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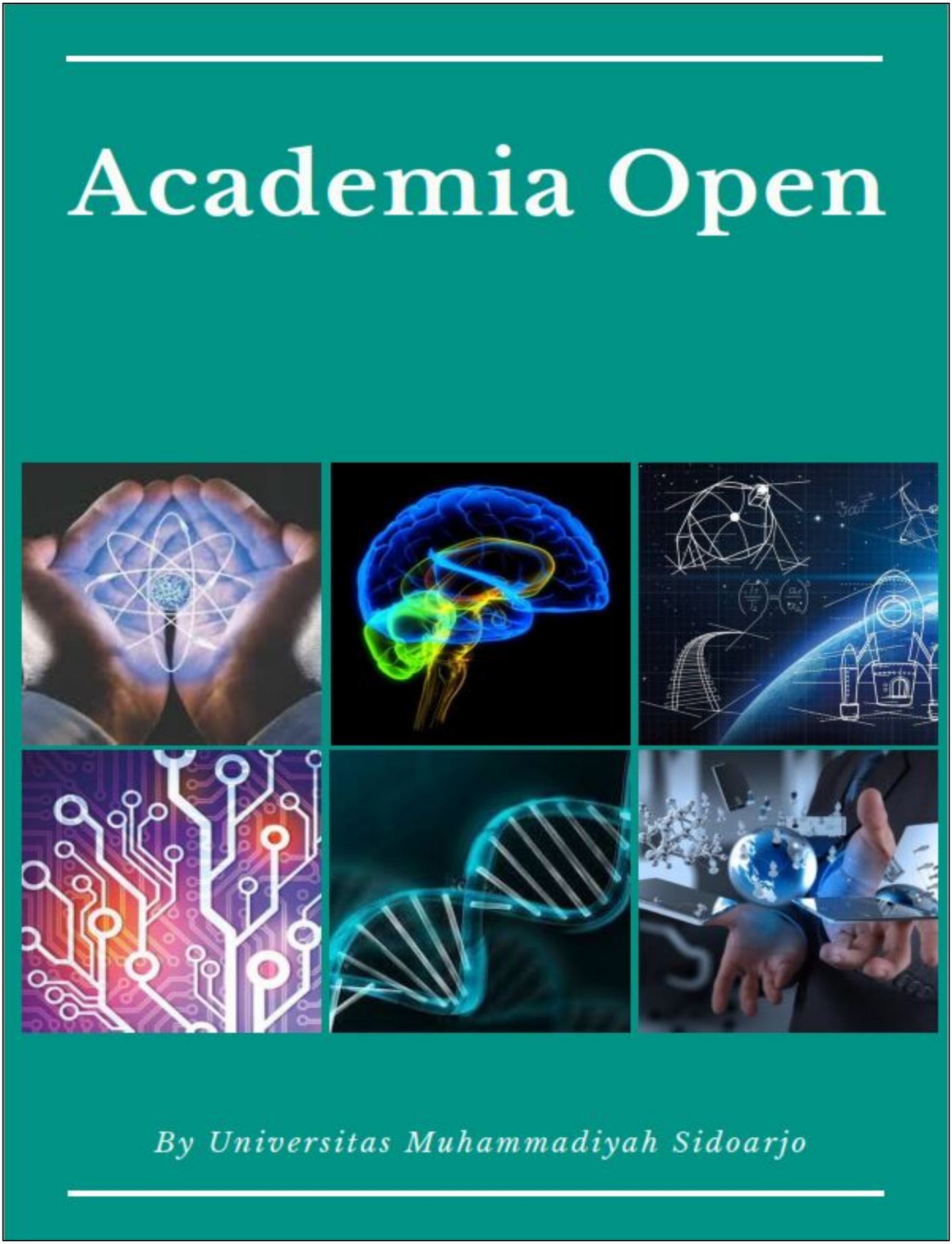

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Immunological Changes and Complement Proteins in Major Thalassemia Patients Proteins Post-Splenectomy

