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Evaluation The Immune Status Of Blood Transfusion-Dependent Thalassemia In Thi-Qar Province/Iraq

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Abstract:
The present study was conducted at the Center of an inherited blood disorder in Dhi-Qar Province, during the period from October 2018 to January 2019. The aim of this study was to evaluate the immune status of patients with beta-thalassemia by measuring (IgM, IgG, IgA, C3 and C4) complement by used single radial immune diffusion (SRID. The study included a total of 150 patients with beta-thalassemia and included (66 males) and (84 females) and their age from (2 to 33 years). The patients divided into three age groups. The second age group(11-20) years the most dominant. All patients over 20 years have been exposed to spleen removal operations. When compared with similar age groups of control groups consisting of 50 healthy persons, there was a significant increase of serum IgM, IgG, and IgA in all age groups of beta-thalassemia patients. The levels of serum IgG, IgM and IgA increased significantly with increased age in patients with beta thalassemia. The levels of C3 and C4 were significantly reduced in serum in all age groups of patients with beta-thalassemia except the first age group for C4 compared to similar age groups of healthy individuals.

Keywords: Beta-thalassemia, Immune abnormalities, immunoglobulins.

1. Introduction

Thalassemia syndromes are the most common form of chronic hemolytic anemia due to impaired globin chain synthesis, thalassemia syndrome is a distribution in Mediterranean region, the Middle East, Indian subcontinent, and Southeast Asia (Honar, 2014;Farashi & Harteveld, 2018). It results from qualitative and quantitative reductions in globin chain synthesis, so it can be classified according to which globin chains are produced in the reduced amount. Those with diminished β-globin chains are termed β-thalassemia, whereas those with decreased α-chain production are termed α- thalassemia. The severity of clinical manifestations in these disorders relates to the amount of globin chain produced and the stability of residual chains present in excess (Tadmouri, 1999). Thalassemia minor syndrome is characterized clinically by mild anemia with persistent microcytosis. Thalassemia intermedia is typified by a moderate, variably compensated hemolytic anemia that may present with clinical symptoms during a period of

physiologic stress such as infection, pregnancy, or surgery and may or may not need a regular blood transfusion (Ioana, 2018). The normal humans HbF (α2γ2) is the dominant hemoglobin at birth, which is gradually decreased with increasing age and changes to adult hemoglobin HbA (α2β2), while in patients with thalassemia major, defective β chain gene causes decreased HbA synthesis as well as increased expression of HbF (Sankaran & Orkin, 2013). Several studies has been shown that HbF expression is affected by several factors, including post-transcriptional regulatory mechanisms as well as single nucleotide polymorphisms (SNPs) (Danjou et al., 2012). Over 600 mutations have been reported in the beta-globin locus, approximately 200 of which are related to beta-thalassemia in some capacity. Most mutations altering HbF levels happen in the promoter of Aγ and Bγ genes between upstream -114 and -205 and the transcription origin. There also are a number of mutations in GATA1 junction, NF-E4 and the upstream CAAT box (Gedara, Megalatha, Unit, Medicine, & Lanka, 2012). Thalassemia patients are always exposed during the period of blood transfusion into antibodies and performed antigens and this leads to weak immune status, therefore immune abnormalities have been suggested as a precipitating factor for the fourth most common cause of death in β -thalassemia, i.e. malignancies, especially leukemia and lymphomas (Muncie & Campbell, 2009). Several studies on immune competence in â-thalassemia have revealed numerous quantitative and functional defects, involving T and B lymphocytes, increased immunoglobulin production particularly immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA), deficient activity of the complement system with reduced levels of complement 3 (C3) and complement 4 (C4), increased of liver enzyme with increased ferritin (Salman & Al-mousawi, 2014). The present study aimed to estimated serum immunoglobulin IgG, IgM, IgA, C3and C4.

