Evaluation of the Serum Levels of Immunoglobulin and Complement Factors in b-thalassemia Major Patients in Southern Iran

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ABSTRACT

Background: Beta-thalassemia major is one of the major health problems in our country. Many studies have confirmed the fact that, these patients have an increased susceptibility to bacterial infections. **Objective:** In this study, we have assessed the humoral immune system in 68 thalassemic patients by measuring their serum concentration of Immunoglobulin G (IgG), IgM, IgA, C3 and C4 in order to find out a responsible immune defect. **Methods:** Sixty eight β-thalassemia major patients were enrolled randomly from referrals to Dastgheib clinic of thalassemia. The same number of case controls with matched age and sex were selected from healthy people without any history of recent or recurrent infections. Serum IgG, IgM, IgA, C3 and C4 levels were assessed using Single Radial Immunodiffusion (SRID). **Results:** Serum levels of IgG, IgM & IgA were significantly higher ($P<0.01$) and those of C3 and C4 were significantly lower ($P<0.01$) in thalassemic patients than the controls. Considering the result of analytic tests, it was revealed that, thalassemia patients show much more increase in serum immunoglobulin levels as they get older. Splenectomized patients had higher serum IgG and IgA levels than non-splenectomized patients but had no difference in serum IgM, C3 and C4. Serum ferritin level had no correlation with the changes of humoral immunity; however, patients with serum ferritin level >2500ng/ml had higher serum IgM level. **Conclusion:** These results can be due to continuous exposure to antigens, repeated infections, chronic liver disease and splenectomy but not iron overload. The only probable cause of humoral immune deficiency found in these patients is a defect in serum complement levels.

Keywords: Thalassemia Major, IgG, IgA, IgM, C3 and C4

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INTRODUCTION

Beta-thalassemia major, a dyserythropoietic hemolytic anemia due to β- globin chain synthesis defect, is a major health problem in our area. They occur at a high gene frequency throughout the Mediterranean populations, the Middle East, India and Southern China through Thailand and the Malay Peninsula into the Island populations of the pacific (1).

Beta-thalassemia major patients suffer from too many problems rather than severe anemia including increased susceptibility to bacterial infections which plays a major role in the patient's morbidity and mortality.

Many studies have been done to evaluate the possible changes of immune system in thalassemic patients, considering the humoral and cellular immune systems; but no consistent defect in white cells or immune function had been documented yet (14,16).Various immunological abnormalities are reported in previous studies such as, decreased opsonization and granulocyte phagocytosis (2), increased serum immunoglobulin levels (3,4) and alterations in B and T cell number and function (5,6,7). Factors such as splenectomy, iron overload, repeated exposure to foreign antigens at the time of blood transfusion and the use of chelating agents, have been suggested to induce profound deleterious effects on the immune system.

Normal concentrations of Immunoglobulin (Ig) levels in β-thalassemia major patients have been demonstrated in some studies (8-11), and in some others, increased levels of IgG and/or IgM (5,9,12) and increased levels of IgA alone (6) have been noticed.

A significant decrease in the serum IgM level with no change in serum IgG or IgA levels have been demonstrated after splenectomy in thalassemia patients (13). On the other hand, an increase in serum IgG and IgA levels with no change in serum IgM level have been demonstrated in splenectomized patients with β-thalassemia intermedia (14).

There are some studies implicating normal serum Ig levels in β-thalassemia patients with no change in relation to splenectomy (15,16).

Serum complement levels were found in some studies to be normal (16,17,18) or decreased slightly (18,19). In some patients a deficiency in the hemolytic activity of the classical (20) or the alternative complement pathways was detected (17).

Normal circulating T-lymphocytes (21,22,23) and decreased numbers have been reported (24,25).

Iran resides on the so called thalassemia belt which has more than 20,000 β-thalassemia major patients (7); to evaluate the change in humoral parameters of immune system in thalassemia patients in our area this project was designed.

