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Study of Serum Level of IL10, CD4, CD8 and Acute Phase Reactants in Thalassemic Children with Effect of Splenectomy

MARWA M.A. KHATTAB, M.Sc.*; SHEBL S. SHEBL, M.D.*; IBRAHIM M. BADRAIA, M.D.*; MAALY M. MABROUK, M.D.** and YASMINE SH. SAID, M.D.*

The Departments of Pediatrics and Clinical Pathology**, Faculty of Medicine, Tanta University, Tanta, Egypt*

Abstract

Background: Thalassemia is one of the most common single gene disorders and widely distributed in the Mediterranean region and inherited as autosomal recessive disorders. Beta-thalassemia major has an increased risk for systemic infections, suggesting that a basic defect in the host defense is present.

Aim of Study: It was to study the level of (interleukin 10, CD4, CD8 subsets) in thalassemic patients and correlation with certain acute phase reactants and as well as effect of splenectomy.

Patients and Methods: The study was carried out on 40 children with thalassemia who attended to Hematology Unit, Pediatrics Department, Tanta University Hospital at the period from June 2016 to February 2017. Also it included 20 healthy children with matched age and sex served as a control group. All patients and controls were subjected to full history taking, clinical examination, routine laboratory investigations, CD4, CD8, CD4/CD8 ratio and interleukin 10 measurements.

Results: This study found that CD4 and CD8 are significantly higher in post splenectomised thalassemic patients compared with non splenectomised thalassemic patients and control group (p-value >0.05). While interleukin 10 was significantly lower in post splenectomised thalassemic patients compared with non splenectomised thalassemic patients and control group (p-value >0.05).

Conclusion: There are significant differences in immune responses among post-splenectomy and non-splenectomy thalassemic patients. There were significant higher level of CD4, CD8 and lower IL10 in the splenectomised thalassemic patients than non splenectomised thalassemic patients and controls. There were significant negative correlation between CD4/CD8 ratio and interleukin 10 (IL10). While there were no significant correlation between CD4, CD8 and interleukin 10 (IL10). There was statistically significant correlation between CD4, CD8 and serum ferritin.

Correspondence to: Dr. Marwa M.A. Khattab, The Department of Pediatrics, Faculty of Medicine, Tanta University, Tanta, Egypt

Key Words: *Thalassemia – Immune response – Splenectomy.*

Introduction

THALASSEMIA is recognized as the most prevalent hereditary disorder all over the world with a significant negative impact on public health and the society especially endemic areas [1]. The most prevalent hemoglobinopathy in Egypt is B-thalassemia major which is a hereditary genetic anemia of hemolytic type. It is considered in our region a problematic health issue [2].

Thalassemia is prevalent in Mediterranean countries, the Middle East, central Asia, India, Southern China and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10, 3%) and South East Asia [3].

Infections are frequent complication of thalassemia (12-13%) and hemoglobinopathies and they can be fatal. Beta-thalassemia major has an increased risk for systemic infections, suggesting that a basic defect in the host defense is present [4].

Furthermore, a research on cellular immunity on thalassemia patients showed increase amount and activity of suppressor cell CD8+, decrease ratio of CD4+/CD8+, decrease of T lymphocyte proliferation and increase T lymphocyte activation whereas humoral immunity research showed similar result between thalassemia and normal person [5].

Interleukin-10 (IL-10) is a key immunosuppressive cytokine that is produced by a wide range of leukocytes, as well as non -hematopoietic cells [6].

IL-10 influences three important functions of the monocytes/macrophages: The release of immune mediators, the antigen presentation, and the phagocytosis [7].

The exact molecular mechanisms of immunosuppressive effects of IL-10 on APCs (the inhibition of cytokine production and antigen presentation) and T cells (the suppression of cytokine production and proliferation) [8].

Aim:

It was to study the level of (interleukin 10, CD4, CD8 subsets) in thalassemic patients and correlation with certain acute phase reactants and as well as effect of splenectomy.

Patients and Methods

The patients had been randomly selected from Hematology Unit, Pediatric Department, Tanta University Hospital at the period from June 2016 to February 2017.

