Research article

The expression level of FOXP3 and CD25 in Iraqi patients with post burn injuries

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ABSTRACT

Introduction and Aim: Regulatory T cells (Treg) are characterized by $CD25^+$ expression and $Foxp3^+$ transcription factors which is a characteristic marker for these cells. This study aimed to investigate the expression of Foxp3 in patients with post-burn injuries in Baghdad, Iraq.

Materials and Methods: The study samples included 38 persons divided into two groups: the first group included patients with Total Body surface area (TBSA) of burn less than 45% (TBSA <45 %), and patients with TBSA of burn more than 45 % (TBSA >45%). The control group included healthy individuals (n=10). The expression of FOXP3 and CD25 of Treg cells was measured by using flow cytometry and data obtained was statistically analyzed.

Results: The result of the study showed a significant increase in Foxp3 and CD25 expression in Treg cells that were isolated from peripheral blood in patients with TBSA <45 % and TBSA >45% following day 1 and day 5 of the burn injury in contrast to the control group, and the expression level of Foxp3 was (35.674± 4.640, 63.768 ± 7.857,16.147±3.2846) respectively after the first day of the burn, while the expression level of Foxp3 after the fifth day of the burn was (39.588±10.533, 47.275±7.4610, 16.1470± 3.2846) respectively. Similarly, the levels of CD5 recorded for patients in TBSA <45%, TBSA>45% and control group on day1 was 29.747±2.943, 43.447±5.669 and 16.793±3.268 respectively, while on day 5 it was 33.870±3.956, 56.197±7.460 and 16.793±3.268, for the three groups, respectively.

Conclusion: The increase of Foxp3 and CD25 expression in Treg cells isolated from burn patients has an important effect in enhancing the activity of Treg cells in inhibiting the immune response.

Keywords: Treg; CD25+; CD4+; FOX P3; flow-cytometry; TBSA; burn injuries.

INTRODUCTION

Regulatory T cells (Treg) are T lymphocytes that play an important role in maintenance of the body's self-tolerance to antigens and immune homeostasis. Any change or malfunction of Treg cells have been demonstrated to result in autoimmune disorders and diseases, including allergy and cancer (1, 2). Natural Treg cells $CD25^+$ and $CD4^+$ are components of the IL-2 receptor that are involved in active suppression of human autoimmune diseases. $CD25^+$ the first marker of Treg cells have many cellular roles, increased expression of which can result in several autoimmune diseases as well as inflammation (3).

The FOXP3 (Forkhead box P3), a member of the Forkhead\winged helix family, is a nuclear transcriptional factor crucial for the development and function of natural Tregs (4). Sustained Foxp3 expression is required for maintaining stability of several key functions, including cytokine signaling, epigenetic regulation of the Foxp3 locus, and FOXP3 interaction with other proteins (5,6). Mutation in the FOXP3 gene in humans has been shown to lead to Treg dysfunction resulting in IPEX (Immune Dysregulation Polyendocrinopathy Enteropathy X-linked syndrome) due to inflammation of organs and lymphocyte infiltration (7).

Studies with rats have shown the activity of Treg cells to be increased in post-burn wounds (8,9). Other studies indicated increased gene expression of IL-10 in burns patients resulting in sepsis due to the suppression of lymphocyte-dependent immunity (10). Studies have also indicated that increasing the size of the burn leads to a higher mortality rate for burned patients thus indicating that the extent of the burn size may be associated with the development of sepsis (8).

Sepsis is one of the most serious complications involved with burns, bacterial infections, and poisoning (11). Globally, the sepsis rate is seen to increase by 1.5% per year, with fatality rate fluctuating between 30% and 70% (12). The activity of the host's immune system is responsible for the excessive inflammatory response which results in sepsis (13). This is due to activation of Treg cells to generate an immune response to foreign antigens. The subsequent activation of Treg cells, which results in the inhibition of T and B lymphocytes, finally leads to the inhibition of the immune response (14, 15).