Perubahan Imunologi dan Protein Komplemen pada Pasien Talasemia Mayor Protein Pasca-Splenektomi

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Abstract

Background: Thalassemia is one of the most prevalent genetic disorders worldwide, with infections being a leading cause of mortality due to compromised immune function.**Specific Background:** Prior studies suggest that major thalassemia patients are highly susceptible to microbial infections, possibly due to altered immunological profiles, particularly immunoglobulin (IgG, IgM) and complement (C3, C4) levels. **Knowledge Gap:** However, the specific immunological changes pre- and post-splenectomy in these patients remain underexplored. **Aims:** This study aims to assess the levels of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) in major thalassemia patients both before and after splenectomy compared to healthy controls. **Results:** Our analysis of 50 thalassemia patients (34 males, 16 females) and 30 healthy individuals revealed that thalassemia patients exhibited significantly lower levels of C3 and C4 (88.52±24.49, 21.20±6.66) compared to healthy controls (123.50±19.04, 32.87±9.77). IgG and IgM were elevated in patients (1288.12±467.87, 153.46±51.29) compared to controls (1129.93±295.96, 148.67±50.17). Post-splenectomy, patients showed a significant decline in IgG (1001.56±154.14) and IgM (110.08±25.83) levels, along with further decreases in C3 (83.28±24.13) and C4 (17.48±4.86). **Novelty:** This study provides novel evidence of the immunological shifts in thalassemia patients post-splenectomy, demonstrating significant reductions in both immunoglobulins and complement proteins, thereby elevating the risk of infection. **Implications:** These findings highlight the spleen's crucial role in maintaining immune competence and suggest that splenectomy in thalassemia patients requires careful post-operative immune monitoring to mitigate infection risks.

Highlights:

1. Splenectomy lowers IgG, IgM, C3, and C4 levels in thalassemia patients.
2. Post-splenectomy patients face higher infection risk due to immune weakening.
3. Highlights spleen's crucial role in immune defense for thalassemia patients.

Keywords: Thalassemia, Splenectomy, Immunoglobulins, Complement Proteins, Immune Competence

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Introduction

One of the most common genetic disorders worldwide is thalassemia, which is more common in tropical and subtropical areas including the Middle East, the Mediterranean nations, India, Southeast Asia, and North Africa (1). Reductions in the synthesis of globin chains characterize the autosomal recessive illness thalassemia. Mutations in the globin chain-encoding genes on chromosome 11 are the root cause of it. Mutations in the -globin gene cause the disease beta-thalassemia major (Beta-TM). An imbalance of globin results from decreased or absent -globin gene expression, and the resulting free, insoluble -globin molecules precipitate, harm, and destroy erythroid precursors. Ineffective erythropoiesis is the term used to describe this (2). The most typical thalassemia signs and symptoms include pallor, limited weight gain, and inadequate growth and development. Red blood cell death and inefficient erythropoiesis are the main causes of anemia. Patients with TM experience enlarged liver and spleen, severe anemia, and skeletal abnormalities. Regular blood transfusions and the use of iron-chelating medications are part of the treatment for thalassemia (3).

One of the affected organs in TM patients is the spleen, and the severity of splenomegaly is correlated with the degree of thalassemia and the frequency of blood transfusions. In order to prevent excessive increases in spleen activity and to lessen the mechanical pressure brought on by an enlarged spleen, splenectomy is necessary (4). Increased blood transfusion requirements in TM patients are caused by excessive splenic activity, which enhances the destruction of transfused red blood cells. One of their most frequent issues is this, particularly in underdeveloped nations where patient compliance is low. Splenectomy may be accompanied by the increased risk of thrombotic complications and acute infections following surgery. However, it could be useful in reducing the need for blood transfusion (5).

