Immunological Evaluation of Patients with B-Thalassemia Major in Kerbala City Using Single Radial Immunodiffusion (SRID) Technique

Zuhair Mohammed Ali Jeddoa; Ph.D*, Sattar jabbar Rahi; M.Sc.*, Adel Ateia Abed Ali; M.Sc. *

Department of medical microbiology, College of Medicine, Kerbala University, Kerbala, Iraq.

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, assumed to be the result of immunological changes.

Objective: This study aimed to evaluate some of humoral immunological parameters of thalassemic patients by measuring their serum concentration of Immunoglobulin (IgG), IgM, IgA, and serum complement components C3 and C4.

Patients & Methods: A total of forty five beta-thalassemia major patients who were attending to the thalassemia center in Kerbala teaching pediatrics hospital (24 males and 21 females) from January to August of 2009 and fifty of control children with matched age and sex were randomly selected from apparently healthy individuals without any history of recent or recurrent infections, were enrolled in the study. Serum IgG, IgM, IgA, C3 and C4 levels were measured using Single Radial Immunodiffusion (SRID).

Results: The mean of serum IgG, IgM &IgA showed a significant increment ($P<0.01$) in thalassemic patients as compared with apparently healthy control group, while the serum levels of C3 and C4 were significantly decreased ($P<0.01$) in thalassemic patients as compared with control group.

Conclusion: The elevation of serum levels of studied immunoglobulins can be due to continuous exposure to antigens, repeated infections, and chronic liver disease, while the only probable cause of humoral immune deficiency were found in these patients were due to the decline of serum complement levels (C3 and C4).

Recommendations: The determination of serum levels of immunoglobulins and complement components have a beneficial value for evaluation of immunological status of thalassaemia major patients and further work need to be done to evaluate another immunological parameters for example the serum level of cytokines to manipulate the immunological aspects of thalassemic patients.

Key word: Beta-Thalassemia major, Immunoglobulin G, IgM, IgA, C3 and C4, (SRID).

الخلاصة

الهدف : ان الهدف من هذه الدراسه هو لتحديد المستويات المصليه للغلوبيولينات المناعيه الثلاثه (غلوبيولين G وغلوبيولين IgM و غلوبيولين IgA وكوني المتمم C3 و C4 في المرضى المصابين بفقر دم البحر المتوسط نوع بيتا من اجل معرفه اسباب التغييرات المناعيه ان وجدت.

طريقه الدراسه : شملت الدراسه 45 مريض مصاب بفقر دم البحر المتوسط نوع بيتا بالاضافه الى 50 شخصا سليما انضموا الى الدراسه لغرض المقارنه كمجاميع ظابطه وباستخدام تقنيه الانتشار المناعي الشعاعي المفرد تم تحديد المستويات المصليه للغلوبينات المناعيه (غلوبيولين مناعي G غلوبيولين مناعي IgM غلوبيولين مناعي IgA ومكوني المتمم C3 وC4 بين مجاميع الدراسه .

لقد تم اجراء هذه الدراسه بين كانون الثاني 2009 وشهر اب 2009 في مركز الثلاسيما التابع لمستشفى الاطفال التعليمي في كربلاء المقدسه .
النتائج : كشفت الدراسه على ان هنالك ارتفاعا معنويا في مستويات الغلوبينات المناعيه الثلاثه لدى المضى مقارنه بالمجموعه السليمه , اما مكوني المتمم فقد ابديا انخفاظا معنويا في مستوياهما لدى مرضى فقر الدم البحر المتوسط مقارنه بامجموعه السليمه
الاستنتاج :تستنتج هذه الدراسه ان هنالك تاثيرات يتعرض لها مرضى فقر دم البحر المتوسط مما ادى الى صعود المستويات الغلوبيولينا ت المناعيه وانخفاظ في مستويات مكوني المتمم , وتحتاج هذه الدراسه مستقبلا الى دراسه المستويات السايتوكينات لمعرفه المناعه الخلويه لهذا المرض .

Introduction

Thalassemias describe a group of autosomal inherited disorders character-ized by defect in globin chains of hemoglobin, these genetic defects are mutations in beta-globin gene causing a beta-thalassaemia while, the alpha thalassemia result from deletion in α-globin gene(s). These two basic groups of thalassemia disorders: alpha thalassemia and beta thalassemia are causing varying degrees of anemia, which can range from insignificant to life threatening [1,2,3]. Many families have thalassemia carriers, but the trait often goes undiagnosed because it produces no or few symptoms. Frequently, thalassemia is not diagnosed in a family until a baby is born with it [4].

The immunological defects were observed in patients with thalassemia major make them susceptible to different kinds of infections due to immunodeficiency, both before and after splenectomy, the reasons for these immunodeficiency are:

A-The immunosuppressive effects of increased ferritin concentrations due to multiple blood transfusions.[5]

B-Cytomegalovirus (CMV) infection is responsible for increased susceptibility to infection because of the immunosuppressive properties of CMV [6]. C-Abnormalities in humoral immunity such as defects in alternative complement pathways (C3 and C4) and abnormal immunoglobulin levels leading to change in the levels of different kinds of immunoglobulins (IgG, IgM and IgA), abnormalities in cell mediated immunity (CMI) such as decreased natural killer (NK) cell activity, defective neutrophil function, decreased T-helper/ T-suppressor ratio and T-cell subset abnormalities [7]. Recent studies on immune competence in beta-thalassemia have revealed numerous quantitative and functional defects, involving T and B lymphocytes, immunoglobulin production, neutrophils and macrophages, chemotaxis, and phagocytosis, as well as the complement system. [7]. This study aimed to evaluate the immunological status of some immunological parameters (IgG, IgM, IgA, C3 and C4) of beta-thalassaemic major patients in comparisons with apparently healthy individuals.

