Research Article

Comparison of the Immunity Status in-Between Children with β-Thalassaemia Major Receiving Different Treatment Modalities: A Single Egyptian District Study

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Abstract

Background: β-thalassaemia major is one of the chronic hemolytic anemias resulting from defect in β-globin chain. It requires frequent blood transfusion plus other treatment modalities. These treatment modalities may be associated with certain immunologic modulations.

Objective: To assess the immunity status in children with β-thalassaemia major under different treatment regimens within El Minia, Egypt.

Subjects and Methods: One hundred forty-four children were enrolled and classified into four groups. Thirty-six β-thalassaemia patients treated only with blood transfusion (group I). Thirty-six patients treated with transfusion and iron chelation (group II). Thirty-six patients treated with transfusion, iron chelation and subjected to splenectomy (group III). Group IV involved thirty-six apparently healthy age and sex matched children. CBC plus serum levels of ferritin, IgA, complement C3 and C4 were measured along with detection of CD3+, CD4+, CD8+, CD19+ and CD56+ lymphocyte percentages and absolute counts.

Results: IgA levels were significantly higher in thalassaemia patients compared to controls (p<0.001) plus highly significant increase in IgA levels in splenectomized patients than non-splenectomized (p<0.001). Levels of C3 were significantly decreased in all patients compared with controls (p=0.001) with a highly significant decrease in C3 levels in splenectomized patients than non splenectomized ones (p<0.001) but no statistical difference between their C4 levels. Significant statistical differences were revealed regarding CD3+, CD4+ and CD8+ T lymphocyte percentages within thalassaemia groups when compared to each other's and to controls. Splenectomized patients had higher significant levels regarding serum ferritin (p=0.02) along with CD3+ (p=0.05), CD4+ (p=0.05) and CD8+ (p=0.037) lymphocyte percentages compared to non-splenectomized. CD19+ lymphocyte percentages were significantly higher while CD56+ lymphocyte percentages were significantly lower in all patients compared with controls (p=0.02 and 0.05).

Conclusion: Immune modulation occurs in thalassaemia patients with regional specific variations and is related to variations in treatment modalities.

Keywords: Thalassaemia; Immunity; Treatment modalities; Egyptian children; El Minia region

Abbreviations: CBC-Complete Blood Count; IgA-Immunoglobulin A

Introduction

Thalassaemia is defined as a group of inherited disorders that arise as a result of certain mutations in hemoglobin (Hb) genes. β-thalassaemia major (βTM) has a high prevalence in the Mediterranean region, including Egypt, Middle East, Indian subcontinent, and South East Asia [1]. Though, it is a growing global health problem due to extensive population migrations. About 1.5% of the world populations are carriers of β-thalassaemia gene [2]. βTM patients present with many problems rather than severe anemia, including increased susceptibility to infections which constitutes the second most common cause of mortality and a major cause of morbidity in β-thalassaemia after heart failure [3]. The underlying causes of their increased liability to infections may be attributed to anemia per-se, reticuloendothelial system dysfunction as well as therapeutic regimens-related infections [4].

These therapeutic approaches incorporate repeated blood transfusion alone or in combination with iron chelators. Splenectomy is added when indicated. Recently, gene therapy and bone marrow transplant or stem cell therapy are promising hopes [5]. Volumetric

and multiple transfusions lead to alloimmunization, accumulation of iron in different tissues and are associated with increased risk of infections. These infections are caused by lots of bacterial and viral infections such as cytomegalo virus, Ebstein-Barr virus and hepatitis C virus [6]. Likewise, Yersinia enterocolitica is usually associated with the use of deferoxamine (DFO) as an iron chelator [7]. As well, the risk of sepsis in splenectomized patients is as high as 7% over a 10-years period and almost 25% of splenectomized patients are at the risk of severe infections [8]. Hence, it is crucial to identify the impact of these treatment modalities on the immune status of these patients.

Several studies about the immune competence in β-thalassaemia had revealed numerous quantitative and functional defects, involving T and B lymphocytes, immunoglobulins in addition to the impairment in components of the complement system [9,10]. Additionally, immunologic disorders in patients with βTM comprise decreased absorption and phagocytic ability of segmented neutrophils, changes in cytokines production as well as dysfunction of macrophages, properdin and lysozymes [11]. As well, it has been reported that the distribution of β-globin gene mutations differs among ethnic groups plus its regional and individual variation [12]. Furthermore, there is increasing evidence suggesting that clinical features or complications of βTM plus their immune characters and even their response to various therapies are population dependence. Also, the immunity status of βTM patients varies in relation to the type and extent of treatment approaches [8].

