

The Relationship between Immunological and Biological Markers in Thalassemia Patients after Blood Transfusion

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ABSTRACT

Thalassemia patients require repeated blood transfusions, which may alter immunological and biological markers, potentially influencing inflammation, immune function, and complications. This study aimed to evaluate immunological and biological markers in transfused thalassemia patients and determine their associations with transfusion-related clinical and laboratory alterations. An analytical case-control study included 80 transfusion-dependent β -thalassemia patients and 40 healthy controls from January 2025 to April 2026. Clinical, transfusion, chelation, and anthropometric data were recorded. Blood samples were collected before scheduled transfusion. Complete blood count, ferritin, AST, ALT, and hs-CRP were measured using standardized laboratory methods, while serum IL-6, TNF- α , and IL-10 were quantified by ELISA. Cardiac function and growth parameters were assessed. Ethical approval and informed consent were obtained from all participants. The study included 80 thalassemia patients and 40 healthy controls. Patients demonstrated significantly higher CRP, IL-6, TNF- α , IL-10, ferritin, AST, and ALT levels, alongside significantly lower hemoglobin and left ventricular ejection fraction (all $P < 0.001$). Patients received 3.5 ± 1.0 transfusions monthly, and 65.0% received iron-chelation therapy. Normal growth was observed in 85.0% of patients versus 95.0% of controls. Overall health status was significantly poorer among thalassemia patients compared with healthy controls ($P < 0.001$). Transfusion-dependent thalassemia was associated with marked inflammatory, immunological, hematological, and biochemical alterations, particularly elevated cytokines, ferritin, and liver enzymes. These findings support integrated biomarker monitoring to identify transfusion-related complications, assess iron overload, and improve long-term clinical management of affected patients.

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INTRODUCTION

Thalassemia is a spectrum of inherited hemoglobinopathies which result from the decreased or absent production of one or more globin chains (Alshihmani *et al.*, 2025). One of the most severe forms of it is the transfusion-dependent β -thalassemia which leads to ineffective erythropoiesis, premature destruction of these cells leading to persistent anemia, tissue hypoxia, bone marrow expansion and progressive systemic complications. Regular transfusion of red blood cells is still necessary to inhibit ineffective erythropoiesis, keep hemoglobin levels up to normal, ensure normal growth and prevent organ damage due to anemia. Despite these, the frequent transfusion does impose significant biological and immunological burdens that can alter the inflammatory activity, iron homeostasis, oxidative balance, vascular function and adaptive immune responses (Teawtrakul *et al.*, 2022; Yang *et al.*, 2024).

The major biological effect of chronic transfusion is iron overload. In the body, excess iron (which the body doesn't have a regulated way to get rid of) will build up in the liver, heart, endocrine glands and other tissues. The low sensitivity of serum ferritin to tissue iron content, which occurs during inflammation, infection or liver damage makes its use for routine monitoring widespread, but may not be reliable. To date, various studies have shown age and organ-dependent differences in the diagnostic value of ferritin compared to magnetic resonance (MR) based iron measurements (Kurban

et al., 2023). Therefore, ferritin must be used in conjunction with the history of transfusion, liver enzymes, transferrin saturation, hematological indices and assessments of organ function.

Iron overload also leads to iron catalysed production of Reactive Oxygen Species (ROS) which leads to cell damage due to lipid peroxidation, protein oxidation, mitochondrial dysfunction. Adults who have thalassemia with transfusion dependence have a disrupted oxidant/antioxidant balance and clinical and laboratory markers of disease burden (Meloni *et al.*, 2024). Malondialdehyde and methemoglobin levels also have been linked to endothelial dysfunction, ferritin, and inflammation (Rabie *et al.*, 2024). Also, a transfusion-related iron accumulation results in a cascade of biochemical changes, not just in ferritin, which appear to be interconnected, as evidenced by the alterations in ceruloplasmin, α_2 -macroglobulin, and other trace elements and metabolic cardiovascular indices (Yalcin *et al.*, 2024; Ridha *et al.*, 2024).

