RESEARCH ARTICLE

Journal of Anatolian Medical Research
JAMER 2023; 8(4):60-6
DOI:10.55694/jamer.1391740

The Relationship of Prognostic Factors with Regulatory T Cells in Langerhans Cell Histiocytosis

Langerhans Hücreli Histiositozda Prognostik Önemi Olan Faktörlerin Düzenleyici T Hücreleri ile İlişkisi

Ganime Çoban[1], Büşra Çoşanay Tekden[1], Beril Güler[1], Fatma Betül Çakır[2], Özlem Toluk[3], Zühal Gücin[1], Şahande Elagöz[1]

[1]Department of Pathology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[2]Department of Child Health and Diseases, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[3]Department of Biostatistics and Medical Informatics Bezmialem Vakif University Faculty of Medicine, Istanbul, Turkey

ÖZET

Amaç: *Langerhans hücreli histiositoz (LHH), dentritik hücrelerin anormal proliferasyonu ile karakterize klonal bir hastalık olup, yoğun inflamatuar bir mikroçevreye (TM) sahiptir. TM'nin, nadir görülen bu hastalığa olan katkısı hakkında bilgiler sınırlıdır. Bu çalışmada, TM deki regülatuar T hücreleri ve $BRAF^{V600E}$ mutasyonunu prognostik veriler ile karşılaştırmayı amaçladık.*

Gereç ve Yöntemler: *Çalışmaya 26 olgu dahil edildi. Olguların yaş, cinsiyet, lokalizasyon, tekli-çoklu lokalizasyon, riskli organ tutulumu ve nüks durumlarına göre immünohistokimyasal olarak FOXP3+ regülatuar T hücre (Treg) sayısı ve $BRAF^{V600E}$ mutasyon varlığı değerlendirildi.*

Bulgular: *Çalışmada yetişkin olgu sayısı çocuk sayısından daha fazla idi. En sık lokalizasyon kemik olup, olguların %81'i tek lokalizasyonlu idi. Riskli organ tutulumu ise 3 olguda mevcut olup, bunlardan ikisinde nüks görüldü. FOXP3+ Treg hücrelerinin sayısı; yetişkinlerde, tek lokalizasyon tutulumu olanlarda ve kemik tutulumu olanlarda yüksek olarak bulundu. Ayrıca nüks olan grupta FOXP3+ hücre sayısı nüks olmayan gruba göre daha yüksekti. $BRAF^{V600E}$ mutasyonu çocuklarda yetişkinlere oranla daha yüksekti ($p=0.003$), fakat prognostik parametreler ile karşılaştırıldığında istatiksel olarak anlamlı sonuç bulunamadı ($p>0.05$).*

Sonuç: *$BRAF^{V600E}$ mutasyon varlığı çocuk hastalarda daha sık olmakla birlikte yetişkin hastalarda da görülmektedir. FOXP3+ Treg sayısı CD3+ ve CD4+ T hücre sayıları ile orantılıdır. TM de yer alan farklı oranlara sahip T hücreleri LHH patogenezinde önemli yere sahiptir.*

Anahtar Kelimeler: *Langerhans, histiositoz, düzenleyici, FOXP3, BRAF, mutasyon*

ABSTRACT

Aim: *Langerhans cell histiocytosis (LCH), is a clonal disorder characterized by abnormal proliferation of dendritic cells, which has an intense inflammatory microenvironment. There is limited information about contribution of microenvironment to this rare disease. We aimed to compare regulatory T cells in microenvironment and $BRAF^{V600E}$ mutation with prognostic data.*

Material and Methods: *Overall, 26 cases were included to the study. The number FOXP3+ regulatory T cell (Treg) and presence of $BRAF^{V600E}$ mutation were assessed according to age, gender, localization, unifocal or multifocal, involvement of organ at risk, and recurrence status in a histochemical manner.*

Results: *The number of adult cases was higher than pediatric cases. Bone was the most common localization, and 81% of cases were unifocal. Risk organ involvement was observed in 3 cases, 2 of which showed recurrence. It was found that the number of FOXP3+ Tregs was higher in adults, those with unifocal localization, and those with bone involvement. In addition, the number of FOXP3+ Tregs was higher in the group with recurrence than those without recurrence. $BRAF^{V600E}$ mutation was higher in children when compared to adults ($p=0.003$), but, no significant correlation was found when compared with remaining prognostic parameters ($p>0.05$).*