The study was carried out on the following groups:

Group (I): 20 cases of β-thalassemia major who were splenecomized.

Group (II): 20 cases of β-thalassemia major who were not splenecomized.

Group (III): 20 healthy children serving as a control group.

Exclusion criteria were: Other types of hemolytic anemia as sickle thalassemia, autoimmune hemolytic anemia, sickle cell anemia and G6PD.

Both patients and control groups were subjected to the following: Full history taking, clinical examination and laboratory investigations include: Complete blood count.

- Hb electrophoresis by HPLC.
- Serum ferritin by ELISA.
- ESR & C-RP.
- CD4, CD8 and CD4/CD8 ratio by flow cytometry.
- Serum level of IL10 by ELISA.

Statistical presentation and analysis of the present study was conducted using the mean, standard deviation, student *t*-test, Chi-square by SPSS V20 with $p<0.05$ means significance [9].

Results

This study was carried out at the Pediatric Department of Tanta University Hospital on three groups of children (20 post splenectomised thalassemic patients, 20 non splenectomised thalassemic patients, 20 healthy children who served as control group).

Table (1) presents the demographics data of the case control study population as regard age, sex, family history of similar condition and family history of positive consanguinity. It shows that there are significant difference between thalassemic patients and control group as regard age, family history of similar condition and positive consanguinity (p-value >0.001). While there are no significant difference between thalassemic patients and control group as regard sex (p-value=0.760).

Table (2) shows significant difference between the studied groups regarding serum ferritin (p-value >0.05). As serum ferritin increase in splenectomised thalassemic patients (group I) than non splenectomised thalassemic patients (group II) and control group.

Table (3) shows significant difference between the studied groups regarding C-reactive protein (p-value >0.05). As C-reactive protein increase in splenectomised thalassemic patients (group I) than non splenectomised thalassemic patients (group II) and control group.

Table (4) shows significant difference between the studied groups regarding ESR (p-value >0.05). As ESR increased in splenectomised thalassemic patients (group I) than non splenectomised thalassemic patients (group II) and control group.

Table (5) shows significant difference between the studied groups regarding CD4 and CD8 (p-value >0.05). As CD4 and CD8 increased in splenectomised thalassemic patients (group I) than non splenectomised thalassemic patients (group II) and control group.

Table (6) shows no significant difference between group (I) and group (II) regarding CD4: CD8 ratio (p-value >0.05). While there was significant difference between group (I) and controls regarding CD4: CD8 ratio (p-value >0.05).

Table (7) shows significant difference between the studied groups regarding interleukin 10 (IL 10) (p-value >0.05). As interleukin 10 (IL10) decreased in splenectomised thalassemic patients (group I) than non splenectomised thalassemic patients (group II) and control group.

Table (1): Demographic and laboratory data of the studied groups.

	Groups						ANOVA or Chi-Square	
	Group I		Group II		Controls			
	N	%	N	%	N	%	F or χ^2	*p*-value
Age (years):								
Range	5-17		2-10		3-16		18.879	<0.001 *
Mean ± SD	10.800±3.708		5.175± 1.764		10.050±3.576			
Median	11		5		11			
Sex:								
Male	13	65.00	11	55.00	11	55.00	0.549	0.760
Female	7	35.00	9	45.00	9	45.00		
FH of similar condition:								
Negative	7	35.00	7	35.00	20	100.00	22.941	<0.001 *
Positive	13	65.00	13	65.00	0	0.00		
FH of positive consanguinity:								
Negative	6	30.00	7	35.00	20	100.00	24.646	<0.001 *
Positive	14	70.00	13	65.00	0	0.00		

FH: Family History.

Table (2): Comparison between studied groups as regard Serum ferritin.

Groups	Serum ferritin ng/ml			ANOVA	
	Range	Mean ± SD	Median	F	*p*-value
Group I	1853-7581	4886.200±1400.293	4820	109.057	<0.001 *
Group II	850-5160	2309.050±1105.624	2130		
Gontrols	35-223	76.950±58.601	48		
	TUKEY'S Test				
	I & II	I & III	II & III		
	<0.001 *	<0.001 *	<0.001 *		

Table (3): Comparison between the studied groups as regard C-Reactive Protein (CRP).