When the annual transfusion volume surpasses 250 mL/kg, splenectomy is advised. Patients with splenectomies run the risk of developing sepsis and having a strong immunological reaction to bacterial infections. About 25% of these people are susceptible to severe illnesses. This may have occurred as a result of anomalies in the complement system, disruptions in B and T lymphocyte activity, disturbances in macrophage and neutrophil function, and abnormalities in the generation of immunoglobulins (6). Numerous studies have been conducted on immunoglobulin levels in TM patients. However, conflicting results have been noted. Higher IgA levels were noted in a study on splenectomized and non-splenectomized patients, although Dwyer et al. (1987) (7). reported increased IgA and IgM levels. Additionally, other investigations have noted an increase in B cells but a disruption in their differentiation (8). B lymphocytes are greatly impacted by splenectomy. As a result, it was discovered that the amount of circulating B cells was three times higher in patients who had not undergone splenectomy. Additionally, these patients had a 10-fold increase in immunoglobulin secretion (5).

The present study aimed to detect levels of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) in major thalassemia patients at pre and post-operation splenectomy.

Methods

Data collection

The present study was conducted in Baquba / Diyala province for the time period from the beginning of June to the end of July 2022. 50 blood samples were collected from major thalassemia patients (34 males and 16 females within age group 1-30 years) who were visiting Blood Diseases Center in Baquba Teaching Hospital after the patients were diagnosed by specialist doctor. Also, 30 blood samples were collected from healthy people and considered as a control group .A form was filled out containing information on gender and age by the two groups (patients and healthy).

Methods

To isolate the serum, 5 ml of human blood were spun at 3000 rpm for 5 minutes. The levels of immunoglobulins (IgG and IgM) and complements proteins (C3 and C4) were measured by Cobas integra 400 plus machine.

Statistical analysis

The levels of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) markers were first tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk test). All parameters were fit both tests (no significant difference), therefore it given as Mean ± SD, and significant difference between means were assessed by student T and F (ANOVA) test. The other parameters were given as percentage frequencies, and significant differences between frequencies were assessed by Pearson-Chi-square test. Pearson correlation (R) accounted to explain type and strength of relationship between variables. The statistical package SPSS version 25.0 and Graph pad prism v.6 was employed to carry out these analyses.

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Result and Discussion

Result

Baseline characters of patients

Results of present study showed there is significant different ($p<0.05$) among age groups and gender of patients. 11-15 years age group scored highest percentage (38.0%), while 1-5 and >20 years scored least percentage (12.0% and 8.0%) in patients. The males patients scored highest percentage (68.0%) than females (32.0%) (table 1).

		Count	%	P value
Age groups (years)	1-5	6	12.0%	p<0.01**
	6-10	11	22.0%	
	11-15	19	38.0%	
	16-20	10	20.0%	
	>20	4	8.0%	
Gender	Males	34	68.0%	p<0.01**
	Females	16	32.0%	

Table 1. *Frequency and percentage of age groups and gender of patients were calculated by chi-square test.*

*Relation of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) with study groups

The conducted results show high significant different ($P<0.05$) between C3 and C4 complements parameters and study groups .We noticed low levels of C3 and C4 complements parameters in patients (88.52±24.49 and 21.20± 6.66) than healthy (123.50±19.04 and 32.87±9.77) respectively. In contrast, the levels of IgG and IgM were high in patients (1288.12±467.87 and 153.46± 51.29) than healthy (1129.93±295.96 and 148.67±50.17) with non significant different ($P>0.05$) (table 2 and figure 1).

groups		N	Mean	SD	P value
IgG (mg/dl)	Patients	50	1288.12	467.87	P>0.05
	Healthy	30	1129.93	295.96	
IgM (mg/dl)	Patients	50	153.46	51.29	P>0.05
	Healthy	30	148.67	50.17	
C3 complement (mg/dl)	Patients	50	88.52	24.49	P<0.001***
	Healthy	30	123.50	19.04	
C4 complement (mg/dl)	Patients	50	21.20	6.66	P<0.001***
	Healthy	30	32.87	9.77	

Table 2. *mean levels of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) with study groups were calculated by student t test.*