MATERIALS & METHODS

It is a case-control, cross-sectional single-center study of β-thalassemia major patients in Southern Iran, and the results were compared with the case control group during a period of 6 months.

68 thalassemic patients were selected randomly from referrals to Dastgheib clinic of thalassemia, Shiraz University of Medical Sciences, as a representative sample of β-thalassemia major patients in Southern Iran.

Splenectomy had been performed on 19 patients and the interval between splenectomy and enrollment in this study was 3-6 years. At the time of blood sampling, all

patients were free of infection.

Sixty-eight age/sex matched controls were selected from healthy school children. The controls were in complete state of health without any history of repeated infection or any immune system dysfunction. All controls had normal hemoglobin level for their age and sex with normal red blood cell indices. Consent form was taken before blood sampling.

Blood was taken from each thalassemic patient just before a scheduled transfusion. Blood sampling from the controls were taken at their schools. Blood samples were centrifuged immediately; serum was obtained and frozen at -70^{oc} until used. Serum levels of immunoglobulins and complements were determined using Single Radial Immunodiffusion (SRID) method.

Using age-matched controls, paired t-test was used to compare the mean serum level of different classes of immunoglobulin and complement factors between patients and the controls (Table 1, 2). The age subgroups considered were as follows: age < 5y/o, 5y/o ≥ age < 10y/o, 10y/o ≥ age <15y/o and age ≥ 15y/o.

To evaluate the effect of iron overload, patients were divided into two groups: one with ferritin level ≥ 2500 ng/ml and the other with ferritin level < 1500 ng/ml. The serum immunoglobulins and complement factors of nineteen splenectomized patients were also compared with non-splenectomized patients. A P value, less than of 0.05 was considered significant.

Table 1. Comparison of serum immunoglobulin levels of thalassemic patients in various age groups with their age matched controls

Age	IgG		IgA		IgM	
	Patients	Controls	Patients	Controls	Patients	Controls
age<5y/o	10.25	14.51	0.72	2.61	0.73	0.95
5y/o≥age<10y/o	14.45	12.38	2.15	2.36	2.34	1.70
10y/o≥age<15 y/o	16.31	11.66	3.17	1.91	2.76	1.51
age≥15y/o	19.81	11.11	4.83	1.46	2.86	0.88

Table 2. Comparison of serum complement factor levels of thalassemic patients in various age groups with their age matched controls

Age	C3		C4	
	Patients	Controls	Patients	Controls
age<5y/o	0.76	0.81	0.16	0.26
5y/o≥age<10y/o	0.60	0.86	0.198	0.23
10y/o≥age<15 y/o	0.67	0.87	0.22	0.26
age≥15y/o	0.52	0.84	0.12	0.29

RESULTS

The serum levels of Ig and complements C3 and C4 in patient and control groups are shown in Table1.

As indicated serum IgG and IgM levels were significantly higher in thalassemic patients than controls (P value < 0.001), while serum levels of C_3 and C_4 were significantly lower in thalassemic patients than controls (P value < 0.001). In the age group of < 5y/o, there was only one patient who had less IgM, IgG, IgA, C_3, and C_4 levels

than his age-matched control. Serum IgM and IgG were significantly higher than the control in age group > 5y/o (P< 0.001). Serum levels of C_3 and C_4 were significantly lower in all the thalassemia patients regardless of age, but data analysis showed that patients ≥ 15y/o had significantly lower C_4 and C_3 levels than those in the range of 10-15 yrs (P value <0.001). This trend was also observed when comparing the patients in the age group of 5-10 yrs with those in the age group of 10-15 yrs (Table2).
Regarding ferritin levels analysis of data demonstrated no significant difference in IgG, IgA, C_3 and C_4 levels between the two groups .Patients with high ferritin level had higher IgM (P Value =0.01).
Nineteen splenectomized patients among a total of 68 had significantly higher serum IgG level, when compared with control (P value < 0.001). However, there was no significant difference between the splenectomized and nonsplenectomized patients as far as IgM, C_3 and C_4 levels were concerned (Table3).

