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Prevalence and Hematological Profiling of Thalassemia Traits in Males from Bahawalpur, Pakistan

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Abstract:
Thalassemia, a hereditary blood disorder, is highly prevalent in Pakistan, where the carrier rate ranges from 5-7%. However, the lack of reliable epidemiological data limits effective healthcare planning. This study aimed to determine the frequency and characteristics of β-thalassemia trait among the male population of Bahawalpur. A descriptive prospective study was conducted with a sample size of 50 individuals, from three regions of Bahawalpur (Noor Mehal, Faisal Colony, and Main City). Blood samples were collected and analyzed using complete blood count (CBC), hemoglobin electrophoresis, and G6PD screening. An HbA_2 level >3.5% was used as the cut-off for β-thalassemia trait. This study highlights a measurable presence of β-thalassemia trait, anemia, and G6PD deficiency among the male population of Bahawalpur. The results showed that 8% of participants were β-thalassemia carriers, 18% were anemic, and 6% had G6PD deficiency. The highest frequency of thalassemia trait and anemia was observed in the Noor Mehal region, while G6PD deficiency was more prevalent in Faisal Colony. These findings indicate regional variation in hematological disorders and emphasize the need for targeted screening and awareness programs. Moreover, incorporation of advanced diagnostic techniques, such as molecular screening, is recommended for precise detection of thalassemia and G6PD mutations.
Keywords: Thalassemia trait, Bahawalpur, Males, G6PD, CBC, HbA2.

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INTRODUCTION

Thalassemia, a hereditary blood disorder, is classified into α and β-forms based on the location of the genetic defect affecting the expression of α- or β-globi/n genes, respectively. The heterozygous forms, α- and β-thalassemia minor or trait, are generally asymptomatic, while the homozygous states, α- and β-thalassemia major, are marked by severe anemia and other hemoglobinopathies (Iyevhobu *et al*., 2023).

Different forms of thalassemia are mainly diagnosed by screening hematological factors, such as the number and morphology of red blood cells (RBCs), and hemoglobin (Hb) fractions, particularly HbA2 level. Common clinical features of thalassemia include pallor, anemia, delayed puberty and growth, splenomegaly, and increased susceptibility to infections. Additionally, patients may also suffer from numerous psychological and mental issues, such as anxiety and depression (Meri *et al*., 2022). Since thalassemic patients undergo multiple blood transfusions, there is an increased risk of transfusion-transmissible infections like hepatitis B &C, and HIV/AIDS (Batool *et al*., 2022a).

Global rates of thalassemia traits vary depending on geographical location and population demographics; however, it is more prevalent in the Mediterranean region (the Greek term Thalassa translates to "sea"), such as tropical Africa, the Indian subcontinent, the Middle East, and parts of Asia (Kumar and Subash, 2021).

The prevalence of thalassemia in Pakistan also varies across different regions & ethnic groups and is strongly associated with consanguineous marriages (Zaheer *et al*., 2020). A study reported that more than 10 million individuals (5.0 to 7.0%) may be carriers of β-thalassemia trait across Pakistan, while 5000 children are born with β-thalassemia major annually, having a short life expectancy (Khaliq, 2022). Currently, approximately 25,000 children are registered with the Thalassemia Federation in Pakistan. The actual number is likely higher as many individuals residing in rural areas are not registered with the thalassemia centers (Khan *et al*., 2018). Moreover, the thalassemia crisis and its associated strain on Pakistan's national health care and blood transfusion systems require more attention. Unfortunately, there is no reliable clinical or epidemiological data on thalassemia in Pakistan because a national-level survey has never been conducted. Regional studies and clinical estimates outline a severe, preventable genetic health crisis in the country, and the exact burden remains unmapped. This gap needs to be filled for healthcare planning & resources allocation.

Although several studies in Bahawalpur have explored the clinical complications and genetic mutations causing thalassemia (Abbas *et al*., 2021; Batool *et al*., 2022a, 2022b) there is no report on the prevalence of thalassemia trait in the local population of Bahawalpur. Rather, researchers relied on statistics from other cities in Pakistan and neighboring countries.