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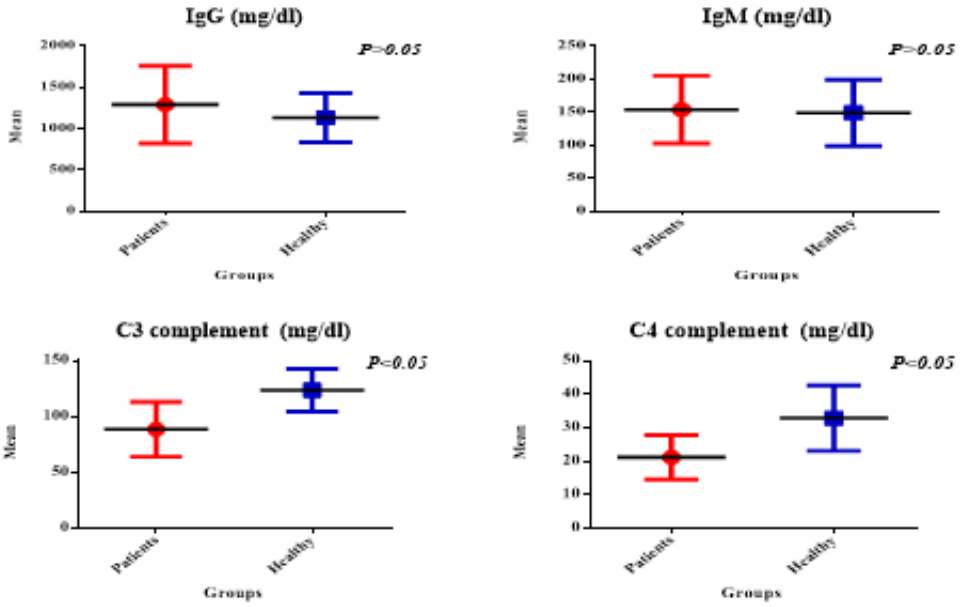

Figure 1. *mean levels of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) with study groups.*

Relation of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) with splenectomy surgery.

The present results show high significant different (P<0.05) between IgG, IgM, C3 and C4 complements parameters and study groups .We noticed highest levels of IgG and IgM parameters in patients before splenectomy (1574.68 ±502.09 and 196.84 ± 27.97), while least mean levels were in patients after splenectomy (1001.56±154.14 and 110.08±25.83) respectively. Additionally, we noticed highest levels of C3 and C4 parameters in healthy (123.50 ±19.04 and 32.87 ± 9.77), while least mean levels were in patients after splenectomy (83.28 ±24.13 and 17.48 ±4.86) respectively (table 2 and figure 3).

		N	Mean	SD	Statistics
IgG (mg/dl)	Before	25	1574.68 a	502.09	P<0.001*** LSD=184.82
	After	25	1001.56 b	154.14	
	Healthy	30	1129.93 b	295.96	
IgM (mg/dl)	Before	25	196.84 a	27.97	P<0.001*** LSD=22.91
	After	25	110.08 c	25.83	
	Healthy	30	157.67 b	50.17	
C3 complement (mg/dl)	Before	25	93.76 b	24.19	P<0.001*** LSD=11.21
	After	25	83.28 b	24.13	
	Healthy	30	123.50 a	19.04	
C4 complement (mg/dl)	Before	25	24.92 b	6.18	P<0.001*** LSD=3.61
	After	25	17.48 c	4.86	
	Healthy	30	32.87 a	9.77	

Table 3. *mean levels of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) with splenectomy surgery and healthy groups were calculated by F test.*

Small different letters refer to significant different (p<0.05)

