Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i4.4964

EVALUATION HAEMATOLOGICAL PARAMETERS WITH LIVER ENZYMES AND RENAL DYSFUNCTION MARKERS IN BETA-THALASSEMIA MAJOR PATIENTS

Asma Arshad[1], Sumera Zaib*

[1*]Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore-54590, Pakistan.

***Corresponding author:** Sumera Zaib
*E-mail: sumera.zaib@ucp.edu.pk

Abstract
Thalassemia is one of the most common genetic diseases, and beta-thalassemia major is its severe form. The present study deals with the analysis of liver function, estimation of important serum ions and hematological characteristics in beta-thalassemia patients and controls. The study included 54 patients with beta-thalassemia major each matched with 54 healthy individuals of corresponding sex and age. The levels of alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) were assessed in order to evaluate the liver function. Hemoglobin (Hb), ferritin, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), creatinine and uric acid level were also measured. Importantly, beta-thalassemia patients had higher ALT ($P < 0.001$), AST ($P < 0.05$), and ALP ($P < 0.001$) levels and lower creatinine levels ($P < 0.001$) compared to healthy controls. Higher iron levels ($P > 0.05$) were found in patients compared to healthy individuals. Hematological parameters such as Hb ($P < 0.001$), ferritin ($P < 0.05$), HCT ($P < 0.001$), MCV ($P < 0.05$), MCH ($P < 0.05$), except MCHC ($P > 0.05$) significantly measured in patients. A great difference was observed between the patients and the control group for uric acid ($P < 0.05$). Our study showed differences in liver function, renal function, serum iron content, and hematological characteristics between beta-thalassemia patients and controls.

Keywords: Beta-thalassemia major, liver function, renal function, hematological characteristics

Introduction
Thalassemia is a group of inherited blood disorders characterized by decreased synthesis or complete deficiency of one or more of the globin chains, leading to diverse clinical presentations (1). Each year 60,000 children are born with thalassemia wherein; 80% of those youngsters are born in Asian countries (2). On the basis of affected globin chain and specific alterations in amino acid sequence, thalassemia is categorized into alpha (α-), beta (β-), δβ- and δβγ- thalassemia (3). Among these types, beta-thalassemia is the most common form specifically resulting from defect in the production of the beta globulin chain and can range from clinically silent, mild to severe cases requiring regular blood transfusions (4,5). The affected individuals are unable to produce enough healthy red blood cells due to different mutations in the β globin genes, most of which involve single nucleotide substitutions, deletions, or insertions of oligonucleotides leading to frameshift mutations. In rarer cases, β-

thalassemia results from gene deletions and severity of the condition can be influenced by the type of mutation (4,6). There are three main forms of β-thalassemia syndromes based on clinical and hematologic severity: β-thalassemia carrier (trait) state, thalassemia intermedia (non-transfusion-dependent thalassemia/ thalassemia minor), and thalassemia major (transfusion-dependent thalassemia/ thalassemia major) with unresolved laboratory issues (2). This inherited blood disorder is characterized by a decrease in (β^+) or absence of (β^0) the β globin chains of the hemoglobin (Hb) tetramer (6). These unstable α-globin molecules can be harmful, causing damage to the cells and impairing the production and survival of new red blood cells. This leads to ineffective production of red blood cells and their premature destruction, resulting in anemia and reduced lifespan of red blood cells (7). The body responds to the chronic anemia caused by thalassemia by increasing the production of a hormone called erythropoietin, which stimulates the formation of red blood cells.