Gouirard *et al.*, (16) demonstrated that Treg cells play an important role in normal inflammation control by suppressing T helper (Th) subsets such as Th1, Th2, and Th17, which play an important role in the development of immune response and autoimmunity. In Iraq, studies investigating the gene expression of FOX p3 in patients with bladder cancer (17), multiple sclerosis (18), and breast cancer (19) revealed an elevated amount of this transcription factor,

implicating FOX p3 to play an important role in these diseases. This study aimed to analyze FOXP3 expression and CD25+ cells in peripheral blood of individuals with post-burn wounds to better understand the role of burn injuries on Treg cell activity.

MATERIALS AND METHODS

The current study included 38 Iraqi individuals in the age range of 15-55 years. The participants were divided into two groups: The first group consisted of 28 patients (10 male and 18 females) with post-burn injury with total body surface area (TBSA) >45% as well as TBSA <45%. The second control group involved 10 normal individuals (3 male and 7 female). Ethical approval (No:4624; 9/7/2019) was obtained from the Medical College Board, Baghdad University, Baghdad, Iraq prior to the study.

Blood (2 ml) was drawn from each participant and placed in EDTA tubes. Centrifugation was used to separate the cells. The cells were washed with PBS and the number of cells was adjusted to 10^6, then the washing process was repeated, and the cells were resuspended with 100 µl of Flow cytometry buffer solution until further use.

Evaluation of FOXp3 and CD25 by flow-cytometry

The expression levels of FOXP3 and CD25 Treg cells was measured by using a Human Regulatory T Cell Multi-color flow cytometry kit (kat.no: FMC013, R and D system com., USA), according to the manufacturer's instruction. The results were calculated by Flow cytometry Cube 6 and C4 view software.

Statistical analysis

Data obtained was analyzed using the SPSS (Statistical Package for the Social Sciences) software. Comparison of the effect of different parameters was done by one-way ANOVA.

RESULTS

The results of the current study indicated a significant increase ($p<0.05$) in the expression of Foxp3 and CD25 levels in the peripheral blood of burn patients with TBSA<45% and TBSA >45% in comparison to the healthy controls (Fig.1). The expression level of Foxp3 on day 1 of burn injury was recorded as 35.674±4.640, 63.768 ± 7.857 and 16.147±3.2846, in patients with TBSA<45%, TBSA>45% and control respectively (Fig.1). The Foxp3 expression levels showed a significant difference ($p\leq0.05$) TBSA< 45% and TBSA>45%) (Fig.1, 3). The expression levels of Fox p3 increased up to day 5, with levels recorded for patients in the TBSA< 45%, TBSA >45% and control groups as 39.588±10.533, 47.275±7.46 and 16.1470± 3.285 respectively (Fig.2). No significant differences in the Foxp3 expression levels between patients with TBSA<45% and TBSA >45 groups was seen (Fig. 2, 3).

Similarly, the expression level of CD25 on day 1 following burn injury showed a significant increase ($p<0.05$) in blood of patients with TBSA>45% and TBSA >45% groups in comparison to controls (Fig.1). The levels of CD5 recorded for patients in TBSA <45%, TBSA>45% and control group on day 1 was (29.747±2.943, 43.447±5.669 and 16.793±3.268) respectively (Fig.1). Like FoxP3, CD 25 levels significantly increased on day 5, with expression levels in the TBSA <45%, TBSA >45%, and control groups being 33.870±3.956, 56.197±7.460 and 16.793±3.268, respectively (Fig. 2, 3).

