

The Immunodeficiency Evaluation of Iraqi major β -Thalassemia (β -TM) Patients

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ABSTRACT

Background: β -TM is most hazardous among the other types of disorder in our country. Iron overload monitor are necessary for the survival of these patients. Iron excess causes immune deficiency and major organs dysfunction due to hemosiderosis. **Study Aims:** It designs to evaluate immunodeficiency in β -TM patients. **Materials and Methods:** The study was carried out between November 2022 and February 2023. The samples included 120 subjects. The samples involved the non-splenectomy, splenectomy, modern infection, and control. IgG, IgA, IgM, iron overload (ferritin), GPT, GOT, and TSB were estimated for patients. **Results:** The findings recorded a significant rise in serum IgG, IgA, and IgM and a decline in C3, and C4. High levels of immunoglobulins and iron overload in splenectomy than non-splenectomy. This may be due to the pressure on the other secondary lymphoid organs to compensate the deficiency. The results showed a positive correlation between IgA and a negative correlation of C4 with each of the serum iron overload and liver functions assays studies. Also, it observed a positive association between IgG and each of the GPT and GOT. **Conclusions:** Immunoglobulin increasing, complement consumption and iron overload elevating may be a sign of immunodeficiency in β -TM patients. Therefore, the present study suggested delaying splenectomy to prolong their survival.

Keywords: β -TM; Immunodeficiency; Iron Overload; Splenectomy

INTRODUCTION

Major beta-thalassemia (β -TM) is an autosomal recessive condition. It is distinguished by a hereditary defect in the chain synthesis of the hemoglobin molecule [1]. There are 771 variants of the β -hemoglobin dispersed globally, with over 116 classified as unstable variants [2]. Red blood cells become damaged as a result of an accumulation of free alpha globin chains inside these cells [3]. Nonfunctional erythropoiesis can lead to iron overload (ferritin) an important factor in morbidity and mortality due to organ harm and pathogenic infections [4]. Therefore, transfusion therapy is necessary for the treatment of hemolytic anemia throughout a person's entire life [5]. This disorder is common in the Mediterranean Sea region, involving Iraq [1]. The issue is harsh due to the financial hardship that the Iraqi population has endured over the past few decades [6]. Adolescent's β -TM has underweight, nutritional wasting and increased energy expenditure due to frequency hemolysis [7]. It works to hasten the aging of the patient's immunity as a result of the generation of reactive oxygen radicals that result in per_ oxidative harm in tissue [8]. It can play a role in immunogenicity through the movement of T-helper cells to lymph nodes. Thus, a sharp rise in immunoglobulin levels has been observed in β -TM disorder [3]. Many studies tried to investigate in the immunological abnormalities of these patients [9]. Immunological abnormalities were recorded due to repeated blood transfusion, splenectomy, liver damage, and foreign allogeneic antigens [10].

The complement system is activated by these complexes of antigen and antibody [11]. C3 and C4 were reduced in the β -TM patients, due to synthesis reduction or increased consumption [12]. Numerous observations mentioned the lower C3 and C4 levels, which may act as

indicators of immune deficiency in these patients [13] that can be responsible for increasing their susceptibility to infections [9]. This study aims to assess the immunological disorder in β -TM patients. Also, it's to investigate the relation of iron overload, liver functions on the humeral immune of these patients[14].

MATERIALS AND METHODS

Design

A total 120 of β -thalassemia major patients, whose ages range between 10-35 years (yrs.). Patients were recruited from Center for Hereditary Blood Disease in Baghdad, Iraq. The sample enrolled in four groups: 30 non-splenectomy, 30 splenectomy, 30 modern infections and 30 healthy participants for statistical comparison purposes. The study was carried out during the period between November 2023 and February 2024. Venous blood samples (5ml) were collected in gel tubes.

The sera gain by centrifuged for 10 minutes at 3000 RPM. Sera patients were frozen at -20 °C, and then they liquefied when the test was done. This study included the immunological (IgG, IgA, IgM, and C3, C4 complements) and biochemical laboratory assays (iron overload, TSB, glutamic pyruvic transaminase GPT, and aspartate aminotransferase GOT). Patients' samples were collected just before monthly blood transfusion.

