Evaluation of Some Humoral Immune System Parameters in Beta Thalassemia Major Patients at Wasit Province / Iraq

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Abstract

The present study aimed to investigate the humeral immunity in Beta Thalassemia Major (BTM) patients by measure the serum levels of immunoglobulins (IgM), (IgG), (IgA), and complement system (C3, C4). A total of 80 BTM patients were enrolled in the study their age ranged between (6-25) years .The patients were subdivided according to their age into three age groups. The most predominant age group was 11-20 years 38:80 (47.5%). While the patients below 10 years were 29:80(36.25%) and more than 20 years 13:80 (16.25%).It has been shown through a questionnaire that all patients more than 20 years were splenectomies patients. In comparison with 45 ages matched healthy control groups there were a significant increase ($p \leq 0.05$) in serum IgM, IgG, and IgA in all groups of BTM patients. The serum levels of immunoglobulins M, G, and A increased significantly ($p \leq 0.05$) with age in BTM patients. Serum C3and C4 decreased significantly in all groups of BTM patients compared with age matched healthy control. It has been observed that sreum C3 reduced in 11-20 years a and more than 20 years BTM patients age groups compared with patients below 10 years, whereas no significant difference in serum C4 among BTM patients age groups when compared with each other.

Introduction

Thalassemia is a hereditary anemia resulting from defects in hemoglobin production (Higgs *et al*., 2001). It results from quantitative reductions in globin chain synthesis due to gene deletion or mutations. It can be classified according to which globin chainsis produced in reduced amount. Those with reduced α-globin chains are termed α-thalassemias, whereas those with decreased β-chain production are called β-thalassemias. The World Health Organization (WHO) recognizes thalassemia as the most prevalent genetic blood disorder in the world found in more than 60 countries with a carrier population of up to 150 million (Cao and Galanello , 2010). Thalassemia are classified according to which particular globin chains are produced in a reduced amount, which may lead to an imbalance in globin chains synthesis, ineffective erythropoiesis, hemolysis, and eventually to a variable degree of anemia. The main types of thalassemias the α and β thalassemia. β thalassemia is the most important and widely spread type which causes severe anemia in the homozygous and compound heterozygous states (Weatherall, 2004; Galanello and Origa, 2010). β - Thalassemias are clinically classified according to their severity into thalassemia major requiring a regular blood transfusion throughout life, thalassemia intermedia characterized by anemia but not of such severity as to require regular blood transfusion, and thalassemia minor or trait which is the symptomless carrier state

(Lahiry *et al*., 2008 and Cao and Galanello, 2010). The management of severe forms of the β - thalassemia diseases depends on three regimens: regular blood transfusion, removal of overloaded iron with chelating agents such as deferoxamine and Exjade, and splenectomy when rate of transfusion is increasing (Peters *et al*., 2012 and Vichinsky *et al*., 2011). Transfusions lead to iron overload and also to immune derangements, both of which exert a negative effect on the functional integrity of the immune system in multitransfused patients with thalassaemia(Salsaa and Zoumbos,1997). There are no reports about the immunoglobulin and complement system in β thalassemia major in wasit province. There for the aims of the present study to determine serum immunoglobulin G, M, A and complement C3and C4.

Material and Methods

A total of 80 β-thalassemia major patients (45 males and 35 females) attended to thalassemia center at AL-Kut Hospital in Wasit Province for regular blood transfusion were enrolled in this study. They were subdivided according to the age into three groups (less than 10 years, 11-20 years, more than 20 years). It has been shown through a questionnaire that all patients more than 20 years suffer from the removal of the spleen [splenectomized β-thalassemia major patients (SβTM)]. Control groups were age and sex matched with β-thalassemia major patients groups.

Blood samples (5 ml) were taken from patients and controls to obtain the serum by centrifugation to assess the immunoglobulins (IgG, IgM ,IgA) and complement C3 , C4 levels using the commercially available single radial immunodiffusion plates (SRID) from LTA, Italy. The serum samples were frozen at -20 °C until used. All values were expressed as means ± standard deviation (μ±S.D). The data were analyzed by using a computerized SPSS program. Student's t –test was used to examine the differences between any two age matched groups. The comparisons between β-thalassemia major patients groups were performed with analysis of variance (ANOVA). $P<0.05$ was considered to be the lest limit of significance.