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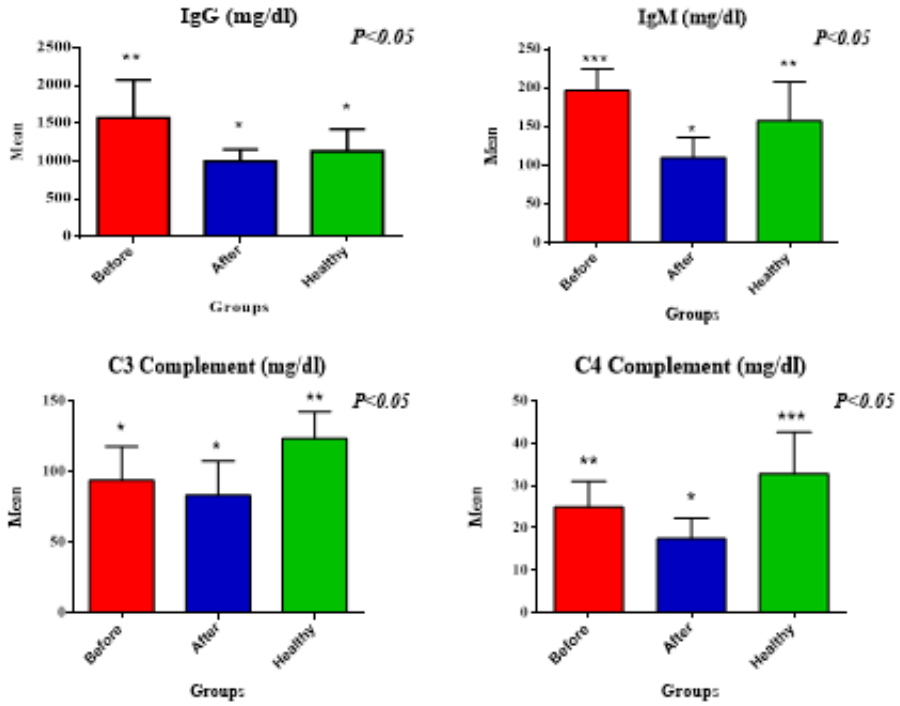

Figure 2.

*Correlation relationship among immunoglobulins and complements proteins

Figure 2; mean levels of immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) with splenectomy surgery and healthy groups.

The present study showed there is a significant positive correlation ($p<0.05$) between IgG and IgM (0.589**), and IgM with C4 complement (0.497**). Other positive correlations among parameters but were no significant ($p>0.05$) (table 4).

		IgG (mg/dl)	C3 (mg/dl)	C4 complement (mg/dl)
IgG (mg/dl)	Pearson coefficient	1	.250	.218
	Significant		.080	.128
IgM (mg/dl)	Pearson coefficient	.589**	.207	.497**
	Significant	.000	.149	.000
C4 complement (mg/dl)	Pearson coefficient	.218	.136	1
	Significant	.128	.347	

Table 4. *Correlation relationship among immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) were calculated by Pearson correlation test.*

Discussion

The present study showed there is significant variation among age groups of patients, where the 11-15 years age groups scored highest percentage than others age groups. Al-Ali and Faraj, (2016)(9) showed most thalassemia patients with the 10-15 years age group, and these results matched with present study. As similar, Xu et al., (2019)(10) showed most thalassemia patients with age groups >10 years and these results matched with present study. In contrast applied in Iran, Kosaryan et al., (2018)(11). showed 70% of thalassemia patients were lied within age group >30 years, and these results disagree with the present study. In Cairo/Egypt, Hamdy et al., (2021) (12) showed 85% of patients in the age group 16-20 years, and these results were higher than a present study that showed 20% of patients in the age group 16-20 years. These differences appeared due to sample size, age group, and geographical regions.)13)

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Based on gender, the present study showed most thalassemia patients were males, and these results were not compatible with the results of Meloni et al., (2022) (14) which showed no differences between thalassemia patients and gender. Husna et al., (2017) (15). all thalassemia types occur in females than males, and these results disagreed with the present study showing that beta thalassemia is more common in males than females. The authors found that females were more anaemic than males, but there was no significant difference in the prevalence of common complications among genders, except for hypogonadism (3). The differences in studies appeared due to sample size, hormones, and genetic disorders.

