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AN OUTLOOK OF HETEROZYGOUS BETA-THALASSEMIA IN FAMILIES OF TRANSFUSION DEPENDENT THALASSEMIA PATIENTS.

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ABSTRACT:

BACKGROUND: Thalassemia is an autosomal recessive illness with chronic hemolytic anemia. One of the most prevalent single-gene hereditary disorder in the world is beta-thalassemia. Prevalence of β-thalassemia patients is on rise because of the increase trend of cousin marriages, illiteracy, and younger age marriages with lack of awareness about family planning. Out of these, consanguinity is the most important factor responsible for this rising trend. **OBJECTIVE:** To determine the heterozygous status of β-thalassemia in family members of transfusion dependent thalassemia patients. **METHODOLOGY:** This cross sectional study was conducted from December 2022 to May 2023 in Pathology and Pediatric Departments of Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, and Pakistan. Convenient sampling technique was used. Blood samples of the study subjects were analyzed to determine HbF, HbA2 and HbA using Hb Electrophoresis. Peripheral blood film and Serum Ferritin were done. Carrier and non-carrier status of each subject was determined. Status of heterozygosity was determined by polymerase chain reaction and genetic analyses. The data was analyzed using SPSS version 25. **RESULTS:** Out of 200 studied family members, 98 were male and 102 were female. 143 71.5% were unmarried and 57 28.5% married. Whereas 155 77.5% were absolutely normal with non-carriers status while 45 22.5% were beta thalassemia carrier with heterozygous status. Hemoglobin status among studied individuals showed an increase in HbF 2110.5%, HbA2 4522.5% and HbA 55 27.5%. Pattern of heterozygosity revealed, 6231% in paternal cousin, followed by 4924.5% in maternal cousin, 2613% in niece, 157.5% in paternal uncle, 147% in paternal aunt, 105% in maternal aunt, 84% in maternal uncle, 63% in sister, 52.5% in nephew, 42% in sister-in-law, and 0.5% in brother. **CONCLUSION:** A high number of heterozygous beta thalassemia family members of a transfusion dependent thalassemia patient strongly suggest the screening for β-thalassemia trait and heterozygous status in all family members. Young people need to learn about their carrier status and heterozygosity as early as possible so that they can consider all of their options, including getting married and undertaking pregnancy.

KEYWORDS: Thalassemia, Heterozygous, Families, Carriers, Transfusion dependent thalassemia

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INTRODUCTION

The most prevalent inherited blood disorders that affect the structure, synthesis, and function of the hemoglobin molecule are the hemoglobinopathies.[1] Hemoglobin disorders especially thalassemia affect millions of individuals worldwide.[2] This disease is genetically inherited from parents to theirChildren, and is not a contagious. It is an autosomal recessive disorder characterizing with chronic hemolytic anemia.[3] Basic etiology is the quantitative reduction of beta globin chains of hemoglobin.[4] Almost every possible defect affecting gene expression at transcription or post-transcriptional level, including translation, have been identified in beta thalassemia. These genetic defects result in varied reductions in globin production, ranging from a slight deficiency β^+ thalassemia to complete absence β° thalassemia.[5] In β-thalassemia, the unaffected α globin genes continue to produce normal α globin chains, causing an accumulation of extra unmatched α globin in the erythroid precursors.[6] Free α globin chains cannot form functional tetramers; instead, they precipitate in the bone marrow's red cell precursors and create inclusion bodies. Thus, this illness leads to anemia and ultimate life time medical care.[7] When two carriers with heterozygosity of the thalassemia gene marry, in each pregnancy there is a 25% chance that the child will have thalassemia major, a 25% chance that the child will be healthy, and a 50% probability that the

