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Original article

Assessment of T lymphocyte subsets in children with beta thalassemia major with iron overload

Background: *Beta thalassemia is an inherited hemoglobin disorder resulting in chronic hemolytic anemia that requires lifelong transfusion therapy. Repeated blood transfusions and RBCs hemolysis are the major causes of secondary iron overload which in addition to immune abnormalities that occur in patients with β- thalassemia are predisposing factors to infection.* ***Objective:*** *To study T lymphocyte subsets in children with beta- thalassemia major and their correlation with iron overload.* ***Methods:*** *The present study was conducted on 40 children with beta thalassemia major followed up at Hematology unit, Pediatric Department, Tanta University including 24 males and 16 females with mean age of 9.22 + 3.9 and 20 healthy children of matched age and sex as a control group. CD3, CD4, CD8 counts and CD4/CD8 ratio were assessed in all children.* ***Results:*** *CD3, CD4 and CD4/CD8 were significantly lower but CD8 was significantly higher in patients than controls. The CD3 and CD4 counts correlated negatively and CD8 counts correlated positively with iron overload.* ***Conclusion:*** *Some abnormalities of lymphocyte subsets, including CD3, CD4, CD8 counts and CD4/CD8 ratio, were found in Egyptian children with beta thalassemia major with significant correlation with iron load.* ***Recommendations:*** *Regular follow up of thalassemic patients for detection of iron overload and its proper management to avoid its impact on cell mediated immunity.*

Keywords: Beta Thalassemia, T lymphocyte, CD3, CD4, CD8, Iron overload.

Adel A. Hagag, Mohamed A. Elgamasy, Enaam S. Abd Elbar*

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

Correspondence:
Adel A. Hagag, 16 Elemam Moslem street branched from Elhelw Street, Tanta, Gharbia, Egypt.
E-mail: adelhagag20 @yahoo.com

INTRODUCTION

Beta thalassemia is hereditary blood disorder which is due to a defect in beta globin gene with excess of free alpha globin chains which become abnormal components in maturing red blood cells (RBCs) leading to destruction of RBCs by the spleen with subsequent anemia[1].

Patients with beta-thalassemia major require regular blood transfusions to survive[2]. The primary long term complication of chronic RBCs transfusions is iron overload with resultant parenchymal organ damage[3]. Also there is increased incidence of infections as it reduces phagocytosis by neutrophils, reduces opsonization and increases bacterial activity. It also causes alterations in T-lymphocyte subsets, with modification of their distribution in different compartments of the immune system[4].

The aim of this work is to study T lymphocyte subsets in children with beta- thalassemia major and their correlation to iron overload.

METHODS

After research ethical committee approval and informed written parental consent from all participants in this research, this study was carried out on 40 children with beta thalassemia major who were attendants to Hematology unit, Pediatric Department, Tanta university hospital. They were 24 males and 16 females with their ages ranging from 2.6 – 15 years and mean age of 9.22 + 3.9. Complete description of the patients is presented in table 1and 2. This was in addition to a control group of 20 healthy children including 11males and 9 females with their ages ranging from 2.2 – 15 years and mean age of 8.38 + 4.48. The study was done in the period between December 2012 and May 2014.

Inclusion criteria

Children with beta thalassemia major with serum ferritin levels of more than 1000 ng/ml (significant iron overload for better assessment of the effect of iron overload on T cell subsets) and who were maintained on regular use of chelating agent during this study. (All patients were treated with

Deferasirox in dose of 20-30 mg/kg/day once daily preferably before meals[17], but in patients with persistently high serum ferritin levels above 3000 ng/ml, Deferasirox is combined with Desferrioxamine 20-40 mg/kg in 8- 12-hour subcutaneous infusion using infusion pump[17] or continuous intravenous infusion for 8-10 hours per day for 10 days per month[18]).

Exclusion criteria

- ♦ Thalassemic children with serum ferritin level less than 1000 ng/ml.
- ♦ Splenectomized thalassemic children.

All the children were subjected to the following:

- Complete history taking and thorough clinical examination
- Laboratory investigations:

Specimen collection and handling:

Five ml of venous blood were collected using sterile needles through gentle venipuncture after sterilization of puncture site by alcohol, and collected samples were divided into; one ml was delivered on 20 uL EDTA solution for complete blood counts including differential white blood cells count which was done on Leishman stained peripheral blood smear with evaluation using ERMA PCE-210 N cell –counter[7], one ml on 20 uL EDTA solution for assay of T cell populations including CD3, CD4, CD8 counts and CD4/CD8 ratio using Becton-Dickinson FAC Scan flowcytometer (BD FACS) (Becton-Dickinson Biosciences Immunocytometry Systems, San Jose, CA, USA). The FAC Scan™ system is an automated flow cytometer which analyzes cells as they pass through a focused laser beam one at a time in a moving fluid stream[8] and the rest of blood was put in a plain tube and serum was separated for estimation of other investigations including Hb electrophoresis[9], assessment of serum iron status including serum iron, serum total iron binding capacity (TIBC) and serum ferritin[10, 12].

