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Serum Immunoglobulins and Complement Factors levels in splenectomized and nonsplenectomized β-thalassemia major patients.

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Abstract

Background: Beta-thalassemia major (β-TM) is the most common hereditary hemoglobin synthesis disorder, often requiring blood transfusions, which, along with splenectomy and iron supplementation, increases the risk of bacterial infections and alters the immune profile of affected individuals. Beta-thalassemia major (β-TM) is the most common hereditary hemoglobin synthesis disorder, often requiring blood transfusions, which, along with splenectomy and iron supplementation, increases the risk of bacterial infections and alters the immune profile of the affected individuals.
Objective: This study aimed to assess the serum concentrations of immunoglobulins (IgG, IgM and IgA) and complement components (C3 and C4) in both splenectomized and non-splenectomized β-TM patients and; correlate these levels with ferritin, blood transfusion and iron chelation therapy.
Methods: A cross-sectional comparative study involving 100 β-TM patients in Sana'a City-Yemen, was conducted, categorizing the participants into splenectomized and non-splenectomized groups. Sociodemographic data were collected via a structured questionnaire„ ferritin levels were assessed using the Cobas e411 method, and immunoglobulins and complement factors were quantified using the immunoturbidimetry method.
Results: The results indicate that β-TM patients who have undergone splenectomy exhibit higher serum ferritin and immunoglobulin (IgM, IgG, IgA) levels than non-splenectomized β -TM patients, with stable C3 and a non-significant decline in C4. Additionally, significant correlations were found between IgG, IgA, blood transfusions and iron chelation therapy, whereas C3 and C4 levels were not significantly associated with these treatments.
Conclusion: The findings indicated that splenectomy alters the immunological profile of β-TM patients, although the precise mechanism underlying this alteration remains ambiguous; thus, analyzing serum immunoglobulins and complements levels may prove beneficial in assessing infection severity in these patients.

Article Info

Keywords:
β-thalassemia major, complement 3, Complement 4, Ferritin, Iron chelation therapy, Serum complements, Serum Immunoglobulins

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1. INTRODUCTION

β-TM arises from a lack of β-globin chains in hemoglobin A, resulting in an accumulation of α-globin chains that disrupt erythropoiesis and cause chronic hemolytic anemia in children and adolescents worldwide, necessitating frequent blood transfusions from early childhood [1, 2]. Although historically prevalent in the Mediterranean, Middle East, and Southeast Asia, migration has led to an increased incidence in non-endemic regions, with prevention initiatives aiding in reducing its prevalence in high-carrier areas [3]. Yemen, with a population of 30 million, suffers from prevalent hereditary hemoglobinopathies, notably thalassemia. This has led to tragic consequences for Yemeni patients, with 50,000 cases of thalassemia blood disorder recorded and 700 new cases

diagnosed annually [4]. A recent study revealed that the incidence of hemoglobinopathies in Yemeni blood donors was 21.7%, comprising 3.8% with β-thalassemia trait, 16% with suspected -thalassemia trait, and 1.9% with sickle cell trait [5]. The effects of splenectomy on immune function in β-TM patients involve not only serum immunoglobulin levels but also complement regulatory proteins. Research indicates that splenectomized patients have notably reduced CD55 and CD35 positive red blood cells, which are essential for safeguarding erythrocytes against complement-induced lysis [6]. A prevalent complication of β-TM is heightened susceptibility to infections, chiefly attributed to functional asplenia in patients who have undergone splenectomy, as the spleen is integral to the immune response, notably in eliminating encapsulated bacteria and modulating immunoglobulin synthesis [7]. Elevated immunoglobulin concentrations indicate compromised secretion and diminished opsonization, granulocyte phagocytosis, and complement system efficacy, while reduced C3 and C4 levels further reflect attenuated complement system activity [8, 9, 10]. Immunological changes post-splenectomy significantly affect patient care and quality of life, necessitating tailored management strategies to mitigate infection risks, particularly in β-TM patients, while balancing the benefits of alleviating hypersplenism and reducing transfusion needs against the potential for increased vulnerability to encapsulated bacterial infections [8]. This study evaluates serum immunoglobulin (IgM, IgG, IgA) and complement component (C3, C4) levels in β-TM patients, with and without splenectomy, while correlating these levels with ferritin, blood transfusions, and iron chelation therapy.

2. PATIENTS AND METHODS

2.1. Study design

This cross-sectional study was conducted from May to December 2023 at the Yemeni Society of Thalassemia and Genetic Blood Disorders (YSTH), in Sana'a City. All patients were previously identified as having β-TM, based on clinical and laboratory evaluations at the YSTH.