Immunological Assays

This study was assessed the immunoglobulins (IgG, IgA, IgM) and C3, C4 complement factors by Single radio-immunodiffusion (SRID)

technique according to the protocol Lambda antigen tray (LAT) company, Italy. Serum (5µl) was put in each well once all plates had been opened. Then, the plates were covered and kept in a damp chamber. The immunoviewer was used to measure the precipitating ring. Finally, the reference table gives the concentration value corresponding to precipitating ring diameter. Also, iron accumulation was detected by using an auto-analyzer Cuba's device according to the electro_chemiluminescence immunoassay (ECLI) technique. While, the GPT, GOT and TSB were detected by the auto analyzer Flexor (EL200) device based on the photo_absorption technique.

Statistical Analysis

The study computed the mean, standard deviation, and T-tests. The difference between the studied groups was considered significant when the p-value was less than 0.05.

Results

Table (1) showed a strong correlation in studied immunoglobulins and complements between general β -TM patients and control. IgG immunoglobulin recorded a highly significant increase ($P<0.01$). Additionally, IgA and IgM immunoglobulin recorded a significant correlation ($P<0.05$). While C3 and C4 complements have a significant decrease ($P<0.05$) amongst general β -TM patients than control. Splenectomy was reported as the maximum level of serum IgG, IgA, and IgM. Also, the same group contained the C3 and C4 minimum titers. The findings showed highly significant rates of serum iron overload ($P<0.01$), TSB, GPT, and GOT ($P<0.05$) amongst the β -TM patients than control. Table (2) refers to that patients who underwent β -TM splenectomy had significantly greater ($P<0.01$) of IgG immunoglobulin levels than the control. IgG levels in β -TM splenectomy patients and controls were 2278.95 ± 623.49 and 902.90 ± 35.43 , respectively. Studied group sera have statistically increased ($P<0.05$) in other immunoglobulin levels including IgA, and IgM. IgA has 518.89 ± 38.59 vs. 220.14 ± 21.85 , and IgM has 218.34 ± 12.98 vs. 146.60 ± 7.32 in splenectomy patients compared to control, respectively.

Additionally, the C3 and C4 levels in the sera of β -TM patients who underwent splenectomy were significantly lower ($P<0.05$) than those of the control group. In contrast to controls, the current data demonstrated significant amounts of iron overload in the sera of β -

TM patients who underwent splenectomy. The medians for iron overload were 3307.47 ± 299.82 and 37.47 ± 2.08 . The median TSB, GPT, and GOT were highly significant ($P<0.01$) in the above group than the control. IgG and IgA levels have somewhat increased in β -TM non-splenectomy patients. While IgM was significantly higher, at 275.92 ± 18.2 , in these patients than in the control. This study (Table 2) showed that the complement levels were significantly lower ($P<0.05$) in β -TM non-splenectomy compared to the control.

The results were 70.20 ± 3.69 vs. 119.46 ± 6.17 for C3 and 10.81 ± 0.96 vs. 27.48 ± 1.23 for C4 in the mentioned groups. While a significant increase ($P<0.001$) in iron overload 1830.35 ± 151 in sera's non-splenectomy than 37.47 ± 2.08 in sera's healthy. Also, TSB has a significant increase ($P<0.01$) among non-splenectomy patients. The TSB median was 35.21 ± 3.86 vs. 14.66 ± 0.77 . The IgG and IgM do not record any significance among β -TM modern infection cases than in healthy control. While, immunoglobulin type A (IgA) (56.93 ± 3.619 vs. 0.14 ± 21.85), C3 (81.64 ± 2.92 vs. 73.07 ± 2.99), and C4 (24.29 ± 1.39 vs. 16.00 ± 0.60) were recorded significant lowering ($P<0.05$) among above groups. Also, the current investigation found a significant difference in iron overload ($P<0.05$) between modern infection 743.22 ± 73.17 and control 1830.35 ± 151.11 . While, there was no statistically significant difference between the modern infection and control groups in liver function testing. The table (3) demonstrates that individuals who underwent splenectomy as opposed to those who did not do. So, they had significantly lower serum C3 complement ($P<0.05$), IgG ($P<0.001$), immunoglobulin type A, M, and C4 complement ($P<0.05$), and increased levels of IgG. Iron overload has a significant increase ($P<0.01$) in these groups. The serum levels of IgG, IgA, IgM, C3, and C4 have significantly increased in splenectomy compared with infection modern ($P<0.001$, $P<0.001$, $P<0.05$, $P<0.05$, and $P<0.05$, respectively). Iron overload demonstrated extremely significant ($P<0.01$) in the above-studied groups. IgA and IgM reported a significant increase ($P<0.05$), C4 reported a significant reduction ($P<0.05$) while, C3 non-significant ($P<0.05$) among modern infection than non-splenectomy groups. So, the current data performed a substantial reduction ($P<0.01$) of serum iron overload in modern infection than in non-splenectomy. The result of liver function assays was significant in two comparatives. Only, GPT and GOT do not record any significant differences between non-splenectomy and modern infection.

