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Gender-Based Analysis of Anemia Prevalence and Hematological Indices in Individuals with Beta Thalassemia Trait

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ABSTRACT

Background: Beta thalassemia trait (BTT) is the heterozygous condition involving mutation in the HBB gene, characterized by hypochromic, microcytic anemia. Although hematological features of beta thalassemia trait are well-described but gender-specific variations in these features, influenced by many physiological factors remain less explored.

Objective: This study aims to identify the role of gender in defining anemia and hematological indices in BTT individuals.

Methodology: This cross-sectional study, involving 200 individuals (84 males and 116 females) was conducted at the department of hematology, Noor Thalassemia Foundation, Lahore. Blood samples were taken, and hemogram was performed and repeated hemoglobin analysis using HPLC were done for confirmation of BTT. WHO guidelines were taken in consideration while defining anemia in both genders and in different age groups. The number of males and females in different age groups and number of anemic individuals in different age groups was noted and presented as frequency (n) and percentage (%). Chi-square and Odd ratios with 95%CI were used to test the association between gender and anemia status. Independent sample t-test was used to compare the hematological indices (Hb, RBC, HCT, MCV, MCH, MCHC) between males and females.

Results: The overall prevalence of anemia in our study population was 62%. Anemia prevalence was relatively higher in females (68.9%) than males (52.3%). Hemoglobin concentration was significantly lower in females (9.75±1.40 g/dL), than males (11.35±2.12 g/dL) $p<0.001$. In the same way, females had statistically lower hematocrit (32.95±4.66%) than males (38.13±6.89%), $p<0.001$.

Conclusion: Anemia is not the defining feature of beta thalassemia trait as substantial number of BTT individual were non-anemic. The diagnosis should not rely on anemia alone rather it must involve comprehensive evaluation based on red cell indices and hemoglobin fractions.

Key words: Beta Thalassemia, Anemia, Microcytosis, Hypochromia.

Introduction

Beta thalassemia trait is the most commonly inherited monogenic disorder worldwide especially in the Mediterranean, South Asian, African and Middle Eastern Population.[1] Beta thalassemia is a disorder caused by mutation in *HBB* gene that leads to reduced (β^+) or absent beta globin chain (β^o) of adult hemoglobin (HbA).[2] This imbalance between alpha and beta chain leads to the ineffective erythropoiesis, hemolysis and varying degree of anemia.[3] Depending upon the number of mutated genes carried by the person, beta thalassemia is classified as beta thalassemia minor / beta thalassemia trait and beta thalassemia major.[4]

Beta thalassemia trait (BTT) is the heterozygous state of disease in which a person carries only one mutated beta globin gene, genetically represented as (β/β^+) or (β/β^o).[5] Individuals with beta thalassemia trait are clinically asymptomatic, often characterized by mild hypochromic microcytic anemia.[6] Diagnostic hallmarks of beta thalassemia trait involves reduced hemoglobin <12g/dL for non-pregnant females, and Hb <13g/dL

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for adult males, mean corpuscular volume (MCV <80fL) and mean corpuscular hemoglobin (MCH <27pg), and increased HbA_2 (>3.5%) on High-Performance Liquid Chromatography (HPLC).[7]

Beta thalassemia trait is the autosomal recessive disorder and affects both genders equally.[7] However, phenotypic expression of hematological indices can primarily be affected by many factors such as type of mutation inherited, age and gender of the individual. Gender is the key biological factor in influencing hematological parameters of beta thalassemia trait individuals due to varying physiological demands between males and females.[8] There are many studies reporting the role of gender in defining the hematological parameters in healthy individuals.[9] There are several physiological differences between healthy males and females that influence hemoglobin levels and red cell indices. These factors include hormonal differences, iron metabolism and nutritional status.[10] Keeping in view the normal ranges of hemoglobin in BTT males, they are usually not picked for further investigations despite having low MCV and MCH as males don't have physiological factors that contribute anemia. Females, especially those of reproductive ages are susceptible to anemia due to menstrual blood loss, pregnancy and increased iron demands. These factors may also influence the hematological indices of individuals with beta thalassemia trait and can potentially worsen the severity of anemia. A study revealed that approximately 1/3rd of the individual with beta thalassemia trait are at the risk of iron deficiency that can aggravate anemia in them.[11]

