

Serum Levels of Tumor Necrosis Factor- Like Weak Inducer of Apoptosis (TWEAK) in Patients With Psoriasis Vulgaris

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ABSTRACT

Background: Psoriasis is a chronic, immune-mediated, systemic inflammatory disease that is defined by a characteristic skin reaction produced when elevated levels of inflammatory cytokines that alter the growth and differentiation of skin cells. Tumour necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) has been implicated in the pathogenesis of variety of inflammatory disorders and autoimmune diseases. However, studies conducted on the relationship of TWEAK and psoriasis patients are limited.

Objective: We aimed to assess the serum level of TWEAK in psoriatic patients and determine whether levels are related with clinical variables, such as disease severity and duration.

Patients and Methods: The study was conducted on 30 patients with psoriasis attending Dermatology, Venerology & Andrology Outpatient Clinic, Faculty of Medicine, Zagazig University Hospitals spanning the period from January 2019 to March 2019. They were divided into mild, moderate and severe according to Psoriasis Area and Severity Index (PASI) score, 10 patients in each group. The patients' age ranged from 10 to 60 years old. 10 healthy subjects with matched age and sex served as control group.

Results: There was non-significant difference between the studied groups regarding age, BMI, sex, family history, DM and smoking. However, the difference between them was highly significant regarding itching and presence of stressors. The mean TWEAK levels were significantly higher in psoriasis patients than in control subjects, which was significantly associated with severity and duration of the disease. There was positive correlation ($P < 0.001$) between serum TWEAK levels and severity of psoriasis with highly significant level in severe cases.

Conclusion: The serum TWEAK levels significantly correlated with the clinical state, as represented by the PASI score. Also, it was found that TWEAK measurement in serum of patients with psoriasis provides a tool for monitoring disease activity and its use as a marker of disease severity. Thus, they have prognostic significance.

Keywords: PSORIASIS, TWEAK.

INTRODUCTION

Psoriasis is a common, chronic, relapsing /remitting, immune-mediated skin disease characterized by red, scaly papules and plaques, which are usually pruritic ⁽¹⁾. The disease affects 0.09 -11.4% of the general population ⁽²⁾. In psoriasis skin cells are replaced every 3–5 days rather than the usual 28–30 days ⁽³⁾. These changes are believed to stem from the premature maturation of keratinocytes induced by an inflammatory cascade in the dermis involving dendritic cells, macrophages and T cells ⁽⁴⁾. These immune cells move from the dermis to the epidermis and secrete inflammatory chemical signals (cytokines). These secreted inflammatory signals are believed to stimulate keratinocytes to proliferate ⁽⁵⁾.

Psoriasis has been considered a T helper type 1 (Th1)-mediated disease for many years ⁽⁶⁾. T cells infiltrating psoriasis skin lesions display a T-helper/cytotoxic cell (Th/c) 17 phenotype producing the Th/c17 signature cytokines interleukin (IL)-17A, IL-22, and IFN- γ ⁽⁷⁾.

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a member of the TNF-ligand superfamily and promotes the expression of pro-

inflammatory mediators from a variety of cell types, including endothelial cells and keratinocytes ⁽⁸⁾.

TWEAK acts through binding to its receptor expressed on endothelial cells: fibroblast growth factor-inducible 14 (Fn14) ⁽⁹⁾. This substrate–receptor complex regulates many physiological and pathological processes. TWEAK promotes the pro-inflammatory activities of other cytokines such as TNF- α , IL-1, IL-6, and IFN- γ , which also participate in the pathogenesis of psoriasis ⁽¹⁰⁾. TWEAK might play a role in IL-17 signal pathways and has been suggested as a potential targeted treatment in inflammatory diseases ⁽¹¹⁾.

Only limited evidence has been available about the effect of anti-TWEAK treatment in inflammatory process. **Park et al.** ⁽¹²⁾ observed that blockade of Fn14 significantly down regulated IL-17 production related to a variety of inflammatory diseases, including psoriasis. Although increased TWEAK levels have been evaluated in various inflammatory and autoimmune diseases, limited studies have reported the serum TWEAK levels in small samples of psoriasis patients ⁽¹³⁾.



