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Original Article

Evaluation of EOS Gene Expression and IL-6 Serum Levels in Iraqi Patients with Psoriasis

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Abstract

Background: EOS (encoded by the *IKZF4* gene) is a member of the zinc finger transcription factor IKaros family, and plays a critical role in Treg suppressor functions, and maintaining Treg stability. IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis. ***Aim***: To estimate serum IL-6 level and EOS gene expression in Iraqi patients with psoriasis. ***Method***: Twenty-two patients with psoriasis (8 females, 14 males) with age ranged 18-64 years, were recruited from Baghdad Teaching Hospital, Dermatology Clinic, Baghdad, and 24 healthy donors. The serum levels of IL-6 by ELISA and the gene expression of *IKZF4* (EOS gene) by RT-qPCR technique. ***Results***: The results showed a non-significant difference in the level of IL-6 in those treated with topical therapy and others treated with Etanercept compared to control. A non-significant increase in patients treated with topical therapy was reported compared to patients treated with Etanercept. There was a higher significant percentage of *IKZF4* gene expression folding in psoriasis patients treated with Etanercept compared to control group, while no significant differences reported between patients treated with topical therapy, Etanercept, and the control group. ***Conclusion***: Activation of Regulatory T cells (Tregs) with Etanercept enhances EOS expression and decreases IL-6 production more than topical treatment in patients with psoriasis.

Keywords: Biological therapy, EOS, *IKZF4* gene, IL-6, psoriasis

تقييم التعبير الجيني EOS ومستويات IL-6 في مصل الدم لدى المرضى العراقيين الذين يعانون من الصدفية

الخلاصة

الخلفية:EOS المشفرة بواسطة الجين (*IKZF4*) هو عضو في عائلة عامل نسخ إصبع الزنكIkaros، ويلعب دورا حاسما في وظائف القامع Treg، والحفاظ على استقرار مستوى IL-6 الذي هو وسيط قابل للذوبان مع تأثير متعدد على الالتهاب والاستجابة المناعية وتكوين خلايا الدم. **الهدف**: تقدير مستوى المصل IL-6والتعبير الجيني EOS في المرضى العراقيين الذين يعانون من الصدفية. **الطريقة**: تم اختيار 22 مريضا مصابا بالصدفية (8 إناث و14 ذكرا) تتراوح أعمارهم بين 18-64 عاما، من مستشفى بغداد التعليمي وعيادة الأمراض الجلدية في بغداد و24 متبرعا أصحاء. تم قياس مستويات المصل من IL-6 بواسطة ELISAوالتعبير الجيني لموروثة (EOS, *IKZF4*) عن طريق تقنيةRT-qPCR. **النتائج**: أظهرت النتائج فرقا غير كبير في مستوى IL-6 في أولئك الذين عولجوا بالعلاج الموضعي وغيرهم ممن عولجوا بإيتانرسيبت مقارنة بالسيطرة. تم الإبلاغ عن زيادة غير كبيرة في المرضى الذين عولجوا بالعلاج الموضعي مقارنة بالمرضى الذين عولجوا بإيتانرسيبت. كانت هناك نسبة مئوية كبيرة أعلى من التعبير الجيني *IKZF4* قابلة للطي في مرضى الصدفية الذين عولجوا بأيتانيرسيبت بالمقارنة مع مجموعة التحكم ، في حين لم يتم الإبلاغ عن اختلافات كبيرة بين المرضى الذين عولجوا بالعلاج الموضعي ، أيتانيرسيبت، ومجموعة التحكم. **الخلاصة**: تنشيط الخلايا التائية التنظيمية (Tregs) مع أيتانيرسيبت يعزز التعبير عن EOS ويقلل من إنتاج IL-6 أكثر من العلاج الموضعي في المرضى الذين يعانون من الصدفية.