Conclusion: *Although $BRAF^{V600E}$ mutation is more common in pediatric patients, it can also be seen in adult patients. The number of FOXP3+ Tregs is proportional to CD3+ and CD4+ cells. T cells, which present at varying rates in microenvironment, play an essential role in the pathogenesis of LCH.*

Keywords: *Langerhans, histiocytosis, regulatory, FOXP3, BRAF, mutation*

Corresponding Author: Ganime Çoban, Department of Pathology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey ***email:*** drgcoban@hotmail.com
Cite this article as: Çoban G, Coşanay Tekden B, Güler B, Çakır F.B, Toluk Ö, Gücin Z, Elagöz Ş. The Relationship of Prognostic Factors with Regulatory T Cells in Langerhans Cell Histiocytosis. JAMER 2023;8(4)60-6.

Received: 17.11.2023
Accepted: 20.11.2023
Online Published: 05.12.2023

INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare disease characterized by abnormal proliferation of myeloid dendritic cells (1,2). Although it is primarily a childhood disease, it can also be seen in adults. Its annual incidence is estimated as 1-1.5 cases per million people (3,4). LCH has a wide clinical spectrum from lesions with spontaneous recovery to chronic disease, and even it may be life-threatening (3). It most commonly involves bone and skin, followed by lymph nodes, lungs, bone marrow, spleen, liver, and brain. The liver, spleen, and bone marrow involvements are associated with the highest risk, and mortality is around 15% in case of liver, spleen, or bone marrow involvement. LCH can be seen in one or more than one organs and can be unifocal or multifocal (2,5,6). Skull involvement is also considered as a risky localization since it comprises risk for diabetes insipidus and degenerative disease (7,8). LCH manifests as an inflammatory lesion in any tissue. Classical morphology includes Langerhans cells and an inflammatory microenvironment. The Langerhans cells are immature dendritic cells originating from early myeloid cursors, which have a "coffee bean-shaped" nucleus and abundant eosinophilic cytoplasm. These cells exhibit immune reaction with Langerin (CD207), CD1a, CD68, and S100 (4,6). In LCH, many immune cells are present together with Langerhans cells, including T cells, neutrophils, eosinophils, B cells, plasma cells, myeloid-derived suppressor cells, macrophages, mast cells, and multinuclear giant cells. Currently, it remains unclear whether these cells play a role in the pathogenesis of LCH and the origin of the mechanism underlying such accumulation (9).

Although viral infectious agents were proposed in the etiology, the immune system is mainly implied in the etiology today (10). Since the disease is a rare entity manifesting with different clinical presentations, there is relatively limited information regarding contribution of tumor microenvironment to the disease pathogenesis. T cells in the tumor have a pivotal role in cell-mediated immunity, and it is well known that they have several potential roles in the LHH pathogenesis (11). The role of the regulatory T cells (Tregs) in the immune escape has become the focus of interest, given that Tregs have a wide effect network (1, 6). Forkhead box protein 3 (FOXP3) is an "inhibitor trans cription factor" present in Tregs and plays a role in T cell activation. Tregs is an immunosuppressive subpopulation of tumor-infiltrating lymphocytes (TIL), which decrease the activation of traditional T cells expressing FOXP3 (9). The $BRAF^{V600E}$ is a pivotal kinase of the RAS-RAF-mitogen activated protein kinase (MPAK) pathway. It plays role in many cell functions, including cell proliferation and migration, and often undergoes mutation in several cancers. It has been proposed that $BRAF^{V600E}$ mutation, detected in 50-65% of LHH patients, supports the immune escape of tumor cells by disrupting immune surveillance (12).

In this study, it was aimed to determine demographic characteristics, recurrence rate, and $BRAF^{V600E}$ mutation status in pediatric and adult patients diagnosed as LHH; to evaluate distribution of CD3+, CD4+ T cells, and FOXP3+ Tregs; to address relationship between FOXP3+ Tregs, CD3+ and CD4+ T lymphocytes; to investigate relation ship between these parameters with presence of $BRAF^{V600E}$ mutation and prognosis and their differences in pediatric and adult patients. This will contribute investigation of role of immune system in the disease pathogenesis and T cell involvement in the disease mechanism. Although data from pediatric patients are commonly addressed in the literature, our data will contribute limited information on adult patients.

MATERIAL and METHODS

Study Population

We identified patients diagnosed with LHH between January 2014 and September 2022 from pathology archives using the electronic database of Bezmialem Vakif University. The study included 26 cases, regardless of localization and age, which underwent surgery in our hospital and had blocks and slides in pathology archives.