According to the findings of the current study, compared to healthy individuals, major thalassemia patients have significantly higher levels of IgG and IgM in patients who have undergone splenectomy before surgery and lower levels after surgery. These findings were corroborated by findings from Darzi et al. (2015) (6). Most thalassemia major (TM) patients require frequent blood transfusions with iron-chelating medications to lessen iron overload to survive. When the bone marrow is unable to treat anaemia, and when hypersplenism develops in individuals with -thalassemia, the spleen initiates extramedullary hematopoiesis. As a result, splenectomy is necessary for hypersplenism brought on by extramedullary hematopoiesis. By controlling immunological homeostasis and creating connections between the innate and acquired immune systems, the spleen is crucial in defending the body against infections. Researchers discovered a correlation between changes in many immunological indicators and increased transfusion needs, iron excess, splenectomy, and HCV infection (Kadam et al., 2014). Streptococcus pneumonia, Haemophilus influenza, and Neisseria meningitides are the most dangerous infections in splenectomized patients (Kurtollu et al., 2019).

Patients with TM may need splenectomy due to hypersplenism. It has long been established that splenectomy can lessen these patients' need for blood transfusions and iron overload. A splenectomy can also ease the discomfort brought on by severe splenomegaly. Splenectomy raises the risk of infections since the spleen helps to fight off infections. Therefore, determining the causes of infection and aiding in the treatment of these patients can be accomplished by looking at immunoglobulin levels (15).

B lymphocytes are crucial for the functioning of humoral immunity in thalassemic patients because they contribute significantly to the formation of autoantibodies and alloantibodies against transfused red blood cells. In this group of patients, there is a higher percentage of B cells, notably those with regulatory phenotypes that express CD19, CD38, and CD24, but there is no difference in the ratios of T cell subpopulations (18). According to study results, splenectomized patients had mean blood levels of IgG and IgA that were higher than those of non-splenectomized patients. These variations weren't statistically significant, though. Individuals with splenectomies had considerably lower mean serum IgM levels than patients without splenectomies (5). In this regard, a variety of results have been recorded. Following splenectomy in beta-TM patients, Darzi et al. (2015) (6) showed significant drops in IgA and IgM levels compared to pre-operative values. Since the spleen plays a crucial role in the release of immunoglobulins, splenectomy may result in lower serum levels of immunoglobulins. In addition, Miri-Aliabad et al. (2022) (5) found that splenectomized thalassemia patients had higher blood IgG and IgA levels than non-splenectomized individuals. In contrast, they found no variations in IgM levels between the two groups. Kiani et al. (2011) (16) also noted normal IgM and IgG levels in both the splenectomized and non-splenectomized groups, but significant increases in IgA levels in non-splenectomized patients (children under the age of five). Serum IgG and IgA levels were greater in splenectomized children than in non-splenectomized children, according to Ahluwalia et al. in 2000, however, these changes were not statistically significant. In the study by Konstantoulakis et al., serum IgG, IgA, and M levels were shown to be higher in the non-splenectomized group of patients and lower in the splenectomized group (1978).

Patients with splenectomies had greater serum levels of IgG and IgA than patients in the control group. The mean serum IgM levels were lower in the splenectomized patients than in the control group, and the difference in IgA levels was statistically significant. This distinction, nevertheless, was not statistically significant. Similar findings to the ones of the present investigation were reported by Ghaffari et al. (2011) (19). In the study by Korn et al., IgM levels were significantly lower in splenectomized individuals with hematologic problems (1984) IgG and IgA levels, however, showed no changes. They proposed that the drastic drop in IgM might be brought on by opsonization errors and the rapid onset of acute infection following splenectomy. The non-splenectomized patients in the current study had mean blood IgM and IgA levels that were considerably greater than those of the healthy control group. Although these changes were not statistically significant, their IgG levels were lower. In addition, Shani et al. (2014) (21) found that thalassemia patients had considerably lower IgG levels than the healthy control group, but they found no other immunoglobulins to be significantly different between the two groups.

According to the results, asplenia (abnormal spleen function) and common variable immune deficiency (CVID) were connected with a deficit of intestinal IgA-secreting plasma cells and circulating IgM memory B cell depletion. It is believed that a healthy gut-spleen axis exists because IgM memory B cells play a unique role in mucosal protection (20).