The overall objective of this study was to identify the immunological alterations of βTM patients within Egyptian children in El Minia district. Our aim was to compare the immune competence between those βTM children who receiving multiple blood transfusions alone or with iron chelating agents plus or minus splenectomy. We studied serum immunoglobulin A (IgA) levels beside serum C3 and C4 complements in addition to blood counts of T, B and natural killer (NK) lymphocytes.

Subjects and Methods

Subjects

This multicenter, case-control study was conducted in El Minia city in Egypt. Patients were selected from Pediatric Hematology Outpatient Clinic at Minia University Hospital and from Pediatric Hematology Outpatient Clinic, National Blood Bank, Minia Ministry of Health while controls were selected from apparently healthy children from Pediatric Growth Clinic, Minia University Hospital. This study was carried out between May and December, 2015. One hundred and forty-four children were included in this study, which involved a group of 108 thalassaemia patients and 36 apparently healthy children with matching age and sex as a control group IV. Control children were 18 males (50%) and 18 females (50%). There ages were ranged from 2-16 years old. The thalassaemia patients group was further subdivided into three subgroups according to the difference in their treatment regimens. These subgroups comprised patient groups from I-III. Group I patients (No. 36) were receiving blood transfusion only. These patients were 18 males (50%) and 18 females (50%). There ages were ranged from 2-8 years old. Patients in group II (No. 36) were receiving blood transfusion and iron chelation while patients in group III (No. 36) were receiving blood transfusion plus iron chelation and also subjected to splenectomy. Group II patients were 12 males (33.3%) and 24 females (66.7%). There ages were ranged from 5-12 years old. Group

III patients were 21 males (58.3%) and 15 females (41.7%). There ages were ranged from 10-16 years old.

All included patients and controls were subjected to complete history taking (name, age, sex, residence, family history, consanguinity, age of 1st blood transfusion, amount of transfusion per year, duration of splenectomy in splenectomized patients in addition to history of exposure to infections). Moreover, the involved children were examined carefully (general examination, anthropometric measures which were plotted on percentile growth charts, vital data as well as examination of chest, heart and abdomen). The exclusion criteria were patients who had a bone marrow transplant. Finally, the laboratory investigations were performed to all subjects. These investigations were comprised Complete Blood Count (CBC), blood smears, Hemoglobin (Hb) electrophoresis for controls, serum ferritin, serum level of immunoglobulin A (IgA), complements C3 and C4 plus percentages and absolute counts of CD3, 4, 8, 19 and 56 T lymphocytes.

Blood Sampling

Venous blood samples were collected from both patients and controls under complete aseptic conditions. About 2 ml of blood were withdrawn in K3-EDTA anticoagulant tubes for complete blood counts, peripheral blood smears as well as flow cytometric analysis (immunophenotyping). About 3ml of blood were withdrawn in a plain tube without any anticoagulant. Their serum was then separated and stored at - 70°C till used for serum ferritin, IgA and complements assays.

Blood samples were withdrawn from thalassaemia patients just prior to a scheduled transfusion. The blood samples from the control group were taken while coming for follow-up of their growth. All controls had normal Hb levels for their age and sex with normal red blood cell indices. At the time of sampling all patients and controls were apparently free from infections (normal C-reactive protein).

Laboratory methodology

Complete blood count was performed using an automated blood counter (Sysmex KX-21N). Additionally, peripheral blood smears were stained by Leishman stain. Reticulocyte counts were detected as well. The absolute lymphocytes and neutrophils counts were calculated. Serum ferritin levels were measured using human *in vitro* Enzyme-Linked Immunosorbent Assay (EIA) kit from Abcam Inc, Cambridge, USA. The assay was performed according to the manufacturer's instructions. Ferritin values were expressed as nanogram per milliliter (ng/ml). [13,14]

Levels of IgA were determined using human *in vitro* EIA kit from Abcam Inc, Cambridge, USA according to the manufacturer's instructions. Final IgA values were expressed in milligram per deciliter (mg/dl). As well, Complements C3 and C4 serum levels were detected with human *in vitro* EIA kit from Abcam Inc, Cambridge, USA. Complements C3 and C4 values were expressed in milligram per deciliter (mg/dl).