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INTRODUCTION

Zinc finger protein Eos (encoded by the *IKZF4* gene) is a member of the zinc finger transcription factor IKaros family [1]. EOS is widely expressed, including in the developing nervous system, especially the brain, as well as in the liver. Also, it was expressed in numerous myeloid and megakaryocytic cell lines, with the highest expression in monocytes [2] and in the $CD4^+$ $CD25^+$ and $CD4^+$ $Foxp3^+$ regulatory T cell populations [3], which play a critical role in Treg suppressor functions and maintain Treg stability. EOS interacts with Foxp3 and C-Terminal Binding Protein 1 (CtBP1). This interaction is required to silence IL-2 production in Tregs [4]. Transcription factors regulate gene expression and eventually control important cellular processes, including differentiation, proliferation, and survival, and they are often called "master regulators." The Ikaros family of proteins includes Ikaros (*IKZF1*), Aiolos (*IKZF3*), Helios (*IKZF2*), EOS (*IKZF4*), and Pegasus (*IKZF5*), one of the significant groups of transcription factors [2]. EOS has two zinc finger regions, one at the C-terminus responsible for homodimerization or heterodimerization with itself or other family members and the other at the N-terminus, which is important for DNA binding [5]. Honma *et al*. (1999) identified EOS as a novel member of the Ikaros family in the new born mouse brain, which is primarily expressed in the developing nervous system [6]. By analogy, it is likely that EOS has an important role in the development of both the central and peripheral nervous systems. IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis. The IL-6 receptor system is comprised of two functional membrane proteins: an 80-kDa ligand-binding chain (known as [IL-6R], IL-6R-chain, or CD126) and a 130-kDa non-ligand-binding signal-transducing chain (known as glycoprotein 130 [gp130], IL-6R-chain, or CD130) [7]. IL-6 was able to down-regulate EOS expression, which led to the reprogramming of FoxP3+ Tregs into T helper cells, without changing the level of FoxP3 expression [8]. There are numerous Iraqi studies on the counts of Treg cells. One of these studies was done by Alasady and Mahmood (2017) about the relationship between the levels of cytokines (IL-10, IL-17A, IFN-γ, MCP-1, TNF-α) and T-regulatory Cells (Tregs) count with the total area of burn in post-burn injury patients [9]. Another Iraqi study was done by Al-Faradhi (2015) to investigate the count of total WBC and the count of CD25+ FoxP3+ Treg and the level of types of interleukins (IL-8, IL-17, IL-12, IL-10 and TGF-β) in Iraqi patients with multiple sclerosis (MS) [10].

METHODS

The current study included 22 Iraqi patients with psoriasis ranging in age from 18-64 years, as well as 24 control samples collected from patients at Baghdad Teaching Hospital, Dermatology Clinic/Baghdad from August to November 2019. The inclusion criteria are patients aged 18-64 years; patients treated with DMARD (biological therapy) or topical therapy (Figure 1).

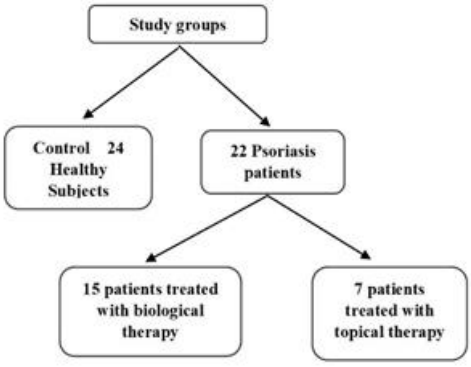

Figure 1: Study design

Exclusion criteria include patients who have hypertension, diabetes, cardiovascular disease or any comorbidity other than psoriasis; smokers or alcohol drinkers; and patients taking any medication other than psoriasis drugs. A questionnaire form was formulated that involved names, age, gender, clinical history, disease duration, smoking, drinking, and the type of therapy at the time of the study (Table 1).

