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ORIGINAL ARTICLE

Significance of hemogram and peripheral smear in diagnosis of suspected cases of thalassemia.

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ABSTRACT… Objective: To estimate the significance of hemogram and peripheral smear in diagnosis of suspected cases of thalassemia presented to the Farooq Hospital Lahore. **Study Design:** Cross-sectional study. **Setting:** Department of Pathology, Farooq Hospital, Westwood Lahore. **Period:** December 2023 to December 2024. **Methods:** Patients from all branches of Farooq hospital Lahore visiting for hemoglobin electrophoresis testing were included. Complete blood count was performed on automated hematology analyzer and blood smears were examined by Hematologist. The hemoglobin electrophoresis was performed on electrophoresis automated system. The IBM SPSS version 27.0 software used to analyze the collected data. **Results:** From total (n=473), majority of the patients were females (73.78%) then males (26.21%). The mean age was 19.99+14.731. The beta thalassemia trait was the most prevalent among the other variants. A substantial number of patients exhibited low MCV levels and moderate hemoglobin deficiency, as well as microcytic hypochromic, anisocytosis, poikilocytosis, and target cells. The beta thalassemia trait was associated with statistically significant red blood cells (p=0.024), poikilocytosis (p=0.001), and target cells (p=0.001). **Conclusion:** These results emphasize the significance of peripheral smear and hemogram in the diagnosis of thalassemia. This study also underscores the importance of targeted screening and diagnostic strategies to effectively identify carriers and manage affected individuals, particularly in light of the high female representation and young mean age of patients.

Key words: Beta thalassemia Major, Beta thalassemia Trait, Hemogram, Hemoglobin Variants, Peripheral Smear.

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INTRODUCTION

Around the world, there are more than forty million people who are carriers of hemoglobinopathies. Thalassemia is the most prevalent form of a single gene disorder that affects people all over the world. It is a serious genetic disease.[1] Alpha thalassemia and beta thalassemia are the two principal varieties of thalassemia. These two types of thalassemia are distinguished by the component of the globin chain that is formed in smaller amounts.[2] There are four genes that make up the alpha globin chain. A reduction in alpha globin chain synthesis is the defining characteristic of alpha thalassemia. This decrease is caused by the deletion or mutation of one or more of the four alpha globin genes that are located on chromosome 16. Mild alpha-thalassemia is another name for this condition. Examples of mild alpha-thalassemia are distinguished by the lack of two alpha-globin genes in the affected individual. Patients who have this disorder do not appear to be experiencing any symptoms; yet, they do have a low red blood cell count and moderate anemia when they are examined. They seem and feel normal, but routine checks may indicate that there is a problem with their health. It is also known as hemoglobin H disease. There was a lack of three alpha globin genes in these affected individuals. Because of the extreme anemia that they suffer from, those who are affected by the disorder typically need blood transfusions in order to continue living.[3]

Beta thalassemia is a hereditary disorder that is extremely prevalent in Pakistan.[4] It has been determined that more than two hundred mutations in the beta globin gene are responsible for the development of beta thalassemia all over the world. A mutation that was found on chromosome 11 is the root cause of beta thalassemia.[5] This disease is quite prevalent along the coast of the Arabian Sea, particularly in the provinces of South and Khyber Pakhtunkhwa, which are located close to the border with Afghanistan.[3]

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Beta thalassemia can result in a wide range of clinical manifestations, such as asymptomatic microcytic hypochromic red cells in the heterozygous state (also known as beta thalassemia trait) and severe anemia in the homozygous state (also known as beta thalassemia major), which can be fatal in the first few years of life if regular blood transfusions are not administered.[6]

According to the findings of studies, the incidence of this specific genetic problem is increased by a number of factors, including poverty, consanguinity, and ignorance of the genetic condition. There are between 90,000 and 100,000 persons in the United States who are affected by this condition. The number of younger patients is growing on a daily basis as a result of the growing burden of disease, but the number of older patients is decreasing as a result of the decreased life expectancy.[7] Some research indicated that low resources and lack of awareness also contribute to the disorder's rising prevalence. Social factors such as consanguineous marriages and marrying within ethnic groups contribute to the disease's increasing prevalence.[8] In a developing country like Pakistan, where many people suffer from beta thalassemia the cost of establishing treatment programs is too expensive. So, the alternate long-term strategy would be able to reduce the number of patients by education, couple screening before marriage, prenatal screening and genetic counseling.[9] The present study was conducted to estimate the significance of hemogram and peripheral smear in diagnosis of suspected cases of thalassemia presented to the Farooq Hospitals of Lahore.

