ORIGINAL ARTICLE

Association between Percentage of TCD4 and TCD8 Lymphocytes with Iron Status in Female Adolescents

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ARTICLE INFO

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ABSTRACT

Background: Iron deficiency impairs the proliferation and function of T lymphocytes. This study was conducted to assess the relationship between serum iron with percentage of TCD4 and TCD8 lymphocytes in peripheral blood of female high school students in Hamadan.
Methods: In this cross-sectional study, 355 female high school students with an age range of 15-18 years were enrolled from January 2016 to March 2017. After approval by the ethics committee of Hamadan University of Medical Sciences, taking written consent of parents, and completion of a questionnaire involving demographic information, serum iron profile, the percentage of TCD4 and TCD8 cells, and TCD4/TCD8 ratio were measured using standard methods. The results were analyzed by SPSS software, version 13.
Results: The prevalence of iron deficiency anemia was 16.1% in 355 female high school students of Hamadan. There was no correlation between transferrin saturation with percentage of TCD4 lymphocytes and TCD4/TCD8 ratio in the two groups of students with and without iron deficiency (P>0.05). However, a significant correlation was found between Tfs with percentage of TCD8 lymphocytes in the group of patients with iron deficiency anemia (P<0.05).
Conclusion: This study indicated an increased percentage of TCD8 lymphocytes with reduced Tfs in patients with iron deficiency anemia. In addition to reduced Tfs, other factors may be associated with the alterations in percentage of TCD4 and TCD8 lymphocytes and TCD4/TCD8 ratio.

Introduction

Iron is involved in multiple cellular processes including erythropoiesis, thrombopoiesis, leukopoiesis, oxidative metabolism, CNS development, and immune responses.[1-7] Negative consequences of iron deficiency on hematopoietic tissues and the nervous system are well proven, while there is controversy regarding the effects of iron deficiency on the immune system.[8] Children, female adolescents, and women of childbearing age are more predisposed to iron deficiency anemia (IDA) because of the increased requirement and loss of Iron. According to a WHO survey between 1993 and 2005, nearly 30.2%

of non-pregnant women aged 15-60 years were anemic.[9]

Low concentrations of serum iron reduce neutrophil bactericidal capacity and proliferation of lymphocytes,[10-12] leading to increased susceptibility to infectious diseases and even development of malignancies.[8] However, the exact role of iron in the regulation of immune system responses, especially in the groups predisposed to iron deficiency anemia has not been fully recognized.

Given the importance of iron deficiency and its health consequences, we aimed to determine the values of serum iron profile, percentage of CD4 positive T cells (TCD4) and CD8 positive T cells (TCD8), TCD4/TCD8 ratio in

peripheral blood, the prevalence of IDA and eventually evaluation of the relationship between serum iron with percentage of TCD4 and TCD8 lymphocytes in female high school students in Hamadan (northwestern Iran) during 2016-2017.

Materials and Methods

This cross-sectional study was conducted during 2016-2017 after obtaining the approval of the Ethics Committee of Hamadan University of Medical Sciences. 355 female students of Hamadan high schools were enrolled in this study using two-step cluster method. First, 16 high schools for girls were randomly selected from the city of Hamadan and the students of each high school participated in the study by stratified random sampling. We included students who had not received iron supplements, hematinics and multivitamin in the past six months, those who did not have a history of acute or chronic bleeding and chronic diseases and infections and were not in the menstruation period, who did not have any symptoms of malnutrition, had no history of receiving immunosuppressive drugs, radiation, and chemotherapy, and were not married.

Written informed consent was taken from the parents and the questionnaire including demographic data was completed. Hematological indices and serum iron profile were measured by standard methods.[13] 5 mL whole blood was drawn using standard methods in tubes without anticoagulant to isolate the serum, as well as in EDTA-containing tubes for complete blood count (CBC). The percentage of TCD4 and TCD8 lymphocytes, as well as TCD4/TCD8 ratio were determined by flow cytometry. CBC was done by Sysmex coulter counter (KX-21N Japan). The serum iron profile, including serum iron, total iron-binding capacity (TIBC), Transferrin saturation (Tfs) was determined by an Autoanalyzer (Hitachi 912, Japan).

EDTA anticoagulated blood samples were used to determine the percentage of TCD4 and TCD8 lymphocytes and TCD4/TCD8 ratio. For this purpose, specific monoclonal antibodies (CD4-FITC & CD8-PE DAKO, Denmark) were used in Attune NxT flow cytometry device (USA). Briefly, 20 λ of relevant antibody was added per 50 λ of blood sample. The level of TCD4 and TCD8 lymphocytes was evaluated by the device after 45 minutes of incubation, adding 100 λ of lyser, and 15 minutes of incubation in the dark. Mouse IgG1 FITC/PE (DAKO, Denmark) was used as the isotype control. IDA was diagnosed upon decreased hemoglobin, MCV, MCH, RBC, serum iron, transferrin saturation with iron and ferritin along with increased TIBC.

