 members from 138 families of first degree relatives of thalassemia major patients from 1 to 50 years of age, irrespective of their genders were done. 7299 members whose MCV and MCH was less than 75fl and 25pg respectively on CBC and had microcytic hypochromic blood picture on peripheral smear, were included in this study. Their 2 cc venous blood sample was taken in EDTA vial, to run on Bio-Rad D 10 fully automated hemoglobin testing system which detect abnormal hemoglobin variant by HPLC. Results were recorded, those with HbA2 levels ≥3.5% were labelled as beta thalassemia carriers, while those with HbA2 Levels 3.0-3.4% were considered inconclusive/ borderline and were screened for iron deficiency by measuring their serum ferritin levels. Iron deficient patients were given iron supplements for six month and Hb electrophoresis was repeated after 6 months. Those with history of previous blood transfusion were not included in the study.

Counseling sessions were held with screened carriers and their parents and importance and implications of being a carrier, inheritance of the disease, its outcomes and counseling regarding premarital screening was explained in detail. Written material in Urdu about a disease was also given to them.

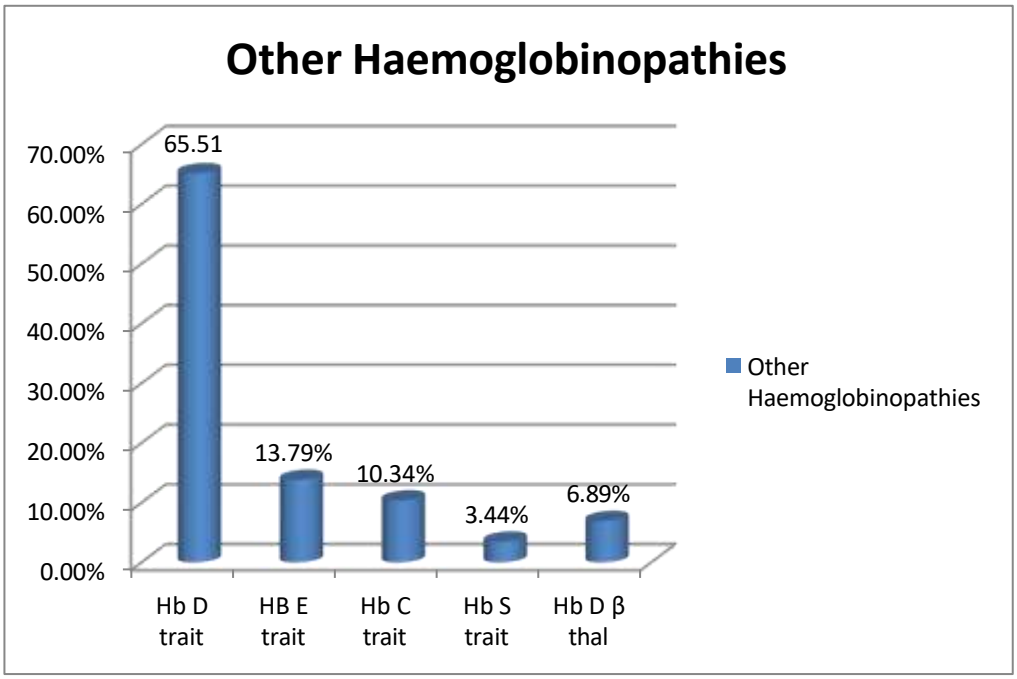
Results:

7299 first degree relatives of patients of Beta thalassemia major were screened for hemoglobinopathies. Out of these 1947 (26.67%) were beta thalassemia carriers, 5021 (68.78%) were non carrier, 302 (4.13%) were repeat or borderline, while 29 (0.39%) have other hemoglobinopathies.

Out of 29 Patients with other hemoglobinopathies 19 (65.51%) had Hb D Trait, 4 (13.79%) had E trait, 3 (10.34%) patients had C Trait, 1 (3.44%) had S trait while 2 (6.89%) patients were compound heterozygous for Hb D and beta Thalassemia.



Screening Results of 7299 First Degree Relatives of Beta Thalassemia Major Patients



Discussion:

Thalassemia major does not only affects the health of the affected child, but also has devastating psychosocial and economic effect on their families. (12)

Targeted screening, parental education, prenatal diagnosis and genetic counseling all plays an important role in reducing the burden of the disease. Cascade screening is a feasible and easy tool for detecting thalassemia carriers in a family with an index case of Thalassemia (12,13).

Our study results show carrier percentage of 27.07%. Frequency for β- Thalassemia trait is 26.67% and other hemoglobinopathies 0.39%. Ahmed *et al* reported 31% carrier rate in extended families of Thalassemia patients (8), which is very close to our study results.

Another study carried out at Thalassemia center, Children Hospital and Institute of Child Health Multan showed slightly high carrier rate of 37% (13). A total of 100 individuals from 10 thalassemia-affected families were selected for this study, while our present study was carried out on 7299 family members from 138 families with index case of thalassemia major.

In the study done in North Maharashtra India 629 (37%) carriers were identified by extended family screening of 1702 family members with index case of thalassemia major (12). Another study carried out in India identified 35% of beta thalassemia carrier in 117 extended family members from 23 families with index case of thalassemia major (13). Our present study identified 26.6% of beta thalassemia trait/carriers and 29 (0.39%) with other hemoglobinopathies in 7299 first degree relatives. Difference in study design, small sample size, regional and cultural differences may be a reason behind the high percentage of carriers detected in studies done in India. Fruitfulness of efforts of thalassemia prevention program in Pakistan is also a reason behind the low percentage of carriers detected in our present study.

Conclusion:

Findings strongly suggest that cascade screening is the most effective, useful and practical approach for detection of carriers. Genetic counseling of these carrier will definitely help to reduce the burden of disease in future.

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