2.2. Sample size

The study was conducted on a sample of 100 patients with β-TM, selected from a total of 6000 cases in Sana'a with a prevalence of β-thalassemia trait of 4.4%, using previous data and Epi-Info software with 80% power, 95% confidence, and ± 5 accuracy. The patients were classified into two groups of 50 patients each, with or without splenectomy, undergoing low-leukopenia red blood cell transfusion along with iron chelation therapy.

2.3. Patients

A cohort of 100 β-TM patients, comprising splenectomized and non-splenectomized patients; undergoing iron chelation and blood transfusions was studied, excluding individuals with positive CRP, hepatitis B or C, HIV infections or heart failure.

2.4. Data collection

Upon admission, verbal consent was acquired from the patient's legal representative, followed by face-to-face interviews employing a validated structured questionnaire tailored for this research, which gathered information regarding the patient's age, annual blood transfusions, iron chelator therapy, recent infections, complications, surgeries, and familial medical history.

2.5. Samples Collection

Five milliliters of venous blood sample was withdrawn from each β-TM patient with splenectomy and from a β-TM patient without splenectomy before the scheduled transfusion. Samples were divided into two vacutainer tubes, 3 ml in tubes with Ethylene Diamine Tetra-acetic Acid (EDTA) for complete blood count using a hematological analyzer Sysmex (KX-21N) and 2 ml in to another vacutainer without anticoagulant was separated within 30 min at 3000 rpm for 10 min for the measurement of immunoglobulin (IgM, IgG and IgA), complements levels (C3 and C4) assays and ferritin levels.

2.6. Determination of Ferritin level using Cobas e411 Method

During the initial 8-minute incubation, a sandwich complex was established by merging a sample with biotin and ruthenium-labeled ferritin-specific monoclonal antibodies, followed by the introduction of streptavidin-coated microparticles that facilitated the attachment of the complex to a solid phase through biotin-streptavidin interactions; subsequently, the reaction mixture was transferred to a measurement cell where the microparticles were magnetically captured by the electrode surface, enabling the monitoring of chemiluminescence emissions induced by voltage application on electrodes via a photomultiplier.

2.7. Determination of IgM, IgG and IgA levels (turbidimetric method)

An immunoturbidimetric assay was employed to quantify serum levels of IgA, IgG, and IgM using the Tina-quant® assay (Roche Diagnostics GmbH, Mannheim, Germany), with results expressed in g/L. Reference ranges for these immunoglobulins in adults, as per the manufacturer's guidance, are 0.7-4.0 g/L for IgA, 7.0-16.0 g/L for IgG, and 0.4-2.3 g/L for IgM, based on the certified reference

material CRM 470, which is internationally acknowledged but does not provide age-specific ranges for adults.

2.8. Determination of C3 and C4 levels (turbidimetric method)

C3 and C4 levels were quantified via turbidimetry using a Cobas Integra 400 analyzer (Roche, Switzerland), with established reference ranges of 0.9-1.8 g/L for C3 and 0.1-0.4 g/L for C4.

2.9. Statistical Analyses

Statistical analyses were performed using SPSS version 23(SPSS Inc., Chicago, IL, USA), with skewed variable parameters normalized via logarithmic transformation, and means presented as mean ± standard deviation (SD) and geometric means with a 95% confidence interval, indicating significant differences at P-value ± 0.05, while percentages represented the correlation among variables.

3. RESULTS

In Table 1, the average age of splenectomized β-TM patients was 13.8 ±4.3 years, contrasting with 9.35 ±3.8 years for non-splenectomized β-TM patients. Of the β-TM patients, 55 were male (33 splenectomized and 22 non-splenectomized) and 45 were female (17 splenectomized and 28 non-splenectomized), with varying usage patterns of iron chelators and blood transfusion therapies among the subgroups. The mean serum ferritin levels were significantly higher in splenectomized patients 4865.74 ng/ml compared to non-splenectomized patients 3953.72 ng/ml (P = 0.022), while the median IgM levels showed no significant higher in splenectomized 138 mg/dl vs non splenectomized 117.5 mg/dl β-TM patients (P = 0.11); however, splenectomized patients exhibited significantly elevated serum IgG levels 2088.76 mg/dl versus non-splenectomized patients 1383 mg/dl (P < 0.001), and similarly, serum IgA levels were significantly higher in splenectomized patients 316.12 mg/dl compared to non-splenectomized patients 199.5 mg/dl (P < 0.001). the median serum C3 levels in splenectomized patients were 121.3 mg/dl, while non-splenectomized patients had levels of 121.9 mg/dl, indicating no significant difference (P = 0.95); additionally, splenectomized patients exhibited lower mean C4 levels of 22.11 mg/dl compared to 25.18 mg/dl in non-splenectomized patients, also showing no significant difference (P = 0.76).