However, this can lead to complications such as bone marrow expansion and the formation of blood cells outside of the bone marrow (8). Iron overloading can also occur causing delayed growth, endocrine system disorders, hypothyroidism, progressive liver failure, and kidney failure. Excessive iron, among other trace metals, is implicated in causing oxidative damage to red blood cells due to the generation of free radicals. The unidirectional nature of iron metabolism in humans means that excess iron is deposited in vital organs such as the heart, liver, spleen, and endocrine organs (1,4,9). In children over 12 months of age, β-thalassemia diagnosis is confirmed by observing specific blood characteristics such as small and pale red blood cells, iron overloading, irregularly shaped red blood cells with some having nuclei when viewed under a microscope, and reduced or lack of hemoglobin A (HbA) along with higher levels of hemoglobin A2 (HbA2) and frequently hemoglobin F (HbF) during testing of hemoglobin (10).

It is particularly prevalent in the eastern and southwestern provinces of Saudi Arabia, where consanguineous marriages exceed 50%. In Pakistan, around 5,000 cases of thalassemia are diagnosed annually, with a carrier rate of approximately 5-8%. Currently, there is no national screening program in place to detect the presence of the β-thalassemia trait in Quetta, Baluchistan, although a few studies have been conducted (11,12). The incidence of the disease can be limited by testing asymptomatic individuals for carrier genes and genetic counseling (13). Newborn screening and prenatal diagnosis are crucial for patient management (14). Moreover, the diagnosis and prognosis of patients can be improved by identifying specific markers. In this research, hematological parameters along with ferritin levels, liver function and renal function markers of beta-thalassemia patients and healthy individuals are compared to evaluate their significance in the pathology of beta thalassemia.

METHODOLOGY

A total of 54 beta-thalassemia patients and 54 controls were included in this study. Patients with beta thalassemia ranged in age from 6 months to 12 years. The control group consisted of healthy children from the age of 5 to 12 years. Informed consent was obtained from the patients and they had the right to withdraw from the study at any time. The research protocol was approved by the Ethics Committee of the Sundas Foundation, Lahore. Patients were interviewed by trained staff using a standardized questionnaire (interviews were conducted with the infant's parents, but the other parents were interviewed in person).

All patients underwent a regular blood transfusion program and received deferoxamine (DFO) as a chelating agent. Moreover, participants had hematological signs of beta-thalassemia such as severe hypochromic anemia, mean corpuscular volume (MCV) <75 fl, and electrophoretic detection of hemoglobin A2 (>3.5% of total hemoglobin). The presence of the disease was also evaluated by genetic analysis, which confirmed the absence or decreased synthesis of alpha or beta chains in hemoglobin.

Sampling

All samples were collected from patients under standardized conditions. A standardized questionnaire was used to obtain information on age and education. Data and blood samples were collected over a period of 8 months (March 2023 to December 2023). Under sterile conditions, approximately 3 mL of blood was collected by venipuncture in a vial with EDTA. An aliquot (2.0 mL) of blood was collected, transferred to another tube, mixed well, and stored at -20°C until the blood count was completed. The tube containing the remaining 3 mL blood sample was then centrifuged at 3000 rpm for 15 min and plasma samples were collected in an eppendorf tube using a Pasteur pipette. For further use, the sample was stored at -20 °C until further use (15).

Biochemical tests

CBC was performed for each sample using an XP-300 (Sysmex) following the manufacturer instructions (16). Other hematological parameters, including hemoglobin (Hgb), hematocrit (HCT), RBC count, and mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell indices were also analyzed. Alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) activities were assessed using Cobas C III (Roche) according to the manufacturer instructions (17). Uric acid and creatinine levels were measured by Cobas C III (Roche) according to the manufacturer instructions (18,19). Serum ferritin levels were measured by the architect (Abbot) according to the manufacturer instructions (20).

Statistical analysis

Results were statistically analyzed using SPSS v 16.0 to find mean and standard error (± S.E.) and P value.