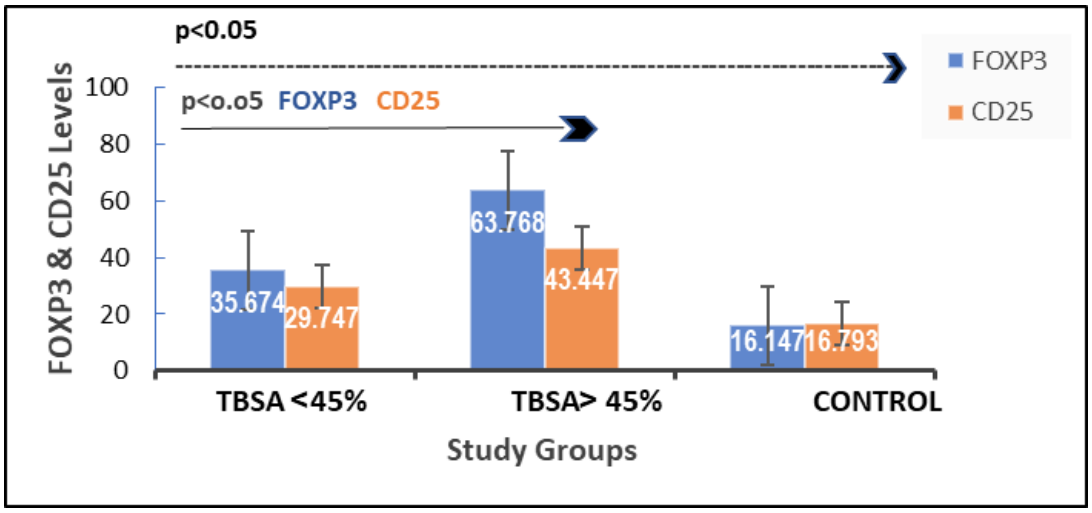

Fig. 1: FOXP3 and CD25 expression levels in Tregs isolated from peripheral blood in burn patients with TBSA 45% and TBSA>45% on day 1 after burning. TBSA: Total Body Surface Area, FOXP3: Forkhead box p3; p<0.05: significance at ($p<0.05$).

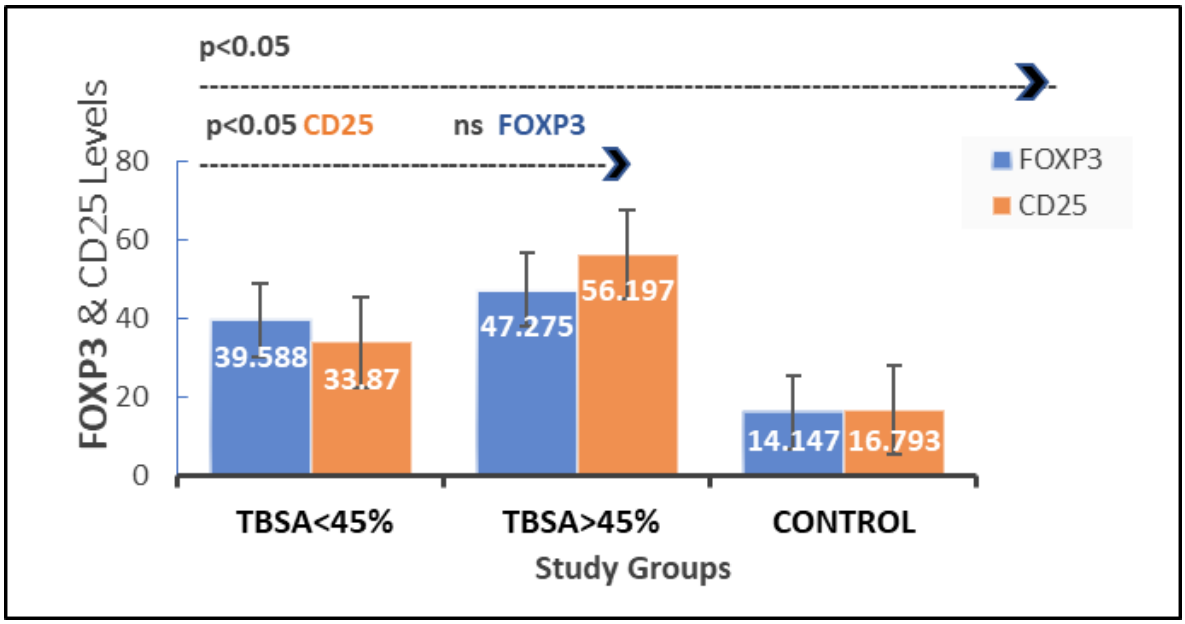

Fig.2. FoxP3 and CD25 expression levels in Tregs isolated from peripheral blood in burn patients with TBSA 45% and TBSA>45% on day 5 following burning. TBSA: Total Body Surface Area, FOXP3: Forkhead box p3, Significance at p<0.05.

