RESEARCH ARTICLE

Detect the Amount of CD4+ Foxp3+ T-regulatory (Treg) Cell in Iraqi Patients with Behcet's Disease

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ABSTRACT

Background:- Behcet's disease (BD) (beh-CHETS) disease also called Behcet's syndrome. Characterized by urogenital ulcers, populopustular and erythema nodosum like skin lesion, uveitis, retinal vasculitis, thrombophlebitis, arterial aneurysms, and arthritis. Treg cells, whose primary roles are to reduce inflammation and decrease effector T cells, aid in maintaining the proper ratio between immunity and auto tolerance. The expression of CD4 + /CD25 + /Foxp3 + mostly identified them.

Objective: To detected the amount of CD4 + Foxp3 + Treg cells in BD patients and to asses their correlation to disease activity.

Patients and methods: This study was applied on 60 patients with BD compared to 30 healthy group as control group from December 2020 to the end of May 2021. Their blood samples were collected to investigate blood concentrations of CD4 + marker and Foxp3 + Treg by using flow cytometer technique.

Results: The (median) of CD4 + Tcells percentage for the patient group was 10.9%, significantly higher than that of the control group 8.30% ranged (0.80–17.30). While there was a highly significant increase in the (median) 26.20% of CD4 + Foxp3 + Tcells in the patients group in comparison with the control group median (4.65%). The Treg cells determined by the proportion of CD4 + Foxp3 + in all CD4 + Tcells showed a significant increase in the (median) for patient group (10.80%) if compared with the control group (8.50%) (p=0.045). A significant decrease CD4 + in monocyte was detected in patient group (8.50%) in comparison to control group (12.70%)(p=0.003).

Conclusion: Patients with BD had more CD4 + , Foxp3 + Treg cells than the control group did. Monocyte levels were lower in BD patients. There was a link between disease activity and Treg concentration.

Keywords: Iraqi patients, BD, Treg, CD4 + and Foxp3 + .

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INTRODUCTION

Behcet's disease (BD) (beh-CHETS), also called Behcet's syndrome, is a chronic auto-inflammatory, relapsing-remitting, multisystem syndrome. Urogenital ulcers, populopustular and erythema nodosum like skin lesion, uveitis, retinal vasculitis, thrombophlebitis, arterial aneurysms, and arthritis characterize it.[1-4] The pathogenesis of BD is assumed to be heavily influenced by immune system dysregulation, altered T cell balance, and in particular, the activation pathway of Th1/ Th17, which suppresses the function of T regulatory cells.[5,6] Treg cells, whose primary roles are to reduce inflammation and decrease effector T cells, aid in maintaining the proper ratio between immunity and auto tolerance. The expression of CD4 + /CD25 + /Foxp3 + mostly identified them.[7,8] Behcet's disease current activity form or the BD CAF form is a well-established tool for the assessment of BD activity in the clinic.[9] epending on the clinical features, accurate history, presenting within the month preceding the assessment date. And because clinical features in BD vary considerably over time;(BDCAF) is applied to document this variation.[10,11]

Patients and Methods

Between December 2020 and May 2021, research on the following groups was done.

- Total 60 patients with Behcet's disease by a specialist attending the Baghdad Medical city, Department of Medicine.
- Thirty healthy people with no other autoimmune disease age and sex were matched. A venous blood sample was collected, then splatted into two tubes using EDTA and used to detect CD4 and Foxp3 markers on T-regulatory cells using flow cytometry.

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Flow Cytometry and Monoclonal Antibodies

The flow cytometry for peripheral blood has been done with 24 hours maximum intervals. [FITC anti-CD4 (RPA-T4)], anti-Foxp3 (PE) were fluorescently labeled monoclonal antibodies (mAbs) acquired from (Biolegand, USA), (Elabscience, USA) and flow cytometry labeling has been done base on the methodology of Biolegend immunofluorescence staining.

Statistical Analysis

By analyzing Kruskal-Wallis test for comparison between different groups and with significant differences at ($p<0.05$).

RESULTS

The flow cytometry marker analysis was utilized to distinguish CD4 + T-lymphocytes in all groups under investigation in this study as shown in Table 1. The (median) of CD4 + Tcells percentage for the patient's group was 10.9% higher than that of the control group 8.30%, so there was a significant increase in the patient's group. It has been noticed that, the CD4 + Foxp3 + Tcells percentage in the patient's group was increased significantly if compared to the control group ($p<0.001$). The Treg cells determined by the proportion of CD4 + Foxp3 + in all CD4 + Tcells. Table 1 showed a significant increase in the patient group (10.80%) in comparison to control group 8.50% ($p=0.045$).

The monocyte cells determined by the presence of CD4 + T cell was significantly higher in the control group median (12.7%) compared to the patient group median (8.50%).