These discrepancies and contradictory results may be caused by a variety of variables, including the quantity of blood transfusions, serum ferritin levels, the amount of iron in tissues, patient ages and genetic backgrounds, as well as the particular mutation responsible for the development of thalassemia phenotype. Numerous variables may be responsible for people with Beta-TM having higher serum levels of immunoglobulins. For instance, receiving frequent blood transfusions may expose Beta-TM patients to a variety of antigens over time, raising

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immunoglobulin levels in the serum. Patients with thalassemia are susceptible to numerous bacterial and viral diseases. Consecutive infections can also boost immune function and immunoglobulin levels by stimulating the immune system. Because the spleen is one of the major lymphoid organs and filters germs and numerous invasive pathogens from the blood, splenectomized patients have been noted to have more infections than non-splenectomized people. It also takes part in the destruction of bacteria that have been opsonized by the complement system. The splenectomy is thought to activate secondary lymphoid organs to make up for the lack of production of the major immunoglobulin types (5).

The results of the current study indicate that the major thalassemia patients have a significant decline in levels of serum C3 and C4 complement in patients with pre-operation and after splenectomy operation compared to the healthy group and these results matched with results of Darzi et al., (2015) (6) and Ghafourian et al., (2017) (21). In addition, •Kuzdan et al., (2023) (23) found no significant differences in C3 and C4 between patients with and without splenectomy, and these results are not compatible with to present study. The decrease in complement proteins can be attributed either to consumption or reduced synthesis (24).

Amin et al., (2015) (24). documented in their research that lower levels of C4 related to iron overload as an important factor in regulatory mechanisms of complement activation or overuses of complement, this change contributes to both classic and alternative pathways, as well as factors like splenectomy, repeated exposure to antigens at the time of blood transfusion and the use of the chelating agents have profound effects on the immune system. Reduced synthesis or increased consumption of complement proteins in patients receiving multiple blood transfusions might lead to continuous contact between the immune system and several antigens, causing nonstop use of complement factors, recurrent infections, and changes in parameters of the immune system due to iron overload as well as exposure to infectious factors such as HBV, HCV, HIV, and HTLV through blood transfusion (22).

The complement system functions as the body's first line of defence, clearing infections, dead cells, and immune complexes by opsonization, triggering an inflammatory response, and creating lytic pores. Red blood cells (RBCs) constantly come into touch with complement proteins in the blood plasma and are crucial for carrying oxygen to tissues. Different complement regulation proteins can be found in plasma and on cell membranes to stop complement activation on RBCs. As unique cells without a nucleus, RBCs differ from nucleated cells in that membrane cofactor protein (MCP) is not expressed and complement receptor 1 (CR1) is strongly expressed. RBCs also have a slightly altered complement regulator composition. Reduced expression and/or function of complement regulatory proteins may result in unwanted complement activation and accelerated removal of RBCs (25). Study results suggested that serum iron was linked with complement C3, and the deficiency of complement C3 may disrupt the regular iron metabolism in the body (26).

Complement pathways are activated in hemolytic anaemias and are closely linked with thrombosis. In acquired disorders such as paroxysmal nocturnal hemoglobinuria (PNH) and possibly cold agglutinin disease (CAD), inhibition of the alternative complement pathway improves clinical outcomes and reduces thrombosis risk. Whether complement inhibition has a similar role in congenital hemolytic anaemias apart from the atypical hemolytic-uremic (aHUS)-type thrombotic microangiopathies remains to be determined (27).

Conclusion

Our study revealed that patients with thalassemia major had increased levels of IgG and IgM immunoglobulins, and decreased levels of complement proteins C3 and C4 than healthy group. In addition, we indicated that splenectomy operation in patients with thalassemia major results in a significant decrease in immunoglobulins (IgG and IgM) and complement proteins (C3 and C4) compared to pre-operative splenectomy and healthy groups, and imposes these patients at greater risks of infection. This indicates a strong link between the spleen and immune competence.

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