Based on localization at the time of diagnosis, cases were classified into 2 categories as unifocal and multifocal groups. The HE (hematoxylin-eosin) and immunohistochemical-stained slides were re-evaluated. In addition, CD1a, S100, CD68, and Langerin stained slides obtained at time of diagnosis were also re-evaluated, confirming the diagnosis. Blocks with adequate tumor tissue were selected to use in immunohistochemical staining. The patient information was retrieved from the electronic database of our hospital. This study was conducted in accordance with the principles of the Declaration of Helsinki. The study was approved by the ethics committee board of Bezmialem Vakif University (2022/357).

Immunohistochemistry

We obtained 4-µm sections from formalin-fixed paraffin blocks with sufficient tumor tissue. The sections were mounted on to slides, which, then, deparaffinized. The slides were subjected to FOXP3 (EP340, EPITOMICS, 1/100 dilution, USA), CD3 (2GVG, Ventana/Roche, ready-to-use, Germany), CD4 (EP204, EPITOMICS, 1/100 dilution, USA) and $BRAF^{V600E}$ (VE1, Ventana/Roche, ready-to-use, Germany) monoclonal antibodies using Ventana Benchmark Ultra and Bench Mark XT automated staining devices. Tonsil tissue was used as the control for CD3 and CD4, whereas Hodgkin lymphoma tissue for FOXP3 and papillary thyroid carcinoma tissue with molecularly proven $BRAF^{V600E}$ mutation for $BRAF^{V600E.}$

Immunohistochemical Staining Assessment

IHC staining was evaluated by two experienced pathologists. Stained specimens were examined using a com-

puterized system consisting of a light microscope (Nicon Eclips).

For immunohistochemical CD3, CD4, and FOXP3 assessment, 3 most densely stained areas were identified at low power magnification (40x). The photographs of the areas identified were captured at high magnification (400x), and cells with CD3, CD4, and FOXP3 expression were counted. Cytoplasmic and membranous staining for CD3 and CD4 were evaluated. Nuclear staining for FOXP3 was evaluated. Tumor cells with strong membranous and cytoplasmic staining were assessed for *BRAF*$^{V600E.}$

All staining results were compared with prognostic parameters such as age, gender, localization, unifocal/multifocal involvement, and recurrence status.

Statistical Analysis

Descriptive statistics were given with median (minimum-maximum) and frequencies with percentages. Comparisons were made with Fisher's exact test, Pearson Chi-square test, and Mann-Whitney U test. SPSS (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp) software was used for analysis. The type-I error rate was taken α=0.05.

RESULTS

Patient Demographics

In this study, data from 26 patients (12 male, 46.2% and 14 female 53.8%) were evaluated. The mean age was 21.85±16.65 years. Of the patients, 12 (46.2%) were aged <18 years, while 14 (53.8%) were aged >18 years. The most common localization was bone (22; 84.6%), followed by lymph nodes (4, 15.3%), skin (3, 11.5%), liver (2, 7.7%), lung (1, 3.8%), bone marrow (1, 3.8%) and adrenal gland (1, 3.8%). The skull was the most commonly involved osseous site, followed by iliac bone, rib, and scapula. There were 3 cases with risky organ involvement, 2 of which had a recurrence. No *BRAF*^{V600E} mutation was in 3 cases with recurrence. Table 1 summarizes demographic, clinical, and pathological data according to age groups (Table 1). No significant difference was detected in clinical and demographic characteristics between age groups (p>0.05). In male cases, number of patients with bone involvement alone (11 cases, 91.7%) was higher than those with bone plus organ involvement (1, 8.3%) while in female cases number of patients with bone involvement (8 cases, 57.1%) and those with bone plus organ involvement (6 cases, 42.9%) was comparable (p=0.81).

BRAF^{V600E} Mutation Status

BRAF^{V600E} mutation was detected in 9 cases (34.6%). The *BRAF*^{V600E} mutation positivity was higher in pediatric patients (8 cases, 66.7%) when compared to adult patients (p=0.003) (Table 1). There was bone involvement alone in 8 cases with mutation (88.9%), all of which were pediatric cases. On the contrary, bone plus lymph node involvement was involved in one case with *BRAF*^{V600E} mutation in adults. No *BRAF*^{V600E} mutation was observed in 3 cases with risk organ involvement. Recurrence was observed in one of 8 pediatric cases with *BRAF*^{V600E} mutation. No significant correlation was observed between *BRAF*^{V600E} mutation and recurrence (p=0.628). The numbers of CD3+, CD4+, and FOXP3+ were higher in the group with *BRAF*^{V600E} mutation when compared to the group without *BRAF*^{V600E} mutation (Table-2). No significant difference was detected in *BRAF*^{V600E} mutation groups according to parameters (p>0.05).