Results

There was a decrease in Hb ($P < 0.001$), HCT ($P < 0.001$), MCV ($P < 0.05$) and MCH ($P < 0.05$) values in beta-thalassemia sufferers in comparison to controls as shown in Figure 1. The creatinine level (0.4 ± 0.2 µg/dL) was significantly lower ($P < 0.001$) than the control group. A significant difference among patients and the control group was observed for uric acid ($P < 0.05$), indicating the renal dysfunction and requiring greater superior evaluation (Figure 2). ALT, AST, ALP levels (Figure 3) in patients with beta-thalassemia are significantly one of a kind ($P < 0.001$, $P < 0.05$, $P < 0.001$, respectively) when compared with control groups, as shown in **Table 1.**

Table 1 Haematological and biochemical parameters in β-thalassemia major patients.

Parameters (Unit)	Beta-thalassemia Mean ± SD	Control Mean ± SD	P value
Hb (g/dL)	7.2 ± 1.5	13 ± 1.4	P < 0.001
Ferritin (µg/L)	1249 ± 59 2	45 ± 17	P < 0.05
HCT (%)	21.5 ± 5.3	38 ± 6.2	P < 0.001
MCV (μm^3)	70 ± 9.5	80 ± 11	P < 0.05
MCH (pg)	23.8 ± 3.8	28 ± 5	P < 0.05
MCHC (g/dL)	34.1 ± 2.8	36.7 ± 4.6	P > 0.05
ALT (IU/L)	81.5 ± 26.8	20 ± 5.7	P < 0.001
ALP (IU/L)	257.5 ± 51.1	136 ± 29.8	P < 0.001
AST (IU/L)	74.8 ± 21.7	16.3 ± 4.1	P < 0.05
Creatinine (µg/dL)	0.4 ± 0.2	0.85 ± 0.26	P < 0.001
Uric acid (mg/dL)	5.3 ± 1.2	3.2 ± 0.7	P < 0.05

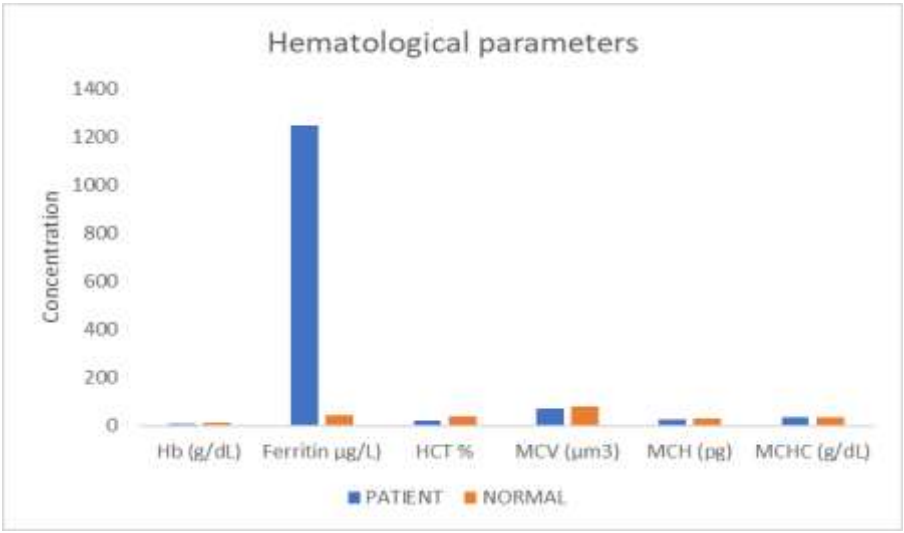

Figure 1. Comparison of hematologic parameters between normal individuals and beta thalassemia major patients.