Results

1. Serum Immunoglobulins

1.1. Serum IgM concentration

The results in table (1) reveals that serum concentrations of IgM increased significantly ($p\leq0.05$) in all age groups of β-thalassemia major (βTM) patients compared with corresponding age control subjects.

Table (1): Comparison of serum IgM concentrations (mg/dL) of the study groups with age matched healthy controls.

Groups	Age	No.	Mean ± SD	Minimum	Maximum
βTM Patients	(≤ 10 years)	29	1916.1 ± 503 A	1325.0	2859.2
Control		15	1010.1±253.1 B	703.7	1451.4
βTM Patients	(11-20 years)	38	2473 ±763.3 A	755.0	3750.4
Control		15	1127.9±245.6 B	755.0	1684.8
SβTM Patients	(> 20 years)	13	2926±741.3 A	1325.0	3750.4
Control		15	1144±245.7 B	703.7	1451.4

Different letters represent significant difference at ($p \leq 0.05$).

1.2 Serum IgG Concentrations:

It seem from table (2) that serum IgG concentrations in all groups of βTM patients raised significantly ($p \leq 0.05$) compared with their concentrations of corresponding age healthy control.

Table (2): Comparison of serum IgG concentrations (mg/dL) of the study groups with age matched healthy controls.

Groups	Age	No.	Mean ± SD	Minimum	Maximum
βTM Patients	(≤ 10 years)	29	159.5 ± 49.5 A	80.1	264.4
Control		15	75.9 ± 22.8 B	41.8	113.0
βTM Patients	(11-20 years)	38	198.3 ± 59.2 A	113.0	349.1
Control		15	95.2 ± 28.0B	46.3	149.5
SβTM Patients	(> 20 years)	13	251.5 ± 77.2 A	124.8	340.2
Control		15	86.7 ± 13.9 B	65.0	107.3

Different letters represent significant difference at ($p \leq 0.05$).

1.3 Serum IgA concentrations

Table (3) shows that all groups of βTM patients have serum IgA concentrations higher significantly ($p \leq 0.05$) than their concentration of age matched healthy control groups.

Table (3): Comparison of serum IgA concentrations (mg/dL) of the study groups with age matched healthy controls.

Groups	Age	No.	Mean ± SD	Minimum	Maximum
βTM Patients	(≤ 10 years)	29	202.7±95.4 A	103.0	443.5
Control		15	133.4±35.9 B	95.3	206.6
βTM Patients	(11-20 years)	38	299.2±133.9 A	124.8	566.6
Control		15	157.9±29.6 B	102.9	206.6
SβTM Patients	(> 20 years)	13	415.9±145.5 A	192.5	581.1
Control		15	152.2±38.5 B	95.3	226.6

Different letters represent significant difference at ($p \leq 0.05$).

Table (4): Comparison of serum immunoglobulins concentrations (mg/dL) in βTM patients age groups.

Groups	Age	No.	Mean ± SD	Minimum	Maximum
βTM Patients	(≤ 10 years)	29	85.2±32.1 B	18.2	139.7
Control		15	151.7±31.2 A	102.5	195.2
βTM Patients	(11-20 years)	38	76.5±26.1 B	22.3	133.2
Control		15	136.2±19.9 A	108.5	180.8
SβTM Patients	(> 20 years)	13	66.9±29.8 B	14.2	126.9
Control		15	142.7±29.9 A	96.7	173.6

The numbers represent the mean ± Standard Deviation.
Different letters represent significant difference at ($p \leq 0.05$).

It seems from table (4) that serum concentrations of immunoglobulins (IgM, IgG and IgA) in βTM patients increased significantly ($p \leq 0.05$) with increasing of age. Splenectomized patients in third age group showed significant ($p \leq 0.05$) increase in serum immunoglbulins (IgM, IgG and IgA) concentrations compared with non splenectomized βTM patients in first and second age groups.

