Research Article

Serum ferritin level affects T lymphocyte CD4, CD8, and CD4/CD8 ratio in transfusion-dependent beta-thalassemia

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ABSTRACT

Background: Chronic blood transfusions and hemolysis are the major causes of secondary iron overloading transfusion-dependent beta-thalassemia. Iron overload can cause several organ complications including immune alteration. Infection due to immune alteration is number two leading cause of mortality in thalassemia. Research on the correlation of ferritin as iron overload marker and T lymphocyte subsets alteration in adult thalassemia patients are still rare and require further study. **Objective:** The objective of the study was to study the correlation of iron overload and T lymphocyte subsets CD4, CD8, and CD4/CD8 ratio in adult transfusion-dependent beta-thalassemia. **Methods:** The study was cross-sectional observational analytic study conducted on 36 subjects with transfusion-dependent beta-thalassemia diagnosed by clinical examination and laboratory high-performance liquid chromatography followed up at Hematology Clinic, Dr. Soetomo Teaching Hospital Surabaya, Indonesia. Ferritin serum was measured by chemiluminescence immunoassay method, while CD4, CD8 counts, and CD4/CD8 ratio were assessed by flow cytometry with antigen for CD3/CD4/CD8. **Results:** There were 36 subjects, 21 males and 15 females with median age of 23 (range 18–48). The mean ferritin serum was 5137.8 ± 2433.5 ng/ml while CD4, CD8, and CD4/CD8 ratio were 695.56 ± 25.17; 822.4 ± 360; and 0.95 ± 0.42, respectively. The analysis of correlation showed that there is correlation of ferritin and CD4 ($r = -0.34$, $P < 0.05$), ferritin and CD8 ($r = 0.43$, $P < 0.05$), and ferritin and CD4/CD8 ratio ($r = 0.34$, $P < 0.05$). **Conclusion:** In this study, there was a correlation between ferritin serum and CD4, CD8, and CD4/CD8 ratio. An increase of ferritin in iron overload will decrease CD4, increase CD8, and decrease CD4/CD8 ratio.

KEY WORDS: CD4, CD4/CD8 ratio, CD8, Ferritin, Transfusion-dependent beta-thalassemia

INTRODUCTION

Thalassemia is a group of congenital anemia disorders caused by disruption of the formation of one or more globin subunits from normal hemoglobin. Genetic defects are characterized by mutations on chromosomes that produce alpha or beta.[1-3] Thalassemia is still a global health problem and is the most common inherited monogenic disorder, especially in developing countries. World Bank data show that 7% of the world's population is a carrier of thalassemia. Every year an estimated 300,000–500,000 babies are born accompanied by severe hemoglobin abnormalities, and 50,000–100,000 children die from β-thalassemia and 80% of that number come from developing countries.[4] Indonesia is a country with a high genetic prevalence of thalassemia which is situated in "thalassemia belt" area. The frequency of genes carrying thalassemia in Indonesia is estimated to be 3–10%.[5]

Transfusion-dependent thalassemia patients require evaluation of iron overload for a lifetime. Serum ferritin examination is a clinical hematological examination standard to monitor iron overload in transfusion-dependent thalassemia patients. The ferritin target of 1000 mg/L is recommended as standard practice in transfusion-dependent thalassemia patients.[6,7]

Progress in the management of thalassemia, namely, transfusion and iron chelation therapy, has improved the quality of life and life expectancy of transfusion-dependent thalassemia. However, this also causes other complications, namely, hemosiderosis and

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immunological changes that cause an increase in infection. A multicenter study in Italy has found that infection is the second most common cause of death in people with thalassemia after cardiac causes.[8] Some factors that allow changes in the immunological response include iron overload, chelation therapy, and exposure to other blood antigens.[9]

Several studies of decreased cellular immune response and its effect on the T lymphocyte subset in thalassemia have been conducted several times. All research is conducted on experimental animals or children. Meanwhile, the research on the growing number of adult thalassemia patients has not been conducted. This study was designed to provide information on alteration in the immune system due to iron overload in adult thalassemia patients.