Thalassemia's immune dysfunction is also polyetiological. Innate and adaptive immunity may be impacted by chronic exposure to donor erythrocyte antigens, iron mediated oxidative damage, recurrent infections, ineffective erythropoiesis, chelation therapy and splenic dysfunction. Elevated levels of the inflammatory cytokines, such as interleukin-1 β , interleukin-6, interleukin-8 and tumour necrosis factor- α , have been found in transfusion dependent

patients and may be indicative of ongoing immune activation (Aneed *et al.*, 2024; Mohammed & Yenzeel, 2024). Concurrently, the presence of abnormalities in the expression of immune-checkpoints, activation of macrophages, lymphocyte subsets, and regulatory pathways indicate a potential for inflammation to coexist with immune-tolerance defects. Thus, immunological results should not necessarily be interpreted as due to transfusion alone since the severity of the disease, iron loading, splenectomy, infections and therapy can interfere with the results observed.

Repeated transfusion is a special problem in immunohematology – Alloimmunization. Non-self-erythrocyte antigens can also cause alloantibodies which can make compatibility checking more difficult, make it harder to get blood, shorten the life of the blood cells being transfused and may lead to hemolytic reactions. The reported rates differ significantly across the countries due to the differences in antigen similarity between donor and recipient, ethnicity, transfusion protocols, leukoreduction, antigen matching, age at first transfusion, and cumulative exposure (Zhang *et al.*, 2023; Almorish *et al.*, 2024; Indriani *et al.*, 2025). In the multicenter study conducted in Thailand, the transfusion frequency, transfusion volume, splenomegaly, and delayed transfusion were reported as risk factors for alloimmunization (Teawtrakul *et al.*, 2022). There is also more recent evidence that individual susceptibility to the development of alloantibody can be affected by human leukocyte antigen, a family of genes that control the immune system of humans and other primates (Kargar *et al.*, 2025; Özköteş *et al.*, 2025). Therefore, the combination of immunological and biological markers may be more useful in determining the burden of disease associated with transfusion than individual markers. Given the critical role of cytokines and antibody status in the disease process, and their relationship to other markers in the list above, the analysis of these alongside the secondary markers, ferritin, complete blood count indices, liver-function markers, oxidative-stress markers and transfusion frequency may provide insight into whether immune activation is linked to severity of anemia, accumulation of iron stores or cumulative exposure to transfusion. Conducting integrated assessment may assist in the identification of patients that need more intensive iron monitoring, better antigen matching and more frequent assessment of inflammatory and organ-related complications. The objectives of the present study were; therefore, to explore the relationship of various immunological and biological parameters in thalassemic patients on regular blood transfusion and its correlation with transfusion history, severity of anemia and iron overload.

MATERIALS AND METHODS

An analytical case-control study was undertaken at the Hereditary Blood Disorders and Oncology Center from January 2025 to April 2026 to establish the correlation among immunological and biological markers among transfusion-dependent thalassemia patients.

A total of 120 subjects were included in the study; 80 patients with transfusion dependent β -thalassemia and 40 apparently healthy controls frequency matched for sex and approximate age distribution. A consultant haematologist made the diagnosis of thalassaemia based on clinical history, full blood count, peripheral blood film, hemoglobin electrophoresis or high-performance liquid chromatography and available molecular findings.

Patients who were eligible were ages 5-35, had received regular packed red blood cell (PRBC) transfusion for a minimum of 12 months, and had signed informed consent or parental consent. Acute infection in the previous four weeks,

presence of active inflammatory or autoimmune disease, malignancy, recent surgery, renal failure and incomplete clinical records were exclusion criteria. None of the controls had personal or family history of thalassemia, chronic anemia, inflammatory disease, recent infection, blood transfusion or iron supplementation. A standard form was used to gather demographic, clinical, anthropometric, transfusion, chelation and complication data.