Table 1: The Comparisons of Immunoglobulins (IgG, IgA, IgM) and Complements (C3, C4) Levels among General β -TM Patients and Control

Variable	Immunoglobulins			Complements		Serum ferritin	Liver Function		
	IgG (Mean $\pm$ SD.)	IgA (Mean $\pm$ SD.)	IgM (Mean $\pm$ SD.)	C3 (Mean $\pm$ SD.)	C4 (Mean $\pm$ SD.)		TSB (Mean $\pm$ SD.)	GPT (Mean $\pm$ SD.)	GOT (Mean $\pm$ SD.)
Control	902.90 $\pm$ 35.43	220.14 $\pm$ 21.85	146.60 $\pm$ 7.32	119.46 $\pm$ 6.17	27.48 $\pm$ 1.23	37.47 $\pm$ 2.08	14.66 $\pm$ 0.77	20.66 $\pm$ 1.11	15.43 $\pm$ 0.67
Splenectomy	2278.95 $\pm$ 623.49	518.89 $\pm$ 38.59	218.34 $\pm$ 12.98	70.20 $\pm$ 3.69	10.81 $\pm$ 0.96	3307.47 $\pm$ 299.82	33.37 $\pm$ 2.20	48.57 $\pm$ 3.85	42.10 $\pm$ 4.67
Non-Splenectomy	1009.24 $\pm$ 50.52	183.00 $\pm$ 13.75	275.92 $\pm$ 18.22	73.07 $\pm$ 2.99	16.00 $\pm$ 0.60	1830.35 $\pm$ 151.11	35.21 $\pm$ 3.86	28.53 $\pm$ 4.60	22.89 $\pm$ 2.19
Modern Infection	812.4 $\pm$ 47.28	56.93 $\pm$ 3.619	133.83 $\pm$ 8.78	81.64 $\pm$ 2.92	24.29 $\pm$ 1.39	743.22 $\pm$ 73.17	16.88 $\pm$ 1.37	23.85 $\pm$ 1.64	26.85 $\pm$ 1.50
P-Value	**	*	*	*	*	**	**	*	*

* $P<0.05$, ** $P<0.01$

Table 2: The Comparisons of Immunoglobulin (IgG, IgA, IgM) and Complement (C3, C4) Levels among β -TM Splenectomy Patients and Control