Examination of more than 120 samples has been preferentially proposed to obtain the reference values in a society. In the present study, 121 normal samples were used to obtain reference values for serum Tfs, percentage of TCD4 and TCD8 lymphocytes and TCD4/TCD8 ratio. The relationship between Tfs with the percentage of T lymphocyte subsets was analyzed using Pearson's correlation test. P-value less than 0.05 was considered significant. Finally, the results were analyzed by SPSS software, version 13.

Results

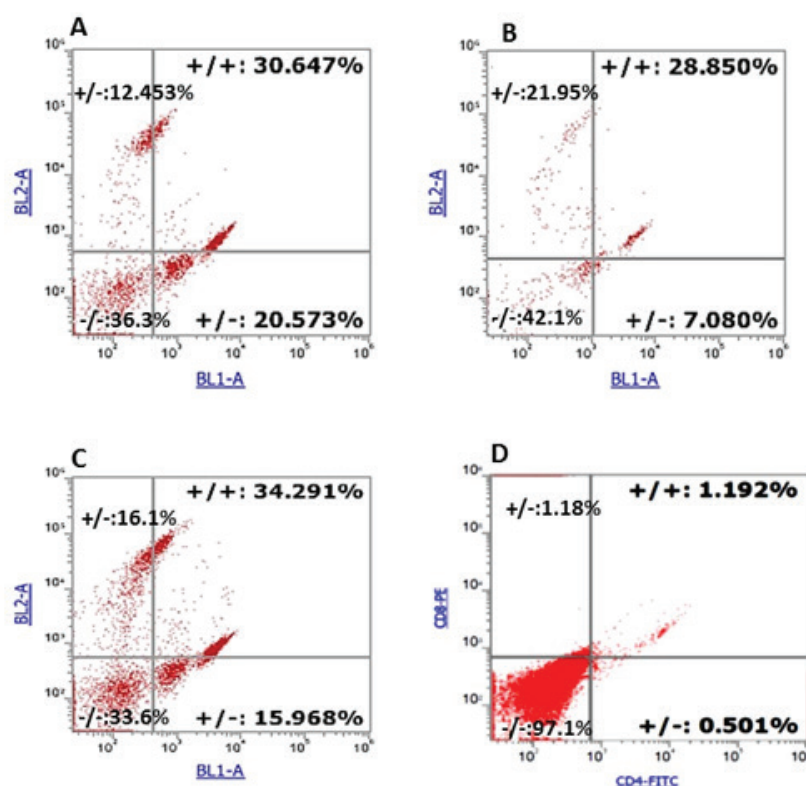In this study, from a total of 382 samples, 360 female

Figure 1: A dot plot of TCD4 and TCD8 lymphocyte populations in a female students. A) Dot plot of percentage of TCD4 and TCD8 in the NID group, B) Dot plot of percentage of TCD4 and TCD8 in IDA group, C) Dot plot of percentage of TCD4 and TCD8 in IDA group, D) Dot plot of isotype control of TCD4 and TCD8 antibodies.

students aged 15-18 were recruited from January 2016 to January 2017. Five out of 360 students were excluded from the study since they were diagnosed to have an anemia other than IDA. Among 355 students, 57 had IDA and 298 were normal. In this study, the students were divided to two groups of iron deficient (ID) and non-iron deficient (NID), including all students with IDA and 121 NID students for descriptive analysis of variables, as well as Pearson's correlation between Tfs and percentage of lymphocyte subtypes, respectively. Among 355 students of this study, the prevalence of IDA was 16.1%. Flow cytometric analysis and iron measurement were conducted for 121 NID students and 57 students with IDA, and the data were analyzed.

In figure 1, a percentage histogram of TCD4 and TCD8 lymphocytes of a female patient is presented. In accordance with international and WHO standards,[13] the group of normal students included those having hematological parameters in a normal range. ID students and those with IDA had Tfs lower than 12%.

The difference in Tfs and percentage of TCD8 lymphocytes was significant between NID and ID groups. As shown in table 1, mean values of TCD8 and Tfs were significantly different between the two groups of NID and those with IDA (in other words, mean TCD8 and Tfs values in students having IDA were significantly lower

than normal students). However, mean TCD4 and TCD4/TCD8 ratio were not significantly different between NID and IDA students (P=0.230).

In NID students, no correlation was found between the percentage of TCD4 and TCD8 lymphocytes, as well as TCD4/TCD8 ratio with Tfs. In group of patients with IDA, there was also no significant correlation between TCD4 percentage and TCD4/TCD8 ratio with Tfs; however, a significant negative correlation was observed between TCD8 percentage with Tfs (P<0.001, table 2).

We found a significant difference in correlation coefficients of Tfs and TCD8 cells between NID and ID students (table 3). In figure 1, a percentage histogram of TCD4 and TCD8 lymphocyte populations of a female student is presented.