Therefore, the current study aims to determine the prevalence of β-thalassemia traits in the male population of Bahawalpur and to assess the disease characteristics in this region.

MATERIALS AND METHODS

Study Design

The study was limited to adult males between 19 and 50 years of age, from various social classes, castes, and religious backgrounds. To comprehend the structure of Bahawalpur City, the whole city was divided into 3 regions, where free camps were established. Based on inclusion and exclusion criteria, a total of 50 samples were taken (Figure 1). Among these, 21 samples were taken from the Noor Mehal area, 15 from Faisal colony, and 14 from the main city region (Model town & Fareed gate).

Ethical Approval

This study was approved by the Institutional Bio-safety Committee (274/ORIC), and the Institutional Human Ethics Committee (245 /ORIC), The Islamia University of Bahawalpur,

and sampling was done with the proper consent of participants, ensuring confidentiality.

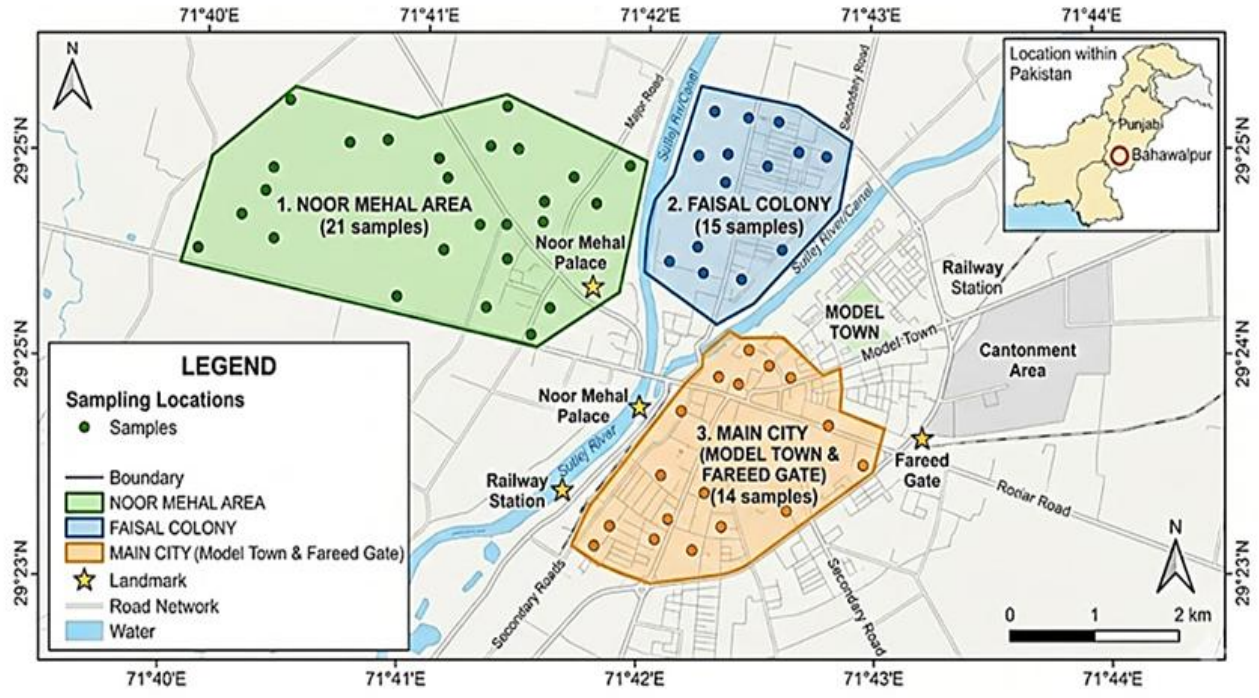

Fig. 1. Study area map showing sampling locations across three areas of Bahawalpur, Punjab, Pakistan.

Blood Sampling

A blood sample of about 4-5cc was drawn in an EDTA tube for hematological testing, including complete blood count (CBC), glucose-6-phosphate dehydrogenase (G6PD), and Hb electrophoresis. After sampling, the tubes were kept in the dark and stored at cold (4 °C).

