Original Article

Relation of Immunoglobulin Level and White Blood Cell Count with Frequency of Infection in Splenectomized and Non-Splenectomized B Thalassemia Major Patients

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Abstract

Background: Beta-thalassemia major is an autosomal recessive condition caused by absent (β0) synthesis of the β globin chains of the hemoglobin tetramer. **Objectives:** Evaluation of immunoglobulin level and white blood cell count in splenectomized and non non splenectomized patient as well as comparison of the levels of Ig and WBC with frequency of infection. **Materials and Methods:** This cross sectional study included a total of 60 patients with beta-thalassemia major and 20 age and sex matched apparently healthy individual as a control, blood taken from them for an evaluation of immunoglobulin level and white blood cell count. **Results:** There is no significant difference in immunoglobulin level between splenectomized and non splenectomized B-thalassemia major patients and control groups. Also, there is significant increase in mean Frequency of infection / year for patients with thalassemia major in comparison to control, Specifically, splenectomized patients are having significant increase in frequency of infection in comparison to non splenectomizd. There is a significant difference in mean of White blood cell count, Neutrophil, Lymphocytes in patients with splenectomy, non-splenectomy and control group. **Conclusion:** Immunoglobulin levels are within normal range in thalassemia major patients whether splenectomized or non splenectomized. Leukocytes count mainly neutrophils and lymphocytes are higher in splenectomised patients. Frequency of infection is higher in splenectomed patients and there is a positive correlation with IgA and IgG.

Keywords: Hite blood cells, mmunoglobulin, thalassemia

Introduction

Beta-thalassemia syndrome are a group of hereditary blood disorders.

Its main features including decreased or absent β globin chain production, yielding in low hemoglobin in red blood cells, low red blood cell production and then anemia. Patients with β0-thalassemia usually have severe anemia early in infancy and then become blood transfusion dependent latter on.[1] They are most predominant in the Mediterranean basin and some other countries.

The "thalassemia distribution belt" extends along the margin of the Mediterranean and throughout the Arabian peninsula, Turkey, Iran, India, southeastern Asia, especially Thailand, Cambodia, and southern China.[2] In Iraq the prevelance of thalassemia had increased from 33.5\100000 in 2010 to 37.1\100000 in 2015,while the incidence rate had decreased from72.4\100000 live births to 34.6\100000 live births between 2010 and 2015[3] Imbalance in α-/non-α-globin chains is the basis of β-thalassemia which trigger a series of events leading to hemolytic anemia and ineffective erythropoiesis.[1,4]

Infectious complications and immune abnormalities affecting both innate and adaptive immunity are considered to be an important cause of morbidity and

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mortality in patients with β-thalassemia (the second most common cause of death in these patients).[5]

Factors, such as splenectomy, repeated exposure to foreign antigens during blood transfusions, iron overload, and the use of iron-chelating drugs have profoundly deleterious effects on the immune system.[6]

Several studies done for evaluation of immune status, Normal concentrations of Immunoglobulin levels in β-thalassemia major patients have been demonstrated in Iranian study,[7] and a study in Thi-qar province\ Iraq[8]which reveal higher mean level of immunoglobulins in B- thalassemia major,increased levels of IgG without increasing in IgM or IgA in a study in Basrah province\ Iraq[9] and increased levels of IgA alone in a study in Egypt[10] have been noticed. The present study aimed to estimated serum immunoglobulin IgG, IgM, IgA, white blood cell count in thalassemic patients.

This study aimed to evaluate the of level of immunoglobulins and white blood cell count in splenectomized and non non splenectomized patient and to compare the level of Igs and WBC with frequency of infection.

Materials and Methods

The present study was done in the Center of an inherited blood disorder in Babylon province, the study was performed over the course of period from January to October2022.