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PATIENTS AND METHODS

This case control study included forty participants who attended the Dermatology, Venerology & Andrology Outpatient Clinic, Faculty of Medicine, Zagazig University, spanning the period from January 2019 to March 2019. Written consents have been taken from all included participants. 30 patients with psoriasis attending Dermatology, Venerology & Andrology Outpatient Clinic, Faculty of Medicine, Zagazig University Hospitals were divided into mild, moderate and severe according to PASI score 10 patients in each group. The patients' age ranged from 10 to 60 years old. 10 healthy subjects with matched age and sex served as control group.

Exclusion criteria:

Other causes of elevated TWEAK and IL17 level as cardiovascular diseases, inflammatory bowel diseases, rheumatoid arthritis, inflammatory lung diseases, osteoarthritis, viral hepatitis, liver cirrhosis, malignancies and any infectious diseases.

Inclusion criteria:

Patients with chronic plaque psoriasis, who were not receiving systemic treatments (e.g., systemic retinoids, cyclosporine, methotrexate or biological therapeutics) for psoriasis for at least three months and with no other comorbid autoimmune or inflammatory diseases, were included in the study. The control group was recruited from the local population and were free from systemic diseases and drug use. The severity of psoriasis was measured by PASI by the same dermatologist⁽¹⁴⁾.

Ethical and Patients' approval:

An approval of the study was obtained from Zagazig University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

All participants were subjected to the following workup:

Thorough history taking and full clinical examination with special stress on complete dermatological examination that was done involving skin, hair, mucous membranes, nails and severity of psoriasis that was assessed by PASI score. Laboratory workup including routine investigation as ESR and CRP and specific investigation included serum levels of TWEAK.

Specimen collection and preparation:

5 ml of venous blood were collected by vein puncture under complete aseptic precautions from every subject divided into two tubes: 1st tube: 1.6 ml of blood were added to anticoagulants tubes for ESR estimation and 2nd tube: 3 ml of blood were

withdrawn into a Serum Separator Tube (SST). The sample was allowed to clot for 30 minutes then the tube was centrifuged for 15 minutes at approximately 1000×g and the serum divided into two tubes, one used for CRP and the second stored at -20°C until assay for TWEAK.

► Human (TWEAK/TNFSF12):

Quantitative analysis of the markers with enzyme-linked immunosorbent assay (ELISA) kits following the protocols of the manufacturers for catalogue no: 201-12-1821 (48T) for TWEAK and Quantitative analysis of the markers with enzyme-linked immunosorbent assay (ELISA) catalogue no: 201-12-0143 (48T)

Evaluation of the disease severity by PASI is the gold standard method to rank the disease severity in patients with chronic plaque-type psoriasis⁽¹⁵⁾. It calculates the affected body surface area and the intensity of the psoriatic plaques. The body is divided into four parts (head, trunk, upper extremities, and lower extremities) by the physician⁽¹⁶⁾.

These areas are scored according to the erythema, induration (thickness), and desquamation (scaling) for each body part. For the final result, weighted average of an area score representing area's proportion of the body is multiplied by the severity score. PASI scores range between 0 and 72. There are no specific laboratory tests for the diagnosis of psoriasis vulgaris or determining severity. Only clinical manifestations guide the diagnosis and allow for choosing the right treatment according to the intensity of the disease.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS version 19. Continuous Quantitative variables were expressed as the mean ± SD & median (range). Categorical qualitative variables were expressed as absolute frequencies (number) & relative frequencies (percentage). Continuous data were checked for normality by using Shapiro Walk test. One-way ANOVA (F test) was used to compare more than two groups of normally distributed data. Kruskal-Wallis (KW) test was used to compare more than two groups of not-normally distributed data. Mann-Whitney test was used to compare two groups of not-normally distributed data. Categorical data were compared using Chi-square test (χ^2 test).