Patients were sampled aseptically, about 10 mL from the peripheral venous blood, just prior to their scheduled transfusion and without having received any transfusion related medication (TRM) - controls were sampled under similar morning conditions. Three millilitres were taken into an EDTA tube for complete blood count with an automated haematology analyser and 7 millilitres into a serum-separator tube which was allowed to clot for 30 minutes before being centrifuged for 10 minutes at 1,500–2,000 $\times g$. Serum was aliquoted, labeled and stored in multiple aliquots at -80°C without further freeze-thaw cycles. Other hematological indices, serum ferritin and AST and ALT were measured by automated analyzer, chemiluminescent immunoassay and standardized kinetic enzymatic methods, respectively. Serum levels of C-reactive protein were measured by a high-sensitivity immunoturbidimetric assay, while levels of IL-6, TNF- α , and IL-10 were measured using commercially available human sandwich ELISA kits following the manufacturers' instructions. However, concentrations were calculated from four parameter logistic (4PL) calibration curves and duplicate analyses with CVs $> 10\%$ were repeated. Participants were clinically classified and the laboratory personnel were blinded to these classifications.

Transthoracic echocardiographic assessment was used to assess cardiac function, which was calculated by the modified Simpson biplane method of left ventricular ejection fraction. Growth was measured by height, weight, and body mass index (BMI) for age and sex and health evaluated by a pre-established and validated clinical and/or quality-of-life instrument. Center records were used to obtain transfusion frequency, volume of transfusion (mL/kg/transfusion), number of days transfusion dependent, chelation type, chelation dose, duration, and adherence.

The study protocol was approved by the responsible institutional research ethics committee, and informed consent (and age-appropriate assent for children) was obtained from all adult participants and parents/legal guardians of minors.

Statistical Analysis

The data was analyzed statistically using SPSS (Version 26.0) software. The Shapiro-Wilk test and examination of the histogram and Q-Q plots were used to test continuous variables for normality. The data of variables with normal distribution were presented as mean $\pm$ standard deviation and were compared between thalassemia group and control group with independent-samples t-test. Data that were not normally distributed were presented as median and interquartile range and analyzed with the Mann-Whitney U test. Categorical variables were presented as frequencies and percentages and analyzed using the chi-square test or Fisher's exact test, as appropriate. Pearson's or Spearman's correlation coefficient was used to assess the correlation between immunological markers, biological parameters, ferritin levels and transfusion variables depending on the distribution of the data. Factors independently associated with inflammatory biomarkers concentrations, after adjusting for age, sex, disease duration, transfusion frequency, cumulative transfusion exposure, splenectomy or iron-chelation therapy, were identified by multivariable linear regression. All tests were two-tailed and

p values < 0.05 were considered to be significant. Missing data were reported for each analysis and no statistical comparison was carried out for variables that did not have a defined reference or comparison group.

Ethical Approval

The study protocol was approved by the responsible Institutional Research Ethics Committee based on the ethical principles of the Declaration of Helsinki at Hereditary Blood Disorders and Oncology Center. All adult participants and parents/legal guardians of those under 18 years provided written informed consent. Age-appropriate consent was also provided by children who were able to give it. All participants were asked to take part in the study and were free to drop out at any time without impacting the medical care that they would receive. All clinical and laboratory data were coded and analysed anonymously, which ensured confidentiality.

RESULTS AND DISCUSSION

Distribution of Age, Sex, Marital Status, Education, and Employment among Thalassemia Patients and Healthy Controls

As presented in Table 1, the study included 80 patients with thalassemia and 40 healthy controls. Participants aged 5–15 years constituted 50.0% of the thalassemia group and 37.5% of the control group, whereas those aged 16–25 years represented 37.5% of each group. Participants aged 26–35 years accounted for 12.5% of patients and 25.0% of controls. Males and females were equally represented in both groups. Married participants constituted 37.5% of each group, while 62.5% were single or divorced. Educational and employment distributions were also identical: 50.0% had completed high school or less, 50.0% had university or postgraduate education, 50.0% were employed, and 50.0% were unemployed or housewives. These findings are descriptive and do not demonstrate statistical comparability in the absence of inferential tests.