Table 1: Demographic characteristics

Age	18-64 years
Gender	Female: 8
	Male:14
Clinical history	Psoriasis without other chronic diseases
Disease duration	Childhood- 41 years
Smoking	Excluded from the study
Drinking	Non
Type of therapy	7 patients under topical therapy
	15 patients under biological therapy (Etanercept)

The patients were divided into 2 subgroups, including: 7 patients were treated with topical therapy (salicylic acid, Betamethasone, Beclomethasone, and Vaseline) for 1 month to 20 years. Patients were treated with the medications together, others were given only one type of the steroids according to the physician advice, and the second group included 15 patients treated with biological therapy such as anti-TNF (Etanercept) 50 mg/week for a period of 2 months to 3 years, and 24 healthy people as a control group. About 5ml of venous blood was collected from the patients, where 4.5 ml was placed in a gel tube and centrifuged at 5000 rpm to obtain serum for analysis using Enzyme-Linked Immunosorbent Assay (ELISA), and 0.4ml was placed in a Trizol-containing tube for RNA extraction for analysis using Reverse Transcriptase-Quantitative polymerase chain reaction (RT-Qpcr). All samples were frozen at -40ºC until used.

Measurement of serum IL-6 by ELISA

The level of IL-6 was measured by the Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer's instructions (Elabscience, USA).

RNA extraction

RNA was extracted from blood samples according to the TRIzol™ reagent protocol (Thermo Scientific, USA).

RNA Detection

Quantus Fluorimeter (Promega, USA) was used to detect the concentration of extracted RNA in order to detect the quality of samples; for 1µl of RNA, 199µl of diluted Quanty Flour Dye (Promega, USA) was mixed. After 5 min of incubation at room temperature and in a darkened room, RNA concentration was detected.

The gene expression level of the IKZF4 gene by RT-qPCR

The *IKZF4* gene was amplified using the GoTaq® 1-Step RT-qPCR System (Promega, USA) in a RT-qPCR program that included the following steps: reverse transcription (37°C for 15 min), initial denaturation (95°C for 10 min), denaturation (95°C for 20 sec), annealing (60°C for 20 sec), and extension (72°C for 20 sec). The gene expression level of the *IKZF4* gene in psoriasis patients under different therapeutic modes was calculated according to the following equation, and the gene expression in control was represented as 100%:

$$\frac{\text{Folding of gene expression in Psoriasis patients}}{\text{Folding of gene expression in control}} \times 100$$

RESULTS

In Figure 2, the results of the current study showed a non-significant difference in the serum level of IL-6 in patients treated with topical therapy (Salicylic acid, Betamethasone, Beclomethasone and Vaseline) and others treated with biological therapy (Etanercept) compared to the control group. The IL-6 serum levels were 9.25±1.62 and 7.17±0.63 pg/ml, respectively, while the level in the control group was 7.60±0.67 pg/ml. The results showed a non-significant increase in patients treated with topical therapy compared to patients treated with biological therapy (Figure 2). The frequency of males was higher than females in psoriasis patients (63.3% male, 36.3% female) while in the control group (62.5% female, 37.5% male). The *IKZF4* gene expression folding results revealed a high percent increase in Psoriasis patients treated with biological therapy (747%) compared to the control percentage of, while there were non-significant differences between patients treated with topical therapy (607.8%) and patients treated with biological therapy as well as the control group (Figure 3).

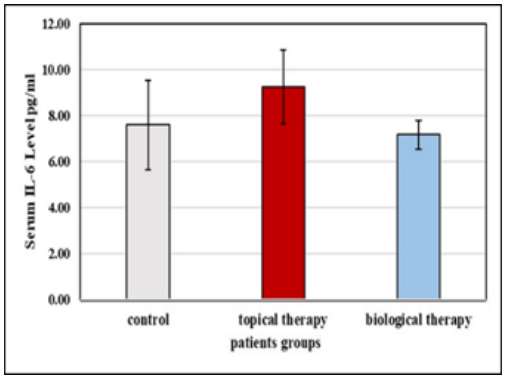

Figure 2: Serum levels of IL-6 in psoriasis patients according to therapy (P<0.05).