2. Material and methods

This study was performed on 150 β-thalassemia major patients who attended to center for inherited blood disorder in Dhi-Qar Province for regular blood transfusion in the period from September 2018 to January 2019 and 50 healthy control. Five ml of venous blood samples were collected by a well-trained nurse from volunteer patients before the next blood transfusion in gel tube and the clinical history was taken from each patient and their parents as in the attached form. 3 ml of Blood has been allowed to clot at room temperature for 30 minutes, then it was centrifuged for 5 minutes at 4000 rpm, hemolysis has been avoided by taking the necessary precautions, however hemolysis samples were ignored, serum was collected and distributed into many Eppendorf tubes and give the same number in the form and in the 3 reported tube . The serum samples were frozen at -20 C until used. Tow ml of blood was added to tow ETDA containing tubes for assessment the hematological examination and for blood group .The serum was frozen for the latter estimation the immunological parameters included (IgG, IgM, IgA; C3, and C4 by single radial immune diffusion(SRID).

3. Result:

3.1. Distribution of thalassemia patient and control according to Age, gender, habitat and endogamy

A total of 150 patients with β- thalassemia major were included in this study (table 4-1) their ages range between (2-33) years(66 males and 84 females). The most predominant age group was 11-20 years (Second age group) 72 patient with the percentage (48.0%). And the patients below 10 years(First age group) were 64 patient with a percentage (42.66%), while the third age group that with more than 20 years (Third age group) 14 patient with the percentage (9.234%). It has been shown through a question that

about (80%) of patients more than 20 years suffer from the removal of the spleen splenectomies β-thalassemia major patients (SβTM)], also it has been shown through a questionnaire that 88 of the patients were inhabited rural areas, while 62 of them were inhabited urban cities, and also it has been shown through the question that 106 patient their parents married their relatives, while 44 patient their parents married strangers

Table (3-1): Distribution of β- thalassemia major patients according to the age, gender, habitat . and endogamy.

Age group / Parameters		< 11 years (1st age group)	11-20 years (2nd age group)	> 20years (3rd age group)	Total
Number of cases	Patient	64	72	14	150
	Control	24	16	10	50
Male	Patient	20	42	4	66
	Control	8	10	6	٢٤
Female	Patient	44	30	10	84
	Control	16	6	4	26
Rural	Patient	38	40	10	88
	Control	12	10	6	28
Urban	Patient	26	32	4	62
	Control	12	6	4	22
Endogamy	Patient	46	48	12	106
	Control	10	4	4	18

3.2. Age of patients in relation to some immunological parameters:

3.2.1. Serum immunoglobulins:

3.2.1.1. Serum immunoglobulin G (IgG):

The level of serum immunoglobulin G was estimated for all samples patients and control Table (4 - 13). The results showed the level serum IgG were correlated with age and there are no significant differences at ($P > 0.05$) between the age group of patient.

The result also shows that serum IgG concentrations raised significantly ($p \leq 0.01$) in all groups of thalassemia patients compared to their concentrations in corresponding age of healthy control. There means in thalassemia patients according to age groups were (1893.12 ± 209.59, 2225.58 ± 358.92, and 2995.42 ± 340.01) respectively, whereas there mean in control subjects were (1117.41 ± 116.84, 1196.0 ± 159.36 and 1442.20 ± 92.31) respectively.

Table (3-2): estimate and comparison the level of serum IgG for both thalassemia patients and control according to age:

Group		NO:	Con of IgG mg/dl Mean ± SD	T-Value	P-Value
G1	Control	٢٤	1117.41 ± 116.84[b]	12.07	0.05
	Patient	٦٤	1893.12 ± 209.59[a]		
G2	Control	١٦	1196.0 ± 159.36[b]	7.88	0.05
	Patient	٧٢	2225.58 ± 358.92[a]		
G3	Control	١٠	1442.20 ± 92.31[b]	11.50	0.05
	Patient	١٤	2995.42±340.01 [a]		

3.2.1.2. Serum immunoglobulin M (IgM):

The level of serum immunoglobulin M was determined for all samples patients and control Table (4 - 14). The results showed the level serum IgM were correlated with age and there are no significant differences at ($P > 0.05$) between the age group of patient.