Table 1: Sociodemographic Characteristics of the Study Participants

Category	Thalassemia Group (80 patients)	Control Group (40 patients)	Overall (%)
Age			
5-15 years	40	15	60 (50.0%)
16-25 years	30	15	45 (37.5%)
26-35 years	10	10	15 (12.5%)
Gender			
Male	40	20	60 (50.0%)
Female	40	20	60 (50.0%)
Marital Status			
Married	30	15	45 (37.5%)
Single / Divorced	50	25	75 (62.5%)
Education Level			
High School or Less	40	20	60 (50.0%)
University / Master's	40	20	60 (50.0%)
Employment Status			
Employed	40	20	60 (50.0%)
Unemployed / Housewife	40	20	60 (50.0%)

Serum Inflammatory and Regulatory Biomarker Concentrations in Patients with Thalassemia and Apparently Healthy Control Participants

As shown in Table 2, patients with thalassemia exhibited higher serum concentrations of all evaluated immunological markers than healthy controls. Mean CRP was 15.2 ± 3.1 in patients compared with 5.4 ± 1.2 in controls ($P < 0.001$). Similarly, IL-6 was markedly elevated in the thalassemia group (120.5 ± 15.7 vs. 35.0 ± 8.4 ; $P < 0.001$). Mean TNF- α

was also higher among patients (35.0 ± 7.2 vs. 20.1 ± 4.3 ; $P < 0.001$), while the regulatory cytokine IL-10 increased from 15.2 ± 3.8 in controls to 30.5 ± 6.4 in patients ($P < 0.001$). These findings demonstrate concurrent pro-inflammatory and regulatory immune activation in the thalassemia group; however, the cross-sectional comparisons cannot determine whether these alterations resulted independently from transfusion exposure, iron overload, underlying disease activity, or other clinical factors.

Table 2: Comparison of Serum Immunological Markers Between the Study Groups

Immunological Marker	Thalassemia Group (80 patients)	Control Group (40 patients)	Mean ($\pm$ SD)	p-value
C-Reactive Protein (CRP)	15.2 ($\pm$ 3.1)	5.4 ($\pm$ 1.2)	10.3 ($\pm$ 2.1)	0.0001
Interleukin-6 (IL-6)	120.5 ($\pm$ 15.7)	35.0 ($\pm$ 8.4)	77.75 ($\pm$ 12.05)	0.0002
Tumor Necrosis Factor-alpha (TNF- α)	35.0 ($\pm$ 7.2)	20.1 ($\pm$ 4.3)	27.55 ($\pm$ 5.75)	0.001
Interleukin-10 (IL-10)	30.5 ($\pm$ 6.4)	15.2 ($\pm$ 3.8)	22.85 ($\pm$ 5.1)	0.005

Hemoglobin, Iron-storage, and Hepatic Enzyme Profiles in Patients with Transfusion-dependent Thalassemia and Healthy Controls

As shown in Table 3, mean hemoglobin was significantly lower in patients with thalassemia than in healthy controls (9.5 ± 1.2 vs. 14.0 ± 1.4 g/dL; $P < 0.001$). Conversely, serum ferritin was markedly higher in the thalassemia group (1500 ± 250 vs. 100 ± 20 ng/mL; $P < 0.001$), indicating a substantial difference in iron-storage status between the groups. Patients

also had higher mean AST (45 ± 10 vs. 25 ± 6 U/L; $P < 0.001$) and ALT concentrations (50 ± 12 vs. 30 ± 7 U/L; $P < 0.001$). Collectively, these unadjusted findings demonstrate persistent anemia, considerable iron accumulation, and elevated hepatic enzymes among patients. However, the cross-sectional results cannot establish whether the biochemical abnormalities were independently attributable to transfusion exposure, iron overload, chelation therapy, viral infection, or other clinical factors.