2. Complement system:

2.1 Complement C3

The results shows that serum concentrations of C3 (table 5) decreased significantly ($p \leq 0.05$) in all three age groups of βTM patients (less than 5 years, 11 -20 years, and more than 20 years) compared with corresponding age groups of healthy control .

Table (5): Comparison of serum C3 concentrations (mg/dL) of the study groups with age matched healthy controls.

Parameter	Age(≤10 year)	Age (11-20)	Age (> 20)
	βTM n = 29	**βTM n = 38**	SβTM n = 13
IgM	**159.5±49.5 C**	**198.3±59.2 B**	251.5±77.2 A
IgG	**1916.1±503 C**	**2473±763.3 B**	2926±741.3 A
IgA	**226.9±106.6 C**	**299.2±133.9 B**	415.9±145.5 A

2.2 Complement C4

The results displayed that βTM patients regardless to their age groups have significantly ($p \leq 0.05$) low concentrations of serum C4 compared with healthy control groups (table 6).

Table (6): Comparison of serum C4 concentrations (mg/dL) of the study groups with age matched healthy controls.

Groups	Age	No.	Mean ± SD	Minimum	Maximum
βTM Patients	**(≤ 10 years)**	**29**	**21.3±9.9 B**	**8.9**	**39.0**
control		**15**	**30.9±6.9 A**	**21.1**	**41.8**
βTM Patients	**(11-20 years)**	**38**	**18.7±7.7 B**	**5.0**	**35.0**
control		**15**	**28.5±4.3 A**	**20.6**	**37.6**
SβTM Patients	**(> 20 years)**	**13**	**16.3±5.3 B**	**2.8**	**25.1**
control		**15**	**35.2±5.8 A**	**26.6**	**50.5**

Different letters represent significant difference at ($p \leq 0.05$).

Whereas, the comparison of serum C3 and C4 concentrations in βTM patients age groups have been presented in the Table (7). The results indicated that the C3 concentration decreased significantly ($P \leq 0.05$) in the second and third βTM patients age groups compared with the first βTM patients age group , whereas there were no significant ($P \geq 0.05$) differences between the second βTM patients age group and third βTM patients age group when compared with each other . The C4 serum concentrations revealed that there were no significant ($P \geq 0.05$) differences among all βTM patients' age groups.

Table (7): Comparison of serum complement (C3, C4) concentrations (mg/dL) in βTM patient's age groups.

Parameter	Age(≤10 year)	Age (11-20)	Age (> 20)
	BTM n = 29	βTM n = 38	SβTM n = 13
C3	85.2±32.1 A	76.5±26.1 A	66.9±29.8 A
C4	21.3±9.9 A	18.7±7.7 A	16.3±5.3 A

Different letters represent significant difference at ($p \leq 0.05$).
The numbers represent the mean ± Standard Deviation.

Discussion

The significantly higher serum IgM (tabe -1-), IgG (tabe -2-) and IgA (tabe -3) in all age groups of βTM patients compared with corresponding healthy control groups 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 (Pardalos *et al* ., 1987; Weatherland *et al*., 2000) Thalassemia patients are prone to many bacterial and viral infections. Repeated infections also stimulate the immune system and may result in increased immunoglobulin levels (Vergin *et al*., 1997; Weatherland *et al*., 2000). Iron over load was suggested by some investigators as an important contributing factor in altering the immune parameters in thalassemia patients (Weatherland *et al*., 2000). 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 (Chalevelakis *et al*.,1975). The results of the present study came in compatible with Tovo *et al.* (1981), Amin *et al.* (2005), Malik *et al.* (2010) Al-Thamir *et al.* (2011) Jeddoa *et al*., (2011). The significant increase in serum levels of immunoglobulins in the elder βTM patients (table -4-) may attribute to increase the frequency of blood transfusions. Splenectomy in patients more than 20 years in the present study may considered as another factor involve in the high levels of immunoglobulins. This can be explained through the fact that in spite of 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 (Abbas *et al*, 2007) . According to Amin *et al.* (2005) who reported that splenectomy increases serum levels of IgG and IgA with no change on IgM level. This might be related to filtration of transfused packed cells resulting in decreasing the chance of repeated exposure to antigens. On contrary Kiani-amin *et al.* (2011) reported that Serum mean levels of IgG and IgM in βTM patients of were normal.