CRP mg/l	Groups								Chi-Square	
	Group I		Group II		Controls		Total		χ^2	*p*-value
	N	%	N	%	N	%	N	%		
Negative	3	15.00	10	50.00	20	100.00	33	55.00	29.495	<0.001 *
Positive	17	85.00	10	50.00	0	0	27	45.00		
Total	20	100.00	20	100.00	20	100.00	60	100.00		

Table (4): Comparison between the studied groups as regard ESR.

	Groups			ANOVA		TUKEY'S Test		
	Croup I	Croup II	Controls	F	*p*-value	I & II	I & III	II & III
ESR 1st mm/h:								
Range	15-100	13-60	4-12	56.585	<0.001 *	<0.001 *	<0.001 *	<0.001 *
Mean ± SD	57.650±22.276	26.400± 13.248	7.650±2.183					
Median	60	22	8					
ESR 2nd mm/h:								
Range	20-125	22-92	11-23	52.152	<0.001 *	<0.001 *	<0.001 *	<0.001 *
Mean ± SD	79.650±28.150	42.550± 18.953	16.150±4.368					
Median	80	35	17.5					

Table (5): Comparison between the studied groups as regard CD4 (T lymphocyte) and CD8 (T lymphocyte).

	Groups			ANOVA		TUKEY'S Test		
	Croup I	Croup II	Controls	F	*p*-value	I & II	I & III	II & III
CD4 cell/mm³:								
Range	1150-7371	423-1898	314-978	36.677	<0.001 *	<0.001 *	<0.001 *	0.585
Mean ± SD	3617.519±2099.646	922.500±416.291	533.400± 181.980					
Median	3039.5	942	538					
CD8 cell/mm³:								
Range	760-5650	310-1915	232-820	33.338	<0.001 *	<0.001 *	<0.001 *	0.723
Mean ± SD	2560.950± 1511.176	658.500±372.562	438.650± 169.583					
Median	2064	630	455					

Table (6): Comparison between the studied groups as regards CD4: CD8 ratio.

Groups	CD4:CD8 Ratio		
	Range	Mean ± SD	Median
Group I	1-2.4	1.705±0.375	1.6
Group II	0.6-2.5	1.466±0.477	1.4
Gontrols	1.02-1.4	1.216±0.121	1.2

TUKEY'S Test		
I & II	I & III	II & III
0.096	<0.001*	0.077

Table (7): Comparison between the studied groups as regard interleukin 10 (IL10).

Groups	IL10 pg/ml			ANOVA	
	Range	Mean ± SD	Median	F	*p*-value
Group I	23-616	258.155± 197.527	295	5.090	0.009*
Group II	126-4740	1366.500 ± 1565.521	387		
Gontrols	13.8-4530	1195.400 ± 1306.415	923		

TUKEY'S Test		
I & II	I & III	II & III
0.0 12*	0.039*	0.891

Discussion

The most prevalent type of hereditary anemia in Egypt is B-thalassemia. The carrier incidence rate is more than 10% [10].

There are various causes of infection including blood transfusion, splenectomy, iron overload in the body, and aberration of function in immunity system. Infections are the first or second cause of death (after heart failure) in thalassemia and hepatic disease is the third most common cause of death [11].

In our study, we compared between thalassemic and control groups regarding age, sex, family history of positive consanguinity, and family history of similar condition, clinical examination, and laboratory investigations.

In our study, there was statistically significant difference between thalassemic patients and control group as regard family history of similar condition (p-value >0.001) and family history of positive consanguinity (p-value >0.001).

This data come in agreement with Rekha and Machado, [12] who demonstrated that; thalassemia is one of the most common single gene disorders and widely distributed in the mediterranean region and inherited as autosomal recessive disorders.

In our study, there was statistically significant difference between thalassemic patients and control group as regard serum ferritin which was significantly higher in thalassemic patients compared with control group (p-value >0.05).