child will develop heterozygosity with thalassemia carrier status.[8] According to the severity of the disease, the clinical signs of thalassemia can be divided into three phenotypes: transfusion-dependent thalassemia TDT, non-transfusion-dependent thalassemia NTDT, and thalassemia minor.[9] Homozygous β^o Thalassemia has severe clinical symptoms and necessitates frequent transfusions of red blood cells. Haemoglobin H illness and a few cases of hemoglobin E/β-thalassemia are examples of mild NTDT forms of thalassemia that present with moderate anemia, splenomegaly, and occasionally require red blood cell transfusions. Heterozygous thalassemia β^+ or thalassemia minor includes individuals who have no clinical symptoms and do not require transfusions.[10] Symptoms of transfusion dependent thalassemia appear between 6 and 24 months after birth. Anemia, poor nutrition, diarrhea, irritability, frequent fever attacks, abdominal distention, and a gradual enlargement of the spleen and liver are all symptoms of this condition in infants.[11] Therefore, patients have skeletal changes such as those to the long bones of the legs and changes to their faces.[12] Thalassemia minor is heterozygous, meaning that only one allele of the gene deficiency on chromosome 11 is present, while the other allele is normal. Carriers of this type of thalassemia are clinically asymptomatic aside from mild anemia.[13] Consanguineous marital trends, high birth rates, fertility rates, poor levels of education, and early marriages without use of family planning have resulted in a very high proportion of transfusion-dependent children in Pakistan.[14] The worldwide prevalence of annually affected beta thalassemia major births are being 128,667,000.[15] Globally, in approximate 1.5% of people are possessing heterozygous status showing beta thalassemia carrier characteristics.[16]

There are 9.8 million beta-thalassemia carriers worldwide, and 9000 newborns in Pakistan are born with beta-thalassemia major each year.[17] With 90,000–100,000 active patients currently suffering from Beta-Thalassemia major in our country, the average life expectancy for a patient is 10 years.[18] It was noticed that the majority of heterozygous moms gave birth to thalassemia-dependent offspring without knowing they were carriers.[19] Although expensive, Hb electrophoresis and genetic analysis are the gold standard for determining heterozygous and homozygous status; however, the cost of treatment with blood transfusions is much higher than the cost of the diagnostic approach.[20] Despite the fact that the rise in thalassemia disorder is currently difficult to stop in Pakistan due to ignorance, a lack of education, and remote health counselling facilities, a program of health education, carrier testing, genetic counselling, and easy access to prenatal diagnosis can give families complete medical information to aid in the birth of healthy children. Taking into account the available resources and inadequate facilities, this study was designed to evaluate the heterozygous status of beta thalassemia in families of transfusion dependent thalassemia patients.

OBJECTIVE

The objective of this study was to determine the heterozygous status of β-thalassemia in family members of transfusion dependent thalassemia patients.

MATERIALS AND METHODS:

This cross sectional study was conducted in Departments of Pathology and Pediatrics of Sheikh Zayed Medical College/Hospital SZMC/H, Rahim Yar Khan during December 2022 to May 2023. Convenient sampling technique was used including 200 family members of known transfusion dependent thalassemia patients. Many variables like age, gender, heterozygous status, history of consanguineous marriage, relation to thalassemia patient, residence. Complete blood count CBC with peripheral blood morphology, Hb electrophoresis and Serum Ferritin were done at SZMC/H. However PCR and genetic analysis were out sourced. The data was analyzed using SPSS version 25. The qualitative data was presented as frequencies and percentage while quantitative data as mean and standard deviation.

RESULTS

A total of 200 family members of known transfusion dependent thalassemia patients were included in the study comprising of 98 49% males and 102 51% females. Table I displayed that 71.5% of study subjects had age group between 0-20 Years. Heterozygous status among study subjects showed 22.5% studied family members were heterozygous for thalassemia gene exhibiting carrier status, area wise distribution unveiled that 14673% of study subjects were belonging to rural area while 5427% were from urban area. Table II displayed the Hb status of the study participants: HbA2 was raised in 45 individuals 22.5%. 15879% of the study subjects were not associated with consanguineous marriages while 4221% were connected with cousin marriages. Pattern of heterozygosity in relationship to patients showed 6231% in paternal cousins followed by maternal cousins 4924.5%, nieces 2613%, paternal uncles 157.5%, paternal aunts 147%, maternal aunts 105%, maternal uncles 84%, sisters 63%, nephews 52.5%, sisters in law 42% and brothers 10.5% table IV.

