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Psoriasis vulgaris and new biomarkers: Tumor necrosis factor- like weak inducer of apoptosis (TWEAK) and Interleukin 17(IL17)

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Abstract

Background and objectives: Inflammatory cytokines such as interleukin 17 (IL-17) and tumor necrosis factor like weak inducer of apoptosis (TWEAK) were implicated in pathogenesis of many immune mediated systemic inflammation, the current study designed for evaluating the serum level of both marker in patients with psoriasis. Another secondary objective was finding any possible correlation between IL-17 and TWEAK with disease severity and duration.

Methods: 30 patients with psoriasis had enrolled in the study, and classified as regarding the disease severity by psoriasis area and severity index (PASI) in to mild, moderate and severe. Another 10 control subjects were involved after ethical approval. The level of IL-17, TWEAK and CRP in serum had been measured, as well as ESR level in patients and control group.

Results: The mean TWEAK, IL-17 levels were significantly higher in psoriasis patients than in control subjects, $P < 0.001$ for both. TWEAK and IL-17 were significantly correlated with disease severity and duration in direct way, $r = 0.34$ and 0.66; $P = 0.03$ and < 0.001, $r = 0.47$ and 0.39; $P = 0.001$ and 0.01 respectively. The performance of TWEAK and IL-17 in predicting psoriasis were excellent, the AUC were 97% and 95% respectively.

Conclusion: The level of both TWEAK and IL-17 were higher in psoriases and had direct correlation with disease severity and duration.

Introduction

Psoriasis is a red scaly skin lesion in form of papules and plaques, itching in characters and commonly chronic with frequent relapsing and remitting attacks (1). The prevalence of the disease ranged from 0.09 up to 11.4% (2). The frequency of psoriasis skin cell replacement was every 3-5 days, while the normal cells replacement was every 28-30 days (3), and that may be duo to premature maturation of keratinocytes by the effect of inflammatory surge in dermis, which contain a lot of immune cells as macrophage, denderitic cells and T cells (4). The preceding cells migrate from dermis to epidermis producing a lot of inflammatory chemical signals (cytokines) that responsible for keratinocytes proliferation (5). Formerly, psoriasis has been considered a T helper type 1 (Th1)-mediated disease, hence the T cells in

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lesion exhibit a T-helper/cytotoxic cell (Th/c) 17 phenotype making the Th/c17 signature cytokines interleukin (IL)-17A, IL-22, and IFN-γ (6,7). The studies supported that psoriasis onset unmolded through interleukin-23/interleukin-17 (IL-23/IL-17) immunologic pathway (8). For this reason, IL-17A and IL-17F stimulate keratinocytes to produce another cytokines as antimicrobial peptides (AMPs) and β-defensins (9).

The family of tumor necrotic factors contained many members; Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) was one of them, and its role was promoting the expression of pro inflammatory mediators that emerged from different cell types as keratinocyte and endothelial cells through binding with fibroblast growth factor-inducible 14 (Fn14) receptors (10, 11). This complex of substrate- receptor pathway was involved in regulation of many physiological and pathological actions of other cytokines as IL-1 and 6, TNF-α and IFN-γ (12). Furthermore, TWEAK might be implicated in IL-17 signal pathway, which made it as a prospective targeted therapy for psoriasis and other immune inflammatory disease (13). Restricteddata suggest the possibility of using anti-TWEAK treatment, thus the authors observed a significant down regulation of IL-17 by blocking Fn14 (14). In psoriasis, a small study evaluating the level of TWEAK level in serum (15). From that point of view, tha current study was designed to evaluate the level of TWEAK and IL-17 in patients with psoriasis, and subsequently correlate its level with clinical characteristics of patients and other inflammatory markers; CRP and ESR.

Methods

Study design, setting and target population

A case control study with convenient sampling methods including 30 patients with psoriasis that attended to Dermatology, Venerology& Andrology Outpatient Clinic, Faculty of Medicine, Zagazig University, spanning the period from January 2019 to March 2019, and 10 healthy control from relatives of patients. A written consent from both cases and control had obtained.

All patients after fulfilling the inclusion and exclusion criteria of the study were classified as regarding the severity index of psoriasis (PASI score) in to three groups; mild, moderated and severe.

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 disease and drug use. The severity of psoriasis was measured with the Psoriasis Area and Severity Index (PASI) by the same dermatologist (16).

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.