The result also shows that serum IgM concentrations raised significantly ($p \leq 0.01$) in all groups of thalassemia patients compared to their concentrations in corresponding age of healthy control. There means in thalassemia patients according to age groups were (162.28 ± 40.89, 199.41 ± 25.98, and 245.14 ± 21.03) respectively, whereas there mean in control subjects were (83.91 ± 13.65, 108.25 ± 6.29 and 112.60 ± 15.35) respectively.

Table (٣-3): estimate and comparison the level of serum IgM for both thalassemia major patient and control according to age.

Group		NO:	Con of IgM mg/dl Mean ± SD	T-Value	P-Value
G1	Control	٢٤	83.91 ± 13.65[b]	9.51	0.05
	Patient	٦٤	162.28 ± 40.89[a]		
G2	Control	١٦	108.25 ± 6.29[b]	18.72	0.05
	Patient	٧٢	199.41 ± 25.98[a]		
G3	Control	١٠	112.60 ± 15.35[b]	11.93	0.05
	Patient	١٤	245.14 21.03[a]		

3.2.1.3. Serum immunoglobulin A (IgA):

The level of serum immunoglobulin A was determined for all samples patients and control Table (4-15). The results showed the level serum IgA were correlated with age and there are no significant differences at($P > 0.05$) between the age group of patient.

The result also shows that serum IgA concentrations raised significantly ($p \leq 0.01$) in all groups of thalassemia patients compared to their concentrations in corresponding age of healthy control. There means in thalassemia patients according to age groups were (210.75 ± 50.16, 292.38 ± 48.44, and 399.85 ± 43.63) respectively, whereas there mean in control subjects were (130.41 ± 11.64, 133.75 ± 14.68 and 151.60 ± 10.62) respectively.

Table (3-4): estimate and comparison the level of serum IgA for both thalassemia major patients and control according to age.

Group		NO:	Con of IgA mg/dl Mean ± SD	T-Value	P-Value
G1	Control	٢٤	130.41 ± 11.64[b]	8.47	0.05
	Patient	٦٤	210.75 ± 50.16[a]		
G2	Control	١٦	133.75 ± 14.68[b]	16.52	0.05
	Patient	٧٢	292.38 ± 48.44[a]		
G3	Control	١٠	151.60 ± 10.62[b]	14.46	0.05
	Patient	١٤	399.85 ± 43.63[a]		

3.2.2. Complement system:

3.2.2.1. Serum complement 3 (C3):

The level of serum complement 3 was determined for all samples patients and control Table (4 -16). The results showed the level serum C3 were decreased with age group and there are no significant differences at ($P > 0.05$) between the age group of patient.

The result also shows that serum C3 concentrations decreased significantly ($p \leq 0.01$) in all groups of thalassemia patients compared to their concentrations in the corresponding age of healthy control. There means in thalassemia patients according to age groups were (88.37 ± 15.94, 76.30 ± 16.97, and 67.71 ± 12.85), whereas there mean in control subjects were (132.33 ± 5.34, 135.87 ± 5.13 and 138.20 ± 4.49) respectively.

Table (3-5): estimate and comparison the level of serum C3 for both thalassemia major patient and control according to age:

Group		NO:	Con of C3 mg/dl Mean ± SD	T-Value	P-Value
G1	Control	٢٤	132.33 ± 5.34[a]	13.67	0.05
	Patient	٦٤	88.37 ± 15.94[b]		
G2	Control	١٦	135.87 ± 5.13[a]	17.71	0.05
	Patient	٧٢	76.30 ± 16.97[b]		
G3	Control	١٠	138.20 ± 4.49[a]	13.40	0.05
	Patient	١٤	67.71 ± 12.85[b]		

3.2.2.2. Serum complement 4 (C4):

The level of serum complement 4 was determined for all samples patients and control Table (4-17). The results showed the level serum C4 were normal in first age group only with the mean (22.18 ± 3.57) but decreased with second and third age group and there are significant differences at (P > 0.05) between first age group and second and third age group of patient.

