Assessment of T Regulatory cells in Egyptian patients with Behcet’s disease as a prognostic marker of Uveitis

Amal H. Eissa MD, Heba M. Selim MD, Abeer M. Zahran MD, Mohamed S. Tawfik PhD, Hussein S. El-Fishawy MD and Karam K. Naguib MD

*Clinical Pathology Department, Faculty of Medicine, Helwan University, Giza, Egypt; †Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Cairo, EGYPT; ‡Rheumatology and Rehabilitation Department, Faculty of Medicine, Cairo University, Giza, Egypt; §Health Radiation Research Department, National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority, Giza, Egypt; ¶Internal Medicine Department, Faculty of Medicine, Cairo University, Giza, Egypt; ††Ophthalmology Department, Nasser Institute Hospital, Giza, Egypt.

ABSTRACT

Behçet’s disease is an autoimmune type of vasculitis presenting by attacks of oral, skin and genital ulcers, as well as ophthalmic disorders that cause blindness in 25% of affected patients. Circulating T regulatory cells suppress atypical autoimmune responses and help developing immune self-tolerance. An X chromosome encoded forkhead transcription factor (Foxp3) is essential for proper T reg cell development and function. Aim of the work: The aim of the study was to determine the correlation between T reg cell percentage of expression, Behçet’s uveitis pathogenesis, and disease manifestations. Materials and Methods: Forty-four subjects were involved in this study: 15 subjects diagnosed with Behçet’s uveitis (including a female) in addition to 29 healthy age and sex-matched controls. The percentage of CD25 and Foxp3 expression in peripheral blood CD4+ T-lymphocytes was determined by flowcytometry. X-rays and magnetic resonance imaging (MRI) were used to assess patients with suspected Behçet’s arthritis. Percentages of CD4+CD25+Foxp3+ and CD4+CD25−Foxp3+ T reg cell expression were higher in Behçet’s uveitis patients compared to the controls without a significant difference. Conversely, CD4+CD25+Foxp3− T reg cell percentage was found to be higher in the controls than in Behçet’s uveitis cases without a significant difference. However, the mean fluorescence intensity of Foxp3 factor necessary for Treg cell function was significantly higher in the control group compared to Behçet’s uveitis cases (p = 0.02). Results of the current work indicate the protective role played by T reg cells (especially the Foxp3+ group) against Behçet’s uveitis that could be used as a valuable prognostic marker.

1. INTRODUCTION

Behçet’s Disease (BD) is recognized as a rarely occurring autoimmune multisystemic vasculitis disease of unidentified pathophysiology accompanied with substantial morbidity and mortality. It presents by recurring attacks of oral, skin and genital ulceration (Emmi et al., 2014; Zeidan et al., 2016). Musculoskeletal affection in the form of joint symptoms and signs such as arthralgia or arthritis is notable in more than 50% and almost up to 75% of Behçet’s disease patients, with 10% suffering joint disease as the first presentation (Bicer, 2012). After a transitory remission, relapsing episodes of varying intensity arise that are difficult to foresee, leading to various complications (Davatchi et al., 2017; Rokutanda et al., 2014).

This syndrome rarely presents with manifestations involving the cardiovascular system, the gastrointestinal tract and the central nervous system. Cardiac symptoms and signs comprise chest pain, myocardial infarction and heart failure, though these normally occur in a very minute portion of patients (Farouk et al., 2016; Hintenberger et al., 2018). Gastrointestinal disorders associated with BD can develop with nonspecific symptoms, such as abdominal bloating and spasms, nausea, vomiting, diarrhea and maybe acute gastrointestinal hemorrhage. Symptoms are usually obvious around 6 years after the first appearance of symptoms and signs (Kim & Cheon, 2016; Skee et al., 2015). Central nervous system affection may occur in the form of motor disorders such as chorea, dystonia or cognitive disorders (Cavaco et al., 2009; Kalra et al., 2014).

All eye compartments may become affected in BD; possible consequences might include anterior uveitis, posterior uveitis, panuveitis, cataract and retinitis. Ocular manifestations of BD, affecting the retina and the uvea, occur between 30% and 70% of BD patients, and they are linked to a high incidence of complications (Cunningham et al., 2017; Karadag et al., 2017). They are considered the prime etiologic loss of vision...
in around a quarter of patients despite thorough corticosteroid treatment (Hussein et al., 2018; Ucar-Comlekoglu et al., 2014).

Ocular symptoms are more commonly seen among males and are related to disease severity, even though prognosis has been recently improved with the current use of intensive chemotherapy. These symptoms usually appear 3 years after the start of oral or genital ulcers but continue to present with the primary disease features in up to 10–20% of patients (Cunningham et al., 2017; Hussein et al., 2018). Ocular affection may occur in the form of a chronic type of intermittent bilateral uveitis that encompasses the anterior or posterior segments, or even both of them, a condition called panuveitis that is linked to a poor prognosis (Ucar-Comlekoglu et al., 2014).

