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Estimation of CD8 Parameter Level in Thalassemia Patients

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Abstract

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 production. Damaged hemoglobin finally causes anemia.
To estimation the levels of CD8 in thalassemia patients. The present study was carried out in the Medical City and Al-Karama teaching hospitals in Baghdad and Al-Kut hospital for maternity and children in Wasit province, Iraq. Including 120 patients suffering from thalassemia from both genders, aged 20–49 years during the period from the beginning of January 2022 to the end of April 2022. It was the measurement of serum CD8 by the ELISA technique. The mean value of CD8 level was statistically significantly different in the studied groups (P<0.001).
The comparison revealed that the mean value of CD8 in patients frequently dialyzed without filter was statistically significantly higher than in both groups of patients dialyzed frequently with filter and dialyzed unfrequently, while when comparing the frequently dialyzed with filter group to the unfrequently dialyzed group, there is no significant difference.

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Keywords: CD8; Intermediate thalassemia; Thalassemia major; B thalassemia; Blood transfusion.

1. Introduction

Thalassemias are classified as either alpha and beta kinds depending on the globin chain that is damaged. Although thalassemia can be found everywhere, it is more common in the Mediterranean region [1]. Transfusion regimen, the quality of life and life expectancy of transfusion-dependent thalassemias has improved due to innovations and iron chelation therapy. Nonetheless, this has resulted in the discovery of various novel problems, such as immunological changes that cause higher infections. Several studies have found that infections are the second leading cause of death in thalassemia, following heart failure [2].Several studies have found cytokine disruption in thalassemia major [3]. Meanwhile, some immune system aging characteristics have been observed in these patients. In certain investigations, the mean value of IFN was found to be considerably greater in thalassemia compared to controls, but lower in others [4]. Despite the expensive and painful medical treatments for patients, complications like endocrine concerns, chronic liver diseases, growth disorders, osteoporosis, etc. continue to pose a threat to various aspects of the patients' and their families' lives and may negatively affect their physical and mental health and quality of life [5]. The most frequent monogenic disorder in the world is thalassemia. In the most severe cases, thalassemia patients experience severe anaemia, requiring regular blood transfusions (major-TM) [6]. Beta globin chains are either reduced (beta+) or absent (beta0), which causes an excess of unbound alpha globin chains to precipitate in erythroid precursors in the bone marrow, causing their early mortality and, as a result, inefficient erythropoiesis. The kind of the mutation at the beta globin gene on chromosome 11 determines the level of globin chain reduction. Insoluble alpha globin chains cause the peripheral erythrocytes to sustain membrane damage, which results in peripheral hemolysis, which contributes to anemia but is less noticeable in thalassemia major than in thalassemia intermediate. Anemia boosts erythropoietin production, which results in an intense but inefficient enlargement of the bone marrow (up to 25 to 30 times normal), which leads to the characteristic bone abnormalities. Hepatosplenomegaly, extramedullary erythropoiesis, and prolonged, severe anemia are all caused by enhanced erythropoietic drive [7]. Major thalassemia is more prevalent in Southeast Asia, the Middle East, and Mediterranean nations. The most prevalent hereditary monogenic disorder and ongoing worldwide health issue is thalassemia, particularly in underdeveloped nations. According to data from the World Bank, 7% of people worldwide are thalassemia carriers [9]. An estimated 50,000–100,000 children die from -thalassemia every year, and 80 percent of those deaths occur in developing nations. Additionally, an estimated 300,000–500,000 babies are born each year with severe hemoglobin abnormalities [8]. Hemoglobin electrophoresis or high-performance liquid chromatography can be used to confirm the diagnosis if -thalassemia is suspected based on the physical examination, personal and family history, and red-cell indexes (low mean corpuscular volume, low mean corpuscular hemoglobin level, and normal red-cell distribution width). It may be necessary to conduct DNA analysis to confirm the HbE diagnosis and determine the precise genotype of -thalassemia [9]. An estimated 50,000–100,000 children die from -thalassemia every year, and 80 percent of those deaths occur in developing nations. Additionally, an estimated 300,000–500,000 babies are born each year with severe hemoglobin abnormalities [8]. Hemoglobin electrophoresis or high-performance liquid chromatography can be used to confirm the diagnosis if -thalassemia is suspected based on the physical examination, personal and family history, and red-cell indexes (low mean corpuscular volume, low mean corpuscular hemoglobin level, and normal red-cell distribution width).

