

# A SURVEY OF CELLULAR IMMUNITY, TOTAL T-LYMPHOCYTE, T-ACTIVE, B-LYMPHOCYTE AND T-CELL FUNCTION IN RELATION TO PHYTOHEMAGGLUTININ (PHA) IN THALASSEMIC PATIENTS

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## ABSTRACT

The aim of this study was to evaluate the immune system and lymphocyte function in 41 Iranian  $\beta$ -thalassemic patients and 50 controls, ages ranging from 2 to 18 years. The patients consisted of 20 splenectomized and 21 non-splenectomized patients. They were treated with Desferal, and had received repeated blood transfusion. Laboratory investigations included measurement of total T lymphocytes, active T lymphocytes, B-lymphocytes and function of lymphocytes treated with PHA. In this study we observed a significant reduction of active T lymphocytes and total T lymphocytes in the patient group compared to controls ( $p < 0.005$  &  $p < 0.001$ ), but there was no significant difference between splenectomized and non-splenectomized patients. Also in both groups, lymphocyte function was reduced against PHA (phytohemagglutinin) compared with the controls, and the numbers of B cells were increased. These results lead to the conclusion that the deficient immune system in  $\beta$ -thalassemia causes infectious diseases, which finally leads to death. Therefore, stimulation of the immune system as well as clinical treatment may prevent infectious disease in patients with  $\beta$ -thalassemia.

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**Keywords:** Thalassemic patient, Immune system, Cytomegalovirus, Blood transfusion.

## INTRODUCTION

Thalassemia syndromes are a heterogeneous group of inherited anemia characterized by defects in the synthesis of one or more of the globin chains which lead to imbalanced globin-chain synthesis, defective hemoglobin, and damage to the red cells or their precursors.

There are two main classes of thalassemia. Beta and Alpha thalassemia.<sup>1</sup>

Beta thalassemia is more important and consists of a

diverse group of disorders of hemoglobin synthesis, all of which result from a reduced output of adult hemoglobin.<sup>2</sup>

Repeated blood transfusion is vital for these patients to have a normal life, but causes increased serum iron levels.

These patients are very sensitive to infections. Khalife et al.<sup>3</sup> studied immunoglobulin levels, opsonizing activity and phagocytic power in patients with thalassemia in order to clarify the causes of sensitivity of their immune system. They reported increased immunoglobulins (IgG, IgM, IgA). The opsonin and phagocytic powers were

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found to be impaired. They also studied the percentage of T and B-lymphocytes and their subgroups in thalassemic patients and reported a decrease in T lymphocytes in major thalassemia compared to normal. Also a decrease in the ratio of T helper/T suppressor was shown.<sup>4,5</sup>

$\beta$ -thalassemia is the most common chronic hemolytic anemia in Iran, and it is associated with frequent infections.<sup>6,7</sup>

In the present study, the immune system and lymphocyte function in 2 groups of thalassemic patients was investigated. One group was splenectomized and the other group was non-splenectomized. The number of T lymphocytes, B-lymphocytes, activated T lymphocytes and lymphocyte activity were measured.

## PATIENTS AND METHODS

Subjects of the present study consisted of 91 persons, composed of 41 patients (26 males and 15 females) and 50 controls. They were classified into 2 groups. 20 patients had previously undergone splenectomy and the other 21 patients had an intact spleen. They had been treated with Desferal, and had repeated blood transfusions.

Lymphocytes were separated from heparinized pe-

ripheral blood by Ficoll-Hypaque density gradient centrifugation method (Boyum).<sup>8</sup> T and B-lymphocytes were separated into sheep erythrocyte rosette forming cells (T lymphocytes) and human cells coated with complement - antibody rosette (B lymphocytes), according to the method of Jondal, with some modifications.<sup>9</sup> Briefly an equal volume of lymphocytes ( $5 \times 10^6$ /mL) and SRBC (2%) were mixed and incubated at 4°C, for 16-20 hours. The percentage of lymphocytes binding with two or more SRBC were determined as T cells.

For T active cells, equal volumes of lymphocytes and SRBC were mixed with 1% fetal calf serum (FCS) and incubated at 37°C for 1 hour, and after centrifugation, the percentage of T active cells was determined.

## RESULTS

Results of the present study are shown in Tables I and II.

As shown in Table I, the percentage of active T lymphocytes showed a significant decrease in 90% of the two groups of patients compared with normal, while total T lymphocytes were decreased in 50% of patients ( $p < 0.005$ ). But there was no significant difference between splenectomized and non-splenectomized patients.