The MHC area on chromosome 6p21 comprises the human leukocyte antigen (HLA) and other important genes required for sound immune reactions (Trowsdale & Knight, 2013). Verity et al. (1999) stated that there was a relationship between HLA, TNF-alpha pleomorphisms and Behcet’s ocular disease (Verity et al., 1999).

Naturally occurring CD25+CD4+ regulatory T (Treg) cells constitute around 1–2% of total circulating peripheral mononuclear cells and jointly express the transcription factor Foxp3. These cells are crucial for safeguarding immune auto-tolerance and homeostasis by inhibiting unusual or unnecessary immune responses injurious to the host (Charbonnier & Chatila, 2018; Lee & Lee, 2018).

There is a considerable amount of studies that affirm that Foxp3, a forkhead transcription factor coded by the X chromosome, exercises an important influence on the maturity and functionality of Treg cells. Several pleomorphisms of human Foxp3 can cause inappropriate maturation or derangement of Treg cells and subsequently, a higher prevalence of immune dysregulation, multiple endocrine disease, enteropathy, X-linked syndrome that complements severe autoimmune diseases such as Behcet’s disease (Lu et al., 2017; Torgerson & Ochs, 2007).

2. AIM OF THE WORK

The current research aimed at exploring the correlation between the percentage of Treg cells (CD4+CD25−Foxp3+, CD4+CD25+Foxp3+ and CD4+CD25+Foxp3− T cells) expression, BD uveitis pathogenesis and disease severity and manifestations.

3. PATIENTS AND METHODS

3.1. Subjects

Forty-four subjects were recruited for this study: They included 15 patients with Behcet’s uveitis fulfilling the international study group (ISG) diagnostic criteria for Behcet’s disease. The ISG criteria for the diagnosis of Behcet’s disease include 5 items as follows: two of them are mucous membrane manifestations including oral aphthosis (OA) and genital aphthosis (GA). The third item is skin manifestations, comprising pseudo-folliculitis (PF) and erythema nodosum (EN). The fourth item is ocular manifestations. They are anterior uveitis (AU), posterior uveitis (PU), and retinal vasculitis (RV). The fifth item is the presence of a pathergy phenomenon (PP) which is detected by the pathergy skin test (Criteria for diagnosis of Behcet’s disease International Study Group for Behcet’s Disease, 1990).

The patients’ group included 1 female and 14 male participants whose ages ranged between 21 and 52 years. Their age at the onset of symptoms ranged between 6 and 47 years while their duration of affection was between 1 and 15 years. All patients were subjected to history taking, physical examination and laboratory investigations. Current disease activity was evaluated by means of Behcet’s Disease Current Activity Form (BDCF) (Choi et al., 2016). In addition, 29 healthy controls who were age and sex-matched to the cases were enrolled in this work.

X-ray radiography followed by magnetic resonance imaging (MRI) was used to diagnose and follow-up all participating patients with suspected Behcet’s arthritis. The radiographic criteria of Behcet’s arthritis searched for in these cases were mainly those related to non-erosive Behcet’s arthritis including minimal joint space narrowing and bony osteophytes as described by Park, 1999 (Park, 1999).

A complete eye examination was carried out for all participants and a diagnosis of uveitis was confirmed by ophthalmoscopic examination (often indirect) as well as slit-lamp examination after appropriate pupillary dilation.

3.2. Sample collection

Three milliliters (3 ml) of venous blood were carefully withdrawn from every participant into sterilized ethylene diamine tetra acetate (EDTA) vacutainer tubes under aseptic conditions for the measurement of CD25+ and Foxp3 expression on peripheral blood CD4+ T-lymphocytes using a flow cytometer (Beckman Coulter, FL, USA) and the blood was processed within a few hours from sampling.

Determination of the percentage of CD25 and Foxp3 expression on peripheral blood CD4+ T-lymphocytes by flow cytometry.

The procedure was carried out by using Phycoerythrin (PE)-conjugated anti-CD25 (eBioscience) and Flourescence isothiocyanate (FITC)-conjugated anti-Foxp3 (eBioscience) expressed on Phycoerythrin-cyanine 5 (PC5) conjugated anti-CD4 (eBioscience). Intracellular staining for Foxp3 was
carried out in accordance to the guidelines provided by the manufacturer using the Foxp3/Transcription Factor-Fixation/Permeabilization kit (eBioscience Catalog Number: 00–5521). The stained samples were mixed and analyzed by flowcytometry. Negative control samples were introduced in the machine. The auto-fluorescence region for FITC, PE and PC5 stains was adjusted for each sample (Figure 1).

### 3.3. Statistics

Data obtained from this study was presented as mean ± standard deviation. Evaluation of correlations between the percentages of T reg cells, mean Foxp3 fluorescence intensity and other Behcet’s disease and uveitis parameters were compared using Pearson’s correlation test. The means of two groups were compared using Student’s unpaired t-test. Statistical analysis was achieved using Statplus (version 7) statistical software for Macintosh operating systems.