METHODS

The Pathology department at Farooq Hospital in Westwood and the College of Allied Health Sciences at Akhtar Saeed Medical and Dental College in Lahore conducted this cross-sectional study and was carried out during the months of December 2023 and December 2024. The study was approved by the institutional review board (CAHS-10/2024-MLT-86). The non-probability convenient sampling technique was used. In this study, four hundred and seventy three patients were enrolled. Patients from all branches of Farooq Hospital Lahore (Farooq Hospital Westwood, Akhter Saeed Trust Hospital EME, Farooq Hospital DHA, and Farooq Hospital Iqbal Town) for hemoglobin electrophoresis testing (advised by the physician) were included in this study. A performa was designed to collect data from every patient. After obtaining verbal informed consent, about 03 ml of whole blood was collected from every patient in EDTA vaccutainer.

The complete blood count (CBC) was performed on MINDRAY (BC-5000) automated hematology analyzer. The blood smears of every patient were examined by the consultant Hematologist. The hemoglobin electrophoresis was performed on MINIPHOR-08 electrophoresis automated system, which follows the principle of separating electrically charged biomolecules in a solution. This instrument quantified the levels of hemoglobin A, hemoglobin A2, hemoglobin F, hemoglobin S/D, and hemoglobin H. To further differentiate between hemoglobin S and hemoglobin D, capillary electrophoresis was performed. The polymerase chain reaction (PCR) was advised for the confirmation of hemoglobin H.

The information collected was entered into an Excel spreadsheet and analyzed using Statistical Package for the Social Sciences (IBM SPSS) version 27.0. The mean and percentages were computed. The chi-square test and multivariate analysis were performed to ascertain statistically significant associations. A p-value of $p<0.05$ was deemed statistically significant.

RESULTS

In this study, the majority of patients were female (n=349, 73.78%) compared to male patients (n=124, 26.21%). The average age (± standard deviation) of the patients was 19.99 ± 14.731. The age range was three months to eighty-five years. The patients were categorized into five age categories. The participating patients were sourced from four branches of Farooq Hospital (Table-I). The majority of patients were from the outdoor (n=257, 54.33%), while others were admitted to the medical unit (n=179, 37.84%), pediatrics (n=30, 6.34%), gynecology (n=5, 1.05%), and the critical care unit (n=2, 0.42%), respectively.

TABLE-I

Characteristics and frequency of different study variables

Study Variables	Frequency (%)
Mean age (+ standard deviation)	19.99+14.731
Age groups	
• Infant (3–12 months old)	53 (11.20%)
• Toddler (1–3 years old)	71 (15.01%)
• Preschool (3-5 years old)	19 (4.01%)
School age (6–12 years old)	16 (3.38%)
Adolescents & Adults (12 to onward years old)	314 (66.38%)
Gender	
Males	124 (26.21%)
Females	349 (73.78%)
Referral branch	
Farooq Hospital Westwood	162 (34.20%)
Farooq Hospital Iqbal Town	67 (14.20%)
Akhter Saeed Trust Hospital	217 (45.90%)
Farooq Hospital DHA	27 (5.70%)
Department	
Outdoor patient	257 (54.33%)
Medical unit	179 (37.84%)
Peads	30 (6.34%)
Gynecology	05 (1.05%)
Intensive care unit	02 (0.42%)

The analysis of the CBC revealed that the mean (± standard deviation) of total red blood cells, hemoglobin, mean corpuscular volume (MCV), and red cell distribution width-standard deviation (RDW-SD) were 4.061 ± 1.931, 8.063 ± 2.863, 67.646 ± 13.044, and 47.216 ± 10.235, respectively. The CBC variables were assessed in patients in accordance with the standard ranges (Table-II). The mean (± standard deviation) values for hemoglobin A, hemoglobin A2, hemoglobin F, hemoglobin H, and hemoglobin D were 94.153 ± 15.543, 2.70 ± 1.88, 1.99 ± 13.51, 0.064 ± 1.40, and 0.77 ± 5.96, respectively.

According to the results of hemoglobin electrophoresis, the majority of patients had normal hemoglobin electrophoresis (n=420, 88.79%). The prevalence of beta thalassemia trait was determined to be 6.97% (n=33), whereas 2.11% (n=10) exhibited beta thalassemia major. 1.90% (n=9) of patients had hemoglobin D disease, while only 0.21% (n=1) had hemoglobin H disease (Figure-1).