Flow cytometric immunophenotypic analysis

T- Lymphocyte subsets in whole blood samples were enumerated using fluoroisothiocyanate (FITC) conjugated CD4 (Becton Dickinson, Bioscience, USA), phycoerythrin (PE) conjugated CD8 (Becton Dickinson, Bioscience, USA) and peridinium-chlorophyll-protein (Per-CP) conjugated CD3 (Becton Dickinson, Bioscience,

USA). B-Lymphocyte subsets in whole blood samples were enumerated using phycoerythrin (PE) conjugated CD19 (Becton Dickinson, Bioscience, USA). Natural killer Lymphocyte subsets in whole blood samples were enumerated using phycoerythrin (PE) conjugated CD56 (Becton Dickinson, Bioscience, USA). Flow cytometric analysis was done by FACS Calibur flow cytometry with Cell Quest software (Becton Dickinson Biosciences, USA). An isotype matched negative control was used with each sample. Forward and side scatter histogram was used to define the lymphocyte population (R1). The absolute counts and percentages of CD3, 4, 8, 19 and 56 subsets of lymphocytes were calculated.

Parameters	Patients			Controls	p-values (patients *vs* controls)
	Group I	Group II	Group III		
Hb level (g/dl)	6.7 ± 1.6	6.8 ± 1.5	6.9 ± 1.1	12.3 ± 0.93	0.015*
WBCs count (× 10^3 cells/μl)	11.3 ± 1.6	9.24 ± 2.0	10.1 ± 2.9	8.8 ± 3	0.08
Platelet count (× 10^3 cells/μl)	510 ± 25	449.8 ± 16	570.9 ± 21	350 ± 14	0.18
ANC (× 10^3 cells/μl)	3.77 ± 0.98	3.57 ± 1.5	3.74 ± 1.6	4.10 ± 1.4	0.05*
ALC (× 10^3 cells/μl)	5.15 ± 2.3	4.15 ± 1.7	5.35 ± 2.2	4.20 ± 1.6	0.38
AOBT/Y (ml/kg/year)	1995 ± 11.6	4585.42 ± 13.6	2907.8 ± 74.3	------	-------
Serum ferritin (ng/ml)	2136 ± 17.6	3031.7 ± 14.2	3974.2 ± 13.7	70.03 ± 18.8	0.013*
Serum Ig A (mg/dl)	295.5 ± 69.7	345.9 ±37.6	469.8 ± 29.9	174.6 ± 78.8	<0.001**
Serum C3 (mg/dl)	113.9 ± 12.6	141.6 ± 31.2	62.7 ± 5.7	152.8 ± 34.8	0.001**
Serum C4 (mg/dl)	19.6 ± 1.4	23.2 ± 4.9	25.0 ± 11.8	27.9 ± 3.4	0.002*
Absolute CD3+ count (× 10^3 cells/μl)	3.3 ± 1.1	2.5 ± 1.3	3.7 ± 1.6	2.3 ± 0.6	0.05*
Absolute CD3+/CD4+ count (× 10^3 cells/μl)	1.8 ± 0.6	1.3 ± 0.7	2.1 ± 0.9	1.2 ± 0.1	0.04*
Absolute CD3+/CD8+ count (× 10^3 cells/μl)	1.5 ± 0.5	1.1 ± 0.6	1.7 ± 0.7	0.9 ± 0.3	0.06
Absolute CD19+ count (× 10^3 cells/μl)	0.8 ± 0.3	0.6 ± 0.3	0.8 ± 0.4	0.5 ± 0.3	0.02*
Absolute CD56+ count (× 10^3 cells/μl)	0.9 ± 0.3	0.7 ± 0.4	0.6 ± 0.3	1.2 ± 0.5	0.05*

Table 1: Comparison between studied groups regarding laboratory data. Results are presented as Mean ± SD; Group I: thalassaemia children receiving blood transfusion only, Group II: thalassaemia children receiving blood transfusion + iron chelation, Group III: thalassaemia children receiving blood transfusion + iron chelation + splenectomy; vs: versus; Hb: Hemoglobin; WBCs: White Blood Cells; ANC: Absolute Neutrophils Count; ALC: Absolute Lymphocyte Count; AOBT/Y: Amount of Blood Transfusion Per Year; Ig A: Immunoglobulin A; C3: Complement C3; C4: Complement C4; *= Significant (p ≤ 0.05), **= Significant (p ≤ 0.001).

Statistical analysis

The clinical and laboratory data were recorded. These data were analyzed using statistical program for social science SPSS software version 17 (SPSS Inc., Chicago, IL, USA). Quantitative variables were presented as mean ± standard deviation (SD). On the other hand, description of qualitative variables was presented as number (No.) and percentage (%). Results were expressed as tables and figures. Graphics were done by Excel, Microsoft Office 2010. Student t-test was used to compare results between groups as regards quantitative variables. The correlation was performed by using Pearson correlation coefficient (r). For all tests, a probability (p) was considered non-significant if >0.05, significant if ≤ 0.05 and highly significant if ≤ 0.001. One Way ANOVA test was used for comparison of quantitative data between more than two groups. The Chi-square test was used to compare qualitative variables between groups.