Many studies involving individuals with beta thalassemia trait focus on genetic mutations and related screening programs. However, there remains limited dataset evaluating hematological differences between males and females with beta thalassemia trait. Anemia is defined as a feature of beta thalassemia trait, while gender-based and age-wise analyses of anemia prevalence in individuals with beta thalassemia trait is limited.

This study aims to determine the prevalence of anemia among beta thalassemia trait individuals. It also aims to evaluate the role of gender in determining anemia, and also to assess how hematological indices differ between male and female individuals with beta thalassemia trait.

Materials and Methods

A prospective, cross-sectional study was conducted at Noor Thalassemia Foundation (NTF), Lahore, a thalassemia referral center. This was studied after obtaining permission from the Ethical Review Committee (ERC) of NTF (ERC reference# 23R/24, 11-08-24). A total of 200 individuals of any age with confirmed diagnosis of Beta Thalassemia trait on the basis of HPLC were recruited between September 2024 and December 2025 using consecutive sampling technique. The exclusion criteria for study included cases of known iron deficiency, other haemoglobinopathies, (HbD trait, HbE trait or HbS trait), chronic inflammatory disorders, or any history of blood transfusion.

After obtaining the informed consent, 5mL of the blood was drawn and subjected to the Complete Blood Count (CBC) using fully automated hematology analyzer (Sysmex XN-1000) and Hemoglobin electrophoresis using HPLC technique (BioRad D-10 Hemoglobin system). Anemia was defined using WHO criteria, Hb <12g/dL for non-pregnant females, and Hb <13g/dL for adult males.

The collected data was then transferred and analyzed using IBM SPSS Statistics version 26.0. Gender frequency, anemia frequency and gender wise prevalence of anemia across various age groups was noted and presented as frequency (n) and percentages (%). Association between gender and anemia status was assessed using Chi-square test. Odds ratios with 95 percent confidence intervals were calculated to evaluate the strength of association between anemia and gender among BTT subjects. The differences in hematological indices between both genders were analyzed using independent sample t-test and parameters were noted as mean±S.D. A *p*-value <0.05 was considered statistically significant.

Results

A Total of 200 individuals, diagnosed with beta thalassemia trait were included in our study. Out of which, males constituted about 42% (n=84) and females comprised of 58% (n=116). The mean age of our study participants was 27.06±14.32years. The largest proportion of participants (almost 42%) in our study population belonged to 25 to 36 years age group. In the pediatric age group, i.e., 0-12 years of age, almost 78.8% of the participants were males.

Table 1: Age group wise distribution of both genders involved in our study.

Age group	Number of Males	Number of Females	Total
0-12 years	26 (13%)	07 (3.5%)	33 (16.5%)
13-24 years	12 (6%)	32 (16%)	44 (22%)
25-36 years	29 (14.5%)	55 (27.5%)	84 (42%)
37-48 years	12 (6%)	12 (6%)	24 (12%)
>49years	05 (2.5%)	10 (5%)	15 (7.5%)
Total	84 (42%)	116 (58%)	200 (100%)

The prevalence of anemia in different age groups was assessed which revealed the highest prevalence of anemia in 0 to 12 years of age group. In the pediatric age group, 93.9% individuals (31 out of 33) were anemic. The lowest proportion of anemic individuals i.e., 46% (11 out of 24 individuals) were observed in 37–48-year age group.

Table 2: Age group wise distribution of anemia prevalence in our study.