### 4. RESULTS

Forty-four subjects were recruited in this study: 15 subjects diagnosed clinically with Behcet’s uveitis including 1 female and 14 male patients whose ages ranged between 21 and 52 years in addition to 29 healthy controls who were age and sex-matched to the cases. Their age at the onset of symptoms ranged between 6 and 47 years while their duration of affection was between 1 and 15 years. The mean ± standard deviation(SD) age of subjects, duration of affection and age of onset are illustrated in Table 1.

The erythrocyte sedimentation rate (ESR) of Behcet’s uveitis cases ranged between 5 and 32 in the first hour while the steroid doses ranged between 0 and 30 mg/day. The total severity score of cases was within the range of 4 and 10. Moreover, the total activity score of cases was within the range of 0 and 3. Table 2 shows the mean ± standard deviation (SD) ESR, steroid dose, total severity and total current activity scores of the Behcet’s uveitis patients participating in the present study.

As regards immuno-suppressive and immunomodulatory medications administered to Behcet’s uveitis cases involved in the current study, the majority of cases received either Cyclosporin (40%) or Infliximab (20%). The number and percentage of cases that received various drugs for the treatment of Behcet’s uveitis are given in Table 3.

Patients with Behcet’s uveitis enrolled in the current study suffered from a group of non-ocular symptoms and signs diagnostic of Behcet’s disease. These included oral ulcers in all of the cases as well as genital ulcers in 80% of them. Skin involvement was observed in 60% of cases participating in the study. Arthralgia or Neuro–Behcet manifestations was presented by 13.3% of cases. Headache and/or skin pustules were presented by 26.7% of cases. The patients enrolled did not suffer any arthritis, GIT bleeding, pleurisy, arterial

| Table 1. Patient Profile Parameters of Participating Behcet’s Uveitis Patients. |
|-----------------------------|-------|--------|-------|-------|
| Parameter                  | Mean  | Standard Deviation | Minimum | Maximum |
| Age                        | 34    | 10      | 21    | 52     |
| Duration (years)           | 7     | 4       | 1     | 15     |
| Age of Onset               | 27    | 10      | 6     | 47     |

| Table 2. Mean ESR, steroid dose, total severity and total current activity scores of participating Behcet’s Uveitis patients. |
|-----------------------------|-------|--------|-------|
| Parameter                  | Mean  | Standard Deviation | Minimum | Maximum |
| ESR mm/hr                  | 16    | ± 8     | 5     | 32     |
| Steroid Dose mg/ day       | 18    | ± 11    | 0     | 30     |
| Total Severity Score       | 6     | ± 2     | 4     | 10     |
| Total Activity Score       | 1     | ± 1.13  | 0     | 3      |

*ESR = Erythrocyte Sedimentation Rate*

| Table 3. Immunosuppressive and Immunomodulatory medications administered to Behcet’s Uveitis patients. |
|-----------------------------|-------|-------|
| Treatment                  | Number of Cases | Percentage (%) |
| Cyclosporine               | No     | 9     | 60.0% |
|                            | Yes    | 6     | 40.0% |
| Cyclophosphamide           | No     | 14    | 93.3% |
|                            | Yes    | 1     | 6.7%  |
| Azathioprine               | No     | 14    | 93.3% |
|                            | Yes    | 1     | 6.7%  |
| Methotrexate               | No     | 13    | 86.7% |
|                            | Yes    | 2     | 13.3% |
| Infliximab                 | No     | 12    | 80.0% |
|                            | Yes    | 3     | 20.0% |
| Mycophenolate Mofetil      | No     | 14    | 93.3% |
|                            | Yes    | 1     | 6.7%  |

Figure 1. Percentage of CD25 and Foxp3 expression on peripheral CD4+ T lymphocytes determined by flowcytometry.
or venous thrombosis. The percentage and number of the different non-ocular symptoms and signs of Behcet's disease cases participating in the study are exhibited in Table 4.

As regards Behcet's disease ocular manifestations, all patients joining the present study did not suffer from either right or left active anterior uveitis. However, 13.3% of the patients suffered from right active posterior uveitis while 26.7% of them suffered from left active posterior uveitis. In addition to this, right non-active anterior uveitis was seen in 13.3% of cases while left non-active anterior uveitis was present in 20% of cases. Right non-active posterior uveitis was seen in 6.7% of cases while left non-active posterior uveitis was seen in 20% of cases. Hence, right pan-uveitis was present in 60% of cases while left pan-uveitis was present in 66.7% of them. Right-sided cataract was diagnosed in 20% of cases, while left-sided cataract was diagnosed in 13.3% of cases. Also, 26.7% of patients involved in the study suffered from vitritis in the right eye while 33.4% of them suffered from vitritis in the left eye. A participating case in the present study presented with retinitis in the right eye and another one presented with retinitis in the left eye. Similarly, two cases presented with corneal opacities. The number and percentage of cases suffering from the different signs of Behcet's ocular disease are shown in Table 5.

