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Frequency of Beta Thalassemia Trait among Pregnant Females

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ABSTRACT

Objective: To determine the frequency of Beta thalassemia trait in pregnant females

Methodology: This cross-sectional study was carried out on 200 pregnant females who presented for antenatal checkup at Shifa International Hospital for the first time from March 2024 till September 2024. Blood counts were analyzed on fully automated hematology analyzer by SYSMEX (XN-9000). High performance liquid chromatography was performed on D-10 instrument by BIORAD. The sample size was calculated with the WHO sample size calculator 2.0. After entering all the relevant parameters, the data were analyzed on SPSS Version-26.

Results: Amongst a total of 200 pregnant females selected for this study, eleven percent were diagnosed as beta thalassemia trait, and 29% had normal hemoglobin studies with concomitant iron deficiency anemia. Additionally, 1.5% were diagnosed as cases of Hemoglobin-D trait, 1% as Hemoglobin-S trait and 0.5% as Hemoglobin-E trait. Among females, who were diagnosed with beta thalassemia trait,17.6% had cousin marriage and 12.5% had positive family history of thalassemia.

Conclusion: This study establishes, that the frequency of β-thalassemia trait amongst pregnant females was alarming (11%), highlighting the importance of routine screening in antenatal clinics. A robust protocol for early identification of thalassemia carriers and to implement appropriate genetic counseling and management strategies is therefore recommended.

Keywords: High performance liquid chromatography, hemoglobinopathies, beta thalassemia trait

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [2,3]Critical analysis and manuscript review; [1,3]Data analysis; Manuscript Editing.

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Introduction

Hemoglobin is a vital component that determines the form, integrity, and half-life of red blood cells, and is essential for the binding and transport of oxygen and carbon dioxide by these cells. Beta thalassemias are the recessively inherited hematological disorders, that result from mutations that reduce or completely stop the production of beta globin chains.

There is a quantitative decrease in structurally normal β-globin chains in β-thalassemia due to a range of mutations. Surplus α globin chains copolymerize into Heinz bodies that make the red cells vulnerable to chronic hemolysis of varying disease severity.[1]

Among all hemoglobin disorders, β-thalassemia is the most prevalent single-gene disorder in Pakistan, with around 9.8 million carriers in the local population. Approximately 50,000 patients are currently receiving treatment at various centers across the country. The prevalence of the β-thalassemia trait in Pakistan is estimated to be between 5% and 8%, leading to approximately 5,000 to 9,000 births of β-thalassemia major cases each

year.[3] Thalassemia being genetic disorder, at least one of the parents must be a carrier for the disease.[1] Children with β-thalassemia major need regular blood transfusions from early childhood due to severe chronic hemolytic anemia. Iron chelation therapy is given along with regular blood transfusion to prevent iron over load, that could result in many fatal complications, such as cardiac morbidity, hepatic damage, and endocrine dysfunctions. β-thalassemia intermedia, a clinically distinct entity falls between Thalassemia trait (heterozygous) and those with β-thalassemia major (homozygous). Patients suffer from moderate anemia due to ineffective erythropoiesis.[2]

According to a report published in 2008 from the World Health Organization, more than 40,000 infants are born with β-thalassemia each year, of whom about 25,500 have transfusion-dependent beta-thalassemia. Worldwide, approximately 1.5% of people are β-thalassemia carriers. The prevalence and carrier rates of β-thalassemia are relatively high in Southeast Asia.[2] According to numerous studies, pregnant females having thalassemia trait are at risk of poor pregnancy outcomes including preterm labor, intrauterine growth restriction and low birth weight babies. Pregnant females also suffer obstetric complications like pre-eclampsia, eclampsia, gestational diabetes and placental abruption. These thalassemia carrier females must first be identified & then closely monitored for worsening of anemia and pregnancy associated complications.[4] It is important to identify couples with thalassemia trait, who are at risk of having thalassemia major child.[5] Conventional procedures including clinical findings, family history, red cell indices, complete blood counts, hemoglobin (Hb) F estimation, sickling test, and hemoglobin electrophoresis, are used to diagnose the majority of hemoglobinopathy cases in neighboring countries. The ability to diagnose specific compound heterozygous situations and to identify hemoglobin variants with the same electrophoretic mobility is limited by these traditional approaches. High-performance liquid chromatography is used to quantify and separate out different normal and aberrant hemoglobin fractions in order to solve this issue. It provides an unmatched laboratory technique for the timely and precise identification of hemoglobinopathy, supporting the management and prevention of these conditions.[6] The identification of an aberrant hemoglobin or increased levels of HbA2 (≥3.5%) for beta thalassemia carriers by HPLC is the recommended method used to diagnose thalassemia.[7] Pregnant women should ideally be tested before 10th weeks of pregnancy. Once abnormal hemoglobin study is detected in mother during antenatal testing, her spouse should also be screened, keeping in consideration of high incidence of consanguineous marriages. It is also important to offer testing to women, who present later in their pregnancy, as the results will be relevant for both their current and future pregnancies, enabling effective genetic counseling.[8] Genomic studies is the next step for molecular testing of β-thalassemia in identified carriers, that might be done to verify the carrier status of the parents, and to document the risk to the fetus and for targeted gene therapy if required.[9]