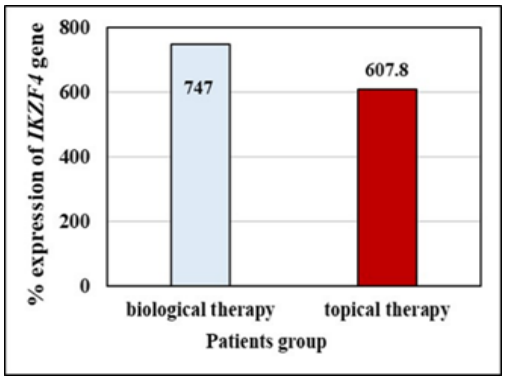

Figure 3: The percentage of gene expression levels of *IKZF4* gene (EOS) in Psoriasis patients according to the type of therapy.

In Table 2, the results showed a significant increase (P<0.05) in the level of IL-6 in males compared to females (8.87±0.80, 6.02±1.01) pg/ml respectively), while there was a non-significant change in *IKZF4* gene expression folding between females and males in psoriasis patients. The levels of the *IKZF4* gene were higher in females compared to males (11.19±7.20 and 4.87±1.95, respectively). In addition, the age of patients ranging from 18-64 years showed a non-significant difference in the level of IL-6 in psoriasis patients at 40> age > 40, and the levels of IL-6 were (8.01±0.85 and 7.22±0.86 pg/ml, respectively), and there was a non-significant difference in *IKZF4* gene expression folding at 40> age >40, where the gene expression levels of *IKZF4* were (8.82±3.62 and 1.56±1.07, respectively) (Table 2).

Table 2: The effects of gender and age on the serum level of IL-6 and expression of *IKZF4* gene in patients with psoriasis

Disease	Clinical characteristics		IL-6 Levels pg/ml	*IKZF4* Levels
Psoriasis	Age (Year)	< 40	8.01±0.85	8.82±3.62
		> 40	7.22±0.86	1.56±1.07
	Gender	Female	6.02±1.01*	11.19±7.20
		Male	8.87±0.80*	4.87±1.95

According to the type of therapy, the results showed a significant positive correlation in this study (P<0.05) between the *IKZF4* gene and IL-6 in patients treated with topical therapy (r=0.445). There was also a significant negative coefficient correlation (P<0.05) in patients treated with biological therapy (r= -0.359) (Table 3).

Table 3: Correlation Coefficient between *IKZF4* and IL-6 in Psoriasis patients under different therapies mode

Parameters	Therapy	Pearson's correlation coefficient	*P*-value
IKZF4 gene and IL-6 in psoriasis	Topical therapy	0.445*	0.023
	Etanercept therapy	-0.359*	0.037