Complete Blood Count (CBC) Analysis

1 to 2 cc of blood was properly mixed on the blood roller mixer and then examined with the CBC Analyzer (Sysmex) to get values of Hb, RBC, HCT, MCV, MCH, and MCHC.

Glucose-6-phosphate dehydrogenase (G6PD) Screening Test

Hemolytic anemia is caused by a deficit in the enzyme G6PD, which regulates the activity of RBCs. For the current study, the G6PD test was performed on a standard rapid test kit. In this procedure, a drop of blood was placed on the test kit. The appearance of purple/violet color shows a normal value of G6PD. If the color does not change (remains plain yellow/colorless), then there may be a deficiency of G6PD (Wu *et al*., 2020).

Hb Electrophoresis (Capillary Method)

Hb electrophoresis is the most common process for the detection of hemoglobinopathies and thalassemia. Capillary electrophoresis effectively separates different types of Hbs in a cation-exchange chamber, as different Hbs have different charges and different bands due to these charges (Srivorakun *et al*., 2009).

For this, 20 µL of blood was mixed with lysing solution (hemolyzing reagent), and incubated at room temperature for 10 minutes to release Hb. Then, 10 µL of the prepared sample was injected into the capillary electrophoresis apparatus, pre-calibrated with separating buffer.

When an electric current was applied, the Hb molecules moved through the capillary at varying rates depending on their size, shape, and charge. The type and amount of Hb variants (HbA, HbA2) were quantified and tracked as peaks, and the results were compared with those of the normal control.

Statistical analysis

Quantitative analysis of hematological parameters, HPLC & G6PD, was done via SPSS (version 24). All the values were evaluated in triplicate to avoid error.

RESULTS

Hematological findings from the Noor Mehal Region

Noor Mehal region comprises Hashmi Garden, Bahawalpur cantonment, Muhammadia colony, Basti Malook & Trust colony. A total of 21 cases were screened from this area, of which 3 were found to have thalassemia trait, with HbA2 levels >3.5%, used as the cut-off. Their HbA2 values were 5.9%, 4.3%, and 4.9%, respectively (Table 1). Moreover, this area also has the highest number of anemia cases (4/21) whose Hb was less than 12mg/dl.

Table 1. Complete Blood Count, HPLC & G6PD Values of Noor Mehal area.

Case No.	Hb (g/dl)	Hct (%)	RBCs (*10^12/l)	MCV (f1)	MCH (pg)	MCHC (g/dl)	HbA (%)	HbA2 (%)	G6PD
1	11.2	35.6	3.7	91	33.9	32	96.1	2.9	N
2	12.1	37	3.9	98	33.2	31.9	97.1	2.8	N
3	13.91	42	4.1	96	32.8	33.7	95.7	2.2	N
4	14.0	42.6	4.6	88	29.1	32.8	95.9	1.9	N
5	13.2	40	3.9	92.8	30.1	32	96.0	2.0	N
6	8.9	30.6	3.73	80.3	35.2	29.8	97.9	2.5	N
7	12.8	38	4.1	95.1	29.5	32.1	96.2	1.9	N
8	15.1	42	4.0	100.2	34	33.8	95.1	1.8	N
9	13.6	40.3	3.9	99.7	33.5	32.1	95.6	2.6	N
10	12.4	36	5.9	61	21	34	91.9	5.9	N
11	12.9	42.1	3.7	91	30.4	31.6	95.3	2.5	N
12	12.3	40.6	3.9	92.2	31.3	32	95.7	2.2	N
13	7.4	22.4	2.81	79.9	36.8	33	95.7	4.3	D
14	13.5	43.1	4.2	101.3	37.1	34.5	96.4	2.1	N
15	12.2	39.8	3.7	96.4	28	29.3	95.0	2.3	N
16	12.7	40.1	3.9	87.0	31	30.9	94.9	3.0	N
17	15.1	43.0	4.4	102.5	35.2	34.8	98.0	1.9	N
18	13.0	42.8	4.1	97.3	34.3	32.6	96.0	2.5	N
19	8.4	39.0	3.7	81	32.4	33	95.2	2.8	N
20	12.8	39.9	4.0	92	33.2	31.5	94.9	2.8	N
21	12.9	41.1	6.8	87.3	19.6	30.4	95.3	4.7	N

Anemia (Hb = <12mg/dl), Thalassemia trait (HbA2 >3.5%), D: Deficient, N: Normal, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration. HbA: Hemoglobin A; HbA2: Hemoglobin A2.