Pearson's correlation was used (r: coefficient correlation) to detect degree of association between two numeric variables. All tests were two sided. P-value ≤ 0.05 was considered statistically significant (S), p-value < 0.001 was considered highly statistically significant (HS), and p-value > 0.05 was considered statistically insignificant (NS). Validity of

the screening tests (IL, TWEAK) were assessed in the terms of sensitivity, specificity, predictive value positive, predictive value negative and accuracy.

RESULTS

Serum TWEAK concentrations:

As shown in table (1), there was highly statistically significant difference between the studied groups regarding TWEAK. It was found to be higher among patients with severe psoriasis compared to those with mild and moderate disease and in the control group (295.6 versus 260.3, 293.7 and 84.8 respectively).

Correlations of TWEAK and traditional parameters:

Concerning IL-17, our study showed that there was positive significant correlation between TWEAK

level and disease duration, PASI, ESR and CRP (Table 2).

Receiver operating characteristic curves analysis (ROC curve):

Receiver operating characteristic (ROC) curves for serum TWEAK levels were calculated for distinguishing psoriatic patients from healthy controls. The diagnostic performance of serum TWEAK presented high sensitivity and specificity.

Table (3) showed that cutoff point of TWEAK equal to or more than 153.4 can be used as a predictor for presence of psoriasis disease with sensitivity of 93.3%, specificity of 90%, PVP of 96.5% and PVN of 81.8%.

Table (1): Studying of serum TWEAK among the studied groups.

Variable	Control group (n=10)	Mild psoriasis group (n=10)	Moderate psoriasis group (n=10)	Severe psoriasis group (n=10)	KW	P
TWEAK:						
<i>Mean ± SD</i>	87.9 ± 57.9	283.5 ± 100	270.3 ± 94.7	278.6 ± 82.6	19.2	<0.001 (HS)
<i>Median</i>	84.8	260.3	293.7	295.6		

(KW) Kruskal-Wallis test. P-value < 0.001 was considered highly statistically significant (HS).

Table (2): The correlation between TWEAK and different parameters among the studied group

Variable	TWEAK	
	p	r
Age	0.97	0.006
Disease duration	0.001	0.497
BMI	0.78	0.045
PASI	0.03	0.341
ESR	0.03	0.344
CRP	0.02	0.348
TWEAK	---	---

r: coefficient correlation p-value < 0.05 was considered statistically significant (S) p-value < 0.001 was considered highly statistically significant (HS) p-value ≥ 0.05 was considered statistically insignificant (NS)

Table (3): Performance of TWEAK as a predictor of psoriasis among the studied group.

Cutoff point	AUC	Sens.	Spec.	PVP	PVN	accuracy	P value
≥ 153.4	0.967	93.3%	90%	96.5%	81.8%	92.5%	<0.001

Sig.: Significance. (Sens) Sensitivity. (Spec) Specificity. (PVP) predictive value positive. (PVN) Predictive value negative P-value < 0.001 was considered highly statistically significant (HS)

DISCUSSION

The present study demonstrated that there was significant increase in serum TWEAK in psoriasis patients. Furthermore, results showed significant correlations between serum TWEAK levels and psoriasis severity and duration.

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a member of the TNF-ligand superfamily and promotes the expression of pro-inflammatory mediators from a variety of cell types, including endothelial cells and keratinocytes (17).

TWEAK can also induce other pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-15, and IL-18 (18), matrix metalloproteinase-9 (MMP-9) (19), MMP-1, prostoglandin E2 and IL-17 (20), which are produced by Th-17 cells and take a part in host defense against infections, autoimmune diseases, and allergic-specific immune responses (21).

