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ARAŞTIRMA / RESEARCH

T helper, cytotoxic T, and natural killer T cell profiles and their association with clinical prognosis in children with sickle cell anemia

Orak hücreli anemisi olan çocuklarda T helper, T sitotoksik ve doğal öldürücü hücre profili ve klinik prognozla ilişkisi

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Abstract

Purpose: Our aim was to determine the effects of ischemic attacks on T cell profiles, immune functions and clinical prognosis in patients with sickle cell anemia.
Materials and Methods: The study group consisted of 29 sickle cell anemia patients who were either in vaso-occlusive crisis or in steady state. Twenty-four age-matched healthy children served as the control group. All patients underwent complete blood cell count, hemoglobin electrophoresis, and blood chemistry analysis. Flow-cytometry was used to assess the T-cell profiles.
Results: The mean HbS in sickle cell anemia patients during vaso-occlusive crisis was 83±6.6%. The CD3 levels of patients in vaso-occlusive crisis (62.31±7.79%) were lower compared to steady state (65.53±5.72 %) and healthy controls (69.09±9.18%). The NK T cell percentages of patients in vaso-occlusive crisis (13.07±7.67%) were higher than the control group (8.11±4.67%).
Conclusion: Total T lymphocyte levels were found to be significantly lower in sickle cell anemia patients during vaso-occlusive crisis compared to healthy controls. NK T cell levels of the study group were higher than that of the control group.

Key words: Children, natural killer t cells, sickle cell anemia, T cell profile, vaso-occlusive crisis.

Öz

Amaç: Orak hücreli anemide iskemik atakların T hücre profiline, immun fonksiyonlara ve klinik prognoza etkisinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: Bu çalışmaya 29 orak hücreli anemi hastası çocuklar çalışmaya dahil edildi. Çalışma grubu vazo-oklüzif kriz ve kriz dışı döneminde olan hastalardan oluşmaktaydı. Kontrol grubu olarak aynı yaş grubunda 24 sağlam çocuk alındı. Tüm çocuklardan tam kan sayımı, hemoglobin elektroforezi, kan biyokimyası çalışıldı. T hücre profilini belirlemek için akım sitometri yöntemi kullanıldı.
Bulgular: Orak hücre anemili hastaların kriz dönemlerinde bakılan HbS ortalaması %83.8±6.6 bulundu. Orak hücre anemili hastaların kriz dönemlerinde bakılan CD3 değerleri (%62.31±7.79) aynı hastaların stabil dönemlerinde bakılan CD3 değerlerine (% 65,53±5,72) ve kontrol grubunda bakılan CD3 değerlerine (% 69,09±9,18) göre anlamlı olarak daha düşük bulundu. Orak hücre anemili hastaların kriz dönemlerinde bakılan doğal öldürücü T hücre değeri (%13.07±7.67) kontrol grubuna (%8.11±4.67) göre anlamlı olarak daha yüksek bulundu.
Sonuç: Çalışma sonucunda kronik hemoliz ve doku hipoksisi ile seyreden orak hücre anemili hastalarda toplam T hücre sayısını gösteren CD3 değerleri vazo-oklüzif kriz döneminde kontrol grubuna göre daha düşük saptandı.. Doğal öldürücü T hücre seviyeleri çalışma grubunda kontrol grubuna göre yüksek bulundu.

Anahtar kelimeler: Çocukluk çağı, doğal ödürücü T hücre,orak hücreli anemi, T hücre profili, vazo-oklüzif kriz.

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INTRODUCTION

Sickle cell anemia (SCA), first defined by Herrick in 1910, is one of the most common hemoglobinopathies in the World[1]. SCA results from a single amino acid substitution at position 6 of the β chain of hemoglobin. The substitution of valine (Val) for glutamate (Glu) results in Hemoglobin S (HbS), which polymerizes under low oxygen tension to distort red blood cells into a characteristic sickle shape. The presence of anemia in the complete blood cell count with reticulocytosis is typical for the disease and the occurrence of erythrocyte sickling is typical for the disease in peripheral blood. Sickling test should be performed in suspicious cases. The appearance of the HbS band in hemoglobin electrophoresis is diagnostic for SCA[2].

The two key features of SCA are chronic hemolytic anemia and vaso-occlusion. Vaso-occlusion leads to ischemia, infarction and ischemia-reperfusion injury in multiple organs and tissues. Progressive organ damage may affect any organ, with the brain, eyes, spleen, and the hepatobiliary, pulmonary, genitourinary and musculoskeletal systems being the most commonly involved sites[3].