Results

Comparison of laboratory results and immunological parameters between β-thalassaemia major children under different treatment modalities and controls

A significant difference was found on comparison between all thalassaemia patients (group I, II, III) and control group regarding Hb levels (p=0.015), ANC (p=0.05), serum IgA (p<0.001), serum C3 (p=0.001), serum C4 (p=0.002), CD3+ absolute count (p=0.05), CD3+/CD4+ absolute count (p=0.04), CD3+/CD8+ absolute count (p=0.06), CD19+ absolute count (p=0.02) and CD56+ absolute count (p=0.05) (Table 1).

Also, differences within the 3 thalassaemia groups regarding IgA, C3, C4 (Table 2) along with percents of CD3+, CD3+/CD4+, CD3+/CD8+, CD19+ and CD56+ as well as their absolute counts were summarized (Figure 1 and Table 2).

Parameters	Group I vs II	Group I vs III	Group II vs III
	p-values		
Serum IgA	0.04*	<0.001**	<0.001**
Serum C3	0.01*	<0.001**	<0.001**
Serum C4	0.03*	0.12	0.58
CD3+ (%)	<0.001**	<0.001**	<0.001**
Absolute CD3+ count	0.1	0.4	0.04*
CD3+/CD4+ (%)	0.01*	0.04*	<0.001**
Absolute CD3+/CD4+ count	0.05*	0.3	0.03*
CD3+/CD8+ (%)	0.02*	0.05*	<0.001**
Absolute CD3+/CD8+ count	0.1	0.4	0.05*
CD19+ (%)	0.02*	0.7	0.02*
Absolute CD19+ count	0.1	0.44	0.05*
CD56+ (%)	0.22	<0.001**	0.04*
Absolute CD56+ count	0.11	0.02*	0.2

Table 2: Comparison between studied patients regarding immunological markers; Group I: thalassaemia children receiving blood transfusion only; Group II: thalassaemia children receiving blood transfusion + iron chelation; Group III: thalassaemia children receiving blood transfusion + iron chelation + splenectomy; vs: versus; Ig A: Immunoglobulin A; C3: Complement C3; C4: Complement C4; Non sig. >0.05, Significant ≤ 0.05*, High Significant ≤ 0.001**.

The comparison between splenectomized patients (group III) and non splenectomized ones (group I plus II) concerning the studied immune parameters was illustrated in Figure 1. Serum IgA levels were statistically significant high in splenectomized patients compared with non splenectomized groups (p<0.001) (Figure 2A). Moreover, splenectomized patients showed significant decrease in serum C3 levels (p<0.001) and non-significant difference in serum C4 levels in comparison to non splenectomized groups (p=0.282) (Figure 2B).

CD3+, 4+ and 8+ percentages were statistically significant higher in the splenectomized group in comparison to non splenectomized patients (p=0.05, 0.05 and 0.037 respectively). On the other hand, splenectomized children showed non-significant difference from non splenectomized ones concerning CD19+ percentage, but highly statistically significant lower levels regarding CD56+ (p=0.235, <0.001 respectively) (Figure 2C).

Serum ferritin levels within β-thalassaemia subgroups in correlation to immunological characteristics

The mean ± SD of serum ferritin levels within thalassaemia groups as well as controls were shown in table 1. There was a significant increase in serum ferritin levels when patients were compared with controls (p=0.013). Additionally, serum ferritin levels were elevated in splenectomized patients (group III) compared with non splenectomized ones (p=0.02) (Figure 3). Significant moderate positive correlation was found between CD3+ cells and ferritin (p=0.04, r=0.6). As well, significant fair positive correlation was found between CD3+/CD8+ cells and ferritin (p=0.04, r=0.3) in group I. There were no other significant correlations between ferritin and the remaining immune parameters (Table 3).
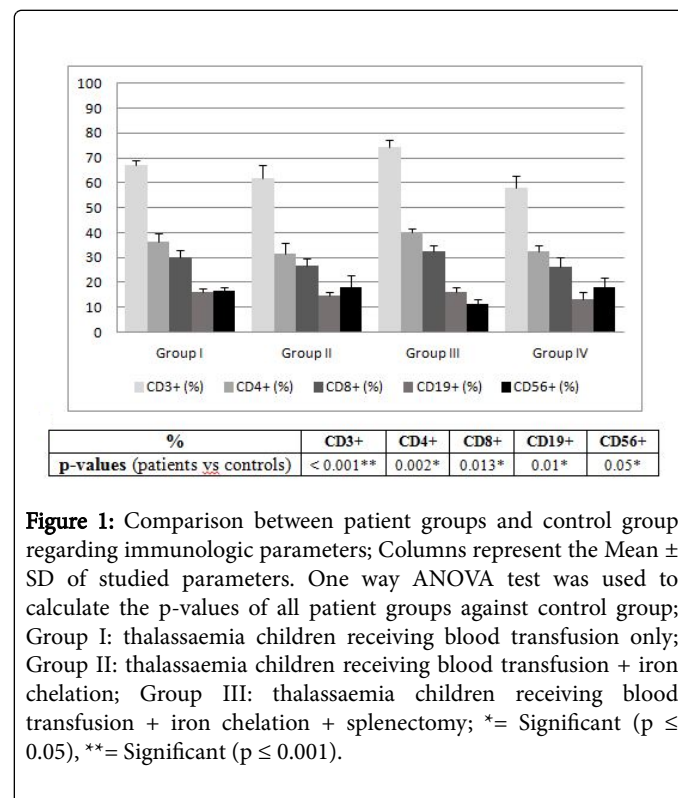