Table 1. Distribution of pediatric and adult cases, demographic characteristics, clinical data and results of immunohistochemical staining. Data were presented with frequencies with percentages and median (min-max).

	Child (<18 years) n (%) n=12	Adult (>18 years) n (%) n=14	p
Gender			
Female	5 (41.7%)	9 (64.3%)	0.249
Male	7 (58.3%)	5 (35.7%)	
Localization			
Bone	10 (83.3%)	9 (64.3%)	0.391
Bone plus organ	2 (16.7%)	5 (35.7%)	
Tumor involvement			
Single localization	10 (83.3%)	11 (78.6%)	1.000
>2 localization	2 (16.7%)	3 (21.4%)	
Risky organ involvement			
No	10 (83.3%)	13 (92.9%)	0.580
Yes	2 (16.7%)	1 (7.1%)	
	(n=11)	(n=11)	
Skull involvement	8 (72.7%)	5 (45.5%)	0.387
Other bone involvement	3 (27.3%)	6 (54.5%)	
Follow-up duration (months)	34.0 (0-84)	30.0 (0-93)	0.817
Time to recurrence (months)	n=4 15.0 (6-25)	n=1 20 months	0.480
Recurrence			
Yes	4 (33.3%)	1 (7.1%)	0.148
No	8 (66.7%)	13 (92.9%)	
BRAF^{V600E} mutation			
Yes	8 (66.7%)	1 (7.1%)	0.003
No	4 (33.3%)	13 (92.9%)	
FOXP3^{+} T cells	12 (1-51)	22.5 (1-200)	0.570
CD4^{+} T cells	230.0 (15-1250)	365.0 (35-790)	0.503
CD3^{+} T cells	296.5 (86-1450)	505.5 (128-1500)	0.700

FOXP3: *Forkhead box protein 3*

Table 2. Comparison of *BRAF*^{V600E} mutation with other parameters Data were presented with frequencies with percentages and median (min-max).

	BRAF^{V600E} Mutation		p
	No (n=17)	Yes (n=9)	
Gender			
Female	10 (71.4%)	4 (28.6%)	0.683
Male	7 (58.3%)	5 (41.7%)	
Localization			
Bone	11 (%57.9)	8 (%42.1)	0.357
Bone plus organ	6 (%85.7)	1 (%14.3)	
Skull involvement	(n=13) 7 (53.8%)	6 (66.7%)	0.674
Other bone involvement	6 (46.2%)	3 (33.3%)	
Tumor involvement			
Single localization	13 (76.5%)	8 (88.9%)	0.628
>2 localization	4 (23.5%)	1 (11.1%)	
Recurrence			
Yes	13 (61.9%)	8 (38.1%)	0.628
No	4 (80.0%)	1 (20.0%)	
Risky organ involvement			
No	14 (82.4%)	9 (100.0%)	0.529
Yes	3 (17.6%)	0 (0.0%)	
FOXP3	25.0 (1.0-200.0)	10.0 (1.0-45.0)	0.317
CD4	330.0 (15.0-790.0)	250.0 (60.0-1250.0)	0.646
CD3	411.0 (86.0-1500.0)	313.0 (160.0-1450.0)	0.746

FOXP3: *Forkhead box protein 3*

FOXP3 Treg, CD3+ and CD4+ Cell Rates

It was found that the median number of CD3+ T lymphocytes was 520.5 (86.0-1500.0), while the median number of CD4+ T lymphocyte and FOXP3+ Tregs were 377.9 (15.0-1250.0) and 25.5 (1.0-200.0) (Figure 1). Figure 1 compares the number of FOXP3+ cells, CD3+ T cells, and CD4+ T cells. The FOXP3+ cells comprised 4.89% of CD3+ T cells and 6.74% of CD4+ T cells. The median number of FOXP3+ cells was higher in patients aged >18 years when compared to pediatric patients aged <18 years (17). The number of FOXP3+ cells was classified according to bone involvement, bone plus organ involvement, unifocal/multifocal localization, and risky organ involvement (Table 3). There was no significant difference in CD4+, CD3+, and FOXP3+ cells according to recurrence status (p=0.068 and p=0.204, respectively).