Nomenclature			
CD8	Cluster of differentiation 8	ELISA	Enzyme-linked immunosorbent assay
CD3	Cluster of differentiation 3	SPSS	Statistical Packages for Social Sciences
CD4	Cluster of differentiation 4	IFNγ	Interferon gamma
TM	Thalassemia major	APC	Antigen presenting cell
HLA	Human leucocyte antigen	LCMV	Lymphatic choriomeningitis virus
TCR	T-cell receptor	HSC	Hematopoietic stem cell

It may be necessary to conduct DNA analysis to confirm the HbE diagnosis and determine the precise genotype of -thalassemia [9]. Since the clinical symptoms and transfusion reliance first develop in infancy, newborn screening to detect thalassemia syndromes early is not very helpful, especially in high incidence areas with comprehensive prevention programs. Some thalassemia homozygote cases and hemoglobin variations can be detected using the same laboratory procedures where neonatal screening for sickle cell disease is established (primarily high-pressure liquid chromatography) [10], and even isoelectric focusing, as well as capillary electrophoresis). However, the numbers are unknown in many nations because patients are not identified and/or there is no national patient register. Neonatal screening (when it is widespread) can be helpful in several instances to gather more precise data than surveys, which frequently comprise tiny cohorts of a population: The ability to correctly identify hemoglobin variants allows for the well-known advantages of screening for sickle cell disease as well as the collection of epidemiological data on additional variants. The detection of Hb Bart's can also provide information on the epidemiology of alpha thalassemia [11]. Using a cutoff value of 1.5 percent HbA, beta can be detected in newborn blood [12].These tests are helpful for epidemiological research and secondary prevention, especially if molecular studies are added on top of them. Tandem mass spectrometry, for example, may even be able to identify thalassemia heterozygotes with greater sensitivity than other types of technology [13]. If neglected, severe hepatosplenomegaly, severe bone abnormalities, and death may occur in TM patients, who typically present within the month of life with failure to thrive, feeding, and pallor. Numerous organ impairments, including heart malfunction, endocrine dysfunction, bone degeneration, severe infections, and other related consequences, have been linked to iron overload [14]. Defects in all four genes are fatal unless treated with blood transfusions in utero because haemoglobin without alpha chains does not transport oxygen. Another type of thalassemia is hemoglobin E (Hb E) linked. Hb E, one of the most frequent hemoglobinopathies, is caused by a missense mutation in codon 26 of the Beta globin gene's splicing sequence [14].This mutation alters beta globin's structure as well as its rate of production [8].treating a small portion of the world's patients[15]. By inserting the normal b-globin or g-globin gene into hematopoietic stem cells (HSCs), gene therapy holds hope for permanently restoring one's bone marrow cells. A recent study confirmed the efficiency of globin gene transfer for clinical exploration and revealed safe mobilization of CD34+cells in adults with beta thalassemia [16]. For the first time, a patient with severe beta-thalassemia has been transfusion-free for five years as a result of gene therapy [17]. Three patients are participating in the first phase of the first gene therapy clinical trial in the US. Despite still needing blood transfusions, the patients handled cyto-reduction well and restored their blood counts [18]. The following problems can result from :
High output cardiac failure caused by severe anemia, cardiomyopathies, and arrhythmias is the leading cause of death in thalassemia patients, along with jaundice and gallstones brought on by hyperbilirubinemia, cortical thinning and bone distortion brought on by extramedullary hematopoiesis, and high output cardiac failure caused by extramedullary hematopoiesis [19]. Excess iron can cause primary hemochromatosis symptoms such endocrine abnormalities, joint issues, skin discoloration, etc. due to extramedullary hematopoiesis and hepatosplenomegaly from recurrent blood transfusions. Slow growth and postponed puberty are both neurological issues, as are an increased risk of parvovirus B19 infection.[20] Aims of the study to Determining the CD8 T cells and side effects of blood transfusion on thalassemia patients.