This data come in agreement with Martin and Thompson, [13] who demonstrated that; serum ferritin levels in those with thalassemia major may be elevated, reflecting the presence of iron overload primary from repeated blood transfusion, but to lesser extent from increased absorption of dietary iron from the gastrointestinal tract. Also in another study, Eissa and El-Gamal, [14] reported that older patients with thalassemia (>12-year old) had lower BMI, higher ferritin levels compared to younger patients with thalassemia. This data are also in agreement with Hershko [15] and Ghone et al., [16] who demonstrated that iron overload in thalassemia patients is the main outcome of multiple blood transfusions which added about 100-200ml of pure RBCs/kg/year (equivalent to 108-216mg of Fe/kg/year) that increased iron stores to many times than the normal range unless regular chelation therapy was given. It also results from increased iron absorption as iron absorption in patients with thalassemia increases several folds than the normal daily intestinal iron absorption which is about 1-1.5mg/day. Our results are also in agreement with

Hagag et al., [17] who demonstrated that, significantly higher serum ferritin and iron levels and significantly lower total iron binding capacity were found in thalassemic patients compared with control group.

In our study, there was statistically significant difference between thalassemic patients and control group as regard CRP and ESR which were significantly higher in thalassemic patients especially post splenectomised compared with control group (p-value >0.05).

This data come in agreement with Ataei and Hashemipour, [18] who found that; thalassemia are classically associated with susceptibility to infections and patients with β-thalassemia major who regularly receive transfusions are at risk of developing Post Transfusion Hepatitis (PTH). Among these infections, hepatitis B and C are the most common so ESR and CRP were elevated in thalassemia. In another study, Sari and Gatot, [5] reported that; high level of C-reactive protein among beta thalassemia was more pathognomonic in case of splenectomized patients.

In the present study, there was statistically significant difference between thalassemic patients and control group as regard CD4 [T-lymphocyte] and CD8 [T-lymphocyte] which were significantly higher in post splenectomised thalassemic patients compared with non splenectomised thalassemic patients and control group (p-value >0.05).

In the current study, there was no statistically significant difference between thalassemic patients either splenectomised or not as regard CD4/CD8 ratio while there was statistically significant difference between post splenectomised thalassemic patients compared with control group (p-value <0.05).

This data come in agreement with Gharagozloo et al., [19] who showed that, CD4+ and CD8+ T lymphocyte were higher on post-splenectomy groups compared to non-splenectomised thalassemic patients. This is due to Increased T lymphocyte count on post-splenectomy which might be associated with antigen that could not be effectively filtered by spleen. This suggests that spleen could play some part in the regulation of lymphocyte counts and act as a reservoir for lymphocytes produced in the body. Aleem et al., [20] who showed that, iron and its binding proteins have immune modulating properties and the effects of the iron overload include alternations in T-lymphocyte subsets and modification of lymphocyte distribution in different compartments of immune system. Iron overload has been associated with increase of CD8 and decrease in CD4 cell counts but use of iron chelators as deferasirox induced reduction in the iron overload and may has direct effect on immune system that cause increased CD8 cells and higher CD4 cell counts resulting in mildly increased or normal CD4/CD8 ratio.

On the other hand, this data are not in agreement with Sari and Gatot, [5] who showed that patients with thalassemia major has increased amount and activity of suppressor cell CD8+ but decrease ratio of CD4+/CD8+, decrease of T lymphocyte proliferation and increase T lymphocyte activation.

Also, this data are not in agreement with Hagag et al., [17] who showed significantly lower CD3, CD4 and IgM and significantly higher CD8, IgG and IgA levels were found in thalassemic patients compared with controls. This is because repeated blood transfusions can lead to continuous alloantigenic stimulation with auto-immune hemolysis, T and B lymphocyte changes and modification of monocyte and macrophage functions.

In our study, there was statistically significant difference between thalassemic patients and control group as regarding interleukin 10 which was significantly lower in post splenectomised thalassemic patients compared with non splenectomised thalassemic patients and control group (p-value >0.05).