In the present study, CD4⁺CD25⁺Foxp3⁺ T reg cell percentage of expression was observed to be higher in Behcet's uveitis patients than the controls (1.24 ± 3.47 vs. 0.3 ± 0.81, respectively) but with no statistically significant difference (p-value: 0.17). Furthermore, CD4⁺CD25⁺Foxp3⁺ T reg cell percentage of expression was higher in Behcet's uveitis patients than that of the controls (0.21 ± 0.63 vs.0.17 ± 0.28, respectively) but with no statistically significant difference (p-value: 0.76). Conversely, CD4⁺CD25⁺Foxp3⁻ T reg cell percentage of expression was found to be higher in the controls than in Behcet's uveitis cases (0.7 ± 0.64 vs. 0.5 ± 0.43, respectively) with no statistically significant difference (p-value: 0.26). However, the mean fluorescence intensity of Foxp3 as detected by flow cytometry was significantly higher in the control subjects compared to the cases with Behcet’s uveitis (1.637 ± 1.867 vs. 0.533 ± 0.72, respectively, p = 0.02). Table 6 compares the mean percentages of the three T reg cell types studied (i.e. CD4⁺CD25⁺Foxp3⁺, CD4⁺CD25⁻Foxp3⁺ and CD4⁺CD25⁺Foxp3⁻) as well as the mean fluorescence intensity of Foxp3 transcription factor in the

Table 4. Non-ocular symptoms and signs of participating Behcet's disease patients.

Table 5. Ocular symptoms and signs of participating Behcet's disease patients.

Table 6. T-Regulatory cells and mean fluorescence intensity of Foxp3 in Behcet's Uveitis cases vs. controls.

---

* GIT = Gastrointestinal Tract.

** SD = Standard Deviation, FLU = fluorescence.
cases and control groups. Figure 1 illustrates the percentage of CD25 and Foxp3 expression on peripheral CD4\(^+\) T lymphocytes as determined by flowcytometry.

In the current study, CD4\(^+\)CD25\(^-\)Foxp3\(^-\) T reg cell percentage of expression was found to be positively correlated with the age of onset of Behcet's ocular disease and the correlation was found to be statistically significant (r = 0.536 and P value = 0.039). In addition to this, CD4\(^+\)CD25\(^+\) Foxp3\(^+\) T reg cell percentage of expression was seen to be positively correlated with the duration of the disease and the correlation was shown to be statistically significant (r = 0.544 and P value = 0.036). The latter group of T cell percentages was positively correlated with the presence of skin pustules in the participating patients in a statistically significant manner (r = 0.528 and P value = 0.043). The percentage of CD4\(^+\)CD25\(^-\)Foxp3\(^-\) cell expression was noted to be negatively correlated with skin affection and the correlation was found to be statistically significant (Pearson’s correlation coefficient = – 0.61, P value = 0.016). No other statistically significant linear correlations were exhibited in the current study.

Table 7 demonstrates the linear (Pearson’s) correlation between the various parameters studied in Behcet’s uveitis cases as mentioned above and the percentage of expression of the Treg cells examined in the current work (CD4\(^+\)CD25\(^-\) Foxp3\(^-\), CD4\(^+\)CD25\(^+\)Foxp3\(^+\) and CD4\(^+\)CD25\(^+\)Foxp3\(^-\)) as well as the mean fluorescence intensity of Foxp3.

### 5. DISCUSSION

Behcet’s disease (BD) is a lifelong severe autoimmune vasculitis disease presenting by intermittent episodes of oral, skin and genital ulcers as well as musculoskeletal disorders and ocular disorders. It rarely affects other systems such as cardiovascular, gastrointestinal, renal or nervous systems. Patients are affected by a variety of symptoms and signs characterized by remissions that are followed by severe exacerbation (Greco et al., 2018; Yazici et al., 2018).

Ocular inflammation occurs in about 15% to 25% of patients with BD during the course of the disease leading to major eye complications, especially pan-uveitis and retinal vasculitis that may cause blindness due to edema, non-perfusion, neovascularization, and retinal or optic atrophy (Desbois et al., 2018; Figus et al., 2015).

### Table 7. Pearson’s correlation between the various parameters in Behcet’s Uveitis cases, T-Regulatory cells and mean fluorescence intensity of Foxp3.