Variable	Immunoglobulins			Complements		Serum Ferritin	Liver function		
	IgG (Mean $\pm$ SD.)	IgA (Mean $\pm$ SD.)	IgM (Mean $\pm$ SD.)	C3 (Mean $\pm$ SD.)	C4 (Mean $\pm$ SD.)		TSB (Mean $\pm$ SD.)	GPT (Mean $\pm$ SD.)	GOT (Mean $\pm$ SD.)
Control	902.90 $\pm$ 35.43	220.14 $\pm$ 21.85	146.60 $\pm$ 7.32	119.46 $\pm$ 6.17	27.48 $\pm$ 1.23	37.47 $\pm$ 2.08	14.66 $\pm$ 0.77	20.66 $\pm$ 1.11	15.43 $\pm$ 0.67
Splenectomy	2278.95 $\pm$ 623.49	518.89 $\pm$ 38.59	218.34 $\pm$ 12.98	70.20 $\pm$ 3.69	10.81 $\pm$ 0.96	3307.47 $\pm$ 299.82	33.37 $\pm$ 2.20	48.57 $\pm$ 3.85	42.10 $\pm$ 4.67
P-Value	**	*	*	*	*	***	**	**	**
Control	902.90 $\pm$ 35.43	220.14 $\pm$ 21.85	146.60 $\pm$ 7.32	119.46 $\pm$ 6.17	27.48 $\pm$ 1.23	37.47 $\pm$ 2.08	14.66 $\pm$ 0.77	20.66 $\pm$ 1.11	15.43 $\pm$ 0.67
Non-Splenectomy	1009.24 $\pm$ 50.52	183.00 $\pm$ 13.75	275.92 $\pm$ 18.22	73.07 $\pm$ 2.99	16.00 $\pm$ 0.60	1830.35 $\pm$ 151.11	35.21 $\pm$ 3.86	28.53 $\pm$ 4.60	22.89 $\pm$ 2.19
P-Value	NS	NS	*	*	*	***	**	NS	NS
Control	902.90 $\pm$ 35.43	220.14 $\pm$ 21.85	146.60 $\pm$ 7.32	119.46 $\pm$ 6.17	27.48 $\pm$ 1.23	37.47 $\pm$ 2.08	14.66 $\pm$ 0.77	20.66 $\pm$ 1.11	15.43 $\pm$ 0.67
Modern Infection	812.4 $\pm$ 47.28	56.93 $\pm$ 3.619	133.83 $\pm$ 8.78	81.64 $\pm$ 2.92	24.29 $\pm$ 1.39	743.22 $\pm$ 73.17	16.88 $\pm$ 1.37	26.85 $\pm$ 1.50	23.85 $\pm$ 1.64
P-Value	NS	*	NS	*	*	*	NS	NS	NS

* $P < 0.05$, ** $P < 0.01$, NS non-significant**Table 3:** Other Comparisons among β -TM Patients Groups Themselves.

Variable	Immunoglobulins			Complements		Serum Ferritin	Liver function		
	IgG (Mean $\pm$ SD.)	IgA (Mean $\pm$ SD.)	IgM (Mean $\pm$ SD.)	C3 (Mean $\pm$ SD.)	C4 (Mean $\pm$ SD.)		TSB	GPT	GOT
Splenectomy (Mean $\pm$ SD.)	2278.95 $\pm$ 623.49	518.89 $\pm$ 38.59	218.34 $\pm$ 12.98	70.20 $\pm$ 3.69	10.81 $\pm$ 0.96	3307.47 $\pm$ 299.82	33.37 $\pm$ 2.20	48.57 $\pm$ 3.85	42.10 $\pm$ 4.67
Nonsplenectomy (Mean $\pm$ SD.)	1009.24 $\pm$ 50.52	183.00 $\pm$ 13.75	275.92 $\pm$ 18.22	73.07 $\pm$ 2.99	16.00 $\pm$ 0.60	1830.35 $\pm$ 151.11	35.21 $\pm$ 3.8	28.53 $\pm$ 4.60	22.89 $\pm$ 2.19
P-Value	***	*	*	NS	*	**	***	*	**
Splenectomy (Mean $\pm$ SD.)	2278.95 $\pm$ 623.49	518.89 $\pm$ 38.59	218.34 $\pm$ 12.98	70.20 $\pm$ 3.69	10.81 $\pm$ 0.96	3307.47 $\pm$ 299.82	33.37 $\pm$ 2.20	48.57 $\pm$ 3.85	42.10 $\pm$ 4.67
Modern Infection	812.4 $\pm$ 47.28	56.93 $\pm$ 3.619	133.83 $\pm$ 8.78	81.64 $\pm$ 2.92	24.29 $\pm$ 1.39	743.22 $\pm$ 73.17	16.88 $\pm$ 1.37	23.85 $\pm$ 1.64	26.85 $\pm$ 1.50
P-Value	***	***	*	*	**	**	**	**	**
Nonsplenectomy (Mean $\pm$ SD.)	1009.24 $\pm$ 50.52	183.00 $\pm$ 13.75	275.92 $\pm$ 18.22	73.07 $\pm$ 2.99	16.00 $\pm$ 0.60	1830.35 $\pm$ 151.11	35.21 $\pm$ 3.8	28.53 $\pm$ 4.60	22.89 $\pm$ 2.19
Modern Infection	812.4 $\pm$ 47.28	56.93 $\pm$ 3.619	133.83 $\pm$ 8.78	81.64 $\pm$ 2.92	24.29 $\pm$ 1.39	743.22 $\pm$ 73.17	16.88 $\pm$ 1.37	23.85 $\pm$ 1.64	26.85 $\pm$ 1.50
P-Value	NS	*	*	NS	*	**	*	NS	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS non-significant