The pathogenesis of vaso-occlusive crisis (VOC) in patients with SCA involves the sickling of the hemoglobin, hyper-inflammatory status and hemolysis. T cell-mediated immunity is impaired in SCA due to splenic dysfunction and the inactivation of the reticuloendothelial system[4]. The deregulation of the immune response and T cell subsets contribute to the development of VOC[5]. Besides having impaired T-cell mediated immunity, patients with SCA have also been shown to have higher levels of natural killer (NK) T cells. The increased level of NK T cells in SCA patients, even in the absence of VOC, is thought to play an important role in the pathogenesis of SCA. Recent studies demonstrate that NK T cells play an important role in the persistence of inflammation and in VOC[6]. Thus, NK T cell-targeted therapies are being investigated as alternatives to conventional treatment in patients with SCA[7-9]. We aimed to investigate this information in pediatric SCA patients by showing the association with clinical status and NK cells.

The purpose of this study was to determine the effects of ischemic attacks on T cell profiles, immune functions, and clinical prognosis. We investigated the effect of the clinical status (VOC or steady state) on immune functions in patients with SCA and compared our results with healthy controls. To the best of our knowledge, although there are some studies in the literature with adult SCA patients, this is the first prospective study about T and NK cells in SCA with only pediatric patients.

MATERIALS AND METHODS

The study was conducted in the Cukurova University, department of Pediatric Hematology in between January 2011 and December 2012. The study group consisted of 29 SCA patients in steady state and in VOC. These 29 patients with SCA diagnosed with hemoglobin electrophoresis and HbSS pattern presented in all patients. Painful crisis (VOC) is described as painful admission of SCA patients without any other significant pain causes. Patients who were presenting with painful crisis and hospitalized, defined as VOC period and blood samples were obtained in the first hour of admission. Same patients with VOC were called for steady state at least three months later after the end of crisis period. Patients who were hospitalized for VOC three months ago, invited to outpatient clinic for examination, and if they have not any complaint blood samples were obtained in this control. All patients were followed by same physician in both VOC and steady state period with the consultant of pediatric hematology. SCA patients who twenty-four age-matched healthy children with hemoglobin genotype AA served as the control group. SCA patients who without VOC and erythrocyte transfused within in three months were excluded.

All patients and healthy children underwent complete blood cell count, hemoglobin electrophoresis, and blood chemistry analysis. The blood samples were analyzed immediately after the blood was taken. Whereas, the blood samples for T cell subsets were obtained from SCA patients and healthy controls, were analysed within six hours in all samples. As far as possible immediately after the sampling, samples should be processed for immunophenotyping. Samples can be stored at +4°C in cases where the blood samples can not be analyzed immediately.

The immunological profile was assessed by flow-cytometry to find the levels of CD3 for total T

lymphocytes, CD4 for T helper cells, CD8 for cytotoxic T cells and CD16+56 for NK T cells in the mentioned groups. In patients with VOC, blood samples were obtained prior to treatment in first hour of admission to emergency department or outpatient clinic. Patients with SCA were questioned for a history of VOC, avascular necrosis, blood transfusion, erythrocytapheresis and use of hydroxyurea. Written informed consent was obtained from the parents of all patients participating in the study. The study was approved by ethical committee of Cukurova University Medical Faculty (20/01/2011-4/2).

Flow cytometric analysis

Flow cytometry was performed using original surface markers to investigate T cell levels in VOC and in steady state period and the control group. For this purpose; fluorescein isothiocyanate (FITC) conjugated anti-CD3, anti-CD4, allophycocyanin (APC) conjugated anti-CD45, Phycoerythrin (PE) conjugated anti-CD8, anti-CD16 + 56 (Becton Dickinson, San Jose, CA, USA) monoclonal antibodies and FACSCalibur (Becton Dickinson, San Jose, CA, USA) flow cytometry device were used.

Statistical analysis

All statistical analyses were performed using the SPSS statistical software (SPSS for Windows, version 19.0; SPSS Inc., Chicago, IL, USA). The data were expressed as means and standard deviation (mean±SD), median and range, n (number of patients) and percentages (%). The data were tested for normality using the Kolmogorov-Smirnov test and histogram. The Independent Samples t-test was used to compare data with normal distribution. Data with abnormal distribution were analyzed using the non-parametric Mann-Whitney U test. A p-value <0.05 was considered significant.