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Method and study tools

All participants were subjected to the following workup: (1)Thorough history and full clinical examination with special stress on Complete dermatological examination was done involving skin, hair, mucous membranes and nails.

(2) Psoriasis Area and Severity Index (PASI score) for evaluation of disease severity; PASI is the gold standard method to rank the disease severity in the patients with chronic plaque-type psoriasis (17), 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 (18), 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.

(3)Laboratory workup; routine investigation as ESR and CRP and specific investigation include serum levels of TWEAK, IL-17.

Specimen collection and preparation

5 ml of venous blood were collected by vein puncture under complete aseptic precaution from every subject divided into two tubes: 1st tube: 1.6 ml of blood was added to anticoagulants tubes for ESR estimation,2nd tube: 3ml of blood was withdrawn in to 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 in two tubes, one used for CRP and the second stored at -20°C until assay for TWEAK and IL17.

Human (TWEAK/TNFSF12) and IL-17

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)

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), and 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.Kruskall-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 (χ2test). 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

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

Results

Demographic and clinical data

The mean age in the studied groups were (42.7 ± 12.9), (39.4 ± 11.9), (38 ± 10.2) and (40.1 ± 11.3) years for groups control, Mild, moderate and severe psoriasis respectively, (p=0.836). The patients and control were matched as regarding the sex and BMI, (p=0.849 and 0.515) respectively. Considering the family history and DM as comorbidity the cases and control were matched, (p=0.265 and 0.17) respectively, while clinical complain of itching was significantly correlated with patient, P < 0.001 **(Table 1)**.

Regarding PASI score and duration of disease, the data informed that the group with severe disease had significantly higher PASI score and longer disease duration; 32.1 versus 6.1 and 17.6 respectively for PASI and 9.4 ± 4.5 versus 4.8 ± 2.6 and 5.4 ± 4.8 years respectively for duration **(Table 2)**.

Serum TWEAK, IL-17 concentrations

As shown in **(Table 2)**, there was highly statistically significant difference between the studied groups as 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). Moreover, there was highly statistically significant difference between the studied groups as regarding IL-17.It was found to be higher among patients with severe psoriasis compared to those with mild and moderate disease and in the control group (47.1 versus 14.3, 24.9 and 6.5 respectively).

Correlations of TWEAK and IL-17 and traditional parameters

As regarding IL-17; **(Table 3)** shows that there was positive significant correlation between IL-17 and disease duration, PASI, ESR and CRP, in addition, as regarding TWEAK, there was positive significant correlation between TWEAK and disease duration, PASI, ESR and CRP.

Validity of TWEAK and IL-17 for psoriasis prediction

The diagnostic performance of IL-17 and TWEAK by Receiver operating characteristic (ROC) curves showed high sensitivity and specificity **(Table 4)** and **(Figure 1)**. The cutoff point of TWEAK ≥ 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%. While the cutoff point of IL-17 ≥ 12.1 can be used as a predictor for presence of psoriasis disease with sensitivity of 90%, specificity of 90%, PVP of 96.4% and PVN of 75%.

Discussion

The family of TNF includes a significant members that play a role in immune mediated inflammation; TWEAK. The later promote the expression of different mediators that released from different cells as well as induction of other inflammatory cytokines as IL-1, 6, 15 and 17 (19-23).

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Immunological pathway through (IL23/IL-17) plays a role in psoriasis onset and unmolding (24). Furthermore, the pathway of TWEAK-Fn14 in psoriasis pathogenesis was controversial (15).

Experimental animal study reporting a skin changes like psoriasis after local injection of TWEAK (25).

The current study reporting that both serum TWEAK and IL-17 were significantly higher in patients with psoriasis than control as well as the more the severity of the disease the higher the level of marker. That came in consistence with *Bilgic et al.*, hence he found that serum TWEAK was higher in psoriasis and had direct correlation with IL23 level in serum, and disagreed in concept of positive correlation with disease severity (26). Additionally, *Zimmerman et al.*, supported that disagreement, and explained with; TWEAK was involved in pathogenesis of disease whatever its activity (27-29). On the other hand, *Xia et al.*, agreed that TWEAK level had a positive correlation with PASI score and could be used as a monitor for disease follow up (30).

Our results were supported by previous studies in point of elevation of IL-17 level in patients with psoriasis than healthy control, while regarding the disease severity; they found no correlation between IL-17 level and PASI (31-33). A discrepancy results reported in different studies, hence, they found insignificant difference between patients and control as regarding serum IL-17 level (34-36).