| Parameters                  | CD4\(^+\)CD25\(^-\) FOXp3\(^-\) | CD4\(^+\)CD25\(^-\) FOXp3\(^+\) | CD4\(^+\)CD25\(^+\) FOXp3\(^-\) | Mean FLU Intensity of FOXP3 |
|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------------|
| Age of onset (R)            | –0.491                        | 0.051                         | 0.536                         | –0.329                       |
| P Value                     | 0.063                         | 0.857                         | 0.039                         | 0.232                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Duration (R)                | –0.037                        | 0.530                         | –0.206                        | –0.262                       |
| P Value                     | 0.897                         | 0.042                         | 0.461                         | 0.346                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Cyclosporin (R)             | 0.544                         | –0.206                        | 0.195                         | 0.312                        |
| P Value                     | 0.036                         | 0.461                         | 0.483                         | 0.258                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Skin (R)                    | 0.214                         | 0.258                         | –0.610                        | 0.047                        |
| P Value                     | 0.444                         | 0.333                         | 0.016                         | 0.868                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Skin pustules (R)           | 0.528                         | –0.166                        | –0.444                        | 0.278                        |
| P Value                     | 0.043                         | 0.555                         | 0.097                         | 0.315                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Arthralgia (R)              | –0.217                        | –0.106                        | –0.070                        | –0.274                       |
| P Value                     | 0.438                         | 0.706                         | 0.804                         | 0.322                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Anterior Uveitis (R)        | –0.276                        | –0.171                        | 0.122                         | –0.350                       |
| P Value                     | 0.319                         | 0.542                         | 0.666                         | 0.201                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Neuro-Behcet (R)            | –0.217                        | –0.134                        | 0.067                         | –0.274                       |
| P Value                     | 0.438                         | 0.633                         | 0.814                         | 0.322                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Genital Ulcer (R)           | 0.091                         | –0.190                        |                                  |                               |
| P Value                     | 0.747                         | 0.498                         | 0.787                         | 0.691                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| ESR (R)                     | –0.010                        | 0.046                         | –0.027                        | 0.043                        |
| P Value                     | 0.972                         | 0.869                         | 0.925                         | 0.880                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Total Severity Score (R)    | –0.225                        | –0.114                        | –0.059                        | –0.435                       |
| P Value                     | 0.420                         | 0.836                         | 0.105                         | 0.105                        |
| N                           | 15                            | 15                            | 15                            | 15                            |
| Total Activity Score (R)    | 0.023                         | –0.280                        | –0.390                        | –0.108                       |
| P Value                     | 0.934                         | 0.312                         | 0.150                         | 0.701                        |
| N                           | 15                            | 15                            | 15                            | 15                            |

* FLU = Fluorescence, ESR = Erythrocyte Sedimentation Rate.
Circulating CD4+CD25+ regulatory T (Treg) cells constitute 1–2% of circulating mononuclear cells and collectively express the forkhead transcription factor known as Foxp3. These cells are crucial for the preservation of self-tolerance belonging to the immune system by suppressing abnormal or severe immune responses considered injurious to the body. The Foxp3 factor that is encoded within the X chromosome, is largely involved in the maturity and functionality of Treg cells. Any mutations affecting the Foxp3 factor may lead to reduced maturity or derangement of Treg cells. Accordingly, these regulatory cells under such conditions are unable to keep the immune response in check resulting in immune system dysregulation and the increased risk of autoimmune disease (Bin Dhurban et al., 2014; McMurchy et al., 2010).

The exact mechanism of how the Foxp3 factor exerts its effect on T reg cells is not yet fully understood. FOX proteins are well-known members of the forkhead family of transcriptional regulators and are thought to exercise control through mechanisms comparable to DNA interactions during transcription. In Treg cell models, the Foxp3 transcription factor has been found to occupy the promoter’s loci for genes contributing to the regulatory T-cell functionality, and may suppress transcription of principal genes following the triggering of T cell receptors (Marson et al., 2007).

In the present study, CD4+CD25−Foxp3+ mean Treg cell percentage of expression was higher in Behcet’s uveitis patients (1.24 ± 3.47) than in the controls (0.3 ± 0.81) but with no statistically significant difference (p value: 0.17). In addition, CD4+CD25+Foxp3− mean Treg cell percentage was higher in Behcet’s uveitis (0.21 ± 0.63) than that of the controls (0.17 ± 0.28) but with no statistically significant difference (p value: 0.76).

Conversely, CD4+CD25−Foxp3− mean cell percent of expression was found to be higher in the controls (0.7 ± 0.64) than Behcet’s uveitis cases (0.5 ± 0.43) without any statistically significant difference (p value = 0.26). Therefore, the present work did not reveal any significant difference between the cases and the controls in relation to the mean percentage of expression of the three types of T reg cell types studied.

On the other hand, the mean fluorescence intensity of Foxp3 as detected by flow cytometry, which was used as an indicator of the function of T regulatory cells, was significantly higher in the control subjects compared to the cases with Behcet’s uveitis (1.637 ± 1.867 vs. 0.533 ± 0.72 respectively, p = 0.02).

In agreement with the current study, Hamzaoui et al. (2006) found that circulating CD4+CD25+ T-regulatory cells, possessing the ability to inhibit native T cells, were higher in active BD patients than control subjects (Hamzaoui et al., 2006). In addition, a study by Nanke et al. (2008) stated that the percentages of CD4+CD25 Treg cells from BD patients with ocular complications (such as uveitis) were significantly decreased before an active ocular attack compared to those after a similar attack. Moreover, these levels before the attack were significantly lower than normal levels, whereas the percentages of Treg cells in both BD with or without inactive ocular affection were also normal (Nanke et al., 2008).