The present results revealed that the mean levels of complements C 3 (table -5-) and C4 (table -6-) were significantly decreased in all patients groups compared with corresponding age group of healthy control. Thalassemic patients age groups in the present revealed no significant differences in serum C3 and C4 when compared with each other (table 7). Similar result was found by James *et al.* (1981) Amin *et al*., (2005). The decrease in complements 3 and 4 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 (Dwyer *et al*., 1987; Corry *et al*., 1981; Weatherland *et al*., 2000).

References

Abbas, A..; Andrew, H.; and Shiv Pillai (2007) Cellular and molecular immunology, Philadelphia. PA 19103-2899 SECTION II Recognition of Antigens pp 77, 82,87,92.

Al-Thamir, S. N. ; and Al-Hussainy S. M. (2011) Effects of Iron Overload and Treatment Methods on Serum Levels of IgG, IgM, and IgA in Beta Thalassemia Major (BTM) Patients Medical Journal of Babylon-Vol. 8- No. 3 .

Amin, A.J ; Amin ,R. S ; Aale ,Y.S ; and Jamalian , N. M. (2005).Evaluation of serum levels of immunoglobulin and complement factors in β- thalassemia major patients in southern Iran. I. journal immunology. Vol (2). NO.4 .

Cao, A. ; and Galanello, R. (2010): Beta-thalassemia. Genetics In Medicine. 12 (2):61-76.

Chalevelakis, G. ; Clegg , J.B. ; and Weatherall , D.J. (1975) Imbalanced globin chain synthesis in heterozygous beta-thalassemic bone marrow. Proc Natl Acad Sci USA;72:3853-3855.

Corry, J.M.; Marshall, W.C.; Guthrie, L.A.; Peerless, A. G.; Johnston , R.B. (1981) Deficient activity of the alternative pathway of complement in beta-thalassemia major. Am J Dis Child.;135:529-31.

Dwyer, J. ; Wood , C. ; McNamara , J.; Williams , A. ; Andiman ,W.; Rink ,L. ; O'Connor, T. ; and Pearson ,H. (1987).Abnormalities in the immunesystem of children with beta-thalassaemia major. Clin ExImmunol.. 68(3):621-629.

Galanello, R.; and Origa , R. (2010): Beta thalassemia. Orphanet Journal of Rare Diseases 5: 11.

Higgs, D.R.; Thein , S.L. ; and Wood , W.G. (2001). The pathophysiology of the thalassaemias. In: Weatherall DJ, Clegg B, eds. The thalassaemia syndromes. 4th ed. Oxford, England: Blackwell Science. 192-236.

Jeddoa, A.; Sattar , J. R. ; and Adel, A. A.(2011).Immunological Evaluation of Patients with B-Thalassemia Major in Kerbala City Using Single Radial Immunodiffusion (SRID) Technique Karbala J. Med. Vol.4, No.1,2.

Lahiry, P.; Al-Attar, S.A.; and Hegele, R.A. (2008): Understanding beta-thalassemia with focus on the Indian Subcontinent and the Middle East. The Open Hematology Journal 2: 5-13.

Malik, A. M.; Emad, M. M.; Al-Shammaa, M.J.; and Al-RubaeiA , Z. M. (2010). Comparative Biochemical Study of Proteins Profile in Iraqi Children and Adolescent with β–Thalassemia Iraqi J Pharm Sci, Vol.19 (2).

Pardalos, G.; Kanakoudi-Tsakalidis , F.; Malaka-Zafiriu , M. ; Tsantali , H. ; Athanasiou-Metaxa , M. ; and Kallinikos , G.(1987). Iron related disturbances of cell-mediated immunity in multitransfused children with thalassemia major. Clin Exp Immunol.;63:138-145.

Peters, M.; Heijboer, H.; Smiers, F.; and Giordano, P.C. (2012): Diagnosis and management of thalassemia. British Medical Journal; 344:e288 doi: 10.1136/bmj.e228.

Salsaa, B.; and Zoumbos, N.C. (1997) A distinct Pattern of Cytokine Production From Blood mononuclear cells in multitransfused patients with beta-thalassaemia.Clinical and Experimental Immunology.107(3):589-592.