2. Materials and Methods

In the present study, a total (180), (60) From healthy (control) and (120) patients suffering from thalassemia (54) males and (66) females with ages ranging between (20-49) years who attended to the Medical city and Al-Karama teaching hospitals in Baghdad province and Al-Kut hospital for gynecology obstetric and pediatrics in Wasit province during the period from the beginning of January 2022 to the end of April 2022. All the above-mentioned hospitals have a special center for Thalassemia patients. These centers contain information about patients, as well as a special card for each patient, on which the date of receiving blood is written. In the morning, samples are taken from these patients to do their own tests, for example, a complete blood picture, serum ferritin etc. the samples are taken in cooperation with the laboratory staff and with the consent of the patients. The number of males was 36, the females 24, and the ages ranged from 21 to 48 in control group.
The number of males was 36, the females 24, and the ages ranged from 21 to 48 in control group.
Patients in this research were divided into three groups:

1. (30) Thalassemia major Patients frequently dialyzed without filter, A group of patients receive blood without using filter in a blood bag.
2. (30) Thalassemia major Patients frequently dialyzed with filter, A group of patients receive blood by placing a filter in a blood bag.
3. (60) Intermediate Thalassemia Patients unfrequently dialyzed.

The CD8 test, which is one of the immunological tests, was measured for all patients by the method of the sandwich ELISA technique.

Table 1 CD8 test

No.	Kit	Company	Origin
1	CD8	Cusabio	USA

3. Statistical Analysis

Data entered with an excel sheet and analyzed with spss 0.28 for Windows Inferential statistics (shapiro-wilk test for normality, anova, and kruskal-wallis h) and descriptive statistics (frequency, mean, standard deviation with tables and graphs) with post hoc analysis were used P-values less than 0.05 are regarded as statistically significant.

4. Results

According to the results in Table 2 the CD8 mean level was statistically significantly different in the studied groups (P<0.001). Patients frequently dialyzed without a filter have the higher mean value of CD8 1042±516 (172-1830), and both the dialyzed with a filter and the unfrequently dialyzed groups have lower mean levels of CD8 562±263 (162-991) and 521±213 (145-1007) (respectively). The mean value of CD8 in patients frequently dialyzed without a filter was statistically significantly higher than in both groups of patients dialyzed frequently with a filter and dialyzed unfrequently (P<0.001), but there was no mean difference in the frequently dialyzed with a filter group in comparison to the unfrequently dialyzed group (P 0.740). The normal range of CD8 is 58-429 U/ml, see Figs. 1 & 2.

Table 2 Comparison of CD8 among the studied groups

Markers	Groups	Number of patient	Mean±SD* (range)	ANOVA	Post hoc**
CD8	Freq. without filter(TM)	30	1042±516 (172-1830)	F=27.43 Sig.<0.001	F1 vs F2 P<0.001 F1 vs U P <0.001 F2 vs U P= 0.740
	Freq. with filter(TM)	30	562±263 (162-991)		
	Unfrequently(intermediate thalassemia)	60	521±213 (145-1007)		

* Standard Deviation.
** Post hoc multiple comparison, Games-Howell Test
F1: Frequent without filter, F2: Frequent with filter, U: Unfrequently.