Our recent data demonstrated that both TWEAK and IL-17 had positive correlation with CRP and ESR, and their performance in predicting disease from healthy subject was higher, thus AUC were 96% and 95% respectively. Moreover at cutoff point ≥153.4 and 12.1, the sensitivity, specificity, PPV and NPV were 93.3; 90, 90; 90, 96.5; 96.4 and 81.8; 75% respectively. That came in harmony with other reports, as they stated that both TWEAK and IL-17 had higher sensitivity and specificity in psoriasis detection, which encourage the suggestion to use anti TWEAK in treatment of psoriases (15, 37).

The study had some limitations; the important one is small sample size, as well as short duration for fulfilling. A larger sample size and longitudinal study was recommended to empower the data and highlighting other predictors for TWEAK and IL-17 level in serum.

Conclusion

The level of both TWEAK and IL-17 levels in serum were significantly correlated with disease severity and duration, so it could be used as a tool for monitoring disease activity. Further studies of TWEAK and its receptor Fn14 levels would help to clarify the mechanism of the disease as well, leading to the discovery of new target molecules for the treatment of psoriasis vulgaris.

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Table (1): Demographic characters of the studied group

Factors	Control (n=10)		Mild (n=10)		Moderate (n=10)		Severe (n=10)		P
Age	42.7 ± 12.9		39.4 ± 11.9		38 ± 10.2		40.1 ± 11.3		0.836§
BMI	28.9 ± 4.5		27.3 ± 4.6		26.3 ± 5.3		29.3 ± 5.3		0.515§
Sex									
Female	5	50	6	60	5	50	4	40	0.849#
Male	5	50	4	40	5	50	6	60	
Family history									
Absent	10	100	9	90	8	80	10	100	0.265#
Present	0	0	1	10	2	20	0	0	
Itching									
Absent:	10	100	0	0	0	0	0	0	<0.001#
Present:	0	0	10	100	10	100	10	100	
DM									
Absent:	7	70	8	80	10	100	6	60	0.17#
Present:	3	30	2	20	0	0	4	40	
Stressors:									
Absent:	10	100	2	20	3	30	1	10	<0.001#
Present:	0	0	8	80	7	70	9	90	

§: One-Way ANOVA test, #: Chi square test, P consider significant if < 0.05.

Table (2): Clinical and Laboratory data of patients

Factors	Control (n=10)	Mild (n=10)	Moderate (n=10)	Severe (n=10)	P§
Disease duration	NA	4.8 ± 2.6	5.4 ± 4.8	9.4 ± 4.5	<0.001
PASI	NA	6.1 ± 1.7	17.6 ± 2.04	32.1 ± 9.4	<0.001
ESR	7.1 ± 2.8	13.6 ± 3.2	24.1 ± 3.3	54 ± 13.9	<0.001
CRP	5.3 ± 2.8	8.1 ± 1.8	18.4 ± 4.5	36.1± 5.9	<0.001
IL-17	7.6 ± 4.3	19.5 ± 11.5	33.4 ± 26.1	49.8 ± 16.9	<0.001
TWEAK	87.9 ± 57.9	283.5 ± 100	270.3 ± 94.7	278.6 ± 82.6	<0.001

§: One-Way ANOVA test, P consider significant if < 0.05.

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Table (3): Correlation between TWEAK&IL17 with clinical and laboratory parameters

Variable	TWEAK		IL-17	
	r	P*	r	P*
Age	0.006	0.97	0.1	0.541
Disease duration	0.497	0.001	0.397	0.01
BMI	0.045	0.78	0.117	0.471
PASI	0.341	0.03	0.642	<0.001
ESR	0.344	0.03	0.662	<0.001
CRP	0.348	0.02	0.641	<0.001
TWEAK	*	*	0.27	0.092

*: Person correlation coefficient, the sign before "r" denoting the direction of relationship, P < 0.05 considered significant

Table (4): Performance of TWEAK and IL-17 in diagnosing psoriasis

Marker	Cutoff point	AUC	Sens.	Spec.	PPV	NPV	accuracy	P
TWEAK	≥ 153.4	97%	93%	90%	97%	82%	93%	<0.001
IL-17	≥ 12.1	95%	90%	90%	96%	75%	90%	<0.001

Sens.: sensitivity, Spec.: specificity, PPV: positive predictive value, NPV: negative predictive value, P considered significant if < 0.05