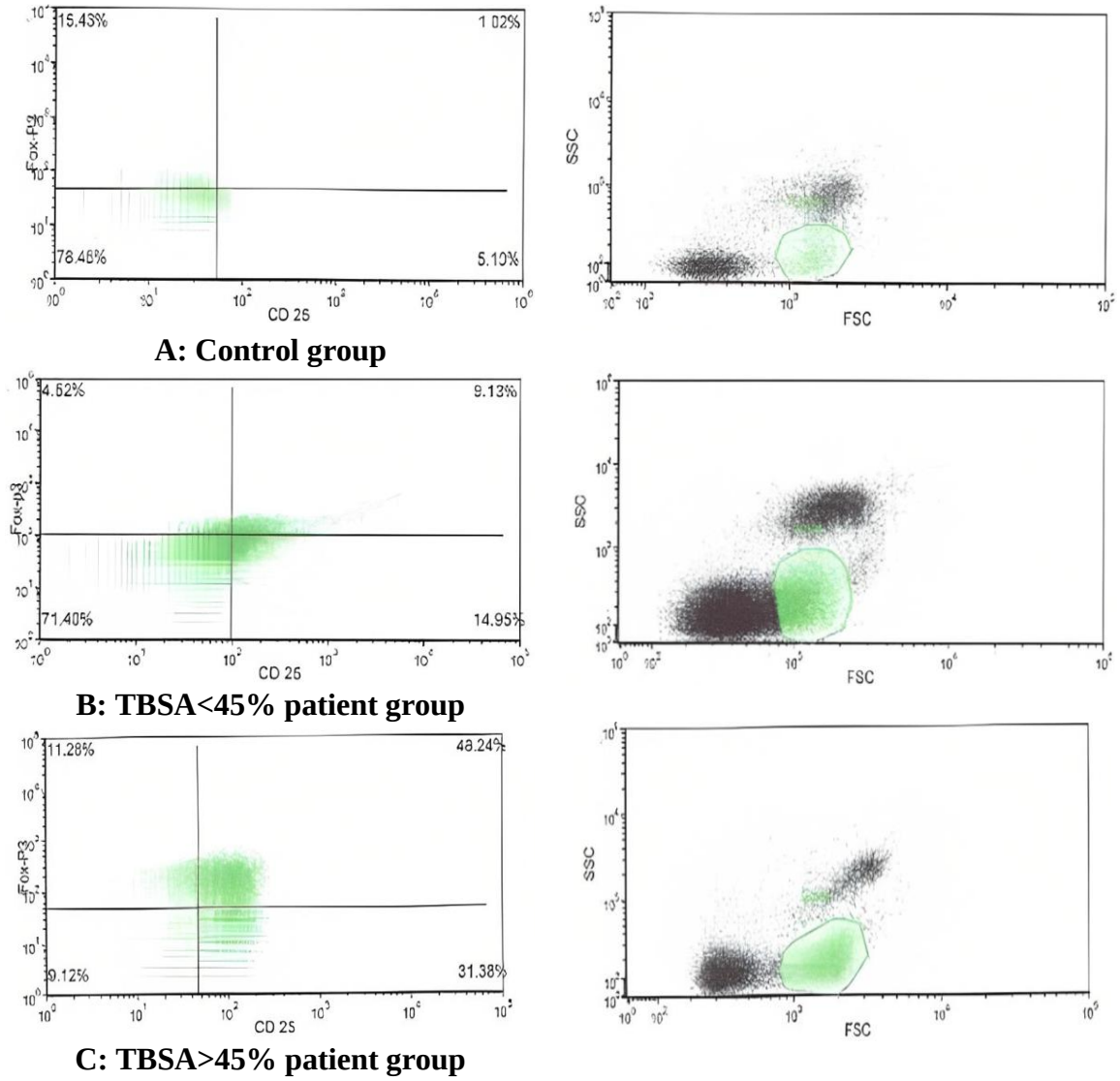

Fig 3: Flow-cytometry for Treg cells $FOXP3^{+}CD25^{+}$ in **A:** Healthy (control); **B:** TBSA<45% and **C:** TBSA >45%

Table 1: Gender differences in the expression of FOXP3 and CD25 in patients with post-burn injury

Treg	Gender	1st day of burn	5th day of burn
FOXP3	Female	42.011±7.371	38.383±7.291
	Male	60.001±8.381	55.752±9.974
CD25	Female	35.834±4.320	49.737±7.007
	Male	38.883±7.041	37.240±6.122

A comparison of expression levels for FOXP3 and CD25 between males and females showed no significant difference in patients belonging to the TBSA >45% and TBSA <45% group on day 1 and day 5 of burn injury (Table 1).

DISCUSSION

Post-burn wounds are one of the most important causes that lead to development of adverse immune system changes, allowing opportunistic microbes to take over, endangering the lives of the injured (20). Numerous investigations and clinical studies revealed the importance of natural T lymphocytes, neutrophils, and macrophages in the immune response to burn injuries and the ensuing complications including sepsis (21). Treg cells play an important role in regulating the balance in the immune system, disruption of which could be harmful to the host (22).

The current study showed a significant increase ($p<0.05$) in the expression level of FOXP3 in Treg cells isolated from peripheral blood on day1 and day 5 following burn injury for patient groups with 45%>TBSA<45% and TBSA>45% compared to healthy controls. Our findings agree with the study by Huang *et al.* (8), who reported that FOXP3 expression increased on the surface of Treg cells and that there was a link between the surface area of the burn, the severity of inflammation, and the function of the afflicted organ. Furthermore, the rise in FOXP3 expression with increasing total body surface area (TBSA) of the burn in this study, is consistent with previous studies (10, 23). This is likely due to the increase in the function of T cells associated with the severity of burn injury, which in turn contributes