METHODS

Research Subjects

This study was a cross-sectional observational study conducted at Dr. Soetomo Teaching Hospital, a tertiary referral center hospital in East Indonesia from July to September 2019. The protocol of the study was approved by the ethics committee of Dr. Soetomo Teaching Hospital. Informed consent was obtained from all subjects. The sampling method was total sampling using sample size equation for correlation studies. This study involved 36 subjects with inclusion criteria of age 18–50 years old, diagnosed with transfusion-dependent beta-thalassemia and already on regular transfusion (more than 10 RBC packs). Exclusion criteria were patients with HIV positive, Hepatitis B, Hepatitis C, and splenectomy.

History taking on transfusion, chelating drug, and splenectomy was noted. Clinical laboratory examination was carried out before regular blood transfusion. Diagnosis of thalassemia was made by history taking, physical examination and laboratory complete blood count, blood smear, hemoglobin electrophoresis, and high-performance liquid chromatography (HPLC). Ferritin serum was measured by DiaSorin Liaison using chemiluminescence immunoassays quantitatively measured in ng/dl. T cell population of CD4, CD8 counts, and CD4/CD8 ratio was measured using flow cytometry method with BD FACSVia flow and FACSLink Next-Gen Lis Software with reagent BD Multitest CD3/CD8/CD45/CD4.

Statistical Analysis

Data were analyzed using SPSS version 20.0. The analysis of the relationship between independent variables and dependent variables was carried out with Pearson test if the data distribution is normal, and the spearman test if the data distribution is not normal. The results are presented in r (correlation coefficient), where the *P* value considered significant if it <0.05 with 95% confidence interval

RESULTS

Data of 36 subjects were analyzed and clinical characteristics are presented in Tables 1 and 2, 21 subjects (58.3%) were male and 15 (41.7%) were female. The age of the subjects ranged from 18 to 48 years with a median of 23 years. The HPLC examination results showed that 15 (41.7%) were people with thalassemia beta and 21 (58.3%) thalassemia beta/HbE disease. The median transfusion duration was 16.14 years, ranged from 3 to 28 years. Mean standard deviation (SD) hemoglobin level was 7.4 (0.94) mg/dl. The mean (SD) packed red cell transfusion requirement in the subjects was 170.8 (28.38) mL/kg/year. All subjects are on chelation therapy, 25 (69.5%) were using deferasirox and 11 (30.5%) on deferiprone.

The results of serum ferritin levels mean (SD) were 5137.83 (2433.53) ng/ml. The results of absolute CD4 count and percentage, CD8 cell count, and percentage and CD4/CD8 ratio are presented in Table 3. The Kolmogorov–Smirnov normality test showed that ferritin, absolute CD4 counts, percentage of CD4, absolute CD8 cell counts, and percentage of CD8 cells were normally distributed, while CD4/CD8 ratio was not normally distributed. Thus, the correlation between serum ferritin levels with absolute CD4 cell counts, percentage of CD4 cells, absolute CD8cell counts, and percentage of CD8 cells was analyzed using Pearson correlation test, while correlation of serum ferritin levels with CD4/CD8 ratio uses the Spearman correlation test [Table 4]. The analysis shows that there is significant correlation between Ferritin serum and CD4 count, CD8 count and CD4/CD8 ratio [Table 5].

There was a significant negative correlation between serum ferritin and CD4 absolute count [Figure 1] while there was a significant positive correlation between serum ferritin and CD8 count [Figure 2] and a negative correlation between ferritin serum and CD4/CD8 ratio showed positive correlation [Figure 3].

Table 1: Clinical characteristics

Characteristics	n	%
Gender		
Male	21	58.3
Female	15	41.7
Chelating agent		
Deferiprone	11	30.5
Deferasirox	25	69.5
Chelating compliance		
Yes	25	69.5
No	11	30.5

Table 2: Clinical characteristics

Characteristics	Mean (standard deviation)	Median	Range
Age	24.7 (6.94)	23	18–48
Hb (pre transfusion)	7.4 (0.94)	7.5	4.1–9.3
Leukocyte	6.73 (3.00)	5.92	2.4–10.1
Thrombocyte	324.97(446.3)	181	104–558
Transfusion duration	16.14 (6.2)	17	3–28
Transfusion volume (ml/kg/year)	160.8 (49.69)	150	90–307

Table 3: Ferritin results

Variable	Mean	Standard deviation	Min	Max
Feritin serum (ng/ml)	5137.83	2433.53	1.360	11.646