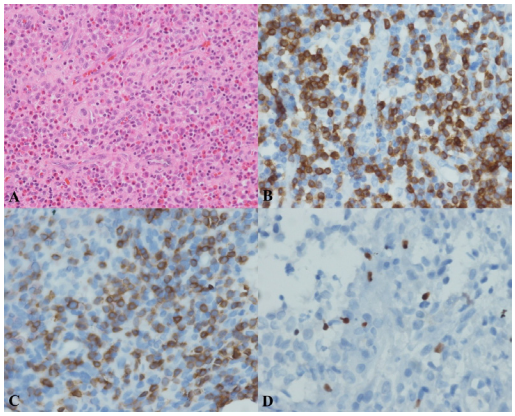

Figure 1. Appearance consistent with Langerhans Cell Histiocytosis with a rich background of eosinophil leukocytes (A, H&E X 200), Membraneous positive staining in lymphoid cells with CD3 and CD4 performed immunohistochemically (brown color positive staining) (B,C,X200) Nuclear positive staining in lymphoid cells with FOXP3 performed immunohistochemically (brown color positive staining) (D X200).

Table 3. Comparison of number of FOXP3+ Tregs with prognostic parameters. Data were presented with frequencies with percentages and mean (min-max).

	Number of FOXP3+ Tregs (n=26)	**p**
Gender		
Female	19.5 (1-200)	0.661
Male	15.0 (1-60)	
Localization		
Bone	17.0 (1-200)	0.561
Bone and organ	7.0 (1-33)	
Skull involvement		
Other bone	17.0 (4-200)	0.087
involvement	2.0 (1-51)	
Tumor involvement		
Single localization	17.0 (1-200)	0.493
>2 localization	7.0 (1-30)	
Recurrence		
Yes	17.0 (1.0-200.0)	0.794
No	14.0 (1.0-33.0)	
Risky organ involvement		
No	17.0 (1-200)	0.519
Yes	7.0 (1-30)	

FOXP3: *Forkhead box protein 3*

DISCUSSION

LCH is a histiocytic disease accompanied by intense inf-

lammatory cells, where mutations are observed in MAP/RRK pathway. The World Health Organization (WHO) accepted as a hematopoietic neoplasm after demonstrating $BRAF^{V600E}$ mutation and its clonality (3). The detection of $BRAF^{V600E}$ mutation in 50-65% of cases and mitogen activated protein kinase (MAP2K1) mutations in 10-20% of cases has indicated that RAS/RAF/MEK/RK signal pathway (1,6). The presence of this pathway has been considered as a strong evidence indicating that the disease is a neoplasia rather than reactive process (12). Several factors, including age, localization, risky organ involvement, and multi-organ involvement have influence on the treatment and prognosis of disease; thus, in this study, we addressed the effects of cells in the tumor microenvironment and $BRAF^{V600E}$ mutation on prognostic factors.

The tumor microenvironment is critical to develop and maintain an effective anti-tumor immune response. Today, it is widely accepted that tumor and microenvironment are functionally linked (12). There is limited information regarding contribution of tumor microenvironment to this rare disease (4,13). In previous studies, the rates of CD3+, CD4+, and CD8+ T cells were studied, showing that number of CD4+ cells was higher than CD8+ cells (6,9). In our study, CD4+ cells comprised 72.6% of CD3+ cells. In the literature, this rate was reported as 74% and 80% in agreement with our study (6,9). Again, number of CD3+ and CD4+ cells were comparable among pediatric and adult cases in agreement with literature (6). Based on this finding, it has been thought that T lymphocytes in tumor microenvironment have similar contribution to disease development in pediatric and adult patients.

The factors suppressing tumor microenvironment include Foxp3+ Tregs, myeloid-derived suppressor cells, tumor-related macrophages and programmed cell death (PD) as well as some immunosuppressive molecules such as PD-Ligand-1. There are studies reporting that higher level of FOXP3+ Tregs are poor prognostic factors in some cancer types and autoimmune diseases such as rheumatoid arthritis or SLE (6,14).