to the pathology brought on by immunosuppression. Studies involving murine Treg cells, demonstrated the expression of FOXP3 to increase by 60% on the first day of stimulation by LPS which further rises to 80% after the tenth day, indicating the role of these cells in inhibiting the immune response (24), as well as in acute and chronic infections, which allows opportunistic organisms to grow and multiply and exacerbate infection (25).

The expression of FOXP3 associated with an increase in the expression of CD25 from day1 to day5 following burn injury in both groups (45%>TBSA>45%) in the current study, indicates an increase in the numbers of Treg cells in the peripheral blood which is line with previous study (23).

The increase in the number of Treg cells is accompanied with the increase in the levels of cytokines that inhibit the immune response, such as TGF-β1and IL-10 (23). In sepsis-enhanced immune suppression, the rate of Treg cells in peripheral blood increases, which is associated with long-term mortality in septic patients, clearly indicates that Treg cells have crucial roles in sepsis (26, 27). Therefore, we assume that the increase in the effectiveness, number of Treg cells, the accompanying release of anti-inflammatory cytokines and hyper-production of pro-inflammatory cytokines cause sepsis symptoms, the progression of which could cause a failure in the function of the innate and acquired immune system, resulting in an immune suppression state in the patient (26,27).

Thus, we conclude that post-burn injuries lead to an increase in Treg cells, which confirms its inhibitory role in the immune response. This probably would allow opportunistic microorganisms to infect the wound areas and cause subsequent harm, such as sepsis and organ function failure.

CONCLUSION

We conclude that post-burn injuries increase the effectiveness and number of Treg cells, confirming their inhibitory role in the immune response.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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