Age group	Number of Anemic Individuals	Number of non-anemic Individuals	Total
0-12 years	31 (15.5%)	02 (1%)	33 (16.5%)
13-24 years	26 (13%)	18 (9%)	44 (22%)
25-36 years	46 (23%)	38 (19%)	84 (42%)
37-48 years	11 (5.5%)	13 (6.5%)	24 (12%)
>49years	10 (5%)	5 (2.5%)	15 (7.5%)
Total	124 (62%)	76 (38%)	200 (100%)

Gender-based analysis of anemia in different age groups revealed that the largest number of anemic males (56.8%) belonged pediatric age group. On the other hand, in females, the largest number of anemic population (48.75%) belonged to 25 to 36 years age group and the second highest percentage of anemic females (25%) were part of 13 to 24 years age group.

Table 3: Gender-based frequency of anemia among different age groups in our study population.

Age group	Number of males		Number of females	
	Number of anemic individuals	Number of non-anemic individuals	Number of anemic individuals	Number of non-anemic individuals
0-12 years	25 (56.8%)	01 (2.5%)	06 (7.5%)	01 (2.7%)
13-24 years	06 (13.6%)	06 (15%)	20 (25%)	12 (33%)
25-36 years	07 (15.9%)	22 (55%)	39 (48.75%)	16 (44%)
37-48 years	04 (9.09%)	08 (20%)	07 (8.75%)	05 (13.8%)
>49years	02 (4.5%)	03 (9.5%)	08 (10%)	02 (5.5%)
Total	44	40	80	36

Out of population of 200, anemia was found in 62% individuals (124 out of 200) while 38% individuals were non anemic (76 out of 200). Out of 116 females, 80 (69%) were found to be anemic while 36 (31%) were non anemic. Among 84 males involved in our study, 44 (52%) were anemics while 40 (48%) were non anemic males. Chi-square was applied to test association between gender and anemia. A statistically significant association was observed between gender and anemia status by applying Chi-square test, (χ^2 = 5.688, df =1, p = 0.017). Males had 50% lower odds of developing anemia as compared to females (OR=0.495, 95% CI: 0.277-0.885).

Table 4: Table of the association between anemia and gender.

	Anemics	Non-Anemics	Total	χ^2	*p*-value
No. of females	80	36	116	5.688	**0.017**
No. of males	44	40	84		
	124	76	200		

Hematological parameters between both genders were compared using independent sample t-test and it revealed statistically significant differences in many parameters. Mean hemoglobin levels were statistically higher in males (11.35±2.12 g/dL) than females (9.75±1.40 g/dL), $p<0.001$. In the same way, statistically higher hematocrit was observed in males (38.13±6.89%) as compared to females (32.95±4.66%), $p<0.001$. Statistically different HbA levels ($p= 0.005$) and HbA_2 levels ($p= 0.002$) were observed between both genders. No statistically significant differences were observed in red blood cell count, MCV, MCH, and MCHC levels in both genders, $p>0.05$

Table 5: Table showing differences in the hematological parameters between males and females' thalassemia minor subjects

Hematological Parameters	Males	Females	*p*-value
Hemoglobin(g/dL)	11.35±2.12	9.75±1.40	**<0.001**
RBC ($\times10^{12}$/L)	6.22±0.96	5.13±0.82	0.573
HCT (%)	38.13±6.89	32.95±4.66	**<0.001**
MCV (fL)	61.93±6.89	64.9±8.46	0.258
MCH (pg)	18.26±3.17	19.43±2.92	0.860
MCHC (g/dL)	29.75±1.81	29.77±1.81	0.388
HbA (%)	91.98±1.39	94.29±1.61	**0.005**
HbA_2 (%)	7.62±1.39	5.16±0.8	**0.002**
HbF (%)	0.38±1.2	0.54±1.37	0.158

Discussion

This study was conducted to evaluate the relative prevalence of anemia in males and females and gender-based differences in the hematological indices (RBC, Hb, HCT, MCV, MCH, MCHC) of individuals diagnosed with beta thalassemia trait. The findings of our study highlighted several important aspects of individuals with beta thalassemia trait such as gender-wise variability of anemia, age-wise patterns in manifestation of anemia in individuals with beta thalassemia trait.