RESULTS

A total of 29 patients with SCA were enrolled in the study group, of whom 13 (44.8%) were male and 16 were (55.2%) female. The median age of the patients in the study and control groups were 11 (5-17) and 13 (5-17) years, respectively. The relevant medical history of patients are presented in Table 1.

The mean leukocyte count of patients in VOC (11,152±3,269/mm³) was higher than that of steady state patients (7,803±1,254/mm³) and healthy controls (7,191±1,807/mm³) (p<0.001). The mean hemoglobin (Hb) and hematocrit levels of patients in VOC were lower compared to patients in steady state and healthy controls (p<0.001). In hemoglobin electrophoresis, the mean HbS level in SCA patients during VOC and in steady state were found to be 83±6.6% and 72.3±8.2%, respectively (p<0.001).

C-reactive protein (CRP) is a well-defined biochemical marker which is widely used to assess inflammation. In our study, the CRP levels were found to be higher in patients in VOC (3.2±3.5 mg/dL) compared to steady state patients (0.2±0.1 mg/dL) and healthy controls (0.2±0.1 mg/dL) (p<0.001). The presence of frequent VOC attacks, hydroxyurea use, avascular necrosis and erythrocytapheresis had no significant effect on the levels of total T lymphocytes, T-helper cells, cytotoxic T cells and NK T cells (p>0.05). The number of blood transfusions received had no effect on the T-helper, cytotoxic T and NK T cell counts.

Table 1. Medical history of patients with SCA.

Variable	n (%)
VOC	
<1 time/year	5 (17%)
1-3 times/year	14 (48%)
≥4 times/year	10 (35%)
Avascular necrosis	7 (24%)
Hydroxyurea use[a]	23 (79%)
Erythrocytapheresis	17 (59%)
Blood transfusion	
No	6 (21%)
1-10 times	18 (62%)
>10 times	5 (17%)

VOC: vaso-occlusive crisis, [a]History of hydroxyurea use for at least 3 months at the time of VOC

The total T lymphocyte, T-helper, cytotoxic T, and NK T cell levels of the study and control groups were compared. Total T lymphocyte levels were found to be lower in SCA patients in VOC compared to the control group (p=0.007). The levels of NK T-cells during VOC and in steady state were found to be higher than that of the control group (p=0.009 and p=0.002, respectively). The immunological findings are shown in Table 2.

Table 2. Immunological findings.

Variables	SCA groups		Control group	p-value*
	VOC	Steady state		
	Mean±SD Median(Min-Max)	Mean±SD Median(Min-Max)	Mean±SD Median(Min-Max)	
CD3 (%)	62.31±7.79 61.80 (44.80-81.90)	65.53±5.72 66.10 (54.70-76.10)	69.09±9.18 70.30 (50.90-84.30)	0.007
CD4 (%)	36.41±8.05 37.10 (18.30-46.90)	37.68±5.68 37.10 (26.50-52.70)	37.92±7.01 38.80 (22.60-48.60)	0.689
CD8 (%)	27.52±6.06 26.90 (12.10-42.90)	27.81±6.17 29.60 (15.90-40.90)	29.76±4.98 29.60 (20.40-41.00)	0.359
CD16+56 (%)	13.07±7.67 11.50 (1.60-33.20)	12.71±5.62 11.40 (4.80-25.30)	8.11±4.67 7.10 (2.30-20.70)	0.009

VOC: vaso-occlusive crisis, *p value: VOC and control group

DISCUSSION

SCA is associated with a chronic inflammatory state, and the hyper-inflammatory response is characterized by elevated white blood cells (WBC), increased levels of inflammatory cytokines, and abnormal activation of endothelial cells[10,11].

In patients with SCA, leukocytosis is observed due to the adhesion of the neutrophils to the site of endothelial injury[12]. Bacterial infection together with leukocytosis is a known predisposing factor for VOC in patients with SCA. In addition, it has been shown that there is a statistically significant relationship between neutrophil levels and the clinical severity of SCA[13]. Most complications of SCA are associated with leukocytosis. Awougu et al. have found significantly higher WBC and mean total polymorphonuclear neutrophil counts in SCA patients in steady state compared to healthy controls[14]. Akinbami and Ojo reported that patients with homozygous sickle cell disease have higher values of WBC compared to healthy controls[15,16]. Omoti et al. found that the total WBC and differential counts of SCA patients in steady state and VOC were significantly higher than healthy controls[17]. In our study, patients in VOC were found to have higher WBC counts than patients in steady state and healthy controls. Leukocytosis is associated with poor prognosis and described as a risk factor for acute chest syndrome and cerebral vasculopathy[18-20].