In β-TM patients, serum IgM, IgG, and IgA levels were not significantly correlated with ferritin, with correlation coefficients of -0.016 (P=0.871), 0.970 (P=0.33), and 0.195 (P=0.052), respectively. The data indicates a non-significant correlation between IgM levels and blood transfusion in patients with β-TM $(r = -0.060; P = 0.552)$, whereas a highly significant correlation exists between IgG levels and blood transfusion $(r = 0.505; P < 0.0001)$, along with a similarly significant correlation for IgA levels $(r = 0.416; P < 0.0001)$. Table 3 reveals a non-significant correlation between serum IgM and iron chelator therapy (r = -0.03; P= 0.978) and IgG levels and iron chelator use in β-TM patients (r = 0.139; P= 0.169), while a significant correlation existed between IgA levels and iron chelator therapy in this population (r = 0.215; P= 0.032). The data suggest a non-significant correlation between C3 and C4 levels and blood transfusions and iron chelation therapy (data not presented).

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4. DISCUSSION

The consequences of splenectomy extend beyond transfusion requirements and; profoundly affect the health-related quality of life (HRQoL). While patients with splenectomized β-TM showed reduced transfusion needs, the lack of significant HRQoL improvements and an increase in post-splenectomy complications highlight the critical need for longitudinal studies assessing both clinical and psychosocial factors to guide treatment choices effectively [11]. In this study, splenectomized β-TM patients exhibit a higher mean age than non-splenectomized counterparts. This age differential underscores the necessity for tailored management strategies, particularly regarding the timing of splenectomy, which significantly influences transfusion needs and health outcomes. Furthermore, while post-splenectomy patients exhibit reduced transfusion requirements, they face heightened risks of infections and thromboembolic events, necessitating careful monitoring and individualized care [12, 13]. The cohort of β-TM patients comprised 55 males (33 splenectomized and 22 non-splenectomized) and 45 females (17 splenectomized and 28 non-splenectomized), revealing distinct patterns in iron chelator and blood transfusion therapies across these categories. The variability in treatment modalities necessitates individualized therapeutic strategies, particularly since splenectomized patients exhibit a heightened risk of thromboembolic events that require vigilant monitoring and potential therapeutic modifications [4]. This highlights the critical need for interdisciplinary collaboration between hematologists and surgical specialists to enhance patient outcomes and address the complex nature of β-TM management [14, 15]. In this study, splenectomized β-TM patients exhibited significantly elevated serum ferritin levels compared to their non-splenectomized counterparts, indicating a height-

Table 1. Demographics and clinical findings of β-TM patients.

Variables		Splenectomized (n = 50)	Non-Splenectomized (n = 50)
Age (years)	(Mean $\pm$ SD)	(13.8 $\pm$ 4.3)	(9.35 $\pm$ 3.8)
Gender	Male	33	22
	Female	17	28
Type of iron chelator	Deferoxamine	26	16
	Deferasirox	24	34
Use of iron chelator	Regular	14	15
	Irregular	36	35
Blood transfusion	Regular	42	46
	Irregular	8	4

Table 2. Association of serum Ferritin, Immunoglobulins, and Complements levels in splenectomized versus non-splenectomized β-TM patients.

Parameters	Splenectomized (n = 50)	Non- Splenectomized (n = 50)	p-value
Ferritin (ng/ml)	4865.74 $\pm$ 1941.859	3953.72 $\pm$ 1988.533	$< 0.022^*$
IgM (mg/dl)	138 (123-158.4)	117.5 (7.7-141.2)	0.11
IgG (mg/dl)	2088.76 $\pm$ 664.042	1383 $\pm$ 550.4	$< 0.001^*$
IgA (mg/dl)	316.12(215-316.2)	199.5(173.8-234.4)	$< 0.001^*$
C3 (mg/dl)	121.3(114.8-144.5)	121.9(114.8-125.9)	0.95
C4 (mg/dl)	22.11$\pm$8.751	25.18$\pm$8.317	0.76

Data are presented as mean$\pm$SD for ferritin, IgG and C4 and as median and range for IgM,IgA and C3. $^*P$-value ≤ 0.05.

Table 3. Correlation between serum ferritin, blood transfusion and use of iron chelator with serum immunoglobulins levels in β-TM patients.

