Original Article

Estimation of CD4 and CD8 T Lymphocyte Cell Count in β- Thalassemia Major Patients

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Abstract

Background: Thalassemia is a group of inherited hemoglobin illnesses characterized by insufficient production of at least one of the globin chains, resulting in irregular globin-chain output. Damaged hemoglobin finally causes anemia. higher incidence of severe infections, indicating the presence of a basic immune system deficiency that may be connected to iron excess, immunological deficiency, splenectomy, and recurrent blood transfusions all cause long-term immune activation. **Objectives:** Estimating the CD4 and CD8 level count in β- thalassemia major patients. **Material and Methods:** The Babylon Province (hereditary blood disease center) conducted a case-control study from January 2023 to April 2023. The study involved 90 patients with thalassemia major and 75 healthy controls. Flow Cytometry was used to detect the CD4 and CD8 T lymphocyte cell count in the blood. **Result**: It was found that the CD4 T cell count in beta-thalassemia major patients was 29.51±4.39 compared to their control group's 36.53±3.76, which showed very significant differences in this study between patients and healthy group (P<0.0001). While the number of CD8 T cells seen in primary patients with beta-thalassemia was 23.15±3.34 compared to the control 32.91±5.36, which also showed a very high significant difference in this study between patients and the healthy group (P<0.0001). **Conclusion**: In patients with β-thalassemia major, a decrease in the count of the CD4 and CD8 compared with the healthy control group due to immune dysfunction in β-thalassemia major patients is caused by a defect in the T-lymphocyte function rather than by the decrease of absolute T-cell count.

Keyword: β-thalassemia major, CD4, CD8, Flow Cytometry.

Introduction

Thalassemia is inherited disease have multiple genetic forms including alpha and beta thalassemia [1]. Thalassemia is the most common monogenic condition worldwide. Patients with thalassemia who develop severe anemia in their most extreme cases need frequent blood transfusions (beta+ TM) [2]. A surplus of unbound alpha globin chains precipitates in erythroid precursors in the bone marrow, resulting in their early death and ineffective erythropoiesis. Beta globin chains are either decreased (beta+) or nonexistent (beta0). The degree of globin chain reduction is determined by the type of mutation at the beta globin gene on chromosome 11. Peripheral erythrocytes suffer membrane damage due to insoluble alpha globin chains. This leads to peripheral hemolysis, which is a contributing factor to anemia but is less apparent in thalassemia major than in thalassemia intermediate.

Anemia increases the synthesis of erythropoietin, which causes the bone marrow to grow up to 25–30 times more than usual. This strong but ineffective enlargement results in the distinctive bone deformities. Enhanced erythropoietic drive is the cause of protracted, severe anemia, extramedullary erythropoiesis, and hepatosplenomegaly [3] between 50,000 and 100,000 children are thought to lose their lives to β-thalassemia each year, with underdeveloped countries accounting for 80% of these deaths. In addition, there are estimated 300,000–500,000 newborns with severe hemoglobin anomalies per year [4]. Patients with thalassemia disease have been found to have a broad range of immunological abnormalities [5]. A weakened innate immune system is one factor that makes the recovery from infection less successful. These deviations in both quantity and functionality affect several elements that make up the immunological reaction. This patient group has been shown to have an altered innate immune cytokine profile as well as a low-grade systemic inflammatory condition, which is indicated by elevated total leukocyte, neutrophil, and lymphocyte counts [6]. A major contributing factor to these patients' susceptibility to infections is their aberrant neutrophil effector function. Furthermore, changes in T lymphocyte subsets in these patients included elevated numbers and activities of suppressor T cells (CD8), decreased helper T cell (CD4) count and activity, which lower CD4/CD8 ratios, T cell proliferation potential, and natural killer (NK) cell activity [7]. Moreover, there are more B lymphocytes and they are more activated, although differentiation is hampered [7]. Reduced immunoglobulin secretion is indicated by elevated levels of IgG, IgM, and IgA immunoglobulins. Inadequate phagocytosis and chemotaxis have also been reported in individuals with thalassemia. Lastly, studies have also documented decreased opsonization, granulocyte phagocytosis, and inhibited complement system activity, which is reflected in lower levels of C3 and C4 [8, 9]. This study aims to estimate the CD4 and CD8 level counts in β- thalassemia major patients.

Materials and Methods

Patients and control

From January 2023 to April 2023, the Maternity and Children's Hospital in Hilla City, Babylon Province, reported 165 cases of major thalassemia. Private labs in Babylon, Iraq, conducted the experiments. We split the study's subjects into two groups using basic randomization: a healthy control group (n = 75) and thalassemia patients as the case group (n = 90). The exclusion criteria included having hepatitis B and C and HIV positive, being pregnant, and receiving chelation therapy other than desferrioxamine within the previous three weeks. Patients' details, including their age, gender, record number, time of disease diagnosis, total number of transfusions, intervals between transfusions, volume of blood transfused in each referral, ferritin and hemoglobin levels, splenecotmy, and desferrioxamine treatment.

