A study of Immunoglobulins,Complements and Some Hematological Parameters Levels in Thalassemic Patients in Related Bacterial Infections

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Abstract

Background: Thalassemia and abnormal hemoglobin are the most common genetic disorders and are considered health problems in many developing countries. Beta-Thalassemia major is the most familiar type, in which the beta-globin chain synthesis is impaired.

Objective: To evaluation the concentrations of immunoglobulins IgM, IgG and IgA also the complements C3 and C4 between β-Thalassimic children patients and control group, and find the differences of some hematological parameters between the two groups, finally, isolation of some bacterial species causes the bacterial infections.

Patients and Methods: The present study was performed on 40 β-thalassemia major children who were registered in the thalassemia center at Al-Karama teaching hospital in Baghdad and 20 healthy children as a control group from the period of September 2015-Augest 2016, the serum immunoglobulins (IgG, IgM, IgA) and complement C3, C4 levels were measured also blood culture was done to identify the causative infection.

Results: The mean serum concentrations of IgM, IgG and IgA in β-thalassemia major children were 1834.1 ± 312.2, 134.5 ± 40.3 and 212.7±89.6, respectively, and they were increased significantly ($p \leq 0.05$). The mean serum concentrations of C3 and C4 were consistently decreased in children and there were no significant differences ($p \leq 0.05$) between the two groups. The mean Hb of β- thalassemic children was 8.32±0.91 g/dl and this lower from the level of control, RBC count is also low 3.7±0.7 but the levels of WBCs was higher in β- thalassemic children 10.4±1.67, several types of bacterial specious were isolated included *Staphylococcus aureus* 30% (12 patients) , *Escherichia coli* 25%(10 patients) , *Streptococcus pneumoniae* 17.5% (7 patients), *Salmonella enteritidis* 10% (4 patients), *Serratia marcescens* 7.5% (3 patients), *Pseudomonas aeruginosa* 5%(2 patients), *Klebsiella pneumoniae* 2.5%(1 patients) and *Haemophilus influenza* 2.5%(1patients).

Conclusion: The alteration in serum immunoglobulins and complements levels in thalassemia major children probably can be due to marked heterogeneity of the patients,.

Key words: Serum immunoglobulins, thalassemia, complements, bacterial infections.

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Introduction

Beta-thalassemia major is considered as a major health problem especially in our area ,the patients suffer from a form of inherited autosomal recessive blood disorder which characterized by abnormal formation of hemoglobin [1].

This disease affects many organs and systems in the body which can causes serious damages to these systems such as reticuloendothelial, immunity and endocrine systems [2]. Different immunological abnormalities factors recorded such as splenectomy, iron overload and frequent exposure to foreign allogenic antigens during the blood transfusion and liver damage after hepatitis, so these variables influence can explain different findings in patients from different geographical places [3]. The frequency of blood transfusion could led to iron overload and many complications such as neurotic problems, growth failure, cardiovascular problems, liver disease and many problems other than severe anemia as well as the increasing of infections chances which may include increased susceptibility to bacterial infections [4].

So this study aims to evaluation the concentrations of immunoglobulins IgM, IgG and IgA also the complements C3 and C4 between β-Thalassimic children patients and control group , and find the differences of some hematological parameters between the two groups, finally, isolation of some bacterial species causes the bacterial infections

Patients and Methods

The study was performed on 40 β-thalassemia major children who were registered in the thalasseamia center at Al-Karama teaching hospital in Baghdad and 20 healthy children as a control group from the period of September 2015 to Augest 2016, the age of the children was 3 months - 10 years and the information's were collected from the hospital records and the parents questionnaire. The serum immunoglobulins (IgG, IgM, IgA) and complement C3, C4 levels were measured by using single radial immunodifussion (SRID) kit from LTA (Italy) . All plates were opened then 5 µl of serum was put in each well of each plate then the plates were covered and stored at a moist chamber. The precipitating ring was measured by immunoviewer then the concentration of each humoral component was detected by using the reference table which contains the concentration value corresponding to precipitating ring diameter [5]. In the EDTA blood samples, the Hb, WBCs and RBCs were analyzed by a hematology analyzer (ADVIA 120, Siemens). In order to show the main bacterial causative agents of infections, gram stain used to identify mixtures of bacteria then appropriate culture media is selected depending upon the bacteria suspected, colonies of bacteria are usually large enough to identify after 18–24 h at 37°C and the biochemical tests used to confirm the diagnosis [6]. The aim of the study was to evaluate the concentrations of immunoglobulins IgM, IgG and IgA and the complements C3 and C4 between β-.