This data are in agreement with Caligiuri et al., [21] who showed that, IL10 production by splenectomised patients are less than non-splenectomised. This is because multi-transfusions could be responsible for a change in the subset of circulating lymphocytes that could contribute to a state of partial immune deficiency in B-thalassemia patients, which is more prominent among the splenectomised patient. Also Jison et al., [22] showed that, IL-10 is a cytokine with potent anti-inflammatory activity which reduces the production of various cytokines including IL-1, IL-6, IL-8, IL-12, TNF-a and GM-CSF to promote uptake and retention of iron in the reticulo endothelial system so patients with iron overload have decrease in interleukin 10 as there is negative correlation between interleukin 10 and serum ferritin which reinforce the existence of a clear inflammatory state in patients with iron overload as a result of excess iron.

The discrepancy between the results in different studies may be due to the difference in the number of sampled patients, frequency of blood transfusion, spelenctomy, serum iron status and iron chelation therapy which were 'proposed as the responsible

factors for alteration of immunoglobulins and T lymphocyte subset in patients with thalassemia'.

Conclusion:

There were significant higher level of CD4, CD8 and lower IL10 in the splenectomised thalassemic patients than non splenectomised thalassemic patients and controls.

Our patients with thalassemia major were susceptible to infection especially after splenectomy and with bad chelation.

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Conflicts of interest:

No conflicts of interest declared.

Authors' contributions:

All authors had equal role in design, work, statistical analysis and manuscript writing. All authors have approved the final article work.

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دراسة مستوى آنترليوكين ١٠ وسى دى ٤ وسى دى ٨ ومتفاعلات المرحلة الحادة فى الأطفال بآنيميا البحر الأبيض المتوسط مع تأثير إستئصال الطحال

المقدمة: الثلاسيميا هى مرض وراثى منتشر فى جميع أنحاء العالم له تأثير سلبى كبير على الصحة العامة والمجتمع خاصة المناطق الموجود بها وتعتبر الثلاسيميا الكبرى بيتا هى أكثر أنواع الأنيميا التكسيرية إنتشارا فى مصر وتمثل مشكلة صحية كبيرة فى مجتمعنا. الثلاسيميا هوالمرض السائد فى دول البحر الأبيض المتوسط والشرق الأوسط وآسيا الوسطى والهند وجنوب الصين والشرق الأوسط.

الهدف من البحث: الهدف من هذا العمل دراسة مستوى (أنترليوكين ١٠ وسى دى ٤ وسى دى ٨) فى الأطفال المصابين بأنيميا البحر الأبيض المتوسط وعلاقتها بمتفاعلات المرحلة الحادة وتأثير إستئصال الطحال.

المواضيع وطرق البحث: تم إجراء هذه الدراسة على ٤٠ من الأطفال الذين يعانون من أنيميا البحر الأبيض المتوسط الذين سوف يتم قبولهم فى وحدة أمراض الدم، وقسم الأطفال، فى مستشفيات جامعة طنطا و٢٠ من الأطفال الأصحاء كمجموعة ضابطة. تم عمل الآتى لكل مريض: أخذ التاريخ المرضى كاملا، الفحص الإكلينيكى، عمل إختبارات معملية تتضمن الآتى: مستوى سى دى ٤ وسى دى ٨ والنسبة بينهما، مستوى آنترليوكين ١٠ فى الدم.

النتائج: هناك علاقة إحصائية بين إستئصال الطحال ومستوى سى دى ٤ وسى دى ٨ حيث أن هناك زيادة فى مستوى سى دى ٤ وسى دى ٨ ونقص فى نسبة مصل آنترليوكين ١٠ فى المرضى المصابين بأنيميا البحر الأبيض المتوسط بعد إستئصال الطحال عن قبل إستئصاله.

الإستنتاج: هناك إختلافات كبيرة فى الإستجابة المناعية بين المرضى المصابين بأنيميا البحر الأبيض المتوسط قبل وبعد إستئصال الطحال حيث أن مرضى الثلاسيميا بعد إستئصال الطحال يظهروا قدرا كبيرا من القابلية للإصابة بالعدوى.