%	CD3+	CD4+	CD8+	CD19+	CD56+
p-values (patients vs controls)	< 0.001**	0.002*	0.013*	0.01*	0.05*

Figure 1: Comparison between patient groups and control group regarding immunologic parameters; Columns represent the Mean ± SD of studied parameters. One way ANOVA test was used to calculate the p-values of all patient groups against control group; Group I: thalassaemia children receiving blood transfusion only; Group II: thalassaemia children receiving blood transfusion + iron chelation; Group III: thalassaemia children receiving blood transfusion + iron chelation + splenectomy; *= Significant (p ≤ 0.05), **= Significant (p ≤ 0.001).

Variables Groups			Ig A (mg/dl)	C3 (mg/dl)	C4 (mg/dl)	CD3+ (%)	CD3+/CD4+ (%)	CD3+/CD8+ (%)	CD19+ (%)	CD56+ (%)
Group I	Serum ferritin (ng/ml)	p	0.9	0.13	0.81	0.04*	0.06	0.04*	0.53	0.14
		r	0.04	-0.47	0.07	0.6	0.2	0.3	0.2	-0.45
Group II		p	0.96	0.58	0.99	0.9	1	0.5	0.35	0.53
		r	0.02	-0.18	-0.04	0.02	0.02	0.2	0.29	-0.2
Group III		p	0.53	0.65	0.51	0.8	0.8	0.2	0.47	0.11
		r	0.19	-0.15	-0.21	0.09	0.07	0.4	0.23	-0.47