**Table I:** Percentage and function of T lymphocytes in thalassemic patients and controls.

Lymphocytes		Controls	Splenectomized	Nonsplenectomized
Total lymphocytes	Mean $\pm$ SD	61.5 $\pm$ 4	44.27 $\pm$ 17.23	44.5 $\pm$ 13.52
	% Decrease	-	50 (a)	50 (a)
	P value	-	$p < 0.005$	$p < 0.05$
Active T lymphocytes	Mean $\pm$ SD	42.1 $\pm$ SD	21.4 $\pm$ 24.04	25 $\pm$ 12.04
	% Decrease	-	90 (a)	90 (a)
	P Values	-	$p < 0.001$	$p < 0.001$
Lymphocyte transformation	RPI	-	0.89 (b)	0.71 (b)
	% Decrease	-	40 (a)	50 (a)

a = % of patients with abnormal immune response

b =RPI = Reactive proliferation index

RPI = Patient stimulation / Normal stimulation

**Table II:** Percentage of B-lymphocytes and ferritin in patients and controls.

		Controls	Splenectomized	Nonsplenectomized
B lymphocytes	Mean $\pm$ SD	19 $\pm$ 3	29 $\pm$ 9.22	26.2 $\pm$ 7.13
	%Increase	-	50	50
	P value	-	0.01 $<$ $p < 0.25$	0.01 $<$ $p < 0.25$
Ferritin (mg/mL)		200-400	7692	5771

Lymphocyte *in-vitro* reactivity to PHA was decreased in patients compared with the normal group ( $0.01 < p < 0.035$ ). The percentage of B-lymphocytes and level of ferritin in two groups of patients and controls are shown in Table II.

Percentages of B lymphocytes were increased in both groups of patients compared with normal ( $0.01 < p < 0.0025$ ) and the level of ferritin was increased in both groups but there was no difference between them.

## DISCUSSION

Human lymphocytes that bind sheep red blood cells to form rosettes are T- lymphocytes. E1-RFC\*, immediate or active rosetting, are those which form rosettes after a brief period of incubation of lymphocytes and sheep erythrocytes.

E2-RFC indicates the total number of rosette forming lymphocytes. The percentage of active E rosettes (E1-RFC) appears to correlate better with *in-vivo* cellular immunity than the total number of E rosettes. These rosettes may represent a subpopulation of T cells that function primarily as effector lymphocytes in cellular immunity.<sup>6</sup>

Researchers have reported that cell mediated immunity (CMI) is depressed in  $\beta$ -thalassemic patients. But in patients with large spleens, decrease of T lymphocytes is more significant, therefore the ratio of T helper to T suppressor is decreased which is similar to patients with AIDS. T helper cells are increased in splenectomized patients, therefore T helpers through releasing Interleukin I will increase immunoglobulin levels in thalassemic patients.<sup>10</sup>

Other reports (1983) showed that IgG, IgM and IgA levels increase especially after splenectomy but serum opsonin activity and killing activity of polymorphonuclears decrease.<sup>5</sup>

In this study, we observed that total T lymphocytes and active T cells are decreased which is compatible with other studies on  $\beta$  thalassemia.<sup>6</sup> It is worth noting that splenectomy did not have any effect on cellular immunity in these patients.<sup>2</sup>

Also, lymphocyte function to PHA in thalassemic patients is decreased which represents a defect in cellular immunity either in T lymphocyte number or their function. This cellular defect in thalassemic patients and also in other patients, who usually have frequent blood transfusions, is probably due to a CMV infection. Gernienis et al. (1989) found that patients with major thalassemia, especially those who have undergone splenectomy, will have a higher prevalence of CMV infections compared to normal.<sup>11</sup> In another study, which was performed on

blood donors from 100 blood banks, it was shown that 5% of these persons have antibody against CMV and repeated blood transfusion may intensify CMV infection in recipients. Thalassemic patients are probably infected with this virus, which could be responsible for CMI deficiency.

This virus also releases a kind of inhibitor factor, which prevents Interleukin 1 secretion in monocytes, and patients infected with this virus are more sensitive to other non-pathogenic microorganisms.<sup>12</sup>

In this study, we observed that B-lymphocytes were increased; this may be explained by CMV infection, because it is reported that CMV is a polyclonal activator for B-lymphocytes, so the number of these cells and immunoglobulin levels are increased.<sup>13</sup>

Increase of immunoglobulins and B lymphocytes in thalassemic patients may occur as a result of CMV infection. Therefore, it can be concluded that in thalassemic patients, cellular immunity is defective which causes increased sensitivity to many infections in these patients, especially CMV infection, which is transmitted by blood transfusion.<sup>2</sup>

To prevent these disorders in thalassemic patients who repeatedly receive blood, we offer the following suggestions:

1- Transfusion must be given with blood which is negative for CMV antibody, especially for young patients who are negative for CMV.

2-In patients who probably need bone marrow transplantation, we must use blood, which is negative for CMV antibody, because the danger of this infection in these patients is very high and may cause graft-versus-host reaction.<sup>14</sup>

3- We must try to use leukocyte-poor blood or use washed red blood cells for these patients.<sup>2</sup>

4- Thalassemic patients who are positive for anti-HIV, must be given CMV-antibody negative blood.

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\*Erythrocyte-rosette forming cell

## CMI in Thalassemia

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