Multivariate analysis was employed to identify the statistically significant correlation between CBC variables and total hemoglobin electrophoresis (Figure-2). Hemoglobin (p=0.001), MCV (p=0.004), and RDW-SD (p=0.016) were shown to be statistically significant.

The majority of patients had microcytic hypochromic red blood cells (n=383, 80.97%). Additional morphologies of red blood cells observed in patients are presented as well (Table-II). The chi-square test was utilized to determine the correlation between red blood cell and total hemoglobin electrophoresis. Microcytic hypochromia (p=0.003), anisocytosis (p=0.003), poikilocytosis (p=0.001), target cells (p=0.001), pencil cells (p=0.001), and tear drop cells (p=0.001) were identified as statistically significant. The chi-square test was utilized to determine the relationship of study variables with beta thalassemia major and beta thalassemia trait. Red blood cells (p=0.024), poikilocytosis (p=0.001), and target cells (p=0.001) were statistically significant in relation to beta thalassemia trait (Table-III).

FIGURE-1.

Estimation of hemoglobin electrophoresis in study patients

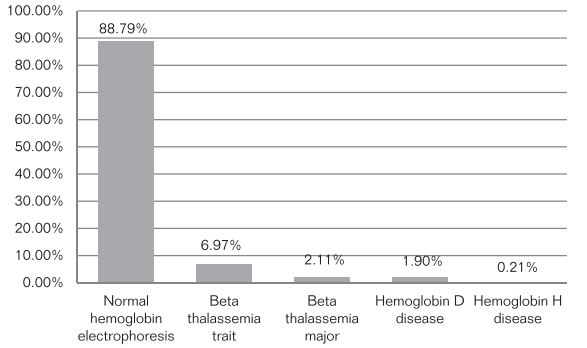

FIGURE-2

Estimated marginal means of complete blood count variables and hemoglobin electrophoresis

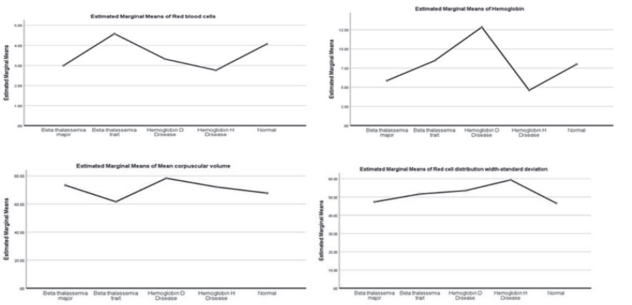

TABLE-II

Frequency and association of clinical variables in overall hemoglobin electrophoresis

Clinical Variables	Frequency (%)	P-Value
Total red blood cells (4.1-5.3 x10^6/uL)		
Normal total red blood cells (4.1-5.3 x10^6/uL)	356 (75.26%)	0.124
Low total red blood cells (< 4.1 x10^6/uL)	95 (20.08%)	
High total red blood cells (> 5.3 x10^6/uL)	22 (4.65%)	
Hemoglobin (12.0-16.0 g/dL)		
Normal hemoglobin levels (12.0-16.0 g/dL)	18 (3.80%)	0.001*
Mild deficiency (10.0-11.9 g/dL)	76 (16.06%)	
Moderate deficiency (6.1-9.9 g/dL)	286 (60.46%)	
Severe deficiency (< 6.0 g/dL)	169 (35.72%)	
Mean corpuscular volume (75-95 fL)		
Normal MCV (75-95 fL)	88 (18.60%)	0.004*
Low MCV (< 75 fL)	385 (81.39%)	
Red cell distribution width-standard deviation (39–46 fL)		
Normal RDW-SD (39–46 fL)	212 (44.82%)	0.016*
Low RDW-SD (< 39 fL)	68 (14.37%)	
High RDW-SD (> 46 fL)	193 (40.80%)	
Red blood cell morphology		
Microcytic hypochromia	383 (80.97%)	0.003*
Anisocytosis	381 (80.54%)	0.003*
Poikilocytosis	56 (11.83%)	0.001*
Target cells	51 (10.78%)	0.001*
Pencil cells	42 (8.90%)	0.001*
Tear drop	17 (3.6%)	0.001*
Macrocytic hypochromia	03 (0.63%)	1.00

*Significant p-value

DISCUSSION

Beta thalassemia, an autosomal recessive hemoglobinopathy, is one of the most common genetically transmitted disorders in the world.[10] Annually, between 5,000 to 9,000 children are born with beta thalassemia, despite lack of a documented registry in Pakistan. The projected carrier rate ranges from 5-7%, with 9.8 million carriers in the entire population.[11] In present study more females (73.78%) were tested for hemoglobin electrophoresis as compared to males (26.21%). The mean age of the patients was 19.99+14.731. The majority (66.38%) of patients were adults (12 to onward years old). The study of Aziz et al. showed male predominance, with 54.0% males and 46.0% females. The patients' ages ranged from 2 to 21 years, with a mean age of 8.33. More patients (36%) were between 6-10 years of age.[12] Another study by Faizan et al. represented more males (51%) than females (49%) with age range from 1 year to 10 years.[3] The difference in these findings represents population difference.