Table 3: Correlation between different immunological markers and serum ferritin levels within patient's groups; Group I: thalassaemia children receiving blood transfusion only: Group II: thalassaemia children receiving blood transfusion + iron chelation; Group III: thalassaemia children receiving blood transfusion + iron chelation + splenectomy; r=0.75-1(strong correlation); r=0.5-0.74(moderate correlation); r=0.25-0.49(fair correlation); r=0.1-0.24(weak correlation); Non Significant >0.05, Significant ≤ 0.05*, Ig A: Immunoglobulin A; C3:Complement C3; C4: Complement C4.
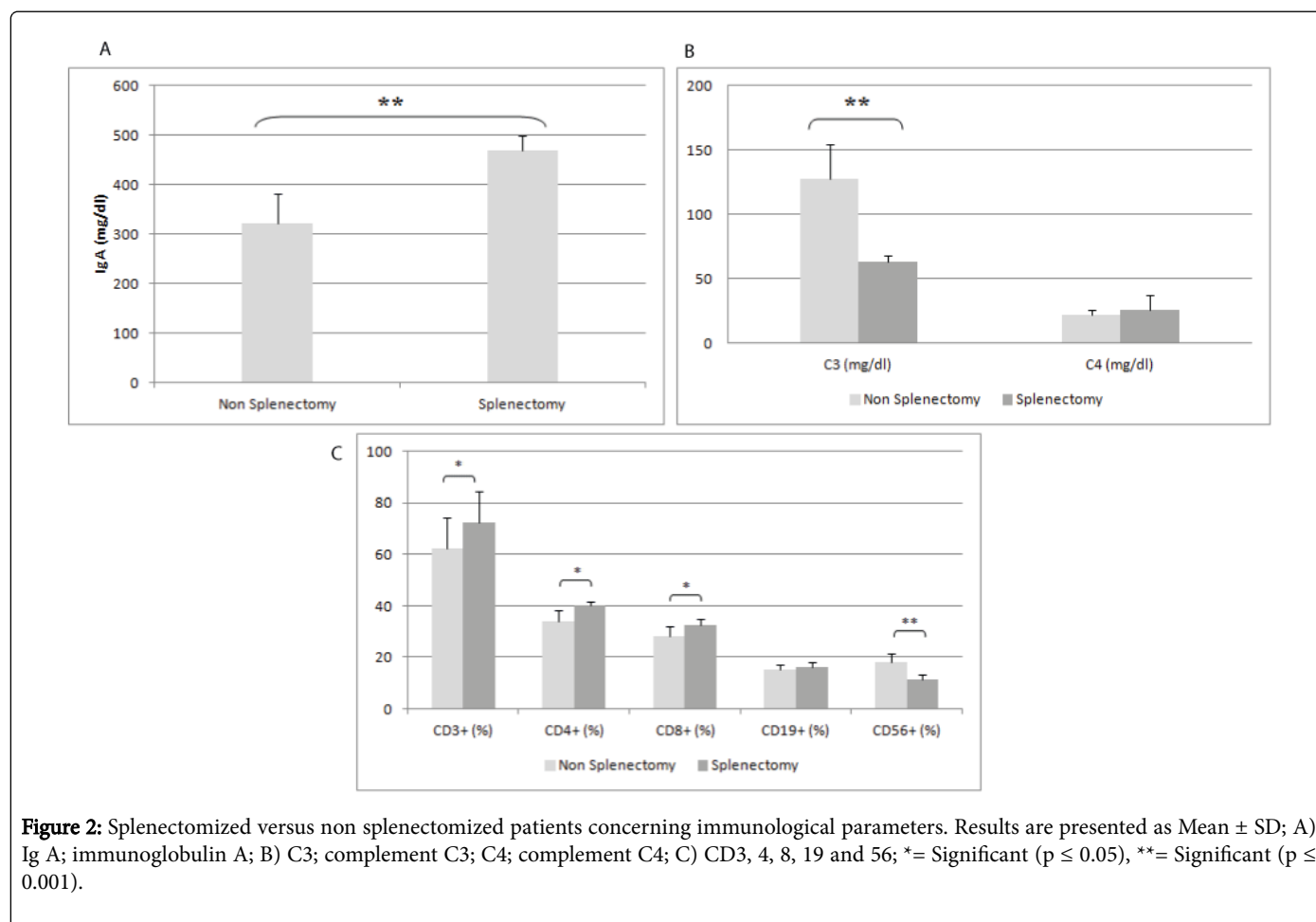

Figure 2: Splenectomized versus non splenectomized patients concerning immunological parameters. Results are presented as Mean ± SD; A) Ig A; immunoglobulin A; B) C3; complement C3; C4; complement C4; C) CD3, 4, 8, 19 and 56; *= Significant ($p ≤ 0.05$), **= Significant ($p ≤ 0.001$).
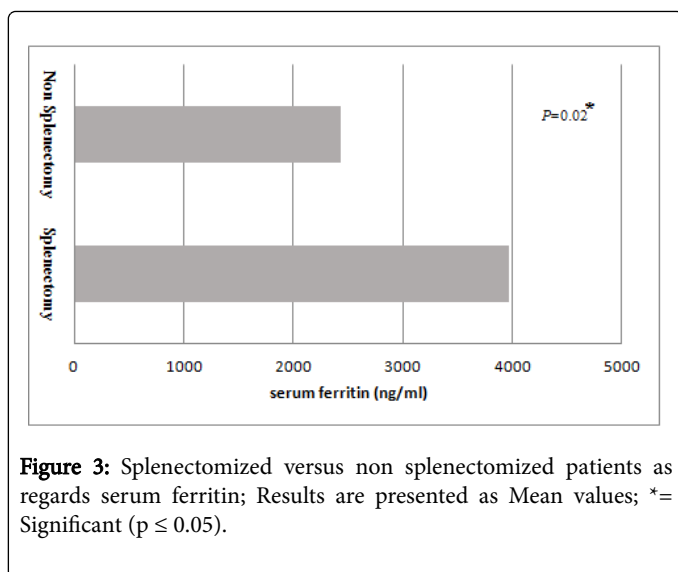
Figure 3: Splenectomized versus non splenectomized patients as regards serum ferritin; Results are presented as Mean values; *= Significant (p ≤ 0.05).

