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Serum TWEAK: A cutoff between segmental and nonsegmental vitiligo

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Abstract

Background: TWEAK/Fn14 is expressed in many tissues including the skin, playing an important role in many inflammatory, autoimmune, and neoplastic cutaneous disorders.

Aims: To assess the serum levels of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in vitiligo patients.

Methods: This case-control study included 100 subjects (50 vitiligo patients and 50 control subjects) recruited from Dermatology Outpatient Clinic, Benha University. All patients were subjected to complete cutaneous examination, to evaluate the clinical type, distribution and severity of vitiligo using the Vitiligo Area Scoring Index (VASI).

Results: TWEAK serum levels were significantly higher in patients than in the control subjects (644.76 ± 688.93 vs 282.75 ± 125.67, respectively). Serum levels were significantly elevated in segmental versus nonsegmental vitiligo. Receiver operating characteristic (ROC) analysis revealed that TWEAK shows 80% sensitivity and 56.67% specificity in diagnosing vitiligo and 100% sensitivity and 80.09% specificity in differentiating segmental from nonsegmental vitiligo.

Conclusion: TWEAK may play a role in vitiligo pathogenesis. It may be used in the differentiation between segmental and nonsegmental vitiligo and represent a promising therapeutic target in vitiligo.

KEYWORDS

segmental, TWEAK, Vitiligo

1 | INTRODUCTION

Vitiligo is a common disorder of pigmentation caused by acquired melanocyte destruction. Multiple theories have been proposed to outline the pathogenesis of this depigmentation; however, the exact pathogenesis is not yet clarified. The most accepted theory is considering vitiligo as an autoimmune disorder affecting genetically predisposed individuals.[1] Among the involved cytokines in this complex disorder, interferon (IFN)-$^{\gamma}$ plays an essential role in recruiting the $CD8^{+}$ T cells, which attack self-melanocytes.[2] Other cytokines that participate in the pathogenesis of vitiligo, with less understood role, include TNF-α, IL-6, and IL-17.[3]

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a multifunctional cytokine, which belongs to the tumor necrosis factor receptor family ligands.[4] At first, it was considered to be an apoptosis inducer only, and then, its role in regulating many vital processes such as angiogenesis, inflammation, and cell proliferation has been discovered.[5] TWEAK exerts its role via binding to its receptor, fibroblast growth factor-inducible 14 (Fn14). This binding triggers a cascade of intracellular events ending in activation of nuclear factor-kappa B (NF-κB) with subsequent expression of multiple inflammatory molecules and enhancing the inflammatory effects of other proinflammatory cytokines, for example, TNF-α, interleukin (IL)-1, IL-6, and interferon-γ.[6]

TWEAK/Fn14 is expressed in many tissues including the skin, playing an important role in many inflammatory, autoimmune, and neoplastic cutaneous disorders.[7]

The role of TWEAK has been discussed in skin diseases including psoriasis,[8] atopic dermatitis,[9] and urticarial vasculitis.[10] However, it is not yet studied in vitiligo.

The present study is a trial to assess the serum levels of TWEAK in vitiligo patients to evaluate its potential role in the pathogenesis of this disorder.

2 | SUBJECTS AND METHODS

2.1 | Subjects

This case-control study included 100 subjects recruited from Dermatology Outpatient Clinic. A written informed consent was obtained from all participants. The study was approved by the local ethics committee on research involving human subjects of Faculty of Medicine. The enrolled subjects included 50 vitiligo patients and 50 apparently healthy and age-, sex-, and skin type-matched vitiligo-free individuals as a control group.

Vitiligo patients were excluded from this study if they were suffering from concurrent significant medical conditions such as malignancy, diabetes mellitus, and hepatic, renal, or cardiovascular diseases. Other excluded patients were those who had other autoimmune diseases, and those who received treatment with phototherapy within one month or applied topical antivitiligo therapy within 2 weeks prior to the study initiation. Pregnant or breastfeeding patients were also excluded from this study.

2.2 | Methods

All patients in this study were subjected to full history taking and complete cutaneous examination in order to evaluate the clinical type, distribution, and severity of vitiligo using the Vitiligo Area Scoring Index (VASI).[11]

2.3 | Laboratory investigations for all enrolled participants

- Complete blood count was performed by utilizing automated cell counter Sysmex XE 5000.[12]
- Fasting blood glucose level, and liver and kidney function tests were performed by appropriate chemical principles using Biosystem A15 auto-analyzer.
- Erythrocyte sedimentation rate and ANA measurements were taken.
- Human ELISA (sandwich technique) kits were used to measure serum TWEAK levels. Kits were provided by SuRed, biotechnology, Shanghai, China (Cat. No. 201-12-1821). The assay range was 15–4200 ng/mL.

2.4 | Statistical analysis

All statistical analyses were carried out in STATA/SE version 11.2 for Windows (STATA Corporation, College Station, Texas). P value <.05 was considered significant.

The collected data were summarized in terms of mean ± standard deviation (SD) and range for quantitative data and frequency and percentage for qualitative data. Comparisons between the different study groups were carried out using the chi-square test ($\chi 2$) and Fisher's exact test (FET) to compare differences between proportions. The Mann-Whitney test (Z) was used to compare two groups as regards nonparametric data, and the Kruskal-Wallis test was used to compare more than two groups. The Spearman correlation coefficient (rho; ρ) was used to examine the correlation between serum levels of TWEAK and estimated parameters.