In our study, only 62% (124 out of 200) individuals exhibited hypochromic microcytic anemia while 38% (76 out of 200) individuals were non-anemic having feature like mild hypochromia and microcytosis. This is one of the key findings of our study that highlights the fact that anemia is not the universal feature of beta thalassemia trait. Although hypochromic microcytic anemia is associated with beta thalassemia trait.[12] This finding reinforces the need that hypochromic microcytic anemia should not be used as the diagnostic hallmark of beta thalassemia trait. Since hypochromia and microcytosis was the feature exhibited by all the individuals rather than anemia, this finding suggests the significance of relying on hematological indices (reduced MCV and MCH) and hemoglobin fraction analysis, increased HbA_2 instead of hemoglobin concentrations.

Taking into consideration the prevalence of anemia in different age groups, the most prevalent age group to have anemia among BTT individuals was pediatric age group, in which 94% of the participants (31 out of 33) were anemic. Age group variation of anemia between males and females' subjects showed that the largest number of anemic males (56.8%) belonged to pediatric age group and the prevalence of anemia decreased in males with increase of age. The decline in anemia prevalence among BTT males may be attributed to improved nutritional status.[13] On the other hand around 73.7% of anemic females fell in 13 to 36 years age group. The highest prevalence of anemia among beta thalassemia trait females in the women of reproductive age group showed that women of reproductive age group are at the higher risk of anemia due to physiological and nutritional factors.[14] These factors involve menstrual blood loss, pregnancies and increased iron demands which aggravates the anemia associated with beta thalassemia trait among females.[15]

The gender-based analysis of anemia prevalence revealed that around 69% of the females in our study were anemic, while the prevalence of anemia in males was noted to be 52%. Chi-square analysis showed a significant association between gender and anemia prevalence. Results of Odds ratios (OR) showed that beta thalassemia males had two times lower odds of getting anemia than beta thalassemia females. It can be inferred from these observations that gender also plays a significant role in defining anemia among individuals with beta thalassemia trait. However, the presence of 38% (31% females and 48% males) non-anemic carriers of beta thalassemia highlighted relying on anemia as the initial diagnostic hallmark of beta thalassemia trait may lead to misdiagnosis and can decrease the efficiency of beta thalassemia screening programs.

Besides gender-based prevalence of anemia, this study also emphasized the difference in hematological parameters between males and females. Hematological parameters (Hb, RBC, HCT, MCV, MCH, MCHC) were also varied by genders. Mean Hb and HCT levels were statistically higher in males (11.35±2.12 g/dL), (38.13±6.89%) than females (9.75±1.40 g/dL), 32.95±4.66%), $p<0.001$ respectively. The observed differences between Hb and HCT levels in males and females may be associated with iron metabolism and beta thalassemia trait, rather than assuming that anemia is the inherent feature of beta thalassemia trait.[16] A study done in 2026, states that concurrent iron deficiency with beta thalassemia trait reduces the hematological parameters in individuals with beta thalassemia trait.[17] So, lower Hb and HCT levels in females were attributed to iron deficiency rather than beta thalassemia trait only.

Beyond its significance, the present study had certain limitations as it was a single center study with limited sample size, which may affect generalizability of the findings. Moreover, iron status and nutritional status of the organisms was not considered. In light of preset finding, it is recommended to conduct large-multicenter study to better evaluate the role of gender in defining anemia and hematological indices in BTT subjects. Moreover, further studies must involve molecular mutation analysis.

Conclusion

In conclusion of present findings, it can be said that anemia is not the defining feature of beta thalassemia trait as substantial number of BTT individuals were non-anemic. Anemia in beta thalassemia trait individuals varies with gender and is attributed to many factors beyond beta thalassemia trait. The diagnosis of beta thalassemia should involve comprehensive evaluation based on red cell indices and hemoglobin fractions rather than anemia. All individuals especially males with low MCV and MCH must have their HPLC done as screening of BTT despite having normal hemoglobin.

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