Patients and methods

Patients

A total of 45 patients (24 male and 21 female), with a mean age (11.60 ± 5.13) years, age range (3 -23) years with β-thalassemia major referred to the thalassemia center in Karbala teaching pediatrics hospital were included in this study.

Control Group:

A total of 50 apparently healthy individuals with matched age and sex who have no history of acute or chronic illness with normal complete blood count and ESR were randomly enrolled in the study as a control group (table 1).

Immunological Examination

Blood samples (5ml) from all participants were collected in labeled tubes (for thalassemic patients just before the scheduled transfusion), the blood samples were immediately centrifuged, serum was obtained and frozen at – 20 until used. The estimation of serum immunoglobulin levels IgG , IgM and IgA, and complement components C3 and C4 levels were adopted in patients and control groups using Single Radial Immunodiffusion (SRID) according to the to manufacturer's instructions (Linear Chemicals, Spain) , the normal (standard) values of the studied immunoglobulins were (800-1800) for IgG , (60-280) for IgM, and (90-450) for IgA, and for C3 and C4 were (91-156) and (20-50) respectively.

Biostatistical Analysis

The calculation of percentages, mean, standard deviation estimation and t-test statistical analysis tools were applied for the analyses of the results using Microsoft Office Excel 2007. P value less than 0.05 regarded as significant.

Table 1. The distribution of studied samples according to the age and gender.

Parameter \ Group		patients N= 45	controls N=50
Age (years)	Mean ± SD	11.60 ± 5.13	12.16 ± 5.82
	Range	3 -23	4-25
Gender	Male	24 (%)	23(46%)
	Female	21 (%)	27(54%)

Results

The study of serum immunoglobulin levels of β- thalassemic patients in comparison to the apparently healthy control revealed that the mean serum immunoglobulin levels were increased in thalassemia patients as compared to apparently healthy controls, and the mean ± SD of IgG,IgM,and IgA for patients were (1751.2±396.9),(258.4±52.2), and (323.3±113.5) respectively, while for control group were (1204.3±203.3) , (120.9±26.8),and(195.6±61.3) respectively.

The statistical analysis showed significant differences between patients and control groups (table 2).

The results of evaluation of serum C3 and C4 complement components were (69.8±16.4) and (17.3±5.2) for patients, while the mean for control group were (91.6±11.9) and (31.02±5.4) respectively, statistically these results were significantly decreased (P value<0.001) in thalassemic patients in comparison to the control group, table (3).

Table 2. The evaluation of serum immunoglobulin levels of β- thalassemic patients in comparison to the apparently healthy control.

Immunoglobulin	serum IgG (mg/dl)		serum IgM (mg/dl)		serum IgA (mg/dl)	
Values	Patients	Control	Patients	Control	patients	Control
Minimum	900	786	100	75	97	105
Maximum	3108	1700	376	200	520	350
Mean	1751.2	1204.3	258.4	120.9	323.3	195.6
SD	396.9	203.3	52.2	26.8	113.5	61.3
P- value	P value<0.001		P value<0.001		P value<0.001	

Table 3. The evaluation of serum complement component levels of β- Thalassemic patients in comparison to the apparently healthy control.

Complement Components	serum C3		serum C4	
Values	Patients	Control	patients	Control
Minimum	38	80	8	20
Maximum	120	140	30	50
Mean	69.8	91.6	17.3	31.02
SD	16.4	11.9	5.2	5.4
P- value	P value<0.001		P value<0.001	

Discussion

Immunoglobulins and complement system play pivotal role in the immunologic defense of the body. It is reasonable therefore to investigate the possible implications of these parameters in beta thalassemia major in which immunologic phenomenon is so important. The results in the current study showed significant elevation of the immunoglobulins (IgG, IgA, IgM) which associated with a significantly decreased level of serum complements (C3 and C4) of thalassemic patients as compared with the control group.

These observations are in accordance with that reported by other investigators [9,10,11,12,13] while disagree with that demonstrated by others [14,15] whom reported normal levels of immunoglobulin's [16, 17]. This discrepancy may be due to marked heterogeneity of thalassemic patients in different studies. This heterogeneity concerns race, socioeconomic class, nutritional status and environmental factors and disease stage [18], and also taking in consideration it is due to the sampling variations and the stage of the disease.

Several factors may involve in explanation of the current study results. Repeated blood transfusion and high susceptibility to infections, which is a feature of beta thalassemic patients, may stimulate the immune system and may result in increased immunoglobulin levels [9,10,12,13].

Iron overload which is also a feature of thalassemic patients, was suggested by some investigators [9,12] as important contributing factor in altering the immune response and results in increased migration of T-helper cells to gut and lymph nodes and this causes an increase in serum immunoglobulin levels in thalassemic patient [12], unfortunately ,iron status did not evaluate in the present study .

Moreover, it has been hypothesized that the removal of spleen, which is one of the major lymphoid organs to clear the blood infection, may stimulate other secondary lymphoid organs to compensate for the synthesis of the major immunoglobulin classes. In the current study, 13 out of 45 thalassemic patients underwent spleenctomy which may be another cause for higher immunoglobulin levels observed in our study.

Finally the reduced levels of complement components in our patients

can be explained on same basis. Repeated blood transfusion in our thalassemic patients may result in a continuous exposure to various antigens and which lead to continuous complement consumption [10, 19, 20]. However, the possibility of deficient complement factors synthesis cannot be ruled out.

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