Table 4 shown a significant increase of IgG particular ($P < 0.01$) and other immunoglobulin's concentrations ($P < 0.05$) with the age progression of β -TM patients. A significantly increasing of immunoglobulins was (1239.87 $\pm$ 126.64) in >21 yrs. of β -TM patients. While, the significance results reported higher reduction ($P < 0.05$) in the complement's concentration C3 (63.99 $\pm$ 4.27) and

C4 (13.51 $\pm$ 1.05) at age range 11-20 yrs. in studies patient. There is significant increasing of iron overload, TSB and GPT among age range >21 yrs. than other ages. The means were (2244.70 $\pm$ 233.49, 29.15 $\pm$ 3.68 and 36.98 $\pm$ 5.62), respectively. GOT does not recorded any significant among β -TM patients according the age as shown in table 4.

Table 4: Distribution of Immunoglobulin, Complements, Ferritin and Liver Functions among β -TM Patients According Age.

Age range (ys)		1-10 (Mean $\pm$ SD)	11-20 (Mean $\pm$ SD)	>21 (Mean $\pm$ SD)	Significant (P Value)
Immunoglobulin	IgG	808.49 $\pm$ 70.01	1157.73 $\pm$ 136.96	1239.87 $\pm$ 126.64	**
	IgA	49.94 $\pm$ 3.71	241.42 $\pm$ 43.06	393.21 $\pm$ 56.63	*
	IgM	137.16 $\pm$ 7.89	237.65 $\pm$ 28.83	268.18 $\pm$ 27.96	*
Complement	C3	86.10 $\pm$ 5.47	63.99 $\pm$ 4.27	73.38 $\pm$ 5.57	
	C4	26.12 $\pm$ 2.93	13.51 $\pm$ 1.05	13.74 $\pm$ 1.17	*
Liver function	Serum ferritin	728.25 $\pm$ 100.57	1777.67 $\pm$ 295.11	2244.70 $\pm$ 233.49	**
	TSB	17.67 $\pm$ 1.60	28.16 $\pm$ 3.23	29.15 $\pm$ 3.68	*
	GPT	29.08 $\pm$ 2.52	22.91 $\pm$ 4.33	36.98 $\pm$ 5.62	*
	GOT	35.49 $\pm$ 5.98	23.25 $\pm$ 4.38	36.86 $\pm$ 6.33	NS

* $P < 0.05$, ** $P < 0.01$, NS non-significant

The results in table 5 showed the positive correlation between IgA and each of serum ferritin and liver functions assays studies. The Pearson's correlation was $r=0.58$, $r=0.28$, $r=0.49$ and $r=0.46$ respectively. Also, the current study founded negative correlation

between each of C4 serum ferritin and liver functions assays studied ($r=-0.19$, $r=-0.08$, $r=-0.15$ and $r=-0.04$ respectively). It was observed the correlation positive between IgG and each of GPT and GOT.

Table 5: Pearson's Correlation between Immunoglobulins and Studied Complements with Iron Overload and Liver Functions

Variable		Serum Ferritin (Pearson's Correlation)	TSB (Pearson's Correlation)	GPT (Pearson's Correlation)	GOT (Pearson's Correlation)
Immunoglobulin	IgG	0.06	-0.18	0.27*	0.28*
	IgA	0.58*	0.28*	0.49*	0.46*
	IgM	0.22	0.30**	-0.13	-0.18
Complement	C3	-0.19	-0.08	-0.15	-0.04
	C4	-0.43*	-0.23*	-0.31*	-0.31*

Discussion

Immunoglobulin and complement are released as a response to repeated blood transfusions, iron overload, and splenectomy among β -TM infections [15, 16]. Our study showed that β -TM had much higher levels of IgM, IgA, and IgG and significantly lower levels of C3 and C4. These observations are in accordance with Amin et al. [17] Malik et al. [18] one reporter mentions that recurrent infections stimulate the immune system and may lead to an increase in immunoglobulins [19].