This study was performed on 60 β-thalassemia major patients who attended to center for inherited blood disorder in Babylon Province for regular blood transfusion and 20 healthy controls. Five mls of peripheral venous blood were collected by appropriate venipuncture technique from each patient, 3 mls of which were placed in an EDTA anticoagulant tube for white blood cell count and two mls for venous blood samples were collected in gel tube and blood has been allowed to clot at room temperature for 30 minutes, then it was centrifuged for 5 minutes at 4000 rpm, hemolysis samples were ignored, serum was collected and distributed into Eppendorf tubes and give the same number in the form. The serum samples were frozen at -20 C until used.

for the latter estimation the immunological parameters included (IgG, IgM, IgA) by chemilumescent method which is a fully automated machine.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 10(including the number and the date in 17/January/2022) to get this approval.

Results

This cross sectional study included 60 patients of beta thalassemia major, of them 30 had β thalassemia major without splenecyomy, 30 had B thalassemia major with splenectomy along with 20 healthy volunteer‟s persons as controls

There is no significant difference in mean of [IgG, IgM, IgA] in patients with splenectomy,non-splenectomy and control group.

There is a significant difference in mean of WBC,neutrophil,and lymphocytes in patients with splenectomy,non-splenectomy and control group. Patients with splenectomy have more increase in level of WBC,neutrophil and lymphocytes than other groups, as showen in [Table 1].

As show in [Table 2] there is significant difference in mean of Frequency of infection / year for patients with splenectomy and non-splenectomy. Patients with splenectomy have more infection times than non-splenectomy patients and control group.

Discussion

β-Thalassemia is a hereditary blood disorder due to reduction in β globin gene in excess of α globin chains which impair maturation of RBCs resulting in their destruction by the spleen with later on development of anemia.[11] Patients with thalassemia major usually suffer from too many problems including splenomegaly and subsequent hypersplenism which is managed by splenectomy and considered as one of the risk factor for increasing risk of infection which plays a major role in the patient's morbidity and mortality.In our study,the results are revealing that there is no significant difference(p value>0.05) in immunoglobulin level between splenectomized and non splenectomized β-thalassemia major and control groups,this result agreed with study in Iran[7] reported that serum mean levels of IgG and IgM, IgA in βThalassemia major patients were normal, their explanation,as the splenic function as a secondary lymphoid organ to engulf infectious agents from the blood, splenectomy will lead to activation of the other secondary lymphoid organs to compensate immunoglobulin synthesis.In contrast to study in Thi-qar[8] province\Iraq which reveal higher mean level of immunoglobulins in B- thalassemia major splenectomized patient in comparison to non splenectomized, due to serial transfusion in β-thalassemic patients may result in a continuously exposure to various antigens with subsequent increasing in the levels of serum immunoglobulins,another study that had been done in Basrah[9] showed increased IgG with out increasing IgM or IgA,this could be due to frequent transfusions of the

Table 1: Difference in mean of (IgG, IgM, IgA, WBC, neutrophil and lymphocyte) for splenectomy and non splenectomy and control groups

Immunological parameters		No	Mean ± SD	P-value
IgG mg\ml	*S*	30	14.46 ± 8.9	
	Non	30	14.41 ± 7	0.93
	control	20	13.70 ± 3.59	
IgM mg\ml	*S*	30	176.73 ± 109.8	
	Non	30	151 ± 77.67	0.42
	control	20	148.55 ± 57.14	
IgA mg\ml	*S*	30	237.93 ± 135.59	
	Non	30	175.87 ± 114.16	0.1
	control	20	235.10 ± 107.16	
WBCx10^9\ml	*S*	30	19.06 ± 5.82	
	Non	30	6.28 ± 2	0.0001
	Control	20	8.58 ± 2.35	
Neutrophil x10^9\ml	*S*	30	11.16 ± 3.3	
	Non	30	3.37 ± 1.45	0.0001
	Control	20	5.22 ± 2.28	
Lymphocytesx10^9\ml	*S*	30	6.35 ± 2.62	
	Non	30	2.33 ± 0.9	0.0001
	Control	20	2.60 ± 1.04	

P-value ≤ 0.05 (significant),s:splenectomy

Table 2: Difference in mean of Frequency of infection / year for patients with splenectomy and non splenectomy and control