The result also shows that serum C4 concentrations decreased significantly at ($p \leq 0.01$) in second and third age groups of thalassemia patients compared to their concentrations in corresponding age of healthy control. There means in thalassemia patients according to age groups were (17.35 ± 3.65, and 16.37 ± 3.18), whereas there mean in control subjects were 30.15 ± 4.07 and 32.0 ± 2.64) respectively.

Table (3-6): estimate and comparison the level of serum C4 for both thalassemia major patient and control according to age:

Group		NO:	Con of C4 mg/dl Mean ± SD	T-Value	P-Value
G1	Control	٢٤	31.40 ± 3.23[a]	7.75	0.05
	Patient	٦٤	22.18 ± 3.57[b]		
G2	Control	١٦	30.15 ± 4.07[a]	8.77	0.05
	Patient	٧٢	17.35 ± 3.65[b]		
G3	Control	١٠	32.0 ± 2.64[a]	8.95	0.05
	Patient	١٤	16.37 ± 3.18[b]		

4. Discussion:

Age, gender, habitat and endogamy distribution of thalassemia patients and control:

The distribution of thalassemia syndrome in our current study according to age the our study showed a significant increase of thalassemia among second age group and first age group compare with third age group table (3-1). The high rate of prevalence of thalassemia in the second and first age group, because the patient who is up to 20 years or more is exposed to many complications, the most important of which is the increase of ferritin, which affects the course of the heart muscle and kidneys and endocrine, in addition to the viral infections, which lead to many deaths (Haghpanah et al., 2018). This result is agree with result obtained by 2014, Mustafa Saber Al-Attar (Al-attar & Shekha, 2014) and 2018, Asal Tawfeeq (Tawfeeq, 2018). The results designate that thalassemia major phenotype can be diagnosed perfectly within the early months of age because the exhibitions of the disease may perform after a complete switch from fetal to adult Hb synthesis occurs. Classically, this switch is complete by the sixth month after birth (Tadmouri, Nair, Obeid, & Gallala, 2009;Sugimoto, 2018). As for severe types of β-thalassemia, the symptoms may not be clear until the second half of the first year of life till that time, the γ globin chains manufacture and their combination into fetal Hb can mask the condition. Frequently, milder forms are discovered by chance and at different ages and many patients whose conditions to be a homozygous may show no significant symptoms or anemia for numerous years(Al-Haddad, Yassin, & Sirdah, 2012,Traivaree, Monsereenusorn, Rujkijyanont, Prasertsin, & Boonyawat, 2018). According to the gender there are no significant difference between gender because thalassemia it is a genetic disease that is transmitted from parents to offspring and to both sexes equally. This result is agree with 2014, Mustafa Saber Al-Attar(Al-attar & Shekha, 2014). A according to habitat the result showed the significant increase of induced of thalassemia syndrome in rural, this is because of some tribal traditions common to rural

people mating them and not being open to other families. This result is agree with 2018, Asal Tawfeeq (Tawfeeq, 2018), and dis agree with 2014, Mustafa Saber Al-Attar(Al-attar & Shekha, 2014), the compatibility between the two studies may be due to the random sampling of models or all ages without discrimination, the lack of consistency between the two studies may be due to several reasons, namely, the large number of rural-urban migration, the study was the study of genetic diseases family.

Immunoglobulins:

The significantly higher serum IgG (table 3-2) , IgM (table 3-3) and IgA (table 3-4) in all age groups of thalassemia patients compared with corresponding healthy control groups can be attributed to numerous factors. As 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 (Kiani-Amin, 2011;Shani, 2014).