Table 4: CD4, CD8, and CD4/CD8 ratio results

Variable	Min	Max	Mean	Standard deviation
CD4 absolute	285.90	1256.90	695.56	253.17
CD4 (%)	20.10	48.00	30.94	6.32
CD8 absolute	286.00	1778.40	822.46	360.04
CD8 (%)	25.72	48.41	34.19	6.20
CD4/CD8 ratio	0.32	2.00	0.95	0.42

Table 5: Correlation of ferritin serum and CD4 absolute, CD4%, CD8, CD8%, and CD4/CD8 ratio

Inter variable correlation	Correlation coefficient	*P*-value
Ferritin↔CD4 absolute	–0.340	0.042
Ferritin↔CD4 %	–0.363	0.030
Ferritin↔CD8 absolute	0.403	0.030
Ferritin↔CD8 %	0.346	0.015
Ferritin↔CD4/CD8 ratio	–0.565	0.001

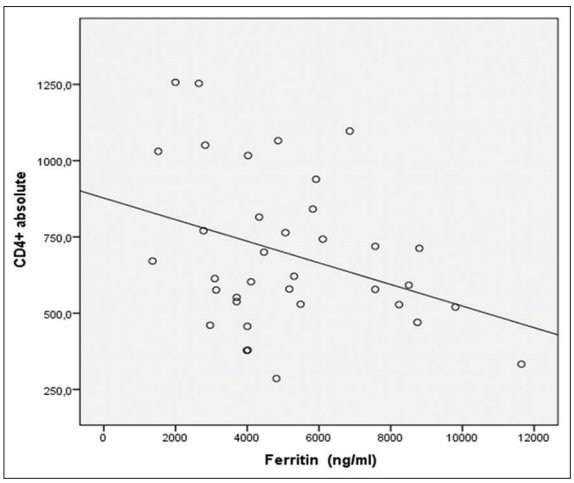

Figure 1: Correlation of CD4 count and ferritin serum (r = –0.34; *P* = 0.042)

DISCUSSION

In this study, the majority of subjects were male, 21 people (58.3%) and 15 (41.7%) were female. This result is not much different from the research from Eissa and El-Gamal, 2019, in Egypt where out of 60 research subjects the number of men was slightly higher, 36 (60%) and 24 (40%) were women.[10] Research on 116 subjects in Jakarta, Indonesia, found more female subjects slightly higher 68 subjects, (58.5%).[11] These results are consistent with the inheritance pattern of thalassemia, where mutations

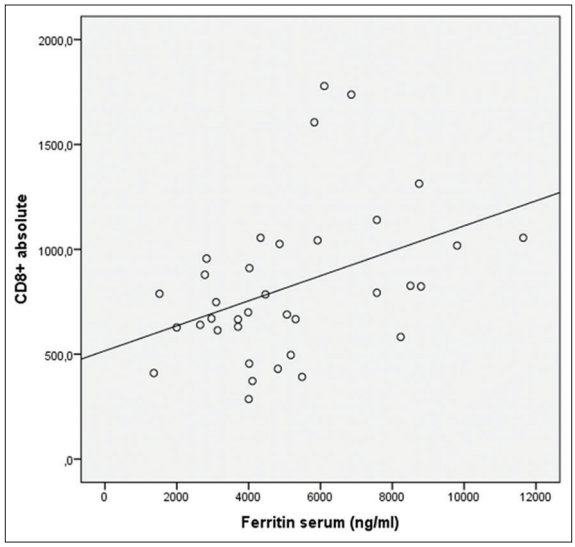

Figure 2: Correlation of CD8 count and ferritin serum (r = 0.43; *P* = 0.03)

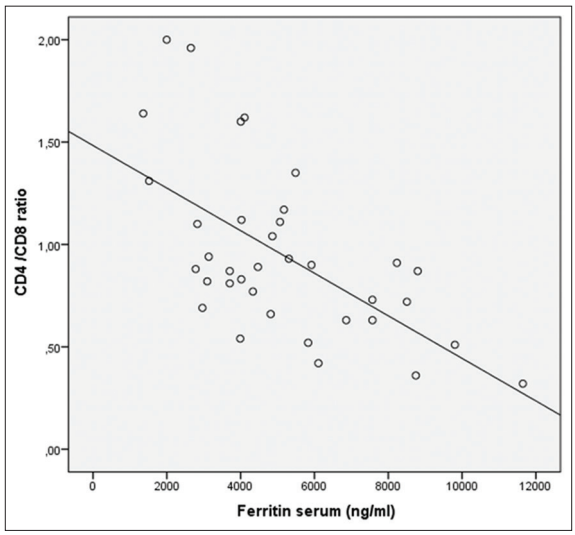

Figure 3: Correlation of CD4/CD8 ratio and ferritin serum (r = –0.565; *P* = 0.001)

occur in heme-forming genes that lie in autosomes and not chromosomal sex.[12]