Moreover, Yeh et al. (2009) reported that T reg cells were decreased in patients with active uveitis in comparison to patients with the inactive form of the disorder. An extreme reduction of CD4+CD25− Foxp3+ T cell populations as well as Foxp3 mRNA derangement was accompanied by severe uveitis suggesting that the loss of T reg cells and Foxp3 dysfunction may be significant causes of uveitis in certain cases (Yeh et al., 2009).

Sugita et al. (2011) confirmed that the percentage of expression of Foxp3+ among CD4+CD25− T cells was significantly lower in patients with chronic active uveitis compared to patients having remitting inactive uveitis. Expression of Foxp3 was found to be at an equal level in CD4+CD25− T cells obtained from healthy controls. Patients having a high percentage of expression of Foxp3+ cells during infliximab administration did not experience any additional attacks of acute uveitis. Contrariwise, patients with a decreased percentage of expression of Foxp3+ cells suffered recurrent episodes of acute uveitis (Sugita et al., 2011).

Gündüz et al. (2013) concluded a similar study by stating that the percentage of expression of CD4+CD25−Foxp3− Treg cells in clinically inactive BD patients or healthy controls was higher compared to active BD patients. The expression of CD4+CD25− Foxp3− Treg cells was observed to be higher in healthy controls compared to clinically active patients but that was not the case when comparing the inactive patients’ group to the healthy control group. The percentage of expression of CD4+CD25−Foxp3+ T reg cells did not vary when comparing active and inactive patients to healthy controls (Gündüz et al., 2013).

6. CONCLUSION

It could be concluded from the present study that the percentages of the Treg cell populations investigated were not significantly different from each other when comparing the BD uveitis cases to the control subjects. Nevertheless, the statistically significant decrease in the measured mean fluorescence intensity of Foxp3 of cases compared to that of control subjects highlighted the importance of the transcription factor Foxp3 as an essential element for the sound development, maturity and subsequently the regulatory function of T reg cells and their protective role against immune system
dysregulation and autoimmune diseases such as Behcet’s disease and Behcet’s uveitis. Therefore, T reg cells (especially the Foxp3\(^+\) group) could be used as potential prognostic markers for the assessment and follow up of Behcet’s uveitis. **Informal Consent Statement:** A written informed consent was taken from every participant involved in the current study prior to carrying out any processes in accordance to the 1964 Helsinki Declaration. The study obeyed the ethical principles required by the National Center for Radiation Research and Technology (NCRRT) ethical committee (Cairo) and the study was granted the authorization code 1 H/19.

**Disclosure statement**

The authors do not have any conflicts of interest to declare.

**Funding**

The authors did not receive any particular funding for this work.

**ORCID**

Amal H. Eissa MD https://orcid.org/0000-0002-9708-9181
Heba M. Selim MD https://orcid.org/0000-0002-6220-2383
Abeer M. Zahran MD https://orcid.org/0000-0003-4022-9355
Mohamed S. Tawfik PhD https://orcid.org/0000-0003-2382-2077

**References**

Bicer, A. (2012). Musculoskeletal findings in Behcet’s disease. Pathology Research International, 2012, 653806. https://doi.org/10.1155/2012/653806

Bin Dhuban, K., Kornete, M., Mason E. S., & Ca, P. (2014). Functional dynamics of Foxp3\(^+\) regulatory T cells in mice and humans. Immunological Reviews, 259(1), 140–158. https://doi.org/10.1111/imr.12168

Cavaco, S., Da Silva, A. M., Pinto, P., Coutinho, E., Santos, E., Bettencourt, A., Pinto, C., Gonçalves, A., Silva, S., Gomes, F., Carvalho, L., Pereira, C., Martins, B., Correia, J., & Vasconcelos, C. (2009). Cognitive functioning in Behcet’s disease. Annals of the New York Academy of Sciences, 1173(1), 217–226. https://doi.org/10.1111/j.1749-6632.2009.04670.x

Charbonnier, L. M., & Chatila, T. A. (2018). Phenotypic and functional characterization of Regulatory T Cell populations. In J. Soboloff & D. J. Kappes (Eds.), Signaling mechanisms Regulating T Cell diversity and function. Boca raton (FL) (Ch. 7, pp. 105–118). CRCPress/Taylor & Francis. https://doi.org/10.1201/9781315371689-7

Choi, H. J., Seo, M. R., Ryu, H. J., & Baek, H. J. (2016). Behcet’s disease current activity form as a Patient’s derived measure. Journal of Rheumatic Diseases, 23(1), 19–22. http://dx.doi.org/10.4078/jrd.2016.23.1.19

Criteria for diagnosis of Behcet’s disease International Study Group for Behcet’s Disease. (1990). Lancet, 335(8697), 1078–1080. https://doi.org/10.1016/0140-6736(90)92643-V