Previous studies on the TWEAK-Fn14 pathway's effect on psoriasis were controversial. According to **Cheng et al.** (13) high expression of TWEAK/Fn14 increased the inflammation and proliferation of keratinocytes. **Sidler et al.** (22) showed that subcutaneous injection of TWEAK in animals led to localized skin inflammation with the histological and molecular features of atopic dermatitis and psoriasis. **Bilgic et al.** (23) showed that serum TWEAK levels were significantly higher in psoriasis patients than in healthy controls and a significant correlation between serum TWEAK levels and serum IL-23 levels existed. **Alaoui et al.** (24) showed that TWEAK induced keratinocyte cell death with TWEAK/Fn14 complex existing in healthy human epidermis and skin appendices. Malignant and benign lesions including psoriasis showed increased epidermal TWEAK/Fn14 expression. However, **Zimmerman et al.** (25) evaluated the TWEAK expression in the psoriatic skin and showed that TWEAK expression in the psoriatic skin was insignificant. **Peternal et al.** (26) showed that TWEAK was downregulated in the proliferating keratinocytes of healthy skin, hyperplastic skin and neoplastic skin.

In alignment with the previous work, we hypothesized that higher serum TWEAK levels induced inflammation and angiogenesis in psoriasis. We investigated the relationship between serum TWEAK levels and PASI scores. The correlation coefficient was calculated as 0.341 and the correlation coefficient with disease duration was 0.497 which is significant. There was a correlation between serum TWEAK level and PASI score and in turn with the disease severity and duration. Previous studies reported different serum TWEAK concentrations in the setting of psoriasis. A standardized range was not accepted, so we could not compare our results to a

standardized range. We found that higher serum TWEAK levels are related with psoriasis.

In our study, TWEAK was found to be significantly ($p < 0.001$) higher in the patients' group. It was higher among patients with severe psoriasis compared to those with mild and moderate disease and in the control group (295.6 versus 260.3, 293.7 and 84.8 respectively). This is consistent with the findings of **Xia et al.** (27) who detected that disease activity scores of psoriatic arthritis are positively correlated with serum TWEAK levels. They mentioned that TWEAK levels correlated well with the clinical state of psoriasis as represented by PASI score and its measurement in serum of patients with psoriasis provides a tool for monitoring disease activity. Our results are not correlated with **Bilgic et al.** (23) and **Zimmerman et al.** (25) who mentioned that no significant correlations were observed between serum TWEAK levels and psoriasis severity or duration and explained these results with that serum TWEAK levels may be related to the pathogenesis of psoriasis regardless of disease severity. Our result may not be similar to the previous studies due to small sample size, so further studies with a large number of patients should be conducted in order to conclude a relationship between the variables.

As revealed in studied results in table (3), cutoff point of TWEAK equaled to or more than 153.4, where sensitivity, specificity and accuracy of serum TWEAK levels were 93.3%, 90.0% and 92.9%, respectively. Therefore, the estimation of serum TWEAK level, as a diagnostic indicator, could be used for early detection of psoriatic patients.

This study support the finding of **Cheng et al.** (13) and **Jadali** (28), who cited that TWEAK is an aneoteric protein biomarker with great specificity and high sensitivity for identification of psoriasis. Moreover, it is essential targeting to signals of activated cells and can be used as potential drug targets of psoriasis. Additionally, when it is expressed in the psoriatic lesional skin, it is associated with anomalous differentiation and hyper-proliferation of keratinocyte and control induction of inflammatory cytokines.

CONCLUSION

In this study, serum TWEAK levels significantly correlated with the clinical state, as represented by the PASI score. Also, it was found that TWEAK measurement in serum of patients with psoriasis provides a tool for monitoring disease activity and a marker of disease severity. Thus, they have prognostic significance.

Thus, it is important to determine a threshold value. However, such a cut-off value of TWEAK could not be calculated in our study similar to previous studies. There is still limited data about TWEAK's role

in the mechanism of psoriasis. If its serum levels were to be standardized in further studies, TWEAK could be used as a follow-up marker of psoriasis patients with the PASI score. Further studies of TWEAK and its receptor Fn14 levels detection would help to elucidate the mechanism of the disease as well as to discover a new target molecules for the treatment of psoriasis vulgaris.

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