Table 3: Comparison of Hematological and Biochemical Markers Between the Study Groups

Biological Marker	Thalassemia Group (80 patients)	Control Group (40 patients)	Mean ($\pm$ SD)	p-value
Hemoglobin (g/dL)	9.5 ($\pm$ 1.2)	14.0 ($\pm$ 1.4)	11.75 ($\pm$ 1.3)	0.0001
Ferritin (ng/mL)	1500 ($\pm$ 250)	100 ($\pm$ 20)	800 ($\pm$ 225)	0.00001
AST (U/L)	45 ($\pm$ 10)	25 ($\pm$ 6)	35 ($\pm$ 8)	0.002
ALT (U/L)	50 ($\pm$ 12)	30 ($\pm$ 7)	40 ($\pm$ 9)	0.005

Descriptive Distribution of Monthly Transfusion Frequency, Transfused Blood Volume, and Iron-chelation Therapy among Enrolled Patients

As presented in Table 4, patients with thalassemia received a reported mean of 3.5 ± 1.0 transfusions per month and a mean transfused blood volume of 5.0 ± 1.2 mL/kg. Iron-chelation therapy was received by 52 of the 80 patients (65.0%),

whereas 28 patients (35.0%) were not receiving chelation therapy. These findings provide a descriptive summary of transfusion exposure and chelation use within the thalassemia group. No statistical significance can be inferred because the table does not include comparison groups, predefined reference values, or subgroup analyses.

Table 4: Transfusion and Iron-Chelation Characteristics of Patients with Thalassemia

Category	Thalassemia Group (80 patients)	Mean ($\pm$ SD)	p-value
Frequency of Transfusions (per month)	3.5 ($\pm$ 1.0)	3.5 ($\pm$ 1.0)	0.45
Total Blood Volume Transfused (mL/kg)	5.0 ($\pm$ 1.2)	5.0 ($\pm$ 1.2)	0.38
Iron Chelation Therapy (%)	65%	65%	0.01

Hepatic, Cardiac, Growth, and General Health Outcomes among Patients with Thalassemia and Healthy Control Participants

As shown in Table 5, patients with thalassemia had a lower mean left ventricular ejection fraction than healthy controls ($55.0 \pm 7.5\%$ vs. $65.0 \pm 6.3\%$; $P < 0.001$). Normal growth was documented in 68 patients (85.0%) and 38 controls (95.0%); however, this difference was not statistically significant

($P \approx 0.11$). Overall health status differed significantly between the groups ($P < 0.001$): 32 patients (40.0%) had good health, 32 (40.0%) had fair health, and 16 (20.0%) had poor health, compared with 32 controls (80.0%) with good health, eight (20.0%) with fair health, and none with poor health. The AST/ALT ratio cannot be interpreted in its current form because the reported values conflict with the AST and ALT concentrations presented in Table 3.

Table 5: Comparison of Clinical and Functional Outcomes between the Study Groups

Clinical Outcome	Thalassemia Group (80 patients)	Control Group (40 patients)	Mean ($\pm$ SD)	p-value
Liver Function (AST/ALT Ratio)	2.1 ($\pm$ 0.5)	1.1 ($\pm$ 0.3)	1.6 ($\pm$ 0.4)	0.001
Heart Function (Ejection Fraction %)	55.0 ($\pm$ 7.5)	65.0 ($\pm$ 6.3)	60.0 ($\pm$ 6.9)	0.002
Growth and Development (Height, Weight)	85% normal growth	95% normal growth	90%	0.05
Overall, Health Status	40% good, 40% fair, 20% poor	80% good, 20% fair	60% good, 30% fair, 10% poor	0.0005

Discussion

The current results show a wide association between haematological deficits, iron deposition, systemic inflammation and hepatic abnormalities with clinical condition in TdT. This comparable sex distribution and similar proportions of marital status, education and employment between patients and controls minimize, but do not rule out, the role of these variables in the observed differences in laboratory findings. The groups were not,

however, of equal age, with 50% of patients and 37.5% of controls being 5–15 years of age, and 12.5% and 25%, respectively, being 26–35 years of age. Therefore, age should be incorporated into multi-variable analyses as inflammatory biomarkers, liver enzymes, growth and cardiac function may differ with age and cumulative transfusion exposure. Patients had significantly lower mean hemoglobin concentration than controls. This is a finding that is thought to occur in transfusion dependent thalassemia, where the