In their study, Parades et al. found that mean number of FOXP3+ was 30.9±24.8, reporting that the FOXP3+ cells are the second most common infiltrative immune cell in tumor microenvironment (6). In another study, it was reported that FOXP3+ Tregs comprised 20% of CD3+ T lymphocytes (10). On contrary, median number of FOXP3+ was around 5% of median number of CD3+ cells in our study. However, flow cytometry in peripheral blood was used in the studies reporting higher rates. Thus, it was concluded that the differences in FOXP3+ Tregs rates are due to different methods, such as peripheral blood and cell counting methods. Tong et al. reported that FOXP3+ Treg rate was higher in LCH when compared to healthy tissue while Senechal et al. reported that FOXP3+ Treg rate was higher within lesions in the pediatric population (2,10). On contrary to the study by Senechal et al., FOXP3+ Treg rate was found to be higher in adult patients compared to pediatric patients. Given the immunosuppressive effects of FOXP3+ Tregs, it was found that the recurrence rate was higher in pediatric patients, although it is anticipated that recurrence would be higher in adult patients. This suggests that immune regulation differs in the LCH pathogenesis of adult and pediatric patients and that other pathogenetic factors are effective in recurrence. More clearly, there is a positive correlation between FOXP3+ Tregs and whole lymphocytes (1,2). This supports the key role of FOXP3+ Tregs in the LCH pathogenesis.

The intensity of inflammatory infiltrate is associated with both increase in the amount of antigen in the lesion and the severity of an individual's defense mechanism (15). This may explain the differences in the defense mechanisms of pediatric and adult patients.

Another hypothesis for LCH pathogenesis is accumulation of Tregs with contact to Langerhans cells and that the contact inhibits immune response that may develop against Langerhans cells. It has been proposed that this contributes to cell survival, granuloma formation, and disease progression (6).

In LCH, there is a cytokine media suggesting that Langerhans cells and T cells contribute localized inflammation by cross-talk between these cells (6,11,13). In LCH, ERC pathway is continuously active together with inflammatory in tumor microenvironment. The fact that mutations aren't necessarily present in all LCH suggests that cytokine media may play role in the activation of ERK pathway (11). Due to such activation, pro-inflammatory cytokines such as tumor necrosis factor, interferon-gamma, interleukin 1-beta, interleukin 2 and granulocyte macrophage colony stimulating factor are increased in cytokine media, leading cytokine storm (11). It has been proposed that cytokine storm is highly effective in the onset of LCH and organ damage (6,11).

Among these cytokines, TGF-ß ad IL-10 are primarily produced by Langerhans cells and macrophages as well as Tregs. Thus, Tregs in LCH lesions are considered as a major component in the LCH pathogenesis. In addition, FOXP3+ Tregs can inhibit anti-tumor immune responses by releasing inhibitor cytokines, producing a suppressive microenvironment that protects tumor cells from immune surveillance through these mechanisms (6,9,13).

It is important to improve our understanding about LCH pathophysiology in order to achieve better management of the disease. LCH can progress with spontaneous remission; however, it may also present as more aggressive and even fatal disease as well (9). This may differ lesion localization, unifocal/multifocal involvement status and presence or absence of solid organ involvement (2).

In our study, bone involvement was most common in agreement with literature. In the study by Nagarjun et al., all 7 patients (mean age: 34.9 years) had pulmonary lesions (16). Although it was reported that LCH is more frequently seen in pediatric age group in the literature, there was 14 adult patients (mean age: 34.5%) in our study and only case with pulmonary involvement was a pediatric patient. The liver, spleen and bone marrow involvement is associated with high risk, leading mortality of 15% (1). In our study, risky organ involvement was detected in 3 cases, 2 of which had recurrence.

It has been suggested that *BRAF*^{V600E} mutation leads disruption of endogenous host immune surveillance, immune escape of tumors, and anti-apoptotic activity (7,10). In many studies, it was reported that *BRAF*^{V600E} mutation is more frequent in pediatric patients among LCH cases (2,6,10,12,16). In our study, *BRAF*^{V600E} mutation was found to be significantly higher in pediatric patients when compared adult patients (p=0.003) in agreement with literature.