Tovo, P.A.; Miniero , R. ; Bardera , C. ; Sacchehi , L. ; And Saita M. (1981): Serum immunoglobulins in homozygous a1-thalassemia. Acta Haematologica Basel, 65:21-25.

Vergin, C. ; Kutukculer ,N. ; Cetingul , N. ; Nisli ,G. ; Caglayan , S. ; and Oztop , S. (1997). Serum immunoglobulins, IgG sub classes,isohemag-glutinins and complement-3 levels in patients with thalassemia major. Indian J Pediatr.; 64:215-219.

Vichinsky, E. ; Bernaudin , F.; Forni , G.L. ; Gardner , R. ; Hassell ,K. ; Heeney , M. M. ; Inusa , B. ; Kutlar ,A. ; Lane , P. ; Mathias , L. ; Porter , J. ; Tebbi, C. ; Wilson , F. ; Griffel , L. ; Deng , W. ; Giannone , V. ; and Coates , T. (2011) Long-term safety and efficacy of deferasirox (Exjade(Æ)) for up to 5 years in transfusional iron-overloaded patients with sickle cell disease. British Journal Haematology. doi:10.1111/j.1365-2141. 08720.x.

Weatherall, D. (2004) The Thalassemias: the role of molecular genetics in anevolving global health problem. American journal of human genetics 74: 385-392.

Weatherland, D.J. ; Clegg , J.B. ; Higgs , D. ; and Wood , W.G.(2000). The hemoglobinopathies. In: The metabolic basis of inherited disease. 8th ed. McGraw-Hill.:4000 - 4006.

Weatherland, D.J.; and Clegg, J.B. (2000). Pathophysiology of Thalassemia. In: The thalassemia syndromes.4 th ed. London: Blackwell Scientific Publications.120-124.

تقييم بعض المؤشرات المناعية الخلطية في مرضى البيتا ثلاسيميا العظمى في محافظة واسط/ العراق

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الخلاصة

هدفت الدراسة الحالية إلى اختبار المناعة الخلطية لدى مرضى الثلاسيميا العظمى نوع بيتا بواسطة قياس مستويات الغلوبيولينات المناعية IgG, IgA, IgM , وبعض بروتينات النظام المتمم C3 وC4 في مصل الدم. تضمنت الدراسة ما مجموعه 80 من مرضى الثلاسيميا العظمى الذين تراوحت أعمارهم بين 6 – 25 سنة. تم تقسيم المرضى إلى مجاميع ثانوية حسب العمر إلى ثلاثة فئات عمرية .كانت الفئة العمرية 11- 20 هي الفئة الأكثر سيادة 38 : 80 (47.5 %). في حين كان المرضى تحت 10 سنوات 29 : 80 (36.25 %) والأكثر من 20 سنة 13 : 80 (16.25 %). اتضح من خلال استمارة الاستبيان أن جميع المرضى الأكثر من 20 عاما مزالين الطحال . عند المقارنة مع الفئات العمرية المماثلة من مجاميع السيطرة والمؤلفة من 45 من الأشخاص

الأصحاء كان هناك زيادة معنوية ($P \leq 0.05$) في غلوبيولينات المصل IgA , IgG, IgM في جميع الفئات العمرية من مرضى الثلاسيميا العظمى نوع بيتا . ازدادت مستويات الغلوبيولينات M, G, A في المصل معنويا ($P \leq 0.05$) مع زيادة العمر في مرضى الثلاسيميا العظمى نوع بيتا. انخفضت مستويات C3, C4 معنويا في المصل لدى جميع الفئات العمرية من مرضى الثلاسيميا مقارنة مع الفئات العمرية المماثلة من الأصحاء. لوحظ بأن مستوى C3 قد انخفض معنويا في الفئات العمرية 11- 20 عاما و الأكثر من 20 عاما في مرضى الثلاسيميا مقارنة مع المرضى الأقل من 10 سنوات, في حين لاتوجد فروق معنوية في C4 المصل بين الفئات العمرية لمرضى الثلاسيميا العظمى عند مقارنتها مع بعضها البعض.