Table No-I: Age wise Distribution of Heterozygosity among Study Subjects

Age Group years	Frequency	Percentage
0-20 Y	143	71.5%
21-50 Y	57	28.5%
Total	200	100%

Table No-II: Distribution of Heterozygous Status among Study Subjects

Relation	Frequency	Percentage
Paternal Cousin	62	31.0%
Sister-in-law	04	2.0%
Nephew	05	2.5%
Maternal Cousin	49	24.5%
Paternal Uncle	15	7.5%
Maternal Uncle	08	4.0%
Niece	26	13.0%
Maternal Aunt	10	5.0%
Brother	01	0.5%
Sister	06	3.0%
Paternal Aunt	14	7.0%
Total	200	100.0%

Carrier Status	Frequency	Percentage
Heterozygous Carrier	45	22.5%
Normal Non-Carrier	155	77.5%
Total	200	100.0%

Table No-III: Hb Status among Study ubjects

Hb Status		N	Frequency	Percentage
HbF	**Increased**	200	21	10.5%
	Decreased		75	37.5%
	Normal		104	52.0%
HbA2	**Increased**		45	22.5%
	Decreased		10	5%
	Normal		145	72.5%
HbA	**Increased**		55	27.5%
	Decreased		41	20.5%
	Normal		104	52.0%

Table-IV: Pattern of Relationship to Patients

DISCUSSION

Affected couples with heterozygous thalassemia status are at high rsik for birth of transfusion dependent thalassemia children. Current study results show the frequency of heterozygosity among study subjects as 4522.5%. A comparative study from Ethiopia reported 35% *prevalence of heterozygosity with thalassemia carrier status.*[21] Ahmed et al reported lower percentage 31% of thalassemia carriers; heterozygosity among extended families of thalassemia patients of Pakistani families.[22] Incidence of β-thalassemia trait among the siblings was 58% in a study conducted in by Khattak et al, which is higher as compared to current study results.[23] A study conducted in Karachi found that 62.2% of the patients' siblings were -thalassemia carriers. Regional differences are present in nearby countries.[24] Another study from Bangladesh reported most common Hemoglobinopathies were β-thalassemia minor 21.3%.[25] According to a study conducted in the Rawalpindi-Islamabad region, there is a 4% carrier frequency heterozygosity in the population. However, compared to worldwide research conducted in nearby nations, the frequency revealed in the current study was higher. According to a research by Xu et al. in southern China, the prevalence was 2.54%. An investigation from Hong Kong found a prevalence of 3.4%.[26] Beta thalassemia trait was present in individuals from Azad Kashmir at a frequency of 5.26%, which is nearly identical to the frequency reported from a research conducted in Muzaffarabad 5.6%. [27] Another study conducted at District Headquarter Hospital DHQ Dera Ismail Khan reported 04 different groups based on age; highest number of individuals had abnormal hemoglobin and beta thalassemia minor. in 18-30 years of age 73.4%, followed by 31-40 years 20.2%, 41-50 years of age group 5.3, and 51-60 years of age group 1.6%.[28]

CONCLUSION:

In families of known transfusion dependent thalassemia patients a number of family members gets heterozygous status of beta thalassemia gene. Findings strongly suggest the screening of the heterozygous status in families of these patients on top priority. The young people need to know about their carrier status as early as possible so they may weigh all of their options, including getting married

and starting a family free of thalassemia disease. Targeted counselling could be an efficient way to reduce the number of children born with thalassemia major. Screening for heterozygous status among extended family members of thalassemia children could result in more carrier instances. The national thalassemia preventive programme could emphasise this tactic to make the country thassemia free.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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CONFLICT OF INTEREST: No competing interest declared.

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