The mechanism of such changes in the immunoglobulins were designated according to hypothesis including the iron overload on skin that lead to (stimulation of IgA produce as a muco-cutaneous antibody(D Asadov, 2014),and frequent exposure to antigens due to frequent transfusions and infection stimulation of IgM and IgG (Farmakis, Giakoumis, Polymeropoulos, & Aessopos, 2003).

Thalassemia patients are prone to many bacterial and viral infections and other infectious agents. frequent infections also stimulate the immune system and may result in increased immunoglobulins levels (Mahdi Muhammad Moosa et al., 2011).Iron overload was suggested by some investigators as an important contributing factor in changing the immune parameters in thalassemia patients(Shah et al., 2010).

It has been suggested that iron overload results in increase migration of T helper cells to the gut and lymph nodes and this causes an increase in serum immunoglobulin levels in thalassemia patients (Chalevelakis, Clegg, & Weatherall, 2006).The results of the present study came in compatible with(R. Amin, Amin, & Alyasin, 2008a;Malik, Malik, Al-Shammaa, & Al-Rubaei, 2010;Jeddoa, Mohammed, Jeddoa, Ateia, & Ali, 2011;Al-Haddad et al., 2012;Javad, Saeid, & Mohammadmehdi, 2016) who reported that serum IgG, IgM and IgA immunoglobulins increased in BTM patients.

The significant increase in the serum levels of immunoglobulins with increased of age may attributed 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 that the spleen acts as one of the major lymphoid organs to clear the blood infections, it is hypothesized that the removal of spleen may force other secondary lymphoid organs to compensate for the synthesis of the major immunoglobulin classes(de Dreuzy, Bhukhai, Leboulch, & Payen, 2016).

According to (R. Amin et al., 2008a)who reported that splenectomy increases the serum level of IgG and IgA without change on serum level of IgM. This might be associated to filtration of transfused packed cells resulting in decreasing the chance of frequent exposure to antigens. On the contrary (Kiani-Amin, 2011)reported that Serum mean levels of IgG and IgM in βTM patients were normal.

Complement system:

The present results revealed that the mean levels of complements C 3 (table 3-5) and C4 (table 3- 6) were significantly decreased in all patients groups compared with corresponding age control group of healthy people. Thalassemia-patients age groups in the present revealed no significant differences in

serum C3 but C4revaled significant difference between first age group and second and third age group when compared to each other (table 3-6). Similar result was presented by (A. Amin et al. 2005;Mahmoud SS,and el all. 2017). The decrease in complements C3 and C4 can be explained on same basis, repeated blood transfusion in our thalassemia-patients may result in a continuous exposure to various antigens and which lead to continuous complement consumption(Dwyer et al., 1997;Mahdi M Moosa, 2014).
Suppressed functioning of the complement system (classic or alternative),with reduced levels of C3 and C4, has also been observed (Dwyer et al., 1997;A. Amin et al. 2005).These defects have been attributed both to the disease itself and the applied therapeutic interventions(Dwyer et al., 1997).

According to(R. Amin, Amin, & Alyasin, 2008b;Javad et al., 2016) who found a negative correlation between serum ferritin and the level of complement system, indicating that high levels of serum ferritin were constantly associated with low serum complement system . So when increased the level of serum ferritin shown in present study could be considered as another reason for the decrement of serum C3 and C4.

5. Conclusion:

The result of current study showed , it is possible to highlight the following conclusions:

- Most people with thalassemia are villages and rural habitats.
- Frequent Blood transfusions in beta-thalassemia patients leads to continuous immune stimulation which affected negatively on cellular and humoral immunity.
- Iron overload has a negative relationship on the humoral and cellular immunity as well as on liver enzymes in beta-thalassemia patients.
- The immune abnormality and their complication increased with age of patient with beta-thalassemia patients .
- serum concentration of immunoglobulins increased with increased of age of beta thalassemia.
- The concentration of serum complement system decreased with increased age of patient with betathalassemia.

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