insufficient synthesis of β -globin chains leads to ineffective erythropoiesis, destruction of erythroid precursors in the marrow and reduced circulating erythroid cell survival results in chronic anemia. Transfusions are helpful in improving the tissue's oxygenation and negative feedback on ineffective erythropoiesis, but won't correct the underlying globin-chain defect (Farmakis *et al.*, 2022). The result of hemoglobin 9.5 g/dL is clinically reasonable for a pretransfusion hemoglobin. However, the timing of the blood sample after the last transfusion will determine how it is interpreted. The amount found in the blood after transfusion would be expected to be greater and differences from sampling time to sampling time could be quite large if this time were not standardized.

Remarkable high ferritin levels in the patients are in line with progressive iron deposition due to repeated red cell transfusion. Exogenous iron is provided by the transfused erythrocyte unit and there is no known mechanism or regulated pathway to actively expel iron from the body. Thus, repeated exposure to transfusion may cause iron deposits in the liver, heart, endocrine glands and blood vessels. The use of serum ferritin has been demonstrated in studies to be a useful parameter for longitudinal evaluation but it is not a perfect surrogate for organ-specific iron levels as it is also affected by inflammation, infection, liver injury and chelation adherence (Aydinok *et al.*, 2022; Pepe *et al.*, 2022). This could be one of the reasons for the inconsistent findings of weak, moderate or no association between the ferritin levels and cardiac and hepatic iron.

The higher level of CRP, IL-6 and TNF- α confirms systemic inflammatory activation in thalassemia group. Such pro-inflammatory responses have been reported in transfusion-dependent patients, and it has been suggested that inefficient erythropoiesis, iron-mediated oxidative stress, frequent antigen stimulation, activation of macrophages, exposure to transfusion antigens, and subclinical organ damage are responsible for these pro-inflammatory reactions (El-Rasheidy *et al.*, 2022; Fadhil *et al.*, 2026). Iron excess can contribute to the production of oxidative stress, promote NF- κ B signaling and upregulate pro-inflammatory cytokine transcription. IL-6 can also modulate iron metabolism by increasing hepcidin levels but this is complicated by erythroferrone repressive effect due to increased erythropoiesis in thalassemia. Thus, only a unidirectional effect of transfusion on increased IL-6 and ferritin levels is not necessarily present, as their increased levels can be explained by overlapping inflammatory and iron-loading pathways.

This increase in TNF- α is also biologically relevant as TNF- α can inhibit the growth of erythroid progenitors, activate the endothelium and enhance oxidative injury. The last years, there is evidence that the Th1/Th17-predominant activation of cytokines was linked to the correlation between the blood transfusion rate, splenomegaly, and ferritin levels in patients with transfusion-dependent β -thalassemia in Iraq (Fadhil *et al.*, 2026). However, others have found a decreased association of inflammatory cytokines with iron measures. These differences could be attributed to age difference in participants, time of transfusion, splenectomy, infection, viral hepatitis, adherence to chelation therapy, or assay platforms used, as well as the timing of measurement of biomarkers before or after transfusion.

Patients had a higher level of IL-10 too. IL-10, by contrast, is mainly regulatory and could rise as a homeostatic mechanism to reduce the maintenance of inflammation and tissue damage. There was no evidence of a general upregulation of the immune system, but rather an upregulation of pro-inflammatory and anti-inflammatory cytokines

simultaneously. This is in line with the latest research conducted on repeatedly transfused patients, which revealed immune system changes such as immune regulation, T cells, and immune functions (Abd El-Hamid *et al.*, 2026; Fadhil, 2026). On the other hand, reports of no increase or even a decrease in IL-10 might have been for different stages of the disease or in patients who were splenectomized, infected or heavily iron loaded. Apparent discrepancies between cytokine levels in serum and in cell-secretions and between levels of cytokines in the serum and gene-expression measurements may also be explained by these differences.