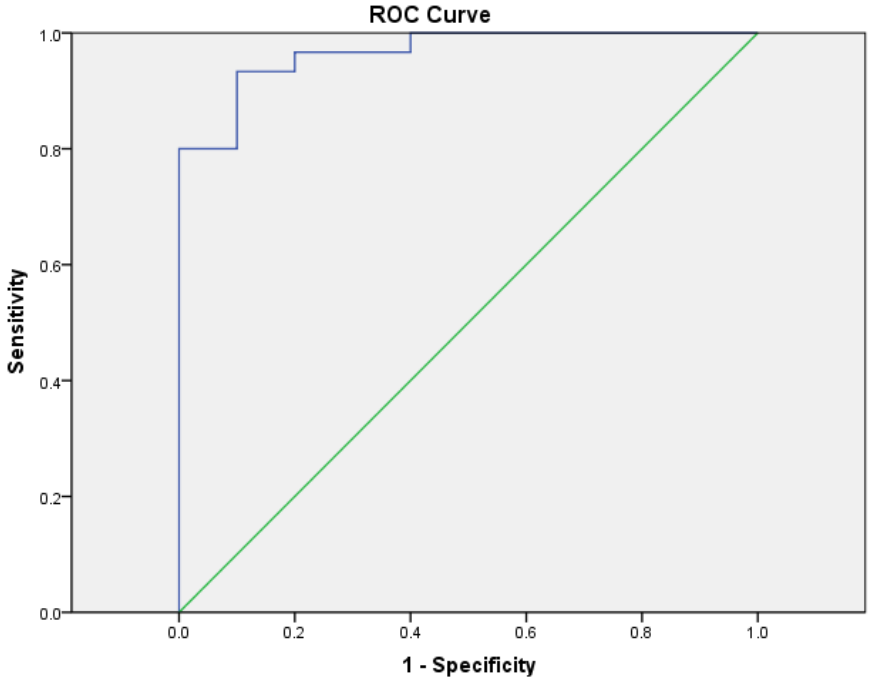

Figure (1-a): ROC curve of TWEAK for selecting psoriasis

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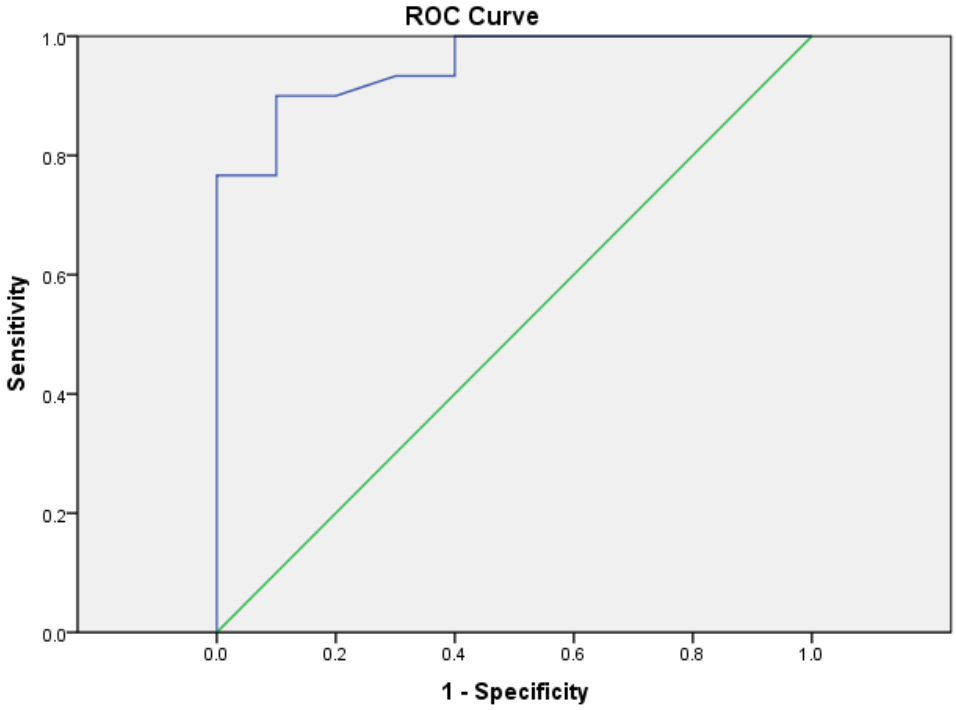

Figure (1-b): ROC curve of IL-17 for selecting psoriasis