Statistical Methods

Data were collected and analyzed using statistical package for social science (SPSS) version for windows (version 12). All Data were expressed as in terms of mean values ± SD. Comparisons of parameters among groups were made using the paired t test. Two-group comparisons were performed nonparametrically using the Mann-Whitney U test. All statistical tests were two tailed, and $P< 0.05$ was considered statistically significant.

RESULTS

Pallor, jaundice, splenomegaly and hepatomegaly were the most common presenting clinical manifestations in studied patients (Table 1) .There were no significant differences between patients and controls regarding age, sex but there were significantly lower weight and height and significantly higher incidence of infective episodes in patients group (Table 2).There were significantly lower mean Hb, MCV and MCH and significantly higher mean reticulocyte %, WBCs, platelets count and lymphocytes count and percentage in patients than controls (Table 3). Significantly higher serum ferritin and iron levels and significantly lower TIBC were found in patients compared to control groups (Table 5). There were significantly lower CD3 and CD4 and significantly higher CD8 levels in patients than controls (Table 5). There were significant negative correlations between serum ferritin and CD3 and CD4 (Figure 1 and 2) while there was a significant positive correlation between serum ferritin level and CD8 (Figure 3).

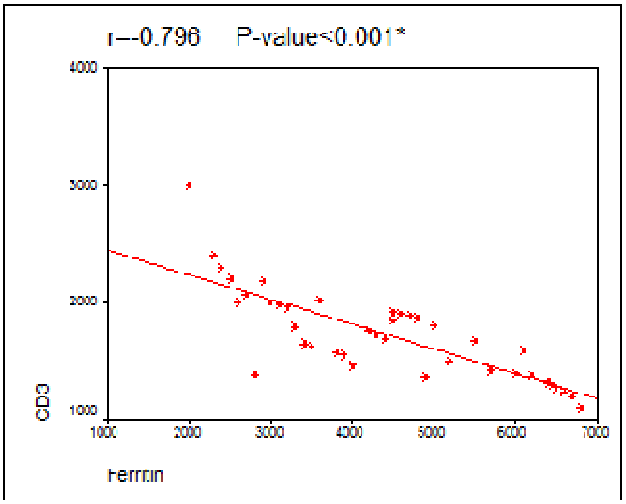

Figure (1): Correlation between CD3 expression and serum ferritin level in studied patients.

Table 1. Clinical data of the study group.

Parameters	Number of patients (%) (n=40)
Pallor	40 (100)
Jaundice	40 (100)
Hepatomegaly	30 (75)
Splenomegaly	40 (100)
Mongoloid facies	18 (40)
Frequency of blood transfusion	
Every 2 weeks	4 (10)
Every 3weeks	10 (20)
Every 4 weeks	26 (70)
Interval of transfusion	
Range (mean± SD)	2 - 8 (4.09 ± 2.652)
Age of diagnosis in months	
Range (mean± SD)	6 -72 (14.92 ± 13.093)
Age of first transfusion (months)	
Range (mean± SD)	7-72 (15±13.081)
Positive Consanguinity	22 (55)
Positive Family history of thalassemia	16 (40)

Table 2. Demographic data of patients versus controls.

	Patients (N=40)	**Control (N=20)**	**t test**	**P**
Age				
Range	2.6 –15 years	2.2 –15 years		
Mean	9.22 ± 3.9.	8.38 ± 4.48.	0.529	0.0601
Sex				
Male	24	11	0.28	>0.05
Female	16	9		
Anthropometric measures				
Weight in kg				
Range	23-44	30-50		
Mean± SD	32.45±6.66	38.55± 5.53	3.14	0.003*
Length in cm				
Range	128-150	140-165		
Mean± SD	148.5±7.72	154.05±8.43	2.17	0.002*
Infective episodes per year				
Mean ± SD	7.5 ± 2.1	3.4 ± 0.9	6.5	0.00*
Type of infections	**Number (%)**	**Number (%)**	**X^2**	**p**
Gastroenteritis				
1-3 attacks /year	12 (30)	14 (70)		
> 3 attacks /year	28 (70)	6 (30)	3.23	0.04*
Respiratory tract infections				
1-3 attacks/year	10 (25)	16 (80)		
> 3 attacks/year	30 (75)	4 (20)	4.11	0.03*
Hepatitis				
Yes	18 (45)	3 (15)		
No	22 (55)	17 (85)	6.11	0.042*

* Significant

Table 3. Complete blood picture in studied patients versus controls.