Thalassimic children patients and control group , and find the differences of some hematological parameters between the two groups, finally, isolation of some bacterial species causes infections.

Statistical analysis

The Chi-square and t tests were used for statistical analysis. A P value less than 0.05 was used for statistical significance to compare the differences between the groups.

Results

The results of the study showed that the newly affected patients registration under one year represented the major group [75%] out of the total number as shown in table 1.

Table (1): Distribution of β-thalassemia major children according to age.

Age /years	N=40 (100%)
Less than 1 year	15 (75%)
1-3 year	11(55%)
4-6 year	9(45%)
7-10 year	5(25%)

The serum concentrations of IgM, IgG increased significantly (p≤0.05) in β-thalassemia major patients children as compared with control group. IgA concentrations very high and there was a significant difference (p≤ 0.05) between the patients and control groups as shown in table 2.

Table (2): Mean concentration of Immunoglobulin IgM, IgG and IgA in β- thalassemic children and control serum**.**

groups	N	IgM	IgG	IgA
		Mean ±SD	Mean ± SD	Mean ± SD
patients	40	1834.1 ± 312.2	134.5 ± 40.3	212.7±89.6
control	20	1212.3±343.4	74.7 ± 19.5	133.4±28.9
probability		P≤0.05	P≤0.05	P≤0.05

The results showed that the complements (C3 and C4) mean concentration of β-thalassemic children patients was less than control group. There was no significant differences between patients and control group (P >0.05), as shown in table 3 .

Table (3): mean concentration of C3 and C4 in β- thalassemic children patients and control serum.

omplements	Patients N=40	Control N=20	probability	
	Mean ±SD	Mean ±SD	t test	P value
C3	214.16± 51.14	232.42±58.61	0.756	-0.418

In the present study the mean Hb of β- thalassemic children was 8.32±0.91 g/dl and this lower from the level of control, RBC count is also low 3.7±0.7 Thalassemic patients show high levels of WBCs 10.4±1.67 than As shown in table 4.

Table (4): Mean differences of some hematological parameters between β-Thalassimic patients and control group.

parameters	β-Thalassimic patients	control	probability	
	Mean ±SD	Mean ±SD	T test	P value
Hb	8.32±0.91	13.4±0.58	1.645	0.741
WBCs	10.4±1.67	8.75±1.43.	1.938	0.582
RBCs	3.7±0.7	5.3±0.31	1.747	0.947

The results showed that there were a bacterial infections by both gram positive and negative bacteria as follows *Staphylococcus aureus* 30% (12 patients) , *Escherichia coli* 25%(10 patients) , Streptococcus pneumoniae 17.5%(7 patients), *Salmonella enteritidis* 10%(4 patients), *Serratia marcescens* 7.5%(3 patients), *Pseudomonas aeruginos*a 5%(2 patients), *Klebsiella pneumoniae* 2.5%(1 patients) and *Haemophilus influenza* 2.5% (1patients) while the control group revealed that the most common infection is caused by *Escherichia coli* 20%[4 patients] followed by *Staphylococcus aureus* 10% (2 patients) as showed in figure 1.