DISCUSSION

The results reveal a non-significant difference in the level of IL-6 in the serum of Psoriatic patients who were treated with topical therapy and others with biological therapy compared to the control group. That agrees with the results of a previous study, which found non-significant differences in the level of IL-6 in the serum of patients with psoriasis after treatment with topical therapy such as (Anthralin) plus UVB (ultraviolet B) and Methotrexate compared to the control group that resulted in successful therapy in all treatment groups [11]. In the study by Mrowietz *et al* (1997), they found that there was a decrease in the level of IL-6 after treatment with topical therapy used (Anthralin), which has an anti-proliferative effect on keratinocytes and some evidence revealed its potent inhibitor of inflammatory activities which are part of the pathogenic features of psoriasis [12]. It depends on the fact that Anthralin is a dose-dependent inhibitor of cytokine secretion from human monocytes. Thus, the non-significant differences in the level of IL-6 may be due to the success of the treatments used to maintain the level of this cytokine in patients under treatment and prevent their exacerbation of the disease. Bonifati *et al* (1994) observed a significant median decrease in the level of IL-6 in the serum of patients with psoriasis after treatment with topical therapy (Betamethasone dipropionate, Salicylic acid plus UVB therapy) [13]. They found that the decrease of this cytokine is correlated with the disease activity and its reduction occurs at the same time as the disease improves. A study on the role of anti-TNF agents and MTX on Th17, Th1 and Treg in including IL-6 in the culture supernatant of peripheral mononuclear cells (PMC) after treatment with anti-TNF agents and Methotrexate found that these treatments have an effect on the activation and production of pro-inflammatory and anti-inflammatory cytokines. Also, these treatments were found to regulate CD^{4+} T-cell activation. Anti-TNF agents appear to have a modulating effect on the activation and production of cytokines by Th1, Th17, and Treg cells in a distinct manner [14]. Therefore, the non-significant level of IL-6 in patients treated with biological therapy may be due to the effect of anti-TNF agents on the production of pro-inflammatory cytokines, in which this therapy blocks TNF-α and that effect on the activation and differentiation of other cells may be the source of IL-6 and other cytokines. In psoriasis patients, the results clarified a significant increase (P≤0.05) in the level of IL-6 in males compared to females in all treatment groups, and these results disagree with those of Arican *et al.* (2005) who observed a non-correlation between gender and cytokine level before treatment [15]. The results of the current study showed higher significant differences in the gene expression folding of the *IKZF4* gene (EOS) in psoriasis patients treated with biological therapy compared to the control group and non-significant differences in patients treated with topical therapy. According to a study on mice, indicated that EOS-/- mice developed Treg normally, displayed normal Treg phenotype, and showed normal suppressor function, and they found that EOS was expressed not only on Treg but also inactivated conventional T cells (T convs), which regulate IL-2 and IL-17 production in CD^{4+} Tconvs (conventional Tcells) [4]. EOS acts with Foxp3 to activate most Treg signals [16]. However, deletion of any transcription factor (IRF4, Satb1, Lef1, and GATA-1), including EOS, was not sufficient to change the gene sign of Treg. In conclusion, they demonstrated that the effective role of EOS lies in the activation and differentiation of Tconvs [4]. Sharma *et al* (2013) proved that losing of EOS (encoded by *IKZF4*), a member of the Ikaros gene transcription factor family, shows a serious role in mediating the conversion of Foxp3+Treg from suppressor T cells to Th cells [17]. The results reported by Pan *et al.* (2009) [3] are compatible with Sharma *et al.* (2013) [17] who mentioned that the loss of EOS in the results of both studies led to loss of suppression function and achievement by the EOS-deficient Treg cells of a pro-inflammatory phenotype. The combination of Foxp3 with any of its cofactors is sufficient to lock in the entire sign of Treg cells, and Treg cells' signs can be maintained after the inactivation of any single cofactor. It thus remains a possible conversion of Treg implies the loss of other cofactors in addition to EOS. Each of these interactions may control a segment of Treg cell sign genes, which in turn order different sides of Treg cell function. For example, that the EOS-Foxp3 complex may selectively control the expression of CD40L and the suppression of some pro-inflammatory cytokines [18], and this agreed with the result of the current study, as there is a significant increase in the gene expression folding of *IKZF4* gene (EOS) in patients treated with biological therapy, and this may indicate the effectiveness of Treg cells in inhibiting pro-inflammatory cytokines and patient's condition improvement, while this study that there is a decrease in the gene expression of *IKZF4* gene (EOS) in patients undergoing topical treatment, which may indicate the loss of Treg cells in their suppressor function and their transformation into effector T cells, thereby stimulating the persistence of symptoms in patients.

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Conflicting interests

Nothing declared by the authors.

Data sharing statement

The datasets analyzed during the current study will be available from the corresponding author on a reasonable request.

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