In a study evaluated *BRAF*^{V600E} mutation using Sanger sequencing and immunohistochemistry (IHC), Sanger sequencing and IHC results were found to be compatible in 94.8% of the cases (65 adult and 32 pediatric cases) (10). In our study using IHC, *BRAF*^{V600E} mutation was detected in 9 cases (34.6%), 89% of which were pediatric cases. In their study, Tong et al. reported no *BRAF*^{V600E} mutation in adult patients(2). On contrary, *BRAF*^{V600E} mutation was detected in one adult patients. In a study on pediatric patients alone, *BRAF* ^{V600E} mutation was detected in 15.8% of the cases and in 66% of the cases with recurrence. Authors found a significant correlation between *BRAF*^{V600E} mutation and recurrence (14). In our study, among 9 cases with mutation, recurrence was detected in one adult case while involvement at >1 localization was detected in one case. No *BRAF*^{V600E} mutation was detected in 3 cases with risky organ involvement.

The association between *BRAF*^{V600E} mutation status and tumor-mediated immunosuppression strategies has been investigated and it was found that FOXP3+ Tregs were significantly higher in cases with *BRAF*^{V600E} mutation when compared to those with out (12,17,18). In our study, the number of FOXP3+ cell was lower in cases with mutation when compared to those without. However, no significant correlation was detected between *BRAF*^{V600E} mutation and FOXP3+ Tregs (p=0.317). We concluded that this may be due to limited sample size. In addition, FOXP3+ Tregs are associated with poor prognosis in many tumors; however, no significant correlation was detected in multivariate survival analysis (12).

The LCH cells harbor a few genetic data beyond *BRAF*^{V600E} mutation and show no progressive accumulation another oncogenic promoter in MAPK pathway (MAP2K1, ARAF) or repeated somatic mutations. Rather, it is thought that a high level of inflammatory infiltration suggests that extracellular immune stimuli may play an important role in the development of LCH lesions (5). The presence of FOXP3+ Tregs may present in all patients regardless of mutation and predisposition to recurrence is greater in the presence of *BRAF*^{V600E} mutation. Thus, the presence of *BRAF*^{V600E} mutation is important regarding use of *BRAF*-^{V600E} inhibitors in the targeted therapy of aggressive cases and may help identification of patients with predisposition to recurrence in the management of the disease.

LCH is assessed with clinical course, age, extent of the disease (one or more organ involvement, in other words single- or multi-system disease) and risky organ involvement (liver, spleen, hematopoietic system) (5). There are many therapeutic approaches in the management, including follow-up, surgery, intra-lesional corticosteroids, chemotherapy and/or radiotherapy, and *BRAF*^{V600E} inhibitors (3,19). The advantages of *BRAF*^{V600E} inhibitors include rapid response, which makes *BRAF*^{V600E} inhibitors choice of treatment of patients who requires rapid reversal of diffuse disease. However, withdrawal of treatment with *BRAF*^{V600E} inhibitors leads recurrences (3). The management of LCH is challenging in adult patients. This is due to lower incidence of the disease in this age group and, therefore, limited experience of clinicians (6). In addition, the presence of heterogeneous groups of inflammatory cells and cytokines and chemokines produced by tumor cells has brought immunotherapy to forefront in controlling inflammation and minimizing damage in the management of the disease.

Conclusion

There is no difference in T cell population between pediatric and adult patients. Tregs at varying rates in tumor microenvironment have an important role in the pathogenesis of LCH, and presence of FOXP3+ Tregs contributes persistency of disease. Although *BRAF*^{V600E} mutation is more frequent in pediatric patients, it is also seen in adult patients. Thus, *BRAF*^{V600E} mutation should be studied in all LCH patients in order to aid in diagnosis and treatment. Further studies with larger series are needed to better understand role of T cells in tumor microenvironment and *BRAF*^{V600E} mutation in the pathogenesis of LCH.

Ethics Committee Approval: The study was approved by the ethics committee board of Bezmialem Vakif University (2022/357).

Conflict of Interest: The authors declare no conflict of interest in this study.

Financial Disclosure: No financial support was received from any institution or organization for this study.

REFERENCES

1. Mitchell J, Jenée M., Stuart P. Berzins, Kannourakis G. A potentially important role for T cells and regulatory T cells in Langerhans cell histiocytosis. Clinical Immunology 2018; 194: 19-25.