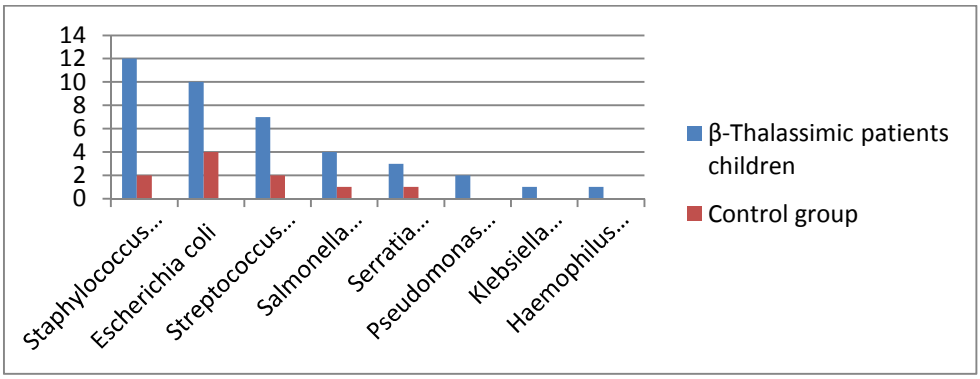

Figure (1): Distribution of bacterial isolations between β-Thalassimic patients children and control group.

Discussion

The results showed that the patient's registration under one year represented the major group (75%) and this may be due to increase awareness of the parents about their child health [7]. In The present study we evaluated only selected immunological parameters. The increased levels of serum immunoglobulins is resulted from continuous exposure to various antigens and the re-infections can also stimulate the immune system which may result in increased immunoglobulin levels, Iron over load was suggested as an important risky factor affecting the immune parameters in thalassemic patients and this agree with other studies in Iran and England [8][9]. C3 and C4 were consistently decreased in the patients children and this might be due to reduction in synthesis or increased consumption; and many observation mentioned the lower levels of C3 and C4 which may act as a serious factor to immune deficiency [10][11]. The Hb was low as comparing with control and this related to disturbances in the haemoglobin synthesis which leading to microcytic and hypochromic anaemia [13]. Which was similar to other studies in India

and USA [14][15]. This could be due to better chelation as well as leuco-depleted red cell transfusions currently in use for thalassemic patients. With increasing number of transfusions, there was an increase in the levels of immunoglobulins. It has been postulated that donor alloantigens lead to development of alloantibodies to the patients' RBCs, leading to conformational changes on the epitope of RBC antigens, thereby stimulating the formation of autoantibodies [16]. The WBCs count was higher than control due to the presence of immature red cells [17]. These results agree with other findings [18][19]. The result was in accordance with other studies, where the percentages of gram-positive and gram-negative organisms isolated from bacteremic children. Similar study in Pakistan reveled that Gram-positive organisms and Gram-negative organisms accounted for causes bacteremic infections, another study pointed out that the etiology may even vary at different times within the same place [20][21].

In conclusion, the present results revealed that the mean serum levels concentrations of IgM, IgG and IgA in β-thalassemia major patients increased significantly and complements C 3, C4 were decreased in all patients groups compared with corresponding age group of healthy control. The levels of WBCs were higher and there were many bacterial species isolated, heterogeneity concerns race, socioeconomic class, nutritional status and environmental factors. The anemia produced by different abnormal hemoglobins and usually is caused by iron deficiency and thalassemia trait

Repeated blood transfusion in β-thalassemia patients will result in a continuous exposure to various antigens and lead to increased levels of serum immunoglobulins and continuous complement consumption, also, increased the bacterial infections.

References

[1] Hahalis G, Alexopoulos D, Kremastinos DT,Zoumbos NC. Heart failure in beta-thalassemia syndromes: a decade of progress. The American Journal of Medicine,2005; 118: 957-967.

[2] Cao A, Galanello R. Beta-thalassemia. Genetics In Medicine, 2010; 12: 61-76.

[3] Alidoost F, Gharagozloo M, Bagherpour B, Jafarian A,Sajjadi SE, Hourfar H, Moayedi B. Effects of silymarin on the proliferation and glutathione levels of peripheral blood mo onuclear cells from betathalassemia major patients. International Immunopharmacology, 2006; 6: 1305-1310.

[4] Morabito N, Russo GT, Gaudio A, Lasco A, Catalano A, Morini E *et. al.* The "lively"cytokines network in beta-Thalassemia Major-related osteoporosis. Bone, 2007; 40: 1588-1594.