	Patients (N=40) Range (Mean ±SD)	**Control (N=20)** Range (Mean ±SD)	**t test**	**P. value**
RBCs (million/ mm^3)	2.9 - 4.1 (3.2 ± 0.65)	4.6 -5.6 (5.1± 0.5)	10.60	0.001*
Hb (gm/dl)	6.5-9 (7.2 ± 0.51)	11-13.1 (12.01± 0.91)	17.82	0.00*
MCV (fl)	61-73.2 (67.1 ± 6)	75.6 – 86 (80.8 ±5.2)	6.2	0.00*
MCH (pg)	23.1-27.3 (25.2±2.1)	26.8 - 30.6 (28.7±1.89)	3.4	<0.05*
Platelets (thousands/mm^3)	264.4 -699.6 (482 ±217.6)	280 -430.5 (335 ±74.5)	2.7	<0.05*
WBCs (thousands/mm^3)	5.8 - 75.6 (45.15 ± 29.54)	8 – 12 (10.22±1.88)	3.70	0.009*
Lymphocyte (%)	41.69- 57.71 (49.7 ± 8.01)	30.3- 39.9 (35.1 ± 4.8)	5.28	0.001*
Lymphocyte count (cells/mm^3)	1570-9000 (4115.75±1958.11)	1500-4350 (2859.5±871.96)	12.97	0.001*
Reticulocytes (%)	4.2 – 8 (6.12±1.8)	0.4 - 1.1 (0.84±0.22)	9.16	0.001*

* Significant

Table 4. Comparison between studied patients and control group regarding iron status.

Parameters	**Patients (N=40)** Range Mean ± SD	**Control (N=20)** Range Mean ± SD	**T test**	**p**
Ferritin (ng/ml)	1039-10467 3418.23±2950.7	35-43 39.48±2.48	4.324	0.00*
Iron (ug/dl)	145-311 222±56.61	50-130 90 ±31.87	6.762	0.00*
Iron binding capacity	170-231 198.38±19.9	274-350 315.7±24.85	-13.643	0.00*

* Significant

Table 5. Comparison of CD3, CD4, CD8 counts and CD4 /CD8 ratio between patients and control group.

Parameters	**Patients (N=40)** Range Mean ± SD	**Control (N=20)** Range Mean ± SD	**T test**	**p**
CD3	1100-3000 1733.25 ± 381.87	1150-3250 1887 ± 390.56	4.95	0.00*
CD4	400-1400 889.67±282.86	720-1700 1003±250.96	2.03	0.048*
CD8	190-1560 779.72±390.63	180-870 663±116.71	2.41	0.02*
CD4 /CD8 ratio	0.87±0.16	1.75±0.14	20.7	0.00*

* Significant

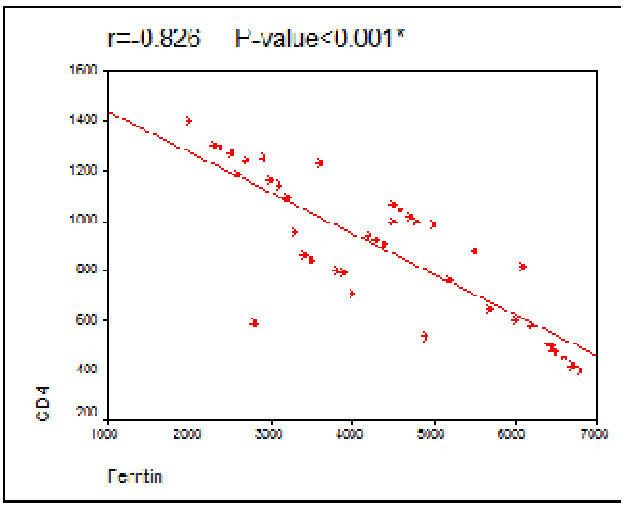

Figure (2): Correlation between CD4 expression and serum ferritin level in studied patients.

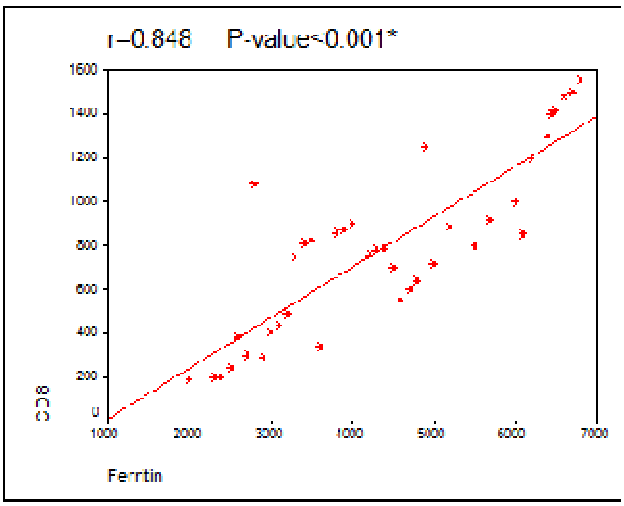

Figure (3): Correlation between CD8 expression and serum ferritin level in studied patients.