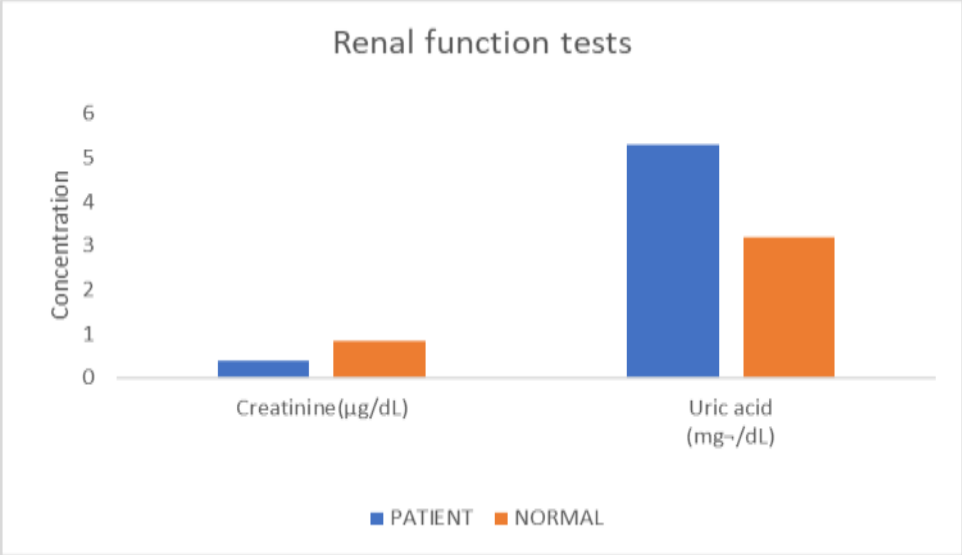

Figure 2. Renal function tests comparison between normal individuals and beta thalassemia major patients.

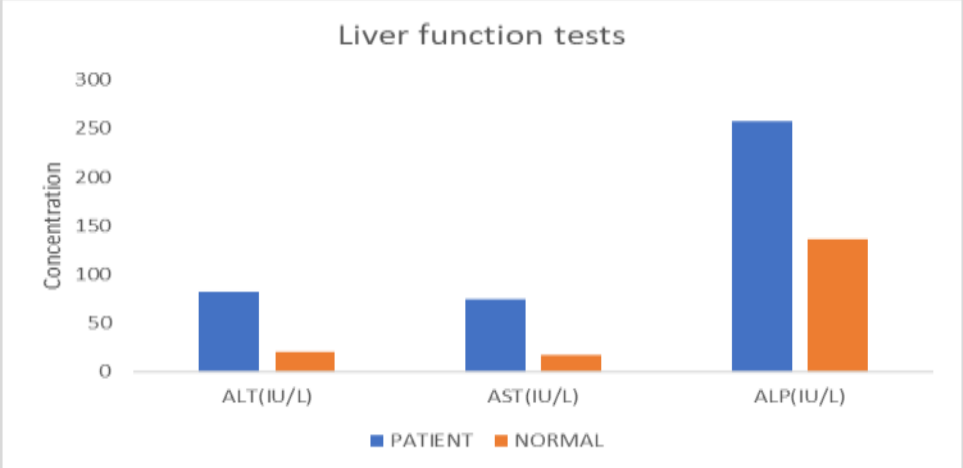

Figure 3. Liver function test comparison between normal individuals and beta thalassemia major patients.

Discussion

Thalassemia syndromes are a collection of hereditary and extreme problems caused by the alterations in thalassemia or hemoglobin Lepore genes in infancy or adolescence. Beta-thalassemia, one of the most not unusual genetic disorders in Asia (8,21), and most of the arena, has already drawn the attention of medical research (22,23). β thalassemia is observed with metabolic dysregulation, iron overloading, continual hypoxia and cell harm. All physiological modifications bring about ineffective erythropoiesis, hemolysis and anemia. Most sufferers are dependent on transfusion and bone marrow transplantation for their survival. Even though patients are actually handled with bone marrow transplantation, most of the sufferers nevertheless depend on transfusions. Normal transfusions and chelation remedy have improved the life span of these patients (24). A higher level of serum AST, ALT and ALP in patients with beta-thalassemia indicates abnormal muscle and liver function. There is a positive correlation between serum concentrations of ALT ($r = 0.315$) and AST ($r = 0.291$) and serum ferritin levels in patients with beta-thalassemia patients compared to control (25). The high creatinine level indicates very low functioning capacity of the kidney, although it needs further evidence to substantiate the present study. All hematological parameters including Hb, HCT, MCV and MCH except MCHC were found to be significantly ($P< 0.001$, $P< 0.001$, $P< 0.05$, $P< 0.05$, respectively) lower than the control (26,27).