[5] Wang LY, Liang DC, Liu HC, Chang FC, Wang CL, Chan YS *et al*. lloimmunization among patients with transfusion-dependent thalassemia in Taiwan. Transfusion Medicine, 2006; 16: 200–203.

[6] Peng CT, Tsai CH, Wang JH, Chiu CF, Chow KC. Bacterial infection in patients with transfusion-dependent beta-thalassemia in central Taiwan. Acta Paediatr Taiwan. 2007; 41(6):318-21.

[7] Prati D. Benefits and complications of regular blood transfusion in patients with beta-thalassaemia major. Vox Sang. 2009; 79(3):129-37.

[8] Amin A, Jalali S, Amin R, Aale-yasin S, Jamalian N, Karimi M, Evaluation of the Serum Levels of Immunoglobulin and Complement Factors in β- thalassemia Major Patients in Southern Iran. Iranian Journal of Immunology, 2005; 2: 220-225.

[9] Vichinsky E, Bernaudin F, Forni G, Gardner R , Hassell K , Heeney M *et al*. Long-term safety and efficacy of deferasirox (Exjade(Æ)) for up to 5 years in transfusional iron-overloaded patents with sickle cell disease. British Journal Hematology. 2011.

[10] Denic S, Souid A, Nagelkerke N, Showqi S, Balhaj G. Erythrocyte reference values in Emirati people with and without α+ thalassemia. BMC Blood Disord 2011; 11:1.
[11] Balgir R. Hematological profile of twenty nine tribal compound cases of hemoglobinopathies and G-6-PD deficiency in rural Orissa. Indian J. Med. Sci. 2008;62:364-73.
[12] Rathod D, Kaur A, Patel V, Patel K, Kabrawala R, Patel V. Usefulness of cell counter-based parameters and formulas in detection of β thalassemia trait in areas of high prevalence. Am J Clin Pathol. 2007; 128: 585-9.
[13] Tritipsombut J, Sanchaisuria K, Fucharoen S, Fucharoen G, Siriratmanawong N, Pinmuangngam C. Hemoglobin profiles and hematologic features of thalassemic newborns. Arch Pathol Lab Med 2008; 132:1739-45.
[14] Tongnoi P. Hematologic parameters and level of HbE for predicting Alpha-thalassemia 1 gene in pregnant women. Hong Kong Med. J. 2008; 32:2 .
[15] Peng CT, Tsai CH, Wang JH, Chiu CF, Chow KC. Bacterial infection in patients with transfusion-dependent beta-thalassemia in central Taiwan. Acta Paediatr Taiwan. 2009, 41(6):318-21.
[16] Prati D. Benefits and complications of regular blood transfusion in patients with beta-thalassaemia major. Vox Sang. 2008, 79(3):129-37.
[17] Shih-Chung Wang, Kai-Hsin Lin, Jimmy P. S. Chern, Meng-Yao Lu, Shiann-Tarng Jou, Dong-Tsamn Lin, and Kuo-Sin Lin. Klebsiella Infection in Patients with Thalassemia. Klebsiella Infection in Thalassemia. 2007; CID 36.
[18] Shih-Chung Wang, Kai-Hsin Lin, Jimmy P.S. Chern, Meng-Yao Lu, Shiann-Tarng Jou, Dong-Tsamn Lin *et al.* Severe Bacterial Infection in Transfusion-Dependent Patients with Thalassemia Major. 2003, CID 37.
[19] Yagupsky P, Giladi Y. Group A Beta hemolytic streptococcal bacteremia in children. Pediatr. Infect. Dis. J. 2010, 6(11):1036-1039.
[20] Orkin S, Nathan G. The thalassemias. In: Hematology of Infancy & Childhood, 5th ed. Philadelphia: WB Saunders. 2008: pp. 811-86.
[21] Campbell GL, Dennis DT. Plague and other Yersinia infections. In Harrison's Principles of Internal Medicine, 15th ed. New York: McGraw-Hill. 2006: p. 993-1001.