Methodology

A total of 200 pregnant females, who presented for the first time for antenatal check-up, from age ranged between 18 - 40 years, and between 6 to 12 weeks of pregnancy were enrolled in the study. Pregnant female who had history of recent transfusion within last three months and the samples received from out-reach clinics or laboratory pickup points were excluded from the study. Venous blood (3 milliliters) in vacutainer (K3-EDTA) was used for the collection of samples. Blood counts were analyzed in fully automated hematology analyzer (XN-9000). Pregnant women,

who were diagnosed as beta-thalassemia trait, their spouses were also advised to undergo screening for the disease. If both parents were found to be carriers of the beta-thalassemia trait on follow-up visits, they were counseled about the option of prenatal diagnosis in future pregnancies to prevent the birth of a child with beta-thalassemia major.

Hemoglobin concentration of less than 11 g/dL in pregnant females was labeled as anemia.[10] Anemia was classified into mild, moderate, and severe categories based on the World Health Organization guidelines: mild anemia was defined as a hemoglobin level of 9.0–10.9 g/dL, moderate anemia ranged from 7.0–8.9 g/dL, and severe anemia was characterized by a hemoglobin level of less than 7.0 g/dL [11]

HPLC was performed on D-10 instrument by BIORAD. The samples were processed within 6 hours after receiving in laboratory, if there was some delay, samples were stored at 2-8 degrees Celsius in accordance with the Bio-Rad protocol, to prevent unwanted peaks in the graph, caused by sample degradation. In cases of low hemoglobin less than 7g/dl, manual pre-dilution was performed on 12 samples. Routine controls and calibrations was performed for each run as part of the laboratory's standard procedures. The procedure is based on the principles of cation exchange chromatography and is fully automated, ensuring reproducibility. It can accurately quantify percentages of Hb A2 and Hb F in a single 6.5-minute assay, making it the most practical method for diagnosing carriers of the beta-thalassemia gene. Additionally, it aids in detecting commonly occurring abnormal hemoglobin's, such as Hb E, S, D, and C. Different hemoglobin variants are identified using specific retention time windows assigned to each variant.[12] A diagnosis of beta thalassemia trait was made when HbA2 was between 4 and 9%. Cases with HbA2 in the range of 3.8%-3.9% were reported as borderline HbA2 level.[17] The sample size was calculated with the WHO sample size calculator 2.0, keeping precision at 3% & confidence level at 95%. Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables were expressed as frequencies and percentages. For numerical data Anova test was applied. p Value <0.05 was considered statistically significant.

Results

Mean age of the study population was 28 years. Out of 200 females, 22 (11%) were diagnosed as beta thalassemia trait, 58 (29%) had normal hemoglobin studies with concomitant iron deficiency anemia.

Table I: Age and Complete blood picture findings (n=200)

CBC Parameters	Mean	Range	Standard deviation	P-value
Age (years)	28.835	20-47	5.801	0.18
WBC Count (10^3/ul)	9.62	10.02-9.64	10.885	0.77
RBC Count (10^6/ul)	4.342	3.12-7.41	.6444	.000
Hemoglobin (g/dl)	10.88	6.3-15.1	1.539	.000
Hct %	33.50	21.70-47.90	4.098	.000
MCV (fl)	78.4	53.80-97.20	43.40	.000
MCH (pg)	25.7	20-35	4.660	.000
MCHC (g/dl)	35.59	22-33.7	29.5	.000
Platelet Count (10^3/ul)	277	61-333	232	0.234
RDW-CV (%)	15.1	11.2-23	2.309	.000

3 (1.5%) were diagnosed as cases of hemoglobin D trait, 2 (1%) as hemoglobin S trait and 1 (0.5%) as hemoglobin E trait (table-I). Out of 22 case of beta thalassemia trait,6 presented with mild anemia, 11 presented with moderate anemia and 2 with severe anemia.

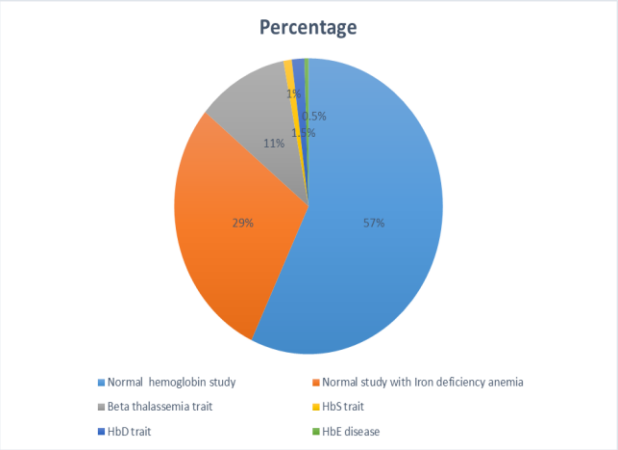

Figure 1. Frequency of hemoglobinopathies in pregnancy (n=200)