Clinical data confirm that the decrease of the haemoglobin level is accompanied by a decrease in the number of erythrocytes and diminished values of their specific indexes (MCV, MCH, HCT, etc). An increase in serum iron and ferritin level in beta-thalassemia patients have been observed in this study, which is consistent with several other studies (28). In case of patients with beta-thalassemia, absence of beta globin chains leads to accumulation of unpaired alpha globin chains. The excess presence of alpha globin chains is a primary reason for cellular oxidative damage and also iron overload. A higher ferritin content was directly linked to the accumulation of reactive iron in the tissues of these patients. Iron overload starts another pathological mechanism leading to oxidative damage of erythrocyte membranes, the so-called "second disease"(29).

Conclusions

A significant correlations between elevated serum levels of AST, ALT, ALP, and ferritin in beta-thalassemia patients has been observed which is an indicative of abnormal liver and muscle function, as well as iron overload. Furthermore, the observed decrease in hematological parameters such as Hb, HCT, MCV, and MCH signifies the impact of beta-thalassemia on erythrocyte indices. Moreover, renal dysfunction, as evidenced by elevated creatinine levels, necessitates further investigation to fully understand its implications in these patients.

Funding

None.

Acknowledgements

None.

References

1. Aziz M, Anwar M. Prevalence Of Beta-Thalassemia Trait In Quetta City, Cross Section Study. J Univ Med Dent Coll 2015;6:21-6.
2. Xu F, Li D, Tang C, et al. Magnetic resonance imaging assessment of the changes of cardiac and hepatic iron load in thalassemia patients before and after hematopoietic stem cell transplantation. Sci Rep 2023;13:19652.
3. Munkongdee T, Chen P, Winichagoon P, et al. Update in laboratory diagnosis of thalassemia. Front Mol Biosci 2020;7:74.