Discussion

It had been shown that the immune-capability of βTM patients has been altered [9, 10]. The presence of abnormal erythrocytes leads to continuous activation of monocytes and immune clearance [15]. Furthermore, it has been shown that βTM-related immunologic changes differ according to the variety and duration of therapeutic procedures and in a population dependence manner [8]. Data relevant to the impact of different treatment modalities on the immune system in thalassaemia patients are yet limited as these data should be adjusted for geographical differences. The aim of this work was to evaluate the immune status of Egyptian children within El Minia region who suffering from βTM and receiving various treatment modalities.

Immunoglobulins and complement proteins play a pivotal role in humoral immunity. Therefore, we assessed the deviations of these parameters among thalassaemia patients according to their treatment regimens [16]. This study has been concerned with assessment of IgA, serum complement C3 and C4 levels. IgA is an important mediator of mucosal immunity along with its involvement in complement activation via alternative and lectin pathways. Additionally, complement C3 and C4 are among the most commonly measured complement components [17].

The present study demonstrated that serum IgA levels were significantly higher in all groups of βTM compared to healthy controls (p<0.001). This was similar to what was detected by Ali N. Salman, et al., Jeddoa et al. and Ghaffari et al. [16,18,19]. On the other hand, Ezer et al. showed that serum levels of IgA were normal within all subgroups of βTM. In Ezer study, all patients with βTM appeared to have an intact humoral immune system whatever their criteria. In addition, our data showed that splenectomized patients had higher significant increase in serum IgA levels than non splenectomized ones (p<0.001) [20]. Many previous studies had supported our data, but Ahluwalia et al. didn't detect any impacts of splenectomy on immunoglobulins [21-24].

The mechanisms of such increase in serum IgA levels can be attributed to iron overload in the skin of thalassaemia patients which leads to stimulation of the mucocutaneous IgA production [25]. As well, it has been suggested that iron overload causes an increased migration of T helper cells to the gut and lymph nodes which increases serum immunoglobulins levels especially IgA as the main immunoglobulin isotype in most mucosal surfaces [18]. Also, iron and its binding proteins have immune regulatory properties. Therefore, iron excess may tip the immune balance unfavorably to allow increased growth rates of infectious organisms followed by increased immunoglobulin levels [8]. Moreover, repetitive transfusions lead to continuous alloantigenic stimulation [26,27]. Additionally, thalassaemia patients are prone to many bacterial and viral infections [3]. Therefore, the immune system is activated and immunoglobulin levels are elevated [8]. Finally, increased serum levels of IgA mostly after splenectomy could be due to the pressure that occurs on other secondary lymphoid organs to synthesis major immunoglobulin classes for compensation of spleen loss [28].

In the current study, the mean levels of complements C3 and C4 were significantly decreased in all patient groups compared with controls (p=0.001, 0.002 respectively). This is in accordance with Jeddoa et al., El Yazji and Amin et al. studies. [16,23,29] Ezer et al. results are unlike ours as their study showed no significant difference in C3 levels between patients and controls [20]. This may be explained on the basis that repeated blood transfusion could result in continuous exposure to various antigens which lead to continuous immune system stimulation and hence complement consumption [8]. The addition of iron chelators in our study led to significant elevation of complement C3 and C4 along with increased serum IgA levels in Group II than Group I (p=0.01, 0.03 and 0.04 respectively). Aleem A et al. showed significant immune abnormalities in βTM patients with oral iron chelators including low C4 level [30]. As well, we found a highly significant decrease in serum C3 levels in splenectomized patients than non splenectomized ones (p<0.001). This was the same as Darzi AA et al. results [21] and unlike Kiani-Amin et al. outcomes [31]. The spleen plays a role in releasing starter proteins of complement activation pathways. So, splenectomy causes reduction in complement components [21]. Also, splenectomized patients have the most increased risk of overwhelming infections which stimulate the immune system, leading to complement consumption [23,32].

Lymphocytes are crucial in cell mediated immunity. Lymphocyte subpopulations include T cells, B cells and NK cells. T lymphocytes contain CD4+ T cells, which become activated upon foreign antigen exposure, such as intracellular pathogens, fungi and protozoa in addition to CD8+ T-cells which destroy virally infected cells. CD4+ cells along with CD8+ cells represent the majority of T lymphocytes [33]. We estimated the absolute counts along with percentages of CD3+ lymphocytes as representative of total T Lymphocytes (pan-T cell marker) besides CD4+ and CD8+ T subsets. Since absolute counts can vary from day to day and according to child age, it is more useful to look at the percentages of these lymphocyte subpopulations [34,35]. We found significant statistical differences regarding percentages of CD3+, CD4+ and CD8+ cells within thalassaemia groups when compared to each other's and to healthy controls. Patients of group I had significantly elevated CD4+ and CD8+ percentages, when compared to controls and to Group II patients. Group II patients had significantly lower CD4+ and CD8+ percentages when compared to other thalassaemia groups but group III had significantly elevated CD4+ and CD8+ percentages when compared to controls and other thalassaemia groups. These findings were in agreement with Kadam et al., Gharagozloo et al. and Vento et al. [3,25,36]. In contrast, Noulsri, et al. and Del Vecchio et al. had reported an insignificant difference in CD3+ cells and in its CD4+ and CD8+ subsets between patients and