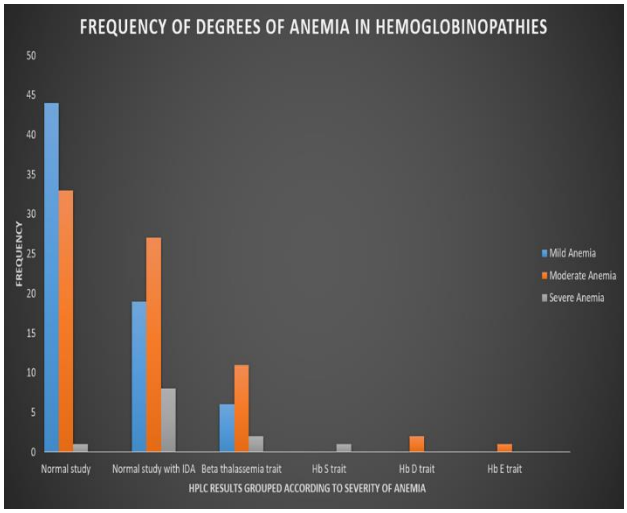

Figure 2. Frequency of degree of anemia in hemoglobinopathies

Complete blood count was performed of every individual included in the study. Mean and standard deviation was calculated (table-3). Amongst females, who were diagnosed with beta thalassemia trait, 6 (17.6%) had cousin marriage and 1 (12.5%) had positive family history of thalassemia. Out of 22 pregnant women identified as thalassemia carriers, 5 (23%) spouses were screened and were found to have normal study. The rest of the 17 (77%) spouses females who were diagnosed with thalassemia trait were counselled about the importance of screening and the option of available prenatal diagnosis, but their samples could not be secured.

Discussion

Pregnancy-related anemia has specific consequences and is unfortunately widespread in this region of the world. One of the common causes of anemia during pregnancy is the beta thalassemia trait. In developing countries including Pakistan, this represents a substantial health challenge. Beta thalassemia trait during pregnancy is related to an increase in child mortality and morbidity in low socioeconomic countries. Our prospective project also highlighted an alarming frequency (11%) of thalassemia carriers among pregnant population in the North of Pakistan

Beta thalassemia is a major public health concern in Pakistan, where approximately 9.8 million people are carriers of the disorder, with an estimated carrier rate of 5-7%.[13] Study done by Iqbal et al in Gurki teaching hospital, Lahore in 2017 showed frequency of beta thalassemia trait in pregnant females was 7.5%. This study was done by using cellulose acetate Hemoglobin electrophoresis.[13] The frequency of beta thalassemia trait in the above mentioned study was relatable with the frequency of our study, that came out to be 11% but the method used in our study was liquid chromatography. In our study mean age of the pregnant females was 28 years, that was comparable with the study done In Mardan In 2020, by Khadija et al that also showed mean age of 28 years [14]. A study led by Diyva Mangla et al in south Haryana, India showed that the prevalence of anemia in the pregnant females was 70.27% (104/148), that was comparable to our study that showed prevalence of 77% (155/200).[15] In a study conducted by Anju Sharma et al, 14 (3%) cases of beta thalassemia trait were diagnosed, 3(0.60%) cases of Hemoglobin-D Punjab, 1(0.20%) case of Hemoglobin-E and Hemoglobin-S trait and 2 (0.43%) cases of Delta Beta Thalassemia of total pregnant females that was quite similar to our variants & carrier population[16]. A study conducted in India in

2023 by Mahson et al showed, that screening of spouses were advised in 313 (7.1%) affected antenatal women, with non-compliance in 33.8% cases. In our study 17 (77%) of spouse were counselled about screening.[17]

In a study conducted by Gayani S et al in Srilanka in 2019, showed mild anemia (14.4%) in pregnant females, but in our study moderate anemia (37.5%) was more prevalent than mild anemia.[18]

A study done on pregnant females by Khan et al in 2021, at Hayatabad medical complex Peshawar revealed that 39.4% of patients, who were married to their cousins and 19.2% of patients who had non-consanguineous marriage, had beta thalassemia trait. 21.8% of individuals had thalassemia trait without a positive family history and 36.8% of patients had thalassemia trait with a family history.[19] In our study females, among the diagnosed cases of β-thalassemia trait, 17.6% had cousin marriages and 12.5% had positive family history of thalassemia. In order to reduce the burden of the disease efficiently, a multidisciplinary and comprehensive approach may be used, including premarital screening, high school and college students screening, and the targeted screening of the extended family of diagnosed cases of thalassemia. Furthermore, prenatal diagnosis of the carriers is to be taken into consideration for this large and ethnically diverse nation.

Conclusion

Our study showed alarming frequency (11%) of beta thalassemia trait among pregnant females, which would be more symptomatic, with superadded pregnancy induced anemia. Thalassemia trait has significant adverse effects on the outcome of pregnancy. Results of our study highlighted the importance of routine screening during antenatal care for early identification of carriers and to implement appropriate genetic counselling and management strategies, especially in communities like ours, with higher rates of consanguineous marriages. Collaborative efforts among obstetricians, genetic counselors, and laboratory specialists will enhance the effectiveness of the preventive screening programs, promoting informed decision-making and personalized care throughout pregnancy.

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