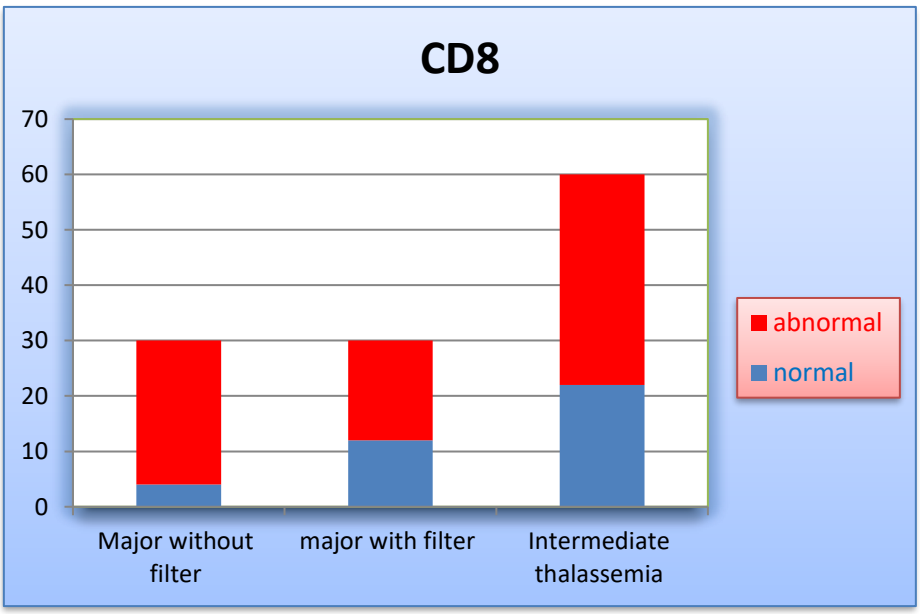

Fig 1. CD8 levels among studied groups

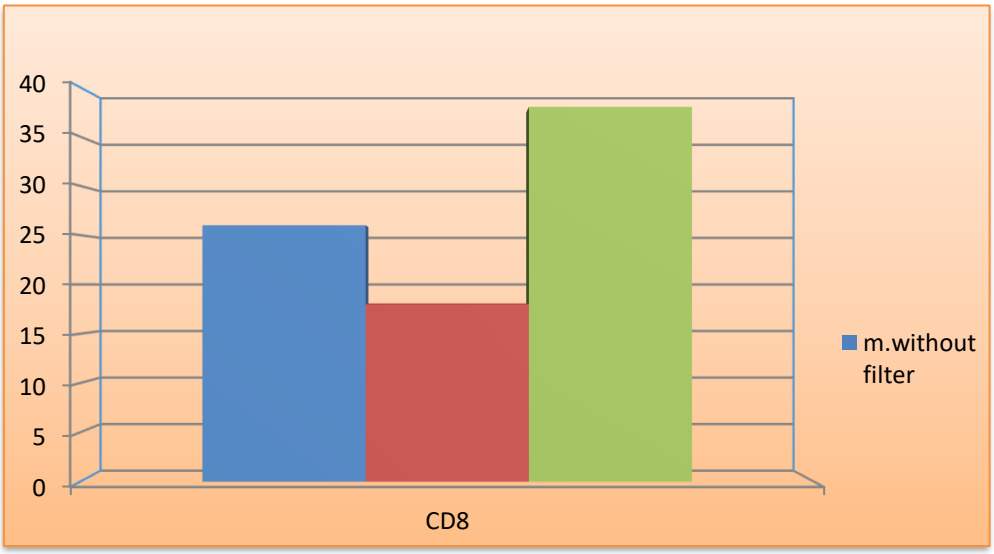

Fig 2. Abnormal levels of CD8 among studied groups

In Table 3, in our study, samples were taken from 120 patients, divided into three groups,
G1 Includes 54 patients, 18 for frequent blood transfusion with filter, 12 for frequent without filter and 24 for unfrequently
G2 includes 59 patients; 12 for frequent blood transfusion with a filter; 16 for frequent without a filter; and 31 for unfrequently.
G3 includes 7 patients: 0 for frequent blood transfusion with filter, 2 for frequent without filter, and 5 for unfrequently.