Table 3.Serum level of immunoglobulins & complement factors in controls & patients

	IgG (g/lit)	IgA (g/lit)	IgM (g/lit)	C_3 (g/lit)	C_4 (g/lit)
Controls	11.74±3.4	1.88±1.0	1.29±1.6	0.86±0.40	0.29±0.27
Patients	16.86±10.0	3.48±4.2	2.63±1.4	0.58±0.34	0.15±0.20
Splenectomized	19.94±4.56	4.55±2.45	2.73±0.83	0.56±0.05	0.14±0.02
Not splenectomized	15.56±4.68	3.02±1.80	2.60±0.66	0.60±0.2	0.15±0.01
Low ferritin	16.52±6.29	3.47±2.33	2.53±0.77	0.61±0.03	0.15±0.01
High ferritin	17.05±4.19	3.48±1.99	2.79±0.63	0.58±0.02	0.15±0.01

The values of the upper two rows are mean±2SD but those in lower four rows are mean±SD.

DISCUSSION

In this investigation comparing with the control group, β-thalassemia patients had higher values of the three major classes of serum immunoglobulins (IgA, IgM and IgG), but lower serum complement levels. This observation can be attributed to multiple factors. For instance repeated blood transfusion in β-thalassemia patients will result in a continuous exposure to various antigens and will lead to increased levels of serum immunoglobulins, while continuous complement consumption may decrease serum complement levels (3, 17, 25). This notice is well correlated with elder patients who in these findings were more prominent. (4). Thalassemia patients are prone to many bacterial and viral infections. Repeated infections also stimulate the immune system and may result in increased immunoglobulin levels (3, 4, 16, 36). HCV infection via blood transfusion is significant in causing a state of chronic hepatitis which also contributes to a rise in serum immunoglobulin levels (3, 4). Our results showed that, splenectomy increases serum levels of IgG and IgA (P value < 0.05) with no change on IgM level. Although, spleen acts as one of the major lymphoid organs to clear the blood infections, it is hypothesized that the removal of spleen may pressurize other secondary lymphoid organs to compensate for the synthesis of the major immunoglobulin classes.
Iron overload was suggested by some investigators as an important contributing factor in altering the immune parameters in thalassemia patients (3, 6, 36). It has been suggested that iron overload results in increased migration of T helper cells to the gut and lymph nodes and this causes an increase in serum immunoglobulin levels in thalassemia patients (6).

According to our results, iron overload does not seem to play any major role in humeral immune system defect.

Serum complement factors (C_3 and C_4) were consistently reduced in our patients. This can be either due to reduced synthesis or increased consumption; the latter is more probable with the rate of infection in our patients which seems to be high. Our observation of significantly lower C_3 and C_4 levels early in life in thalassemic patients, however, points to the possibility of deficient complement synthesis may also act as a contributing factor to immune deficiency.

An important consideration in thalassemia patients is their exposure to blood transmitted viruses such as HTLV and HIV. Infection with these viruses can lead to alterations in humeral immune system secondary to T-cell immune response depression (10).The controversy concerning alterations of serum immunoglobulin and complement levels in β-thalassemia major may be due to marked heterogeneity of the patients in different studies. This heterogeneity concerns race, socioeconomic class, nutritional status and environmental factors (16). Immunoglobulin abnormalities, deficiency of the alternative pathway of complement activation, abnormal phagocytosis and chemotaxis, decrease in the number of T lymphocytes and their malfunction have all been suggested. Our study revealed an increase in immunoglobulin levels and a decrease in complement levels, which is in contrast to some other studies in the literature (8, 9, 10, 11). The observed immune disorder represents mostly a secondary immune system defect rather than a primary problem (4,23). These changes can not fully explain the increased susceptibility to infections among patients, and it seems that, β-thalassemia major patients have got rather normal antibody response to bacterial and viral infections with normal levels of complement factors (8, 11). Changes of complement factors seem to follow an increase in immunoglobulin level; but, the possibility of deficient complement factors synthesis can not be ruled out.

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