Cunningham, E.T.J., Tugal-Tutkun, J., Kairaliath, M., Okada, A. A., Bodaghi, B. & Zeirhut M. (2017). Behcet’s Uveitis. Ocular Immunology and Inflammation, 25(1), 2–6. https://doi.org/10.1080/09273948.2017.1279840

Davatchi, F., Chams-Davatchi, C., Shams, H., Shahram, F., Nadji, A., Akhlaghi, M., Faezi, T., Ghodsi, Z., Sadeghi Abdollahi, B., Ashofteh, F., Mohtasham, N., Kavosi, H., & Masoumi, M. (2017). Behcet’s disease: Epidemiology, clinical manifestations, and diagnosis. Expert Review of Clinical Immunology, 13(1), 57–65. https://doi.org/10.2147/OARRR.S46644

Desbois, A. C., Terrada, C., Cacoub, P., Bodaghi, B., & Saadoun, D. (2018). Ocular manifestations in Behcet’s disease. La Revue De Medecine Interne / fondee par la Societe nationale francaise de medecine interne, 39(9), 738–745. https://doi.org/10.1016/j.revméd.2009.04.014

Emmi, G., Silvestri, E., Squatrito, D., D’Eliaos, M. M., Ciucciarelli, L., Prisco, D., & Emmi, L. (2014). Behçet’s syndrome pathophysiology and potential therapeutic targets. *Internal and Emergency Medicine, 9*(3), 257–265. https://doi.org/10.1007/s11739-013-1036-5

Farouk, H., Zayed, H. S., & El-Chilali, K. (2016). Cardiac findings in patients with Behçet’s disease: Facts and controversies. *Anatol J Cardiol*, 16(7), 529–533. https://doi.org/10.14744/AnatolCardiol.2016.7029

Figueroa, M., Posarelli, C., Albert, T. G., Talarico, R., Nardi, M., & Clinical, A. (2015). Picture of the visual outcome in adamanities-Behçet’s disease. *BioMed Research International*, 2015, 120519. https://doi.org/10.1155/2015/120519

Greco, A., De Virgilio, A., Ralli, M., Ciofalo, A., Mancini, P., Attanasio, G., De Vincentis, M., & Lambiasi, A. (2018). Behçet’s disease: new insights into pathophysiology, clinical features and treatment options. *Autoimmunity Reviews*, 17(6), 567–575. https://doi.org/10.1016/j.autrev.2017.12.006

Gündüz, E., Teke, H. U., Bilge, N. S., Cansu, D. U., Bal, C., Korkmaz, C., & Regulatory, G. Z. (2013). T cells in Behçet’s disease: Is there a correlation with disease activity? does regulatory T cell type matter? *Rheumatology International*, 33(12), 3049–3854. https://doi.org/10.1007/s00296-013-2835-8

Hamzaoui, K., Hamzaoui, A., & Houman, H. (2006). CD4+CD25 + regulatory T cells in patients with Behçet’s disease. *Clinical and Experimental Rheumatology*, 24(5), 571–578. https://www.clinexprheumatol.org/abstract.asp?a=2913

Hintenberger, R., Falkinger, A., Danninger, K., & Pieringer, H. (2018). Cardiovascular disease in patients with autoinflammatory syndromes. *Rheumatology International*, 38(1), 37–50. https://doi.org/10.1007/s00296-017-3854-7

Hussein, M. A., Eissa, I. M., & Dahab, A. A. (2018). Vision-threatening Behcet’s disease: Severity of ocular involvement predictors. *Journal of Ophthalmology*, 2018, 9518065. https://doi.org/10.1155/2018/9518065

Kalra, S., Silman, A., Akman-Demir, G., Bohle, S., Borhani-Haghighi, A., Constantinescu, C. S., Houman, H., Mehr, A., Salvarani, C., Sifakis, P. P., Siva, A., & Al-Araj, A. (2014). Diagnosis and management of Neuro-Behçet’s disease: International consensus recommendations. *Journal of Neurology, 261*(9), 1662–1676. https://doi.org/10.1007/s00415-013-2709-3