Table 3 Distribution of studied groups according to age

Demographics		Dialysis status Number =120			Total
		Freq. with filter (number=30)	Freq. without filter (number=30)	Unfrequently (number=60)	
Age Group (years)	20-29	18 (60)*	12 (40)	24 (40)	54
	30-39	12 (40)	16 (53)	31 (52)	59
	40-49	0	2 (7)	5 (8)	7
Mean±SD of age		28.2±4.4	31.0±5.6	30.9±6.4	30.3±5.8

5. Discussion

Table 1 demonstrates Patients who are frequently dialyzed without a filter have a greater mean level of CD8, while those who are dialyzed with a filter and those who are not frequently dialyzed have a lower mean level of CD8. The mean value of CD8 in patients frequently dialyzed without a filter was statistically significantly higher than in both groups of patients frequently dialyzed with a filter and those dialyzed infrequently ($P< 0.001$), but there was no mean difference in the frequently dialyzed with a filter group compared to the unfrequently dialyzed group (P 0.740). Naive and effector T cells are highly skilled migrators crucial to immune surveillance and the establishment of adaptive immunity against infection and cancer. T lymphocytes efficiently traverse and scan nearly all areas of the body for unwanted or alien material [21]. T-cell differentiation and phenotypes are strictly regulated by transcription factors, cytokines, chemokines, integrins, and metabolic signals, and T-cell lineages are thought to be stable and mutually exclusive [22]. T cell Cytotoxic In order to maintain the CD8+ response and prevent exhaustion, CD4+ T cells are crucial. CD8+ T cells are the primary killers of infections and cancerous cells [23]. Antigen-presenting cells (APCs) and target cells that exhibit antigenic peptide fragments produced by proteasomal degradation of cytoplasmic proteins bound to the corresponding binding grooves interact with CD8+ T cells via major histocompatibility complex class-1 (MHC-1) molecules on their surface [21]. When CD8+ cells interact with an APC or a target cell, they adhere to it and crawl over the surface to search for MHC-antigen-peptide complexes. In order to activate the CD8+ T-cell receptor (TCR) complex, mechanical energy must be converted into biomechanical signals by direct contact and movement of the cells [24]. Activated CD8+ T lymphocytes generate immunological synapses between their supramolecular activation complex and adhesion molecules (such as intercellular adhesion molecule) on the target-cell surface by homing towards chemokine and integrin gradients on APCs or target cells [25]. The TCR and CD8, acting as a co-receptor, interact with the presenting peptide and the MHC-subunit, respectively, to validate the target's identity. Before the killing apparatus is turned on, a co-stimulatory signal from the CD28 co-receptor must be transduced after the TCR-activating signal [21].
CD8 and CD4 are glycoproteins on the cell's surface that take part in molecular complexes important in T cell development as well as T cell antigen recognition Increased intercellular adhesion and improved activation of T cells are the results of CD4 and CD8's interactions with the Class II and class I MHC molecules have nonpolymorphic regions, respectively [26]. The cytotoxins perforin, granzymes, and granulysin are released by T cell Cytotoxic in response to contact with infected or defective somatic cells. Granzymes enter the target cell's cytoplasm by the action of perforin, and their serine protease function starts the caspase cascade, a chain of cysteine proteases that eventually causes apoptosis (programmed cell death). This is referred to as a "lethal strike," because it enables the observation of the target cells' death in waves [27]. TC cells are resistant to the effects of their perforin and granzyme cytotoxins because of the high lipid order and negatively charged phosphatidylserine present in their plasma membrane [28], the increased levels of CD8 in TM patients causes many abnormalities of the immune system may be due to receiving multiple blood transfusions. In addition to the link between blood-borne illnesses and frequent transfusions, co-occurring immunological deficiencies have also been linked to these patients' higher susceptibility to infection. Numerous immunological abnormalities, such as increased immunoglobulin production, reduced opsonization, and granulocyte phagocytosis, have been identified in studies of the immune systems of these patients [6]. Patients with thalassemia have also shown evidence of abnormalities in the cell-mediated immune response (CMI). The reason thalassemia patients are prone to infections is unclear [4].
Regular transfusion therapy has a significant side effect known as alloimmunization, It results in the production of a single or several targeted anti-red blood cell antibodies [7]. It is advised that all patients use packed washed leukocyte depleted/filtered red blood cells to lessen allergic reaction, feigned non-hemolytic transfusion reactions, and CMV infection [8].In fact, patients with IgA deficiency and those who experience frequent allergic reactions should wash their packed red blood cells [9]. Using leukofiltrated blood products is not a routine practice in countries with limited health resources. It reduces cytomegalovirus reactivation, HLAimmunization, and febrile non-haemolytic transfusion reactions [10]. Improvement in pulmonary function in postcardiac surgery patients has been reported with leukodepletion [10]. Patients with B - thalassemia have been discovered to have several immunological abnormalities [11]. They are mostly manifested by reduced neutrophil and monocyte activity, increased polyclonal immunoglobulin synthesis, impaired complement alternative pathway activity, and functional or cellular changes in several subpopulations of peripheral blood lymphocytes. These anomalies are typically assumed to be a side effect of blood transfusions and, to a lesser extent, iron overload or splenectomy. However, it is still unclear if these immunological changes represent a true, acquired immunodeficiency and, more importantly, whether they have any clinical significance [10]. The results of our study showed that the mean age of TM and intermediate patients was 30.3±5.8 years, while the number of males was 54 and females 66. age and gender distribution of the disease has no clinical value, and there is an equal chance of disease inheritance for both genders. Infections are a common (12-13%) complication of thalassemia and hemoglobinopathies, and they can be fatal [12]. Beta-TM has an increased risk for systemic infections, suggesting that a basic defect in the host defence is present [12]. one of the main side effects of routine blood transfusions, especially in individuals with chronic illnesses is alloimmunization [13]. Whether this specific increase in CD8 T cells and the decrease in CD4 is owing to continuous alloantigen stimulation of the immune system with autoimmune hemolysis as a result of iron overload remains elusive [29]. The discrepancy between our results and theirs may be related to the changes in the immune system are time-related and depend on the disease duration and iron overload amount. One of the reasons for the increased proportions of CD8+ cells is increased ferritin levels [30]. Further