	Type	No	Mean ± SD	P-value
Frequency of infection / year	S	30	8.93 ± 2.65	0.0001
	Non	30	5.50 ± 1.27	
	Control	20	2.2 ± 0.95	

P-value ≤ 0.05 (significant),s:splenectomized

patients which may cause persistent exposure to diferrent antigens and this will lead to increased IgG level, also iron overload may be as an causative contributing factor by altering immune defence mechanism in thalassemic patients, suggested that iron overload leading to more traveling of T- helper cells to the GIT and lymph nodes and this may cause an higher level of IgG values. The present study is partielly compatible with another study[10] which show that immunoglobulin A is higher in thalassemia major patients while immunoglobulins G,M were normal in comparison to control. The mechanisms of such changes in the immunoglobulines were explained, according to, hypothesis, including iron overload on the skin leading to stimulation of IgA production as a mucocutaneous antibody, concurrently they found normal level of IgM in thalassemic patients in comparison with controls due to serious control of infections and accurate filtration of transfused packed cells resulting in lowering the chance of exposure to antigens.

Variation in these results of our study and the previous studies can be explained by clinical variation among the patients themselves including many factors such as age, race, frequency of transfusion, spleen state, body iron status, iron chelation therapy which were noted as important factors for alteration of Ig level in thalassemia.

There is significant increase in mean of Frequency of infection / year for patients with thalassemia major in comparison to control that is agreed with study,[12] which can be explained by the effect of iron overload include decreased antibody-mediated and antigen-stimulated phagocytosis by monocytes and macrophages, alterations in T-lymphocyte subpopulation, and modification of lymphocyte distribution in different parts of the immune system.

Specifically, splenectomized patients are having a significant increase in frequency of infection in comparison to non splenectomizd,this result agreed with study,[13] and another study[14] found the rate of infection increased significantly when the period following splenectomy more than 10 years.

The explanation of increasing risk of infection in splenectomized patient, by the splenectomy, the levels of antibody are preserved,but there is a loss of memory B cells directed against pneumococcal and tetanus infection, and also absence of marginal zone monocytes delegated to immunological defense from encapsulated

There is a significant difference the mean of WBC in patients with splenectomy and non-splenectomy and control group. Patients with splenectomy have more

increase in level of WBC than other groups, this is compatible with these studies.[15-17]

There is significant difference in mean of Neutrophil and lymphocytes in patients with splenectomy and non-splenectomy and control group. Patients with splenectomy have more increase in the level of neutrophils and lymphocytes than other groups. This result was compatible with several studies worldwide.[13,17]

All of these findings explained as, post splenectomy there is a elevation in the WBC count. Usually there is neutrophilia in the early postoperative period, later is replaced by a noticeable and permanent increase in lymphocytes and monocytes. Usually the WBC count ranging between 10 and 15×10^9/L, rarely it may rise to twice this level.[18]

There is negative significant correlation between [Age at which splenectomy done, mean age of patients at splenectomy are [9.8 ± 2.5] years] and WBC, Lymphocyte in patients who done splenectomy. As the leukocytes count is usually higher in children, and then decreased until adulthood, consistent with the pattern found in previous studies.[17,18] The lymphocyte count was also higher in early childhood and then decreased with age, which was in apposite to the trend of neutrophil count.There is a positive significant correlation between [frequency of the infection \year] and IgG, IgA in patients who do splenectomy, as most infections that happened after blood transfusion in chronic stage not in acute stage of infection.[9]and iron deposition in the skin induce immunoglobulin A production.[10]

Conclusion

We can conclude from this study that the immunoglobulin levels are within normal range in thalassemia major patients whether splenectomized or non splenectomized. Also, the leukocytes count mainly neutrophils and lymphocytes are higher in splenectomised patients. The frequency of infection is higher in splenectomed patients and there is a positive correlation with IgA and IgG.

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Not applicable.

Conflicts of interest

There are no conflicts of interest.

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