Prevalence of Thalassemia in Males from Faisal Colony

The Faisal colony area comprises Faisal colony sharqi & ghabi & surrounding areas like Darbar mahal, Arif town, Goheer town, etc. In this particular area, no case of thalassemia trait was found, while 2 cases of G6PD deficiency were detected. Moreover, Anemia was seen in 3 cases, making it the second-highest area for this disease (Table 2).

Hematological Parameters, Hb Electrophoresis, and G6PD Values in The Main City Area of Bahawalpur

In the main city area camp, samples were collected from Model town B, Model town C & Fareed gate. Out of the total 14 screened samples, 1 case of thalassemia trait was detected, whereas G6PD values were normal in all samples. This positive patient had Hb of 9.8, while the HbA2 level was 4.3 %. His MCV, MCH, and MCHC were also on the lower side, suggesting anemia associated with thalassemia trait (Table 3). Another male (case no. 12) was diagnosed as anemic with Hb of 9.9 g/dl and an MCV value of 103fl, which is suggestive of Megaloblastic hypochromic anemia. This case was referred to the physician for confirmation of Vitamin B12 or B6 deficiency.

Table 2. Clinical picture of samples from the Faisal Colony region.

Case No.	Hb (g/dl)	HCT (%)	RBCs (*10^12/l)	MCV (f1)	MCH (pg)	MCHC (g/dl)	HbA (%)	HbA2 (%)	G6PD
1	13.1	40.8	4.11	99.3	31.9	32.1	96.1	2.9	N
2	14	42.8	4.16	102.9	33.7	32.7	97.1	2.8	N
3	12.2	37.9	4.02	94.34	30.3	32.29	95.7	2.2	N
4	12.5	40.2	5.35	75.1	23.4	31.19	95.9	1.9	N
5	12.5	30.0	3.77	100	33.2	32.9	96.0	2.0	N
6	15.0	45.4	4.83	94.0	31.1	33	97.9	2.5	N
7	13.2	45	4.7	95	34	35	96.2	1.9	N
8	**9.9**	**34**	**3.7**	**91**	**31**	**34**	**95.1**	**1.8**	**D**
9	12.1	36.4	4.1	88.1	29.3	33.2	95.6	2.6	N
10	12.8	42.6	4.0	89.9	29.1	32.4	96.0	2.7	N
11	13	40.1	4.2	94.1	30.5	32.4	96.0	2.4	N
12	**10.7**	38	4.6	82	29	35	95.2	2.0	N
13	14.6	43.0	4.4	97.5	33.1	34.0	97.1	2.6	N
14	**9.8**	**39.9**	**4.0**	**92**	**33.2**	**31.5**	**94.9**	**2.8**	**D**
15	15.2	43.7	4.2	103.3	35.9	34.8	97.8	2.7	N

Anemia (Hb = <12mg/dl), Thalassemia trait (HbA2 >3.5%), D: Deficient, N: Normal, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular Hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, HbA: Hemoglobin A, HbA2: Hemoglobin A2.

Table 3. Main city area: Complete blood counts, HPLC & G6PD findings.

Case No.	Hb (g/dl)	HCT (%)	RBCs (*10^12/l)	MCV (f1)	MCH (pg)	MCHC (g/dl)	HbA (%)	HbA2 (%)	G6PD
1	13.8	40.7	4.0	100.2	34.0	33.9	97.0	2.7	N
2	13.5	40	4	95.5	32.1	33.5	96.3	2.8	N
3	12.1	42	4.5	97	31	34	95.3	2.1	N
4	12.8	39	4.4	87	29	35	95.5	1.9	N
5	13	46	4.7	94	27	33	96.1	2.0	N
6	14	41	3.9	90	30	32	97.1	2.8	N
7	**9.8**	38	4.1	88	32	33.4	96.2	**4.3**	N
8	14.4	39.3	4.9	88.6	27.2	33.0	97.4	2.4	N
9	13.4	37.3	4.4	83.6	26.2	32.0	96.2	3.0	N
10	12.7	40.1	3.9	87.0	31	30.9	95.0	2.3	N
11	12.8	39.9	4.0	92	33.2	31.5	95.1	2.1	N
12	**9.9**	34	3.7	**103**	31	34	95.1	2.4	N
13	13.1	41	3.3	89	33	35	94.5	2.8	N
14	12.1	38	3.0	91	31.2	34	94.8	2.2	N