2. Tong C, Jia X, Jia Y, He Y. Langerhans cell histiocytosis in Chinese adults: absence of *BRAF*^{V600E} mutations and increased FOXP3(+) regulatory T cells. Int J Clin Exp Pathol. 2014; 7: 3166-73.
3. Goyal G, Tazi A, Go RS, Rech KL, Picarsic JL, Vassallo, et al. International expert consensus recommendations for the diagnosis and treatment of Langerhans cell histiocytosis in adults. Blood. 2022; 139: 2601-21.
4. Mitchell J, Kvedaraite E, von Bahr Greenwood T, Lourda M, Henter JI, Berzins SP, et al. Plasma Signaling Factors in Patients With Langerhans Cell Histiocytosis (LHH) Correlate With Relative Frequencies of LHH Cells and T Cells Within Lesions. Front Pediatr. 2022; 29: 872859.
5. Halbritter F, Farlik M, Schwentner R, Jug G, Fortelny N, Schnöller T, et al. Epigenomics and single-cell sequencing define a developmental hierarchy in Langerhans Cell Histiocytosis. Cancer Discov. 2019; 910: 1406-21.
6. Paredes SEY, Almeida LY, Trevisan GL, Polanco XBJ, Silveira HA, Rab S, et al. Immunohistochemical characterization of immune cell infiltration in paediatric and adult Langerhans cell histiocytosis. Scand J Immunol. 2020; 92: e12950.
7. Gao XM, Li J, Cao XX. Signaling pathways, microenvironment, and targeted treatments in Langerhans cell histiocytosis. Cell Commun Signal. 2022; 19:195.
8. Senechal B, Elain G, Jeziorski E, Grondin V, Patey-Mariaud de Serre N, Jaubert F, et al. Expansion of regulatory T cells in patients with Langerhans cell histiocytosis. PLoS Med. 2007; 4: e253.
9. Mitchell J, Kannourakis G. Langerhans cell histiocytosis: A malignant myeloid neoplasm or disorder of immune regulation? Acta Paediatr. 2021; 110: 2888-91.
10. Zeng K, Wang Z, Ohshima K, Liu Y, Zhang W, Wang L, et al. *BRAF* ^{V600E} mutation correlates with suppressive tumor immune microenvironment and reduced disease-free survival in Langerhans cell histiocytosis. Oncoimmunology. 2016; 5: e1185582.
11. Mitchell J, Kelly J, Kvedaraite E, von Bahr Greenwood T, Henter JI, Pellicci DG, et al. Foxp3+ Tregs from Langerhans cell histiocytosis lesions co-express CD56 and have a definitively regulatory capacity. Clin Immunol. 2020; 215: 108418.
12. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in Caner: Can Treg cells be a new therapeutic target? Caner Science. 2019; 110: 2080-9.
13. Bergamini ML, Mardegan AP, DE Rosa CS, Palmieri M, Sarmento DJS, Nakamura Hiraki KR, et al. Presence of langerhans cells, regulatory T cells (Treg) and mast cells in asymptomatic apical periodontitis. Braz Oral Res. 2020; 34: e108.
14. Özer E, Yüzügüldü RI, Olgun HN, İnce DG, Sevinc A. RAF-MEK-ERK yolağı mutasyonları: pediatrik çağ Langerhans Hücreli Histiositozunda prognostik ve prediktif belirteçler. 27. Ulusal Patoloji Kongresi; 2017; Antalya. Güncel Patoloji Dergisi: 2017. SS30, 1-2.
15. Nagarjun Rao R, Chang CC, Tomashefski JF Jr. Lymphocyte sub-populations and non-Langerhans' cell monocytoid cells in pulmonary Langerhans' cell histiocytosis. Pathol Res Pract. 2008; 204: 315- 22.
16. Leslie C, Bowyer SE, White A, Grieu-Iacopetta F, Trevenen M, Lacopetta B, et al. FOXP3C T regulatory lymphocytes in primary melanoma are associated with *BRAF*^{V600E} mutation but not with response to BRAF^{V600E} inhibitor. Pathology 2015; 47: 557-63.
17. García Díaz MP, De Luca Sologaistoa A, De Paula Vernetta C, Moreno MA, Galeano Paniagua LA, et al. Langerhans cell histiocytosis of the head and neck in the pediatric population in a tertiary center: Clinical presentation, classification and treatment. Int J Pediatr Otorhinolaryngol. 2022; 155: 111073.
18. Heritier S, Emile JF, Barkaoui MA, Thomas C, Fraitag S, Boudjemaa S, et al. BRAF^{V600E} Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy. J Clin Oncol. 2016 1; 34: 3023–30.
19. Haupt R, Minkov M, Astigarraga I, Schafer E, Nanduri V, Jubran R, et al. Langerhans cell histiocytosis (LCH): guidelines for diagnosis, clinical work-up, and treatment for patients till the age of 18 years. Pediatr Blood Cancer. 2013; 60: 175-84.