DISCUSSION

In the present study, there was significant higher level of serum ferritin, serum iron and lower total iron binding capacity in thalassemic patients compared to controls which was in agreement with Hershko[13] and Ghone et al[14] who demonstrated that iron overload is the main outcome of multiple blood transfusions and inappropriately increased iron absorption as with the recommended transfusion scheme for patients with thalassemia major, about 100-200 ml of pure RBCs per kg are transfused per year which is equivalent to 108-216 mg of Fe per kg body weight per year. Thus with regular blood transfusion, iron stores increase to many times the normal unless regular chelation therapy is given[15]. Also, iron absorption increases several folds in patients with thalassemia due to ineffective erythropoiesis[16].

The present study revealed significant increase in the frequency of acute infectious episodes in β-thalassemic patients with significant iron overload compared with controls. This was in agreement with Ahluwalia et al[17] who studied some immune functions in thalassemic children including serum IgG and IgA, Nitroblue tetrazolium dye reduction by stimulated polymorphonuclear leukocytes; Phytohaemagglutinin induced mitogen proliferation, absolute lymphocyte count and CD4/CD8 ratio and concluded that infection is a common complication associated with significant morbidity and mortality in β-thalassemia and this has been thought to be partly due to immunological abnormalities which include increased immunoglobulin production, deficient activity of the complement system, decreased opsonization and phagocytosis and abnormalities in the cell-mediated immune response[17].

In this work there was significantly lower CD3 and CD4 and significantly higher CD8 in thalassemic patients with significant iron overload compared with controls. This was in accordance with Vento et al[18] who found lower $CD4^+$ and higher CD8+ and Gharagozloo et al[19] who found significantly higher $CD8^+$ T lymphocyte in thalassemia patients compared with controls which can explain the higher frequency of acute infectious

episodes in thalassemic non splenectomized patients. The alteration of T-lymphocyte subsets; result in an abnormal immune response in β-thalassemia patients especially in case of iron overload that generates oxygen-free radicals and causes peroxidative tissue injury leading to accelerated ageing of immune system with subsequent gradual decline in responsiveness to antigens and abnormal T cell function[19, 20]. The increase in CD8 T cells and the decreased CD4/ CD8 ratio may be also related to blood transfusion as repeated blood transfusions can lead to continuous alloantigenic stimulation, with autoimmune hemolysis, T and B lymphocyte changes, and modification of monocyte and macrophage functions[21, 22].

Kadam et al[23] found that iron overload in thalassemic children led to decline in CD4 and CD8 levels, Noulsri et al[20] and Al Awadhi, et al[24] reported insignificant differences in T-cell subsets CD3, CD4 and CD8 between patients and controls and they concluded that high iron levels in thalassemic patients have a more significant effect on the function and activity of T cells rather than cell number and percentage. Ahmadiafshar[25] studied immunologic markers including CD8, CD4, CD19, and CD56 in thirty patients with β-thalassemia major under 18 years. They did not found any abnormality in cellular and humoral systems. However, mean CD56 level in thalassemia group were significantly lower than control group and mean CD4 in thalassemia patients with spelenectomy was significantly lower than patients without splenectomy.

The variation between the results of this study and the previous studies may be explained by clinical heterogeneity among thalassemia patients, frequency of blood transfusion, splenectomy, body iron status, and iron chelation, which had been proposed as the factors responsible for alteration of lymphocyte subset in thalassemia. It is for this reason that we confined our study to a more or less homogeneous group of thalassemic patients.

The negative impact of iron overload on T cell subset is further supported in this study by the finding of significant negative correlations between serum ferritin and CD3 and CD4, and significant positive correlation with CD8. This is in agreement with Gharagozloo et al[19] who reached the same results and attributed this to iron overload which generates oxygen-free radicals and causes peroxidative tissue injury. Oxidative injury is a major factor of accelerated ageing of immune system which results in a gradual decline in responsiveness to antigens, abnormal T cell function with block in cell division, shortening of telomere length and decrease in costimulatory receptors which has pivotal role in providing the stimulation required for a full proliferative T cell response[19].

CONCLUSION

Some abnormalities of lymphocyte subsets, including CD3, CD4, CD8 counts and CD4/CD8 ratio, were found in Egyptian children with beta thalassemia major with significant correlation with iron load.

RECOMMENDATIONS

We recommend regular follow up of thalassemic patients for detection of iron overload to avoid its impact on T lymphocytes.

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