Discussion

During their rapid growth period, early adolescents need more iron and other primary materials to generate red blood cells. Ignorance of serum iron profile and not preventing or treating iron deficiency anemia can have unfavorable outcomes on hematopoietic tissue, nervous system, and immune competence, especially the decreased number and function of T lymphocytes, increased risk of infections and even malignancy.[8] In our study the prevalence of IDA was 16.1%. But in other studies in Iran, the prevalence of IDA

Table 1: Descriptive statistics of hematological, immunological and iron storage parameters in ID and NID groups

	Groups	No	Mean	SD	Max	Min	P value
Hb	ID	57	13.1	1.2	15.8	9.7	>0.05
	NID	121	13.8	0.8	17.1	12.0	
Hct	ID	57	40.4	3.0	45.60	30.0	>0.05
	NID	121	41.1	2.0	46.7	36.0	
T CD4+ (%)	ID	57	37.6	22.2	90.0	12	0.122
	NID	121	45.3	19.2	97.0	6	
T CD8+(%)	ID	57	28.5	3.3	32.0	23	0.001
	NID	121	33.8	13.5	66.0	8	
CD4+/CD8+ ratio	ID	57	1.3	0.7	3.0	0.5	0.230
	NID	121	1.5	0.9	5.0	0.37	
Tfs (12%)	ID	57	4.1	2.0	7.5	1.5	<0.001
	NID	121	26.0	9.8	67.1	12.3	
Ferritin	ID	57	6.0	3.2	11.0	2.0	0.001
	NID	121	51	3.3	92.0	15.0	

Table 2: Pearson correlation between serum transferrin saturation and percentage of lymphocyte subsets in NID and IDA patients

Groups		Non-iron deficient Students			Students with Iron deficiency Anemia		
		T CD4+	T CD8+	CD4/CD8	T CD4+	T CD8+	CD4/CD8
Transferrin saturation	Pearson correlation	0.161	0.027	0.015	-0.165	-0.723	-0.092
	Number	121	121	121	57	57	57
	P value	0.109	0.786	0.882	0.501	<0.001	0.707

Table 3: Comparison of Pearson correlation between serum transferrin saturation and percentage of lymphocyte subsets in normal and ID students

	Non-iron deficient Students	Students with Iron deficiency Anemia	P value
Transferrin saturation/TCD4	0.161	-0.165	0.222
Transferrin saturation/TCD8	0.027	-0.723	<0.001
Transferrin saturation/CD4 to CD8 ratio	-0.092	0.015	0.691

was 9.3% and 4.5% in Yazd and Semnan, respectively.[13] The main objective of this study was to determine normal values of serum iron profile, the percentage of TCD4 and TCD8 lymphocytes, TCD4/TCD8 ratio, as well as to detect the correlation between serum iron with percentage of TCD4 and TCD8 lymphocytes.

Recent studies have given conflicting results on the impact of iron deficiency and IDA on cellular and humoral immunity in humans and animals.[14] The impact of iron deficiency on immune system is investigated intensively by different groups. Some researchers have shown that iron deficiency mainly affects the function of the lymphocytes and others showed that iron deficiency primarily affects the number of lymphocytes rather than their functions.[8] One study showed that the percentage of TCD4 lymphocytes and TCD4/TCD8 ratio in children with IDA was significantly decreased compared to the control group.[15] Another study on non-pregnant premenopausal women with IDA showed that absolute count of T-cells, TCD4, and TCD8 cells were significantly decreased in comparison to the control group; however, TCD4/TCD8 ratio was not significantly different between the two groups.[16] The study of Ekiz and colleagues was conducted in Turkey on 2005 to investigate the effect of iron deficiency on immune system function and showed no significant difference in the percentage of CD3/CD4, CD3/CD8, and CD3/CD19 lymphocyte subsets between patients with IDA and the control group.[8] Van Heerden and colleagues in 1981, in line with the findings of Ekiz et al. indicated no abnormality in percentage of B-cells and T-cells neither in children with IDA nor in those only with ID.[17]

We found a significant association between TCD8 with Tfs in students with IDA. In other words, reduced Tfs led to increased TCD8 percentage. No significant difference was found between Tfs with percentage of TCD4 and TCD4/TCD8 ratio between normal students with those with iron deficiency. In our study, there was a significant association between Tfs and percentage of TCD8 in the IDA group in comparison with NID, which was consistent with some studies,[8,17] but inconsistent with Karamati and colleagues' study.[16] Regarding the TCD4/TCD8 ratio, the results of our study were consistent with the findings of Karamati and colleagues' study.[16]

Differences in sample size, location where the study was performed and other factors that affect the immune system function such as zinc and vitamin A deficiency or infections may account for different results of this study which requires further investigation. In this study, the association between serum iron levels with percentage of TCD4 and TCD8 lymphocytes and TCD4/TCD8 ratio was assessed, which was merely a quantitative assessment of cellular immunity. Therefore, it is suggested to evaluate the relationship between iron saturation with T-cell function, B-cell count and function or humoral immunity in another study with a larger sample size.

Conclusion

The findings of our study showed that TCD8 percentage is increased in students with IDA along with reduced Tfs. Therefore, decreased Tfs may be associated with increased cellular immune response or other factors may be involved in the alterations in percentage of TCD4 and TCD8 cells, as well as TCD4/TCD8 ratio, which requires further investigations.

Conflict of Interest: None declared.

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