Blood sample collection and processing

We collected blood samples from each patient and the control group. We collected 5 ml of venous blood from each subject and divided it into 1.0 ml EDTA tubes and 4.0 ml gel tubes. We left the gel tubes for a short time to allow the blood to clot and then centrifuged them at 4000 rpm for 10 minutes to obtain clear serum samples. We placed the separated serum in five tubes, sealed them, and stored them at -20 °C until analysis. Before use, we thawed the frozen

serum samples at 4–8 °C and gently shook them at room temperature.

Monoclonal Antibody Reagents

The monoclonal antibodies used in this study were directly conjugated with phycoerythrin (PE) and allophycocyanin (APC). The monoclonal antibodies utilized included CD4 PE and CD8 APC.

Flow Cytometry

Flow cytometric analysis was performed on a flow cytometer (BriCyte E6). 2×104 - 5×104 cells were collected, and lymphocytes were gated by FSC versus SSC. Data were analyzed using flowmax 2.4 software and displayed as dot plots of CD4 or CD8 versus other phenotyping markers. The expression of each individual marker was analyzed versus CD4 or CD8. The absolute number was calculated by flow cytometric data and the cell counts. Monoclonal Antibodies The following monoclonal antibodies were purchased from Ex bio / china and used in the study: Anti-Hu CD4 PE and Anti-Hu CD8 APC.

Statistical analysis

IBM SPSS v26.0 software analyzed the collected data. Data were presented as mean±SD (standard deviation); P values < 0.05 were regarded as statistically significant; and data were given as mean±SD (standard deviation). Since the current study's results were statistically normal, we compared the mean scores of the experimental groups to see if there were any significant differences. To do this, we employed an independent sample t-test.

Ethical Approval

The College of Medicine at Al-Qadisiyah University's ethical committee carried out the ethical approval for this study, obtaining verbal consent from each patient and control. A local ethics committee reviewed and approved the subject information and consent form, citing document number 30/1237, dated January 10, 2023, as the reason for this approval.

Results

CD4 T lymphocyte cell count result

The comparison of CD4 T cell count between β-thalassemia patients and healthy controls groups has been carried out and the results were demonstrated in table (1). show that the CD4 T cell count in of the β-thalassemia major patients was Mean ± S.D 29.51 ± 4.39 comparison with their controls Mean ± S.D 36.53 ± 3.76. showed significant study difference between patients and healthy group (P= 0.0001).

Table 1: Distribution of CD4 among study population; β-thalassemia major patients and healthy control groups .

Parameter	Patients	Control	T test	P value
	Mean±S.D			
CD4	29.51± 4.39	36.53± 3.76	8.57	0.0001*
*(P<0.05), SD: standard deviation.				

The comparison of CD8 T cell count between β-thalassemia patients and healthy controls groups has been carried out and the results were demonstrated in table (2) and figure (2) . show that the CD8 T cell count in of the β-thalassemia major patients was Mean ± S.D 23.15 ± 3.34 comparison with their controls Mean ± S.D 32.91 ± 5.36. showed significant study difference between patients and healthy group (P< 0.0001).

Table 2: Distribution of CD8 among study population; β-thalassemia major patients and healthy control groups .

Parameter	Patients	Control	T test	P value
	Mean±S.D			
CD8	23.15 ± 3.34	32.91 ± 5.36	10.83	<0.0001*
*(P<0.05), SD: standard deviation.				

Results of Flow Cytometry Analysis of CD4 and CD8 T lymphocyte Immune Markers.

Flow cytometry results expressed as mean concentration of CD4 and CD8 immune markers, as in figures (3) and (4).

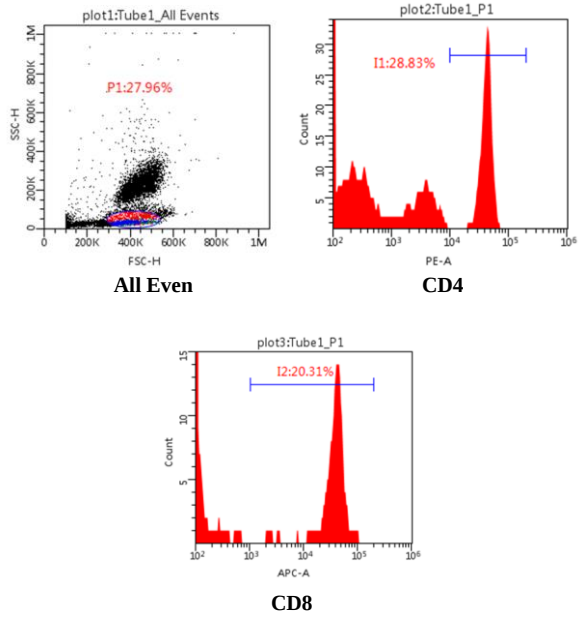

Figure 3: Results of Flow cytometry Analysis for CD4 and CD8 in patients.