4. Al-Kefaei TH, Mahdi AA, Ali HJ, et al. Estimation some of hematological parameters for thalassemia patients residing in the Al-Najaf Province. InAIP Conference Proceedings 2023 (Vol. 2977, No. 1). AIP Publishing.
5. Venou TM, Barmpageorgopoulou F, Peppa M, Vlachaki E. Endocrinopathies in beta thalassemia: a narrative review. Hormones 2023:1-2.
6. Mustafa A, Ali BA, Zulfiqar M, Naseem L. Role of discrimination indices in screening of beta thalassemia trait in low-resourced areas of Pakistan. NJHS 2019;4:21-4.
7. Tripathi N, Soni JP, Sharma PK, Verma M. Role of haemogram parameters and RBC indices in screening and diagnosis of beta-thalassemia trait in microcytic, hypochromic Indian children. Int J Hematol Disord 2015;2:43-6.
8. Kontoghiorghes GJ. The Vital Role Played by Deferiprone in the Transition of Thalassaemia from a Fatal to a Chronic Disease and Challenges in Its Repurposing for Use in Non-Iron-Loaded Diseases. Pharmaceuticals 2023;16:1016.
9. Roth IL, Lachover B, Koren G, et al. Detection of β-thalassemia carriers by red cell parameters obtained from automatic counters using mathematical formulas. Mediterr J Hematol Infect Dis 2018;10.
10. Brancaleoni V, Di Pierro E, Motta I, Cappellini MD. Laboratory diagnosis of thalassemia. Int J Lab Hematol 2016;38:32-40.
11. Jahangiri M, Rahim F, Saki N, Saki Malehi A. Application of Bayesian Decision Tree in Hematology Research: Differential Diagnosis of β-Thalassemia Trait from Iron Deficiency Anemia. Comput Math Methods Med 2021;2021:1-10.
12. Tiwari D, Thapa NB, Devkota K, Gupta SK. Clinical Profile of Children with Thalassemia Admitted for Blood Transfusion at a Tertiary Health Care Center. J Coll Med Sci Nepal 2023;19:294-98.
13. Ali S, Mumtaz S, Shakir HA, et al. Current status of beta-thalassemia and its treatment strategies. Mol Genet Genomic Med 2021;9:e1788.
14. Bonello-Palot N, Benoit A, Agouti I, et al. Transfusion requirements and complication rate in β-thalassemia intermedia due to heterozygous β-globin gene mutation and triplicated α-globin genes. Eur J Haematol 2023;111:742-7.
15. Hu Y, Mulot C, Bourreau C, et al. Biochemically tracked variability of blood plasma thawed-state exposure times in a multisite collection study. Biopreserv Biobank 2020;18:376-88.
16. Van Dievoet MA, Louagie H, Ghys T. Performance evaluation of the Sysmex® XP-300 in an oncology setting: evaluation and comparison of hematological parameters with the Sysmex® XN-3000. Int J Lab Hematol 2016;38:490-6.
17. Latiwesh OB, Younis MY, Shakila S, et al. Hepatic enzymes changes in chronic kidney disease patients-a need for modified reference values. J Evolution Med Dent Sci 2018;7:1949-54.
18. Sarangi R, Varadhan N, Bahinipati J, et al. Serum uric acid in chronic obstructive pulmonary disease: A hospital based case control study. J Clin Diagn Res 2017;11:BC09.
19. Kocak FE, Meral A, Kocak H. Assessment of serum indices implementation on Roche Cobas 6000 Analyzer. Eur J Med Sci 2014;1:43-52.
20. Wang QP, Guo LY, Lu ZY, Gu JW. Reference intervals established using indirect method for serum ferritin assayed on Abbott Architect i2000SR analyzer in Chinese adults. J Clin Lab Anal 2020;34:e23083.
21. Ebrahimi M, Mohammadi-Asl J, Rahim F. The worldwide molecular spectrum and distribution of thalassaemia: a systematic review. Ann Hum Biol 2021;48:307-12.
22. Carla MG, Rafael SP, Isabel FG, et al. New haematologic score to discriminate beta thalassemia trait from iron deficiency anaemia in a Spanish Mediterranean region. Clin Chim Acta 2020;507:69-74.
23. Gluba-Brzózka A, Franczyk B, Rysz-Górzyńska M, et al. Pathomechanisms of immunological disturbances in β-thalassemia. Int J Mol Sci 2021;22:9677.

24. Azizidoost S, Nasrolahi A, Sheykhi-Sabzehpoush M, et al. Signaling pathways governing the behaviors of leukemia stem cells. Genes Dis 2023.
25. Wickramaratne KA, Wijewickrama DC. Screening for beta-thalassemia trait; applicability of red cell indices and parameters–A study in Sri Lanka. Int J Health Sci 2021;15:29.
26. HANAN F, NAHLA AM. Evaluation of oxidative stress and antioxidant status in beta thalassemia major patients: A single-center study. Med J Cairo Univ 2020;88:2147-55.
27. Mottaghi S, Abbaszadeh H. Grape seed extract in combination with deferasirox ameliorates iron overload, oxidative stress, inflammation, and liver dysfunction in beta thalassemia children. Complement Ther Clin Pract 2023;53:101804.
28. Althanoon ZA, Altalib WH, Khalaf MM. The Effects Of Vitamin E Supplementation In Patients With Beta Thalassemia On Iron Chelation Therapy. Nat Vol Essent Oil 2021:11516-28.
29. Rakib NM, Rishad MM, Prince SM, et al. Types of Inherited Hemoglobin Disorders Among the Patients Attending Hematology Outdoor of a Tertiary Care Hospital in Bangladesh. J Med 2023;24.