The age of the subjects ranged from 18 to 48 years with a median of 23 years. These results are not much different from the research in Jakarta, Indonesia, where the age of thalassemia patients ranged from 12 to 38 years.[11] Research in Italy by Fraquelli *et al*. in 2010 has found that the age of thalassemia patients was older with median of 33 years, ranged from 21 to 50 years old.[13] The age of the subjects in this study does not differ from the study of Gharagozloo *et al*., 2007 with the median age was 21 years (14–39 years).[14] At present, with the availability of transfusion, iron chelation and comprehensive care the life expectancy of dependent-transfusion thalassemia are quite high.[12]

HPLC examination results show that 15 (41.7%) are thalassemia beta and 21 (58.3%) are HbE beta-thalassemia. HbE is a variant of the structure of hemoglobin B that arises in a high frequency in Southeast Asia. Hemoglobin E itself will appear as a mild thalassemia phenotype. If coinheritance with beta-thalassemia, the phenotype will be more severe and is more dependent on transfusion. Hemoglobin E beta-thalassemia is often found in Southeast Asia and covers 50% of the severe form of beta-thalassemia. The phenotype interaction of beta-thalassemia and HbE genes can be categorized into three categories based on the severity of mild HbE/beta-thalassemia, found in 10% of HbE/beta-thalassemia cases in Southeast Asia. In this group, the average hemoglobin is 9–12 g/dl, and usually does not require special treatment. Moderate HbE/beta-thalassemia usually has 6–7 g/dl hemoglobin level and is clinically similar to thalassemia intermedia. The final group is severe HbE/beta-thalassemia, which usually has hemoglobin level 6–7 g/dl and is clinically similar to thalassemia major.[12]

In this study, there are no alpha-thalassemia subjects, possibly because all subjects are from the Javanese ethnic group. Thalassemia-alpha is currently a very rare finding in the referral center of thalassemia in Indonesia, around 0.3% and most (69%) are from Chinese ethnic group.[15] On average patients have received transfusion for 16 years. The duration of transfusion of subjects ranged from 13 to 28 years with a median of 16.14 years.

All subjects receive deferasirox or deferiprone for iron chelation therapy. A total of 25 (69.5%) use deferasirox and 11 (30.5%) used deferiprone. Iron chelation treatment requires adherence and commitment from patients and families. In this study, all patients have high serum ferritin levels above 1000 ng/ml, mean ferritin levels are 5137.83 + 2433.53 ng/ml ranged from 1360 ng/ml to 11,646.00 ng/ml. Increased levels of ferritin above 1000 ng/ml in thalassemia-dependent transfusion are found in several studies, including the Arseno *et al*., 2017 study with a mean ferritin level of 3429 ng/dl, lower than this study. This is most likely due to most of the subjects were children so that the duration of transfusion is shorter and the compliance of chelation medication is quite high with parental motivation.[16]

Increased ferritin levels due to chronic transfusions and destruction of red blood cells in patients with thalassemia can cause transferrin capacity to decrease. This will result in the formation of non-transferrin bound iron in plasma and labile iron pools (in the intracellular) that can form hydroxyl molecules. Both of these toxic forms of iron can damage lysosomes, intracellular enzymes, and cell death.[14,17,18]

T cell CD4 counts have age, race, and laboratory instrument variability. A normal CD4 value for a particular population and reagent is obtained as a CD4 value in 95% of the population. In this study, the absolute CD4 cell count had an average of 695.56 + 253.17 cells/µL with a range from 285.90 to 1256.9. The range of CD4 counts from the laboratory reference is 404–1612 cells/µL. For the study subjects, only five people had CD4 lower than the reference value. All five subjects had high ferritin levels >5000 ng/ml. When compared with CD4 cell counts in the normal population of other studies, CD4 cell counts in this study were lower. Afolabi *et al*. examined CD4 levels in the normal adult population in Nigeria to obtain a normal population value of 1023 ± 603 cells/mm^3 (median 842 cells/mm^3, and range 400–1288 cells/mm^3).[19] A study by Hagag *et al*., 2016, showed higher CD4 count (889,6 ± 282) than this study. This could be due to lower ferritin level, younger subjects (age 5-15 years old), and better chelating drug compliance. The percentage of CD4 cells has an average of 30.94% with a range from 20.1% to 48%. This result is lower than the laboratory reference value with 33–58%. Most subjects (67%) had a lower percentage of reference values, while 33% had values in the reference range.[20]