From total (n=10), thalassemia major patients, (80%) were males and (20%) females. The more patients (40%) were 3-12 months old. The findings of Khan et al. study are in consistent with the present study showing the male predominance (56%). The majority of beta thalassemia major patients were within the age range of 5-11 years (70%), with a smaller but significant proportion aged between 12-18 years (30%).[13] The study of Siddiqui et al. represented more males (47.8%) than females (52.2%). The average age was 11±5.2 years.[14]

TABLE-III

Frequency and association of variables in beta thalassemia major and beta thalassemia trait

Variables	Beta thalassemia Major n=10	P-Value	Beta thalassemia Trait n=33	P-Value
Age groups				
• Infant (3–12 months old)	04 (40%)	0.591	-	0.122
• Toddler (1–3 years old)	03 (30%)		06 (18.18%)	
• Preschool (3-5 years old)	-		02 (6.06%)	
School age (6–12 years old)	-		01 (3.03%)	
Adolescents & Adults (12 to onward years old)	03 (30%)		24 (72.72%)	
Gender				
Males	08 (80%)	0.800	07 (21.21%)	0.635
Females	02 (20%)		26 (78.78%)	
Hemoglobin				
Normal hemoglobin levels	-	0.591	01 (3.03%)	0.173
Mild deficiency	-		06 (18.18%)	
Moderate deficiency	02 (20%)		20 (60.60%)	
Severe deficiency	08 (80%)		06 (18.18%)	
Total red blood cells				
Normal total red blood cells	03 (30%)	0.700	18 (54.54%)	0.024*
Low total red blood cells	07 (70%)		10 (30.30%)	
High total red blood cells	-		05 (15.15%)	
Mean corpuscular volume				
Normal MCV	04 (40%)	0.483	01 (3.03%)	0.162
Low MCV	06 (60%)		32 (96.96%)	
Red cell distribution width-standard deviation				
Normal RDW-SD	07 (70%)	0.689	08 (24.24%)	0.194
Low RDW-SD	-		09 (27.27%)	
High RDW-SD	03 (30%)		16 (48.48%)	
Red blood cell morphology				
Microcytic hypochromia	07 (70%)	0.300	32 (96.96%)	0.273
Anisocytosis	07 (70%)	0.300	32 (96.96%)	0.273
Poikilocytosis	06 (60%)	0.600	26 (78.78%)	0.001*
Target cells	06 (60%)	0.600	26 (78.78%)	0.001*
Pencil cells	-	-	07 (21.21%)	0.081
Tear drop	03 (30%)	0.700	01 (3.03%)	0.273
Macrocytic hypochromia	-	-	-	-

*Significant p-value

In thalassemia trait patients of present study (n=33), more females (78.78%) were observed as compared to males (21.21%) and more (72.72%) adult (12 years to onward) patients. The study of Shakoor et al. had same findings of beta thalassemia trait representing more females (59.54%) than males (40.46%) with more patients (25.95%) belonging to 21–30 years age group.[15]

In present study, the frequency of beta thalassemia

major was found to be 2.11% while the frequency of beta thalassemia trait was 6.97%. Shakoor et al. conducted study on 805 suspected cases and found that 131 patients (16.27%) were diagnosed with beta thalassemia trait.[15] According to Mansoor et al. overall hemoglobinopathies was observed in 14.5% patients. The frequency of beta thalassemia major was 42.4% while beta thalassemia trait was observed in 57.6% of patients.[16] The frequency of hemoglobin D and hemoglobin H was 1.90% and 0.21% respectively in present study. According to Kamil et al. findings, 21.5% of patients had hemoglobin disorders. Out of 708 carriers, 19.6% showed traits of thalassemia minor, 0.36% showed Hb S trait, 1.30% showed Hb D trait, and 0.21% showed Hb E trait.[17] The study of Mansoor et al. reported the frequency of hemoglobin D of 1.2%.[16] The present study found fewer frequencies of beta thalassemia major and beta thalassemia trait as compared to the previous studies. This may suggest regional differences, ethnicity difference, and the status of consanguineous marriages, as the present study did not estimate these factors.