Karadag, A. S., Bilgin, B., & Soylu, M. B. (2017). Comparison of optical coherence tomographic findings between Behcet
Nanke, Marson, Lu, Kim, Emm.2017.313, Rheumatism. 703–717. Molecular manifestations ocular ease Levings, Gambineri, 2008). 2007, 2013 (1), 2007 (4), 2016 2014 (2), (3), 2011, 2017 -). 10.1034/j.1999-69–73. https://doi.org/10.5935/0004-2749.20170018 Kim, D. H., & Cheon, J. H. (2016). Intestinal Behcet’s disease: A true inflammatory bowel disease or merely an intestinal complication of systemic vasculitis? Yonsei Medical Journal, 57(1), 22–32. https://doi.org/10.3349/ymj.2016.57.1.22 Lee, W., & Lee, G. R. (2018). Transcriptional regulation and development of regulatory T cells. Experimental & Molecular Medicine, 50(3), e456. https://doi.org/10.1038/emm.2017.313 Lu, L., Barbi, J., & Pan, F. (2017). The regulation of immune tolerance by FOXP3. Nature Reviews. Immunology, 17(11), 703–717. https://doi.org/10.1038/nri.2017.75 Marson, A., Kretschmer, K., Frampton, G. M., Jacobsen, E. S., Polansky, J. K., Maccasac, K. D., Levine, S. S., Fraenkel, E., Von Boehmer, H., & Young, R. A. (2007). Foxp3 occupancy and regulation of key target genes during T-cell stimulation. Nature, 445(7130), 931–935. https://doi.org/10.1038/nature05478 McMurchy, A. N., Gillies, J., Allan, S. E., Passerini, L., Gambineri, E., Roncarolo, M. G., Bacchetta, R., & Levings, M. K. (2010). Point mutants of forkhead box P3 that cause immune dysregulation, polyendocrinopathy, enteropathy, X-linked have diverse abilities to reprogram T cells into regulatory T cells. The Journal of Allergy and Clinical Immunology, 126(6), 1242–1251. https://doi.org/10.1016/j.jaci.2010.09.001 Nanke, Y., Kotake, S., Goto, M., Ujihara, H., Matsubara, M., & Kamatani, N. (2008). Decreased percentages of regulatory T cells in peripheral blood of patients with Behcet’s disease before ocular attack: A possible predictive marker of ocular attack. Modern Rheumatology / the Japan Rheumatism Association, 18(4), 354. https://doi.org/10.1007/s10165-008-0064-x Park, J. H. (1999). Clinical Analysis of Behcet disease: Arthritic manifestations in Behcet disease may present as Seronegative Rheumatoid Arthritis or Palindromic Rheumatism. The Korean Journal of Internal Medicine, 14 (1), 66–72. https://doi.org/10.3904/kjim.1999.14.1.66 Rokutanda, R., Kishimoto, M., & Okada, M. (2014). Update on the diagnosis and management of Behcet’s disease. Open Access Rheumatol,7(1), 1–8. https://doi.org/10.2147/OARRR.S46644 Skew, W., Hamilton, M. J., & Gastrointestinal Behcet’s, A. T. (2015). disease: A review. World Journal of Gastroenterology : WJG, 21(13), 3801–3812. https://doi. org/10.3748/wjg.v21.i13.3801 Sugita, S., Yamada, Y., Kaneko, S., Horie, S., & Mochizuki, M. (2011). Induction of regulatory T cells by infliximab in Behcet’s disease. Investigative Ophthalmology & Visual Science, 52(1), 476–484. https://doi.org/10.1167/iovs.10-5916 Torgerson, T. R., & Ochs, H. D. (2007). Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: Forkhead box protein 3 mutations and lack of regulatory T cells. The Journal of Allergy and Clinical Immunology, 120(4), 744–750. https://doi.org/10.1016/j.jaci.2007.08.044 Trowsdale, J., & Knight, J. C. (2013). Major histocompatibility complex genomics and human disease. Annual Review of Genomics and Human Genetics, 14(1), 301–323. https://doi.org/10.1146/annurev-genom-091212-153455 Ucar-Comlekoglu, D., Fox, A., & Sen, H. N. (2014). Gender differences in Behcet’s disease associated Uveitis. Journal of Ophthalmology, 2014, 820710. https://doi.org/10.1155/2014/820710 Verity, D. H., Wallace, G. R., Vaughan, R. W., Kondeatis, E., Madanat, W., Zureikat, H., Fayyad, F., Marr, J. E., Kanawati, C. A., & Stanford, M.R. (1999). HLA and tumor necrosis factor (TNF) polymorphisms in ocular Behcet’s disease. Tissue Antigens, 54(3), 264–272.http://doi.org/10.1034/j.1999-0039.1999.540307.x Yazici, H., Seyahi, E., Hatemi, G., & Yazici, Y. (2018). Behcet syndrome: A contemporary view. Nature Reviews. Rheumatology, 14(2), 119. https://doi.org/10.1038/nrrheum.2017.208 Yeh, S., Li, Z., Forooghian, F., Hwang, F. S., Cunningham, M. A., Pantanelli, S., Lew, J. C., Wroblewski, K. K., Vitale, S., & Nussenblatt, R. B. (2009). CD4+Foxp3+ T-regulatory cells in noninfectious uveitis. Archives of Ophthalmology, 127(4), 407–413. https://jamanetwork.com/journals/jamaophthal mology/fullarticle/1729837. https://doi.org/10.1001/arch ophthalmol.2009.32 Zeidan, M. J., Saadoun, D., Garrido, M., Klatzmann, D., Six, A., & Cacoub, P. (2016). Behcet’s disease physiopathology: A contemporary review. Auto Immun Highlights, 7(1), 4. https://doi.org/10.1007/s13317-016-0074-1