analysis of the effect of ferritin on immune markers depending on the cutoff of 1000 µg/l could reveal significant difference between patients with ferritin levels of at least 1000 and patients with ferritin less than 1000 when compared with the controls, as the level of T cells and CD8+positive cells are higher in patients with ferritin level of at least 1000 but not in patients with ferritin less than 1000Alloimmunization can also result in a potentially fatal hemolytic transfusion reaction in thalassemia patients. many variables, including the clinical heterogeneity of -thalassemia patients, the frequency of blood transfusions, splenectomy, serum iron status, and iron chelation therapy, can contribute to variation in results [20]. Regardless of the patients' splenectomy status, iron overload has been linked to an increase in CD8+ cells. These findings suggest that patients with chronic iron excess should have a lower CD4+/CD8+ ratio, and patients with thalassemia have been found to have a low CD4+/CD8+ ratio [31], patients who receive blood transfusions on a regular basis, such as those who have hemoglobinopathies, myelodysplastic syndromes, chronic renal disease, and other conditions, frequently develop human leukocyte antigen (HLA) and/or red cell alloimmunization [14]. The study showed, agree with McGinty, E.E., *et al* found higher in the levels of CD8 in thalassemia patients [15]. present study compatible with,Bajwa, H. and H. Basit who uses Patients with TM have been found to have higher numbers of various lymphocyte subsets, such as helper T cells, suppressor T cells, NKCs, and B cells (differentiated by respective phenotypic signatures of CD3+/CD4+, CD3+/CD8+, CD3-/CD16/56+, and CD3-/CD19+) [16]. Cappellini, M.D.,*et.,al* disagree with objective, found a decline of CD8 number in thalassemia patients [17].

6. Conclusion

In this study, CD8 expression was higher in TM. the CD8 level differed statistically significantly between the groups investigated. Patients who are frequently dialyzed without a filter have a higher mean value of CD8, while those who are dialyzed with a filter and those who are not frequently dialyzed have a lower level. This finding is attributed to multiple antigenic stimuli during a blood transfusion.

7. Recommendation

We recommend using the filter during receiving blood for patients who need it frequently, especially thalassemia patients, to avoid immune reaction. The researchers need to develop more comprehensive and systematic research with advanced diagnostic.

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