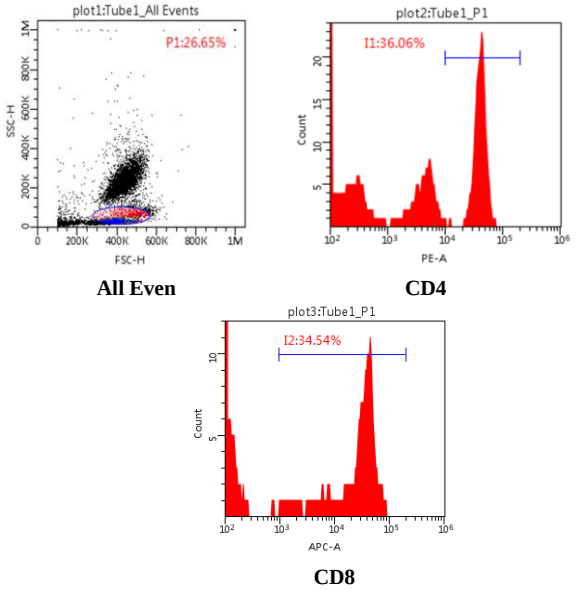

Figure 4: Results of Flow cytometry Analysis for CD4 and CD8 detection in healthy control.

Discussion

Patients with β-thalassemia major have previously been shown to have immunologic abnormalities, such as non-specific immune response, altered synthesis of cytokines, and lymphocyte subsets [10] . While the precise etiology of these disorders is unknown, iron excess, frequent blood transfusions, allogeneic stimulation, and iron chelation therapy are among the possible causes [11, 12].

T-cells can be broadly classified into three primary groups: proinflammatory and anti-inflammatory populations. With the ability to cause cytotoxicity, proinflammatory CD8 T-cells are involved in the immune system's reaction to viral infections, malignancies, and metastatic cells [13]. CD4 T helper (Th) cells govern innate immunity, promote the production of antibodies, and activate immunologic memory in addition to regulating the inflammatory environment. The third group is made up of anti-inflammatory CD4+ cells called regulatory T-cells (Tregs), which reduce inflammatory responses, promote immunological tolerance, and control immune responses to prevent autoimmunity [14]. Several studies have revealed that the primary cause of immunological insufficiency in β-thalassemia is iron excess [10]. Immune system abnormalities associated with conditions involving elevated iron load include reduced phagocytosis by the monocyte-macrophage system, altered T-lymphocyte subsets (represented by the upregulation of CD8 and the downregulation of CD4), impaired immunoglobulin secretion, and impaired complement system function (hemochromatosis, thalassemia) [10]

In the Rakhmanova et al. study, 200 individuals between the ages of 1 and 27 had their ferritin levels, IL-6 and IFN-β levels, and immunological marker levels of CD4, CD8, CD3, and CD16 measured. The number of

patients who fell into the adult age category was not disclosed. Although the CD3+ cell ratio was found to be high in this study (39.5%), immunological markers and lymphocyte subsets were not compared with ferritin levels or splenectomy, in contrast to our findings. Nonetheless, our study's finding that CD3 HLADR+ T cells were elevated, particularly in splenectomy patients, might be seen as a commonality between the two investigations [15].

According to the research by Ehsanipour et al., iron overload is a side effect of both the illness and treatment and is thought to play a significant role in immunological insufficiency in TM. Since iron and its protein components are known to be directly involved in immune regulatory processes, an excess of iron may have a negative impact on the balance of the immune system [16].

T lymphocyte cytotoxicity maintaining the CD8+ response and preventing exhaustion requires CD4+ T cells. CD8+ T cells are the primary immune system component that eliminates infections and cancerous cells [17,18]. CD8+ T lymphocytes are in contact with Major Histocompatibility Complex Class-1 (MHC-1) molecules on the surface of antigen-presenting cells (APCs) and target cells that exhibit antigenic peptide fragments produced by proteasomal degradation of cytoplasmic proteins linked to the proper binding grooves [19]. CD8+ cells adhere to the surface and crawl over it after interacting with an APC or a target cell in an effort to locate MHC-antigen-peptide complexes. To convert mechanical energy into biomechanical signals that activate the CD8+ T-cell receptor (TCR) complex, direct contact and cell movement are necessary [20].

Conclusion

In patients with β-thalassemia major, an decrease the count of the CD4 and CD8 compere with the healthy control group due immune dysfunction in β-thalassemia major patients is caused by defect in the T-lymphocyte function rather than by the decrease of absolute T-cell count.

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