Receiver operating characteristic (ROC) analysis was carried out to evaluate the diagnostic performance of TWEAK levels. The best cutoff point and the corresponding sensitivity and specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) were estimated.

3 | RESULTS

There was no significant difference between patients and control groups as regards gender (74% vs 70% females, $P = .66$) using the chi-square test. Both groups were also matching regarding age (30.74 ± 15.88 vs 30.22 ± 10.63, respectively, $P = .85$) and BMI (25.34 ± 3.02 vs 25.46 ± 3.48, respectively, $P = .85$) using Student's t test. Both groups also were matching regarding skin types (40 patients with type III and 10 with type IV versus 35 control subjects with type III and 15 with type IV, $P = .25$) using the chi-square test.

The mean age of onset of vitiligo in our patients was 8.93 ± 9.55 years. The mean VASI score in the studied patients was 23.39 ± 12.33. The clinical types of vitiligo in our patients were nonsegmental vitiligo in 30 patients (12 acrofacial, 8 focal, and 10 generalized) and segmental vitiligo in 20 patients.

Using the Mann-Whitney test, serum levels of TWEAK were significantly higher in the patients when compared to its levels in the control subjects (644.76 ± 688.93 vs 282.75 ± 125.67, respectively, $P = .01$). Among the collected demographic and clinical data, the clinical type of vitiligo was the only factor affecting TWEAK levels,

TABLE 1 Correlation between TWEAK and estimated parameters

Variable (no.=50)	Spearman correlation coefficient (rho; ρ)	*P*
Age (years)	−0.08	.57
BMI (kg/m^2)	0.09	.52
Duration (years)	−0.20	.16
VASI score	0.04	.76

TABLE 2 Variation in TWEAK by personal and clinical characteristics of cases

Variable		TWEAK (ng/mL) No.	Mean	SD	Mann-Whitney test	*P*
Sex	Female	37	615.11	668.1	0.59	.56
	Male	13	729.12	767.16		
Skin Type	III	40	716.32	751.62	0.46	.64
	IV	10	358.49	149.01		
Clinical types	Nonsegmental	30	624.68	683.07	3.70	<.001
	Segmental	20	1126.5	918.25		
Leukotrichia	Negative	40	716.32	751.62	0.46	.64
	Positive	10	358.49	149.01		

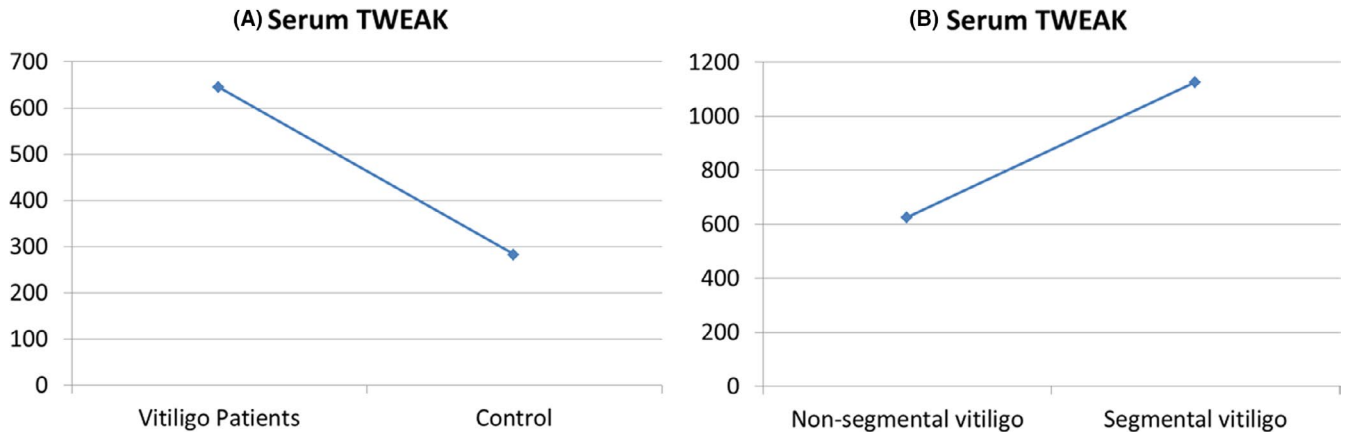

FIGURE 1 Serum TWEAK levels; A, patients versus control, B, nonsegmental versus segmental vitiligo

where serum levels were significantly elevated in segmental versus nonsegmental vitiligo (Tables 1 and 2 and Figure 1).

Receiver operating characteristic (ROC) analysis revealed that TWEAK shows 80% sensitivity and 56.67% specificity in diagnosing vitiligo and 100% sensitivity and 80.09% specificity in differentiating segmental from nonsegmental vitiligo (Table 3 and Figures 2 and 3).

4 | DISCUSSION

In the current work, serum levels of TWEAK in vitiligo patients were significantly higher than in the control subjects. Among the accepted theories that tried to explain the melanocyte loss in vitiligo is melanocyte apoptosis. This could be triggered by different factors, such as molecular mechanisms, exposure to certain exogenous chemicals, and the effect of different cytokines including TNF-α and TNF-related apoptosis-inducing ligand (TRAIL), which are also members of the TNF superfamily.[13-15]

In fact, fibroblast growth