The present study described the significance of hemogram and peripheral smear in diagnosis of suspected cases of thalassemia presented at Hospital. The MCV and hemoglobin levels are used in the preliminary identification of microcytic anemia, which is a characteristic of beta thalassemia. The present study estimated the overall mean of total red blood cells, hemoglobin, MCV, RDW-SD were 4.061+1.931, 8.063+2.863, 67.646+13.044, and 47.216+10.235 respectively. The majority of patients had low MCV (81.39%), moderate deficiency of hemoglobin (60.46%), normal RDW-SD (44.82%), and normal total red blood cells (75.26%). The findings of present study are in consistent with Khanzada et al. study. They also presented that the (41.7%) patients had low MCV, with (46.3%) low MCH. These findings are consistent with typical characteristics of beta thalassemia trait, including a decrease in MCV and MCH due to reduced hemoglobin synthesis and the presence of unpaired globin chains in red blood cells.[18]

In present study, the red blood cells (p=0.024), poikilocytosis (p=0.001), and target cells (p=0.001) were found statistically significant with beta thalassemia trait. In study of Kamil et al. the mean hemoglobin level was 10.3 ± 2.3 in thalassemia minor, 9.3 ± 2.3 in HbS trait, 10.3 ± 3.2 in HbD trait, and 9.3 ± 2.0 in HbE trait. The mean RBC value in thalassemia was 5.1 ± 1.1, while Hb S, HbD, and HbE had values of 4.1 ± 0.9, 4.6 ± 1, and 4.3 ± 0.6. The average MCV was 65.3 ± 8.5 in thalassemia carriers, 71 ± 10.8 in HbS, 72.8 ± 14 in HbD, and 70.4 ± 9.5 in HbE.[17] In the present study, the overall mean of hemoglobin A, hemoglobin A2, hemoglobin F, hemoglobin H, and hemoglobin D was 94.153+15.543, 2.70+1.88, 1.99+13.51, 0.064+1.40, and 0.77+5.96 respectively. According to Kamil et al. the average HbA2 levels for thalassemia minor, HbS trait, HbD trait, and HbE trait were 5 ± 1.5, 2.5 ± 0.9, 1.9 ± 0.4, and 0.9 ± 1.6, respectively.[17]

The present study shows (70%) microcytic hypochromia, (70%) anisocytosis, (60%) poikilocytosis, (60%) target cells, and (30%) tear drop cells seen in thalassemia major patients. The study of Nurjanah et al. demonstrated 100% microcytic anemia, 100% hypochromic, 100% poikilocytosis dominating target cells, and 85% basophil stippling in beta thalassemia major patients.[19] The present study also shows (96.96%) microcytic hypochromia, (96.96%) anisocytosis, (78.78%) poikilocytosis, (78.78%) target cells, (21.21%) pencil cells, and (3.03%) tear drop cells seen in thalassemia trait patients. The findings of Bhabhor et al. study are relevant with the present study findings represented (100%) microcytic hypochromia, poikilocytosis, and target cells seen in beta thalassemia trait patients.[20] The frequency rates and morphological findings of present study align with global and regional trends but also highlight some differences, such as the absence of rare hemoglobin variants. The sample size is limited, and the study is single-centered, involving participants from a tertiary care hospital, therefore the generalizability of the results is constrained. More studies with relevant large sample size and more comprehensive approaches are recommended.

CONCLUSION

The present study highlights the frequency of hemoglobin variants and significance of hemogram and peripheral smear in diagnosis of beta

thalassemia. Beta thalassemia trait was the highest among other variants in present study population. A significant proportion of patients represented moderate hemoglobin deficiency and low MCV levels with microcytic hypochromia, anisocytosis, poikilocytosis and target cells. These findings underscore the importance of targeted screening and diagnostic strategies to identify carriers and manage affected individuals effectively, particularly given the high female representation and young mean age of patients. Enhanced awareness, early detection, and comprehensive care are essential for reducing the burden of beta thalassemia in our population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Atiqa Arshad:** Manuscript writing.
2	**Muhammad Rizwan Gohar:** Conceptualization.
3	**Masooma Jaffer:** Literature search.
4	**Sadia Alam:** Data analysis.
5	**Muhammad Awais:** Data interpretation.
6	**Alia Waheed:** Data collection.