Anemia (Hb = <12mg/dl), Thalassemia trait (HbA2 >3.5%), D: Deficient, N: Normal, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular Hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, HbA: Hemoglobin A, HbA2: Hemoglobin A2.

DISCUSSION

Understanding the nature and prevalence of thalassemia traits in the Bahawalpur region is critical for developing effective preventative & management methods. Furthermore, increasing awareness and education can lead to earlier detection & diagnosis, allowing for more effective disease care and eliminating the stigma associated with thalassemia, resulting in better support for people affected.

In this study, a cut-off value of HbA2 > 3.5 % was used to diagnose a patient as thalassemia trait, which was also used in many other studies (Qazi *et al*., 2014; Sadiq *et al*., 2018). We found an overall thalassemia trait in 8% cases, which aligns with findings from other studies in Pakistan. For instance, a study from Ghurki Teaching Hospital, Lahore, reported a rate of 7.5% (Khalid *et al*., 2018). While another study reported a higher prevalence (19.6%) in a tertiary care hospital setting (Kamil et al., 2021). Similarly, regional variations have been observed, with frequencies reported as 3.68% in Punjab, 5.1% in KPK, 5.35% in Sindh, 13.33% in Balochistan, and 5.26% in Azad Kashmir (Sadiq *et al*., 2018).

Thalassemia and G6PD deficiency are linked with malaria in the Mediterranean regions (Chen *et al*., 2026). Therefore, we tested all samples for G6PD and found an overall deficiency of 6% (3/50). Previously, in Karachi, 29 (8.4%) out of 342 adult male blood donors were identified to be G6PD deficient (Moiz and Brohi, 2022). Another study conducted in Quetta highlighted that 10.06% of the local population was deficient in G6PD, which was slightly higher than our findings. Research conducted between 1996 and 2017 indicated a prevalence of 3.9% (ranging 1.1–8.5%) for G6PD deficiency in Northern Pakistan. The highest incidence was recorded among Pashtun men at 5.3%, followed by Punjabis at 3.3%, Sindhis at 2.7%, and Mohajir males at 2.2% (Moiz *et al*., 2017).

In our investigation, a large population (18%) was anemic, with or without thalassemia trait, which is alarming. Iron deficiency anemia (microcytic hypochromic) and thalassemia are clinically difficult to identify. β-thalassemia minor typically results in lower MCV and MCH, whereas red blood cell counts are typically raised. Thalassemia has a normal to slightly raised RDW, unlike iron deficiency and sideroblastic anemias, which have greater RDWs (Sun *et al*., 2023). This is consistent with our findings. Moreover, the co-occurrence of thalassemia & G6PD deficiency in one case highlights the complex nature of inherited blood disorders in this population. This finding emphasizes the need for comprehensive diagnostic approaches to accurately identify and manage such cases.

CONCLUSION

A total of 50 male participants from three regions of Bahawalpur were analyzed, with the highest proportion from Noor Mehal (42%). The overall prevalence of β-thalassemia trait was 8%, anemia 18%, and G6PD deficiency 6%. Noor Mehal showed the highest occurrence of thalassemia (14.3%) and anemia (19%), while no thalassemia cases were detected in Faisal Colony. G6PD deficiency was most common in Faisal Colony (13.3%) and absent in the main city. Despite these observations, the study is limited by a small sample size (n=50), inclusion of only males, and restricted geographic sampling, which limits generalizability. Future research should involve larger, more diverse populations (including females) while strengthening premarital screening, genetic counseling, and awareness about consanguineous marriages to reduce disease burden.

CONFLICT OF INTEREST

The authors hereby declare that they have no conflict of interest.

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Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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