C3 and C4 concentrations were not significantly lower in β -TM disorder compared to healthy individuals [20]. The other two studies refer to a statistically significant reduction of C3 versus a non-significant result of C4 and immunoglobulin levels among β -TM patients [21, 22]. While Nsaif et al. [23] pointed to non-significant differences in immunoglobulins and complements among the same patients. Also, the IgG and IgM levels have been reported to be normal in β -TM patients at different ages [24]. Current investigation discovered that significantly high ferritin levels are associated with high immunoglobulin levels in the studied patients. These results were logical due to increased IgM levels among those who had ferritin levels >2500 ng/ml in β -TM patients [17].

Another previous study found that 53.3% of these patients had a ferritin (Iron overload) more than or equal to 2500 μ g/L [15]. Also, a comparable study found that ferritin was significantly higher in β -TM patients compared to healthy patients [5]. The ferritin concentration recorded a significant correlation with blood transfusion duration of those patients [13]. GPT and GOT are enzymes found in the liver, red blood cells, heart, muscle tissue, and other organs [25]. The present findings agree with other studies [26-28] due to the GPT, GOT, and TSB rising. The mechanical reason for that rising is still unclear in β -TM patients [29].

For age: the current study revealed to immunodeficiency was accompanied with age progress among β -TM patients. These are clarified due to the studied immunoglobulins, ferritin significant increasing and C3, C4 significant decreasing at age progress of the studied disorder. Our result agree with [15] due to the same degree of significant of both immunoglobulins and complements in the older β -TM patients at aged > 15 yrs. than control. While, Amen et al. refer to the immunoglobulin normal levels in β -TM patients due to aged 5-10 yrs [17]. Also, this study showed splenectomy causes immunodeficiency due to high immunoglobulin and complement consumption in splenectomy compared to non-splenectomized study patients. One study said 38 percent of β -TM underwent splenectomy

surgery [15]. Another study showed that the IgA and IgM had a significant decrease post-splenectomy from β -TM patients [19].

IgM alone was low in patients post-splenectomy [4]. Splenectomized β -TM patients exhibited significantly higher levels of IgG and IgA and non-significant differences in IgM, C3, and C4 compared to non-splenectomized patients [17]. IgA was increased in the β -TM non-splenectomized group aged over twenty years [24]. The current study found a direct relationship between ferritin and immunodeficiency amongst β -TM patients. These are supported due to the iron accumulation in the form of hemosiderin, which is a toxic substance in tissues. Iron chelation therapy may inadvertently influence the gut micro biome, a critical modulator of immunity [4]. The study returns to the immunity due to the migration of T-lymphocyte cells to the lymph nodes, which leads to an immunoglobulin increasing with the consumption of complement factors in β -TM patients [18].

Also, the excessive iron can be explained by reduced growth failure in β -thalassemia children [30]. Ocular manifestations may relate to iron accumulation in β -TM patients' tissues [31]. The splenectomy can cause the pressure on other secondary lymphoid organs to compensate for the deficiency. This may be our enable to design the appropriate therapies of these patients [16]. Therefore, the current study recommended that necessary not removing the spleen in the early stages of the age of β -TM patients as anew therapy of these patients.

Conclusion

The immunodeficiency reason may be due to preoccupation immunity with hemosiderin accumulation in tissue. Therefore, our study suggested that the iron overload (ferritin) has correlated to immunodeficiency. Also, an increase in TSB and GOT greatly affects the immunity of these subjects. This makes the β -TM patients more exposed to hypo_gammaglobulinemia (immunodeficiency) due to any subsequent illnesses.

Ethics Consideration

The β -TM patients' and their parents were informed of the nature of the research and its immunological importance.

Acknowledgements

The research team extends its sincere thanks and appreciation to center of hereditary blood disease administration for their cooperation in facilitating the task of collecting samples to complete the research.

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