Parameters		IgM	IgG	IgA
Ferritin	r	-0.016	0.970	0.195
	P	0.871	0.33	0.052
Blood transfusion	r	-0.060	0.505	0.416
	P	0.552	< 0.0001*	< 0.0001*
Iron chelator	r	-0.03	0.139	0.215
	P	0.978	0.169	0.032^*

P-value ≤ 0.05.

ened iron load and potential complications in long-term management due to impaired hepcidin regulation and increased hemosiderosis [16]. Ongoing surveillance of ferritin and hepcidin levels is essential for refining treatment modalities and alleviating health risks linked to iron overload, particularly in splenectomized β-TM patients who require effective iron chelation therapy because of increased total body iron stores post-splenectomy, which increases the risk of organ damage and metabolic issues [17]. Through the integration of biochemical surveillance and sophisticated imaging methodologies, healthcare practitioners can optimize interventions to meet the specific needs of this patient population, thereby enhancing their long-term prognoses. In this study, IgM levels were non-significantly increased in splenectomized β-TM patients compared to non-splenectomized patients, while splenectomized individuals exhibited significantly elevated serum IgG and IgA levels compared to their non-splenectomized counterparts. This alteration in immunoglobulin levels prompts inquiries into the mechanisms that increase susceptibility to infection in patients with splenectomized β-TM. Elevated IgG and IgA levels indicate a compensatory mechanism, while decreased IgM levels suggests compromised initial immune responses crucial for addressing encapsulated bacteria [18, 19]. An investigation indicated a marked reduction in IgA and IgM levels post-splenectomy, while the increase in IgG levels was statistically insignificant [20]. Furthermore, the absence of the spleen, which is essential for blood filtration and immune activation, may further increase vulnerability, resulting in elevated morbidity from bacterial infections [19]. Conversely, some studies indicate that non-splenectomized patients may also experience immune dysregulation, albeit with different immunoglobulin profiles, suggesting that both groups face unique challenges in managing infections and immune responses [19, 21]. Conversely, research suggests that splenectomized patients, despite heightened immunoglobulin levels, may experience greater morbidity from thromboembolic complications, indicating a complex relationship between immune function and health outcomes in this population [22]. This underscores the importance of implementing preventive measures, such as vaccinations and prophylactic antibiotics, to mitigate the risk of infection in this vulnerable population. In the present study, serum C3 levels in splenectomized β-TM patients were comparable to those in non-splenectomized patients, indicating no significant disparity; furthermore, splenectomized patients demonstrated non-significant lower mean C4 levels when compared to non-splenectomized patients. Studies have indicated that serum C3 levels remain comparable between splenectomized and non-splenectomized patients, suggesting that splenectomy does not significantly impact this component of the complement system [20, 23]. Although splenectomized β-TM patients showed lower mean C4 levels, the difference was not statistically significant, indicating a minimal impact of splenectomy on C4 levels [23]. Additionally, a significant decrease in serum C3 levels was observed, whereas the reduction in C4 levels lacked statistical significance [20]. Splenectomy is often performed to manage hypersplenism and reduce

transfusion requirements in β-TM patients; however, it carries risks such as increased infection rates and thrombotic events [24]. The immune system in β-TM patients is already compromised by factors such as iron overload and chronic immune stimulation from repeated transfusions, which may exacerbate post-splenectomy vulnerabilities [25]. These findings indicate that splenectomy does not significantly compromise the overall complement system's C3 and C4 functionality. This necessitates a reassessment of the immune regulatory role of the spleen, suggesting that the infection risk in splenectomized β-TM patients may be more closely related to other immunological factors than to complement deficiencies.

5. LIMITATIONS OF THE STUDY.

The limited sample size of participants poses a constraint on this region-specific study, although it simultaneously reduces individual variability, leading to relatively uniform study cohorts with comparable infection risk factors. Future research should encompass larger, more diverse populations while also investigating the genetic mutations of participants and their correlational phenotypic characteristics, which are essential for understanding the influence of geographic location on β-TM complications and therapeutic responses. Furthermore, addressing unexamined immunological parameters due to financial constraints is crucial; thus, consistent monitoring of the immune status in β-TM patients is advocated to enhance our understanding of their immune dynamics and ultimately improve their quality of life and survival rates.

6. CONCLUSIONS

Research indicates that splenectomy alters Immunoglobulin levels with elevation of IgM, IgG and IgA and reduction of C4 but not C3 levels in β-TM patients. Furthermore, strong correlations were observed between IgG and IgA levels with blood transfusions and IgA levels with iron chelator treatment, with no significant correlations for C3 and C4 levels in these therapies. These findings suggest that monitoring immunoglobulin levels may be crucial for managing patients with β-TM post-splenectomy, as they could provide insights into the effectiveness of therapeutic interventions and the patient's immune response.

ETHICAL CONSIDERATIONS

The study adhered to the Declaration of Helsinki of the World Medical Association and received approval from the Faculty of Medicine and Health Sciences, Sana'a University ethics committee. The rationale, nature, and potential risks of the experiments were thoroughly communicated to the parents, who provided written informed consent at the onset of the study, with all data maintained confidentially and utilized exclusively for the research objectives.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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