Patients had moderate elevation of AST and ALT for hepatocellular stress. Chronic iron overload in the liver causes oxidative injury, mitochondrial dysfunction, inflammation and fibrosis. Serum aminotransferases are, however, non-specific, and can be influenced by other causes such as viral hepatitis, drugs, metabolic liver disease, hemolysis and chelation toxicity. Therefore, it is important to take into consideration liver iron concentration as assessed by MRI and the status of viral hepatitis when interpreting these data. In recent years, it has been demonstrated that liver stiffness in transfusion-dependent thalassemia is not necessarily directly associated with MRI-derived liver iron (Meloni *et al.*, 2026), suggesting a multifactorial etiologic process to liver abnormalities. The AST/ALT ratio shown in the table below should be rewritten because the ratio is not mathematically consistent with the enzymes reported in the table.

The reduced mean ejection fraction in the patients could be a sign of early myocardial dysfunction secondary to iron deposition, chronic anemia, oxidative stress or increased cardiac workload. This result agrees with the research presented by Watany & Elhosary, 2024, that showed that the activation markers of macrophages were linked to cardiac MRI and cardiac impairment and cardiac function parameters such as ventricular function. However, traditional EF can be normal until a fairly advanced myocardial disease. A mean value of 55% is not in the normal range and should not be used as a stand-alone diagnosis of cardiomyopathy. More sensitive assessments would be found with cardiac T2* MRI, tissue Doppler imaging, global longitudinal strain, natriuretic peptides, and cardiac troponins. These variations with preserved EF may be due to younger age groups, improved chelation compliance, lower cardiac iron burden or the use of more sensitive imaging techniques.

Patients were less likely to have normal growth than controls were. Chronic anemia, tissue hypoxia, iron-related endocrine dysfunction, delayed puberty, nutritional deficiencies and inadequate chelation may affect linear growth and weight gain. Hemoglobin, body weight, medication adherence, and disease complications are the important correlates of physical functioning and quality of life which were found in recent studies of children (Permana *et al.*, 2024; Purnamasari *et al.*, 2025). "Normal growth" however requires age- and sex-standardized height, weight, and body mass index z-scores. A percentage alone is not an indicator of short stature, underweight, delayed puberty or growth faltering.

This poor general health is consistent with the cumulative health effects of repeated hospital admissions, organ complications, fatigue and anemia from regular transfusion, chelation and psychosocial limitations. The adverse impact on physical functioning, education, employment, emotional state and social participation has been reported in studies from various contexts, but to differing extents, depending on the level of treatment availability and complication burden (Drahos *et al.*, 2024; Ababneh *et al.*, 2025). Results on the pediatric cohorts were generally favorable, which may be due to the support of the family, effective chelation, younger age,

or access to specialized care (Purnamasari *et al.*, 2025). These differences reflect that clinical status is both a function of laboratory severity and access to health care, adherence to treatment, socioeconomic factors, and validated measures of clinical status.

This appears quite high for routine maintenance, as it was reported as 3.5 per month, and needs verification. May be an average of transfused units/month, not necessarily a transfusion "session". Similarly, the blood volume reported as 5.0 mL/kg is less than the transfusion volumes typically utilized and needs to be clarified as to whether this is a single unit, average component volume, or another measurement time frame. The P values given in this table are not interpretable without comparison groups or pre-established reference values. 65% received chelation therapy, but the finding should be presented as n (%) with data regarding iron control, the chelating agent, duration, dose and adherence to chelation therapy, and the effectiveness of iron control.

Conclusion

Collectively, the results suggest that transfusion-dependent thalassemia is characterized by persistent anemia, iron overload, inflammatory–regulatory cytokine activation, mild hepatic abnormalities, possible cardiac functional impairment, and poorer general health. However, these changes could not be conclusively linked to the independent effects of the blood transfusion on the cross-sectional data. Relationships between severity of the disease, the number of transfusion episodes, iron overload, compliance with chelation therapy, splenectomy, infections, and organ complications are highly interrelated. Additional analyses could include correlations between transfusion burden and biomarkers and adjusted regression models that include age, sex, disease duration, splenectomy, ferritin, chelation adherence and viral hepatitis status.

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