High sensitive CRP (hs-CRP), a well-established inflammatory biomarker, was strongly associated with VOC, which is an important clinical endpoint of microvessel occlusion in SCA[21]. Krishnan et al. reported that hs-CRP showed an inverse correlation with Hb, suggesting that baseline hemolytic activity may be associated with inflammation[22]. Mohammed et al. reported that SCA patients had higher CRP levels during VOC compared to steady state[23]. Similarly, we found that CRP levels were higher in VOC than in steady state. This result may be explained by chronic inflammation and the infections accompanying VOC.

Although many studies about T cell subset levels were reported, there is still no consensus on the matter. SCA patients in steady state were found to have lower total T cell, T helper, and cytotoxic T cell levels than healthy controls[24]. Koffi et al. reported that total T cell and cytotoxic T cell levels were lower in SCA patients compared to the control group, but there was no significant difference between the T helper cell levels of the two groups[25]. On the other hand, Musa et al. found that T helper levels were lower during VOC compared to steady state and that the total T cell levels were similar during VOC and steady state. Adadeji et al. reported that, compared to healthy controls, patients in VOC had higher cytotoxic T cell levels and lower total T lymphocyte and T helper cell levels[26]. In our study,

total T lymphocyte levels were found to be significantly lower in patients with VOC compared to the control group (p=0.007).This result was in accordance with the previously mentioned reports by Koffi et al and Adadeji et al. The total T lymphocyte levels were found to be lower in SCA patients in steady state compared to healthy controls, but this result was not statistically significant. The asplenia arising from ischemia and infection as a result of the chronic sickling in SCA patients may explain the marked reduction in the T cell levels.

In their study on steady-state SCA patients under 20 years of age, Ojo et al found that these patients had lower T helper cell levels compared to healthy controls[16]. In our study, though the levels of T helper and cytotoxic T cells were found to be lower in VOC compared to steady state and control groups, it was not statistically significant. However, T helper cell and cytotoxic T cell levels were similar between the steady state and control groups.

Wong et al. reported that NK T cells were higher in SCA patients compared to the control group[27]. In contrast to these results, Kaplan et al. reported that NK T cell levels were similar between non-transfused SCA patients and healthy controls[28]. In our study, NK T cell levels of both VOC and steady state groups were found to be significantly higher than the control group. In this context, we believe that even if erythrocytes are in a stable condition, NK T cell activation still occurs with the effect of various inflammatory cytokines. In the literature, no association was found between the frequency of crisis and T cell levels[29]. Also, Kaplan et al. reported that NK T cells were lower in transfused SCA patients compared to healthy controls[28]. These findings may be indicative of a suppression of the inflammatory response. In our study, no significant relationship was found between the number of blood transfusions and total T lymphocyte, T helper, cytotoxic T, and NK T cell levels. A history positive for avascular necrosis, erythrocytapheresis, frequent crises and hydroxyurea use was found to have no significant effect on total T lymphocyte, T helper, cytotoxic T, and NK T cell levels. In the light of these findings, we believe that the increase in NK T cell levels and total T cell depletion affect the clinical prognosis. The increased level of NK T cells in SCA patients, even in the absence of VOC, is thought to play an important role in the pathogenesis of SCA. A recent study showed statistically significant increases in the levels of T cells in patients with SCA who underwent erythrocytapheresis[30].

Treatment strategies for patients with SCA are currently limited to supportive care with the prophylactic use of antibiotics to prevent infections, fluids, pain management, transfusion therapy, and hydroxyurea[2]. With current treatment strategies, the mortality and morbidity rates of patients with SCA still remain high. Therefore, recent clinical trials aim to develop new treatment strategies by illuminating the pathogenesis of SCA. In this study, we tried to determine the levels of T cells (especially NK T cells) in SCA patients with or without VOC and compared the results with healthy controls. This way, we aimed to contribute to the understanding of the pathogenesis leading to chronic inflammation in SCA.

In conclusion, children with SCA may have decreased T cell levels and increased NK T cell levels. This study is limited by its small sample size. For this reason, these findings cannot be generalized. Whereas, there are many studies on T lymphocyte subset levels in SCA and there is still no consensus on the subject. We would like to point out the clinical significance of T lymphocyte subset levels, and especially NK T cells. Further prospective studies involving a larger pediatric SCA patient population are needed to clarify the role of T cells in SCA.

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