controls [37,38]. Blood transfusion causes chronic infections and thus constant stimulation of the immune system which might induce T cells. Besides, the majority of patients in this study were found to have increased CD4+ and CD8+ cell percentages except those in group II when compared to controls and other patient groups. This may be related to DFO induced reduction in iron overload or its direct effect on immunity [30].

When classifying the studied patients into splenectomized and non splenectomized groups; splenectomized patients had higher significant levels regarding serum ferritin, CD3+, CD4+ and CD8+ compared to non splenectomized ones (p≤ 0.05) which was in agreement with Gharagozloo et al. and Lee et al. [25,39]. Increased T lymphocyte counts in post-splenectomy might be associated with antigens that could not be filtered by the spleen. This suggests that the spleen could play a role in the regulation of lymphocytes [25]. Iron overload indicated by ferritin was higher on post-splenectomy group than non-splenectomy group. This condition was due to iron absorption in response to ineffective erythropoiesis [40]. Therefore, iron overload, both from increased iron absorption or chronic blood transfusions, may affect the regulation and redistribution of lymphocyte subsets from the spleen and lymph nodes to the circulating pool and vice versa [41].

T-cells are required for the normal function of B-cells. Accordingly, most T-cells deregulation affects B lymphocytes. Thus, we measured CD19+ B cells. As well, we measured CD56+ cell counts as an indicator of NK cells. We revealed that percentage of CD19+ cells was significantly higher in all patients groups compared with controls (p=0.01). This was in accord with AI- Consolini et al. and Pattanapanyassat et al. [40,42] but Ahmadiafshar et al. and Gharagozloo et al. found insignificant statistical differences regarding CD19+ cells [25,43]. Also, we found that the percentages of CD56+ cells was significantly lower in group III compared with group I, II and controls. This matched the findings in Hagag et al. and Ahmadiafshar et al. [43,44]. The depressed natural killer cell activity in βTM patients may follow repeated blood transfusions and iron overload. Accordingly, this raises the concern of reduced resistance to viral infections and to the development of malignancy [45]. This makes thalassaemia patients susceptible to an increased risk of serious systemic infections [46]. Contrary, Gharagozloo et al. found no statistical difference in CD56+ cells between patient and controls [25].

These contradictory results between our study and the data of some previous studies could be referred to many factors. These factors are including: 1) Clinical heterogeneity, ethnicity and geographic regions variations because thalassaemia patients are different genetically and thus phenotypically due to human migrations. [47] 2) Frequency of blood transfusion [7,8,19,48,49]. Li et al. had reported that the number of NK cells is inversely proportional to transfusion numbers. 3) Body iron status and iron chelating therapy or 4) Duration since splenectomy [45].

The small size of the enrolled children is one of the limitations of this research which is related to the nature of this study as a region-specific one. This could be both limitation and advantage at the same time as this minimized the heterogeneity between individuals resulting in a more or less homogenous study groups. Thus, all patients had quite comparable predisposing risk factors for infections. More studies with greater multiregional populations seem to be required. Additionally, recognizing the genetic mutations of the enrolled patients plus correlating between these mutations and their phenotypic characters are fundamental. This will help in defining the role of location in βTM-induced complications or related responses to different therapies. Furthermore, covering the remaining immunologic parameters which are lacking in our study because of financial issues should be performed. As a consequence of this study along with earlier ones, regular monitoring of the immune status of βTM patients is recommended. This can increase our knowledge of their immune system behavior aiming to improve their quality of life and to prolong their survival rates.

Conclusion

Our results support that thalassaemia patients are immunologically different from normal children and that the difference in treatment approaches are crucial players in this immune alteration. Also, βTM-related immune modulations have a range of regional heterogeneity. Thus, contrasting findings are expected and regular follow-up of βTM immune status is needed aiming to reduce their liability to catch infections.

Ethical Considerations

The study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the research ethics committee of Minia University. The rationale, nature and possible risks of the experiments were fully explained to the parents. All parents gave written, informed consents at the beginning of the study and all data were kept confidential and used for research purposes only.

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