Table 2 shows a correlation between CD4 + Foxp3 + Treg cells and the scoring of BD patients. The median of CD4 + Tcells percentage for patient group was significantly increased in score 3. In contrast, there was a non–significant difference between CD4 + Foxp3 + Tcell and scoring disease activity in the patient's group ($p=0.805$). It also showed a highly significant increase in CD4 + Foxp3 + Treg cells percentage (11.60%) between BD patients at score 4($p<0.002$).

Table 1: Amount of regulatory (Treg) cell and monocyte cell percentage in study groups according to different markers

Percentage(%)	*Patients(60)*	*Control(30)*	*p-value*
⁎CD4 +	10.90(2.30-20.05)	8.30(0.80-17.30)	0.031*
⁎CD4 + Foxp3 + In CD4 + Tcells	26.20(7.90-68.50)	4.65(1.10-34.90)	<0.001**
⁎T-reg CD4 + Foxp3 + In CD4 + Tcells	10.80(2.30-19.80)	8.50(0.90-17.80)	0.045*
⁎CD4 + in Monocyte	8.50(4,40-18,90)	12.70(5.70-27.60)	0.003*

* = significant, NS = non-significant, ⁎ = Presented using their median (interquartile range)

DISCUSSION

The triggering variables in BD patients with genetic predispositions cause immune system malfunction. T-cells are the most common lymphocytes linked to the development of BD. The discovery of novel T-cell subpopulations has provided new light on BD etiology and various disruptions in T-cell homeostasis. T-regs, defined by the expression of CD4 + , CD25 + , and Foxp3 + , play a critical role in preventing autoimmunity and have been examined in various autoimmune illnesses.[12]

In the present study, we measured the amount and type of lymphocyte by the flow cytometry analysis. The CD4 + T-cell in the patient group significantly increased when compared to the control group. This result agree with many studies.[13-16] Since circulating CD4 + Tcells in patients with active BD tend to generate a Th1 response to appropriate mitogenic or antigenic stimulation.[17,18] In the present study, the level of CD4 + Foxp3 + Treg cells among BD patients showed a significantly higher median level difference ($p<0.001$) when compared to the control group. This result agreed with the study.[19] In other investigations, i.e., in patients who were clinically active, the CD4 + Foxp3 + Treg cells level was lower than healthy controls, but not in the inactive group, which explains why patients with low T-reg levels are classified as clinically active. Furthermore, some immunosuppressive drugs are likely to be more or less permissive of Treg formation or activity.[20,21] Other investigations have found an increased frequency of T-regs in BD patients' peripheral blood or cerebral fluid.[22,23] However, higher numbers of T-reg cells were observed in the active period of disease compared with the remission and healthy groups.[24,25] The significant decrease in CD4 + monocyte in BD patients ($p=0.003$) in this study may be due to the predominant role of innate immune response in BD pathogenesis. This result agreed with a study by Talita *et al.* in 2021 where they assessed that no associations were observed with the severity of clinical manifestation or therapy.[26] In the current study, the amount of CD4 + Tcells was significantly higher in score 3 of disease activity in BD patients. This result agree with many studies.[27-29] This is because the active BD-patients may have already developed predisposition to a Th1 response during the cell division on stimulation of T-cell.[11,30]

Table 2: Amount of cell percentage in BD patients according to different markers with disease activity

Percentage%	*Disease Activity*				*p-value*
	Score-2	*Score-3*	*Score-4*	*Score-5*	
⁎CD4 +	3.90 1.10 -11.20	11.50 6.20 -26.30	11.20 2.60-18.20	9.80 9.00-12.50	0.005*
⁎CD4Foxp3 In CD4 + Tcells	19.10 5.90 -70.80	24.45 6.90-48.50	29.45 10.50-68.80	27.00 18.40-36.40	0.805^{NS}
⁎ Treg CD4 + Foxp3 + In CD4 + Tcells	3.90 1.40-11.60	11.05 6.70-27.90	11.60 2.90-18.60	10.00 9.30-12.10	0.002*
⁎CD4 + in Monocyte	15.60 4.30-21.20	7.90 5.10-10.30	8.55 4.50-12.90	8.20 7.20-12.80	0.246^{NS}

* = significant , NS = non- significant, ⁎ = Presented using their median (interquartile range)

In the present study, the CD4 + Foxp3 + Treg cell level increased in score 3 of disease activity in BD patients this result agreed with many studies.[30-32] These cells can suppress immune responses.[33]

CONCLUSION

Patients with BD had more CD4 + , Foxp3 + Treg cells than the control group did. Monocyte levels were lower in BD patients. There was a link between disease activity and Treg concentration.

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