This study showed that there is a relationship between serum ferritin levels of patients with dependent-transfusion beta-thalassemia patients with absolute numbers of CD4 T lymphocyte cells ($r = –0.34$; $P = –0.042$) and CD4% ($r = –0.336$; $P = 0.03$) where the higher the ferritin level the lower the number of positive CD4 T cells. The absolute CD4 cell count of the reference range is 404–1612 cells/µL. Five of the 36 subjects had lower values than the lower limit of the reference value. This patients has a very high ferritin level >5000 ng/dl. From the statistical analysis, it was found that there was only weak correlation between

ferritin and CD4 lymphocyte T cell counts percentage. This was probably due to other factors that could influence CD4 lymphocyte T cell counts besides iron overload, namely, genetic, dietary patterns, physical exercise, and psychological stress.[21] Research in mice by Cardier *et al*., 1997, administration of continuous parenteral iron will cause an increase in ferritin accompanied by a decrease in the number of CD4 lymphocyte T cells in the blood. This study also examined CD4 T lymphocyte cells in mouse spleen and found that there is decreased number and ability of proliferation. After 50 days of parenteral iron administration stopped, the number of CD4 lymphocytes T cell proliferation will return to normal. Research on transfusion-dependent beta-thalassemia children also shows the same relationship, which is that an increase in serum ferritin will be accompanied by a decrease in the number and percentage of CD4 T cells, especially in the group of children who get transfusion <5 years.[16] Research on 40 children in Egypt also showed the same thing where ferritin levels had an effect on decreasing blood CD4 cell levels. This study mentions oxidative stress as a cause of premature aging of the immune system that will decrease the response to antigenic stimulation and the ability of CD4 T cell proliferation.[20]

From this study, it was found that there was a relationship between serum ferritin levels of patients with absolute levels of CD8 T lymphocytes ($r = 0.403$; $P = 0.030$) and CD8 % ($r = 0.346$; $P = 0.015$), where the higher levels of ferritin the higher the number of CD8 T cells. Research on animal mice by Cardier *et al*., 1997, given continuous parenteral iron showed an increase in ferritin accompanied by an increase in the number of CD8 lymphocyte T cells in the blood.[22] Research on transfusion-dependent beta-thalassemia children also shows the same relationship, namely, an increase in serum ferritin will be accompanied by an increase in the number and percentage of CD8 T cells.[16] The proposed mechanism is a repetitive stimulation from donor leukocyte. Another mechanism is early immunosenescence due to oxidative stress from iron overload. Gharagozloo *et al*. showed an increase CD8CD28- T cell in transfusion-dependent thalassemia-beta. These cells are replicative and resistant to apoptosis.[14]

Ratio of CD4/CD8 in normal person ranges from 1.5 to 2.5. Inverse ratio <1 showed immune risk phenotype and immunosenescence. The prevalence of an inverse CD4/CD8 ratio increases with age. In normal people aged 20–59 years, CD4/CD8 ratio can be found in 8% of the population while at 60–94 years it can be found in 16% of the population. In thalassemia where repeated transfusions occur and iron overload caused decrease in the value of the CD4/CD8 ratio with the consequence of a decreased immune response to infection.[20,23] In our subjects, there is decrease in CD4/CD8 ratio and this correlates with the increase in ferritin serum.

CONCLUSION

This study of adult patients with transfusion-dependent beta-thalassemia and HbE beta-thalassemia showed abnormality in T lymphocyte subsets, including CD4 count, CD8, and CD4/CD8 ratio. This T lymphocyte subsets alteration has a significant correlation with ferritin serum as iron overload marker.

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REFERENCES

1. Marengo-Rowe AJ, McCracken AW, Flanagan P. Complete suppression of haemoglobin A synthesis in haemoglobin D Los Angeles--beta thalassaemia. J Clin Pathol 1968;21:508-10.
2. Muncie HL, Campbell JS. Alpha and beta thalassemia. Am Fam Physician 2009;80:339-44.
3. Pignatti CB, Galanello R. Thalassemia and related disorders: Quantitative disorders of hemoglobin synthesis. In: Greer JP, Arber DA, Glader B, List AF, Means RT, editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia, PA: Lipincott and Wilkins; 2013. p. 862-913.
4. De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H. β-thalassemia distribution in the old world: An ancient disease seen from a historical standpoint. Mediterr J Hematol Infect Dis 2017;9:e2017018.
5. Ministry of Health Republic of Indonesia. Keputusan Kementrian Kesehatan Republik Indonesia Nomer HK.01.07/MENKES/1/2018. Tentang Pedoman Nasional Pelayanan Kedokteran Tatalaksana Thalasemia. Available from: http://www.persi.or.id. [Last accesssed on 2019 Feb 24].
6. Fairbanks VF, Klee GG. Biochemical aspects of hematology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. Philadelphia, PA: W.B. Saunders Co.; 1999. p. 1642-710.
7. Viprakasit V, Porter V. Iron overload and chelation. In: Cappelini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors. Guidelines of the Management of Transfusion Dependent Thalassemia. Nicosia (CY): Thalassemia International Federation; 2014.
8. Ricerca BM, Di Girolamo A, Rund D. Infections in thalassemia and hemoglobinopathies: Focus on therapy-related complications. Mediterr J Hematol Infect Dis 2009;1:e2009028.
9. Porto G, De Sousa M. Iron overload and immunity. World J Gastroenterol 2007;13:4707-15.
10. Eissa DS, El-Gamal RA. Iron overload in transfusion-dependent β-thalassemia patients: Defining parameters of comorbidities. Egypt J Haematol 2014;39:164-70.
11. Sari TT, Gatot D, Akib AA, Bardosono S, Hadinegoro S, Harahap AR, *et al*. Immune response of thalassemia major patients in Indonesia with and without splenectomy. Acta Med

Indones 2014;46:217-25.
12. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010;5:1-15.
13. Fraquelli M, Cassinerio E, Roghi A, Casazza G, Colombo M, Massironi S, *et al*. Transient elastography in the assessment of liver fibrosis in adult thalassemia patients Am J Hematol 2010;85:564-8.
14. Gharagozloo M, Karimi M, Amirghofran Z. Double-faced cell-mediated immunity in beta-thalassemia major: Stimulated phenotype versus suppressed activity. Ann Hematol 2009;88:21-7.
15. Setianingsih I, Harahap A, Nainggolan IM. Alpha thalassemia in Indonesia: Phenotyppes and molecular defects. In: Marzuki V, Kluwer S, editors. Tropical Disease. New York: Academic/ Plenum Publishers; 2003. p. 47-56.
16. Arseno B, Setiabudi D, Susanah S. The correlation of ferritin levels with CD4, CD8, and CD4/CD8 ratio in children with thalassemia major. Sari Pediatri 2017;19:76-80.
17. Noulsri E, Lerdwana S, Fucharoen S. Pattamapanyasat K. Phenotypic characterization of circulating CD4/CD8 T-lymphocytes in β-thalassemia patients. Asian Pac J Allergy Immunol 2014;32:261-9.
18. Al-Awadhi AM, Alfadhli SM, Al-Khaldi D, Borhama M, Borusly M. Investigation of the distribution of lymphocyte subsets and zinc levels in multitransfused beta-thalassemia major patients. Int J Lab Hematol 2010;32:191-6.
19. Afolabi JK, Fadeyi A, Desalu OO, Durotoye IA. Normal CD4 count range among healthy Nigerian population in Ilorin. J Int Assoc Provid AIDS Care 2017;16:360-4.
20. Hagag AA, Elgamasy ME, Elbar ES. Assessment of T lymphocyte subsets in children with beta thalassemia major with iron overload. Egypt J Pediatr Allergy Immunol 2015;13:57-63.
21. Somarriba G, Neri D, Schaefer N, Miller TL. The effect of aging, nutrition, and exercise during HIV infection. HIV AIDS (Auckl) 2010;2:191-201.
22. Cardier JE, Romano E, Soyano A. T lymphocytes subsets in experimental iron overload. Immunopharmacol Immunotoxicol 1997;19:75-87.
23. McBride JA, Striker R. Imbalance in the game of T cells: What can the CD4/CD8 T-cell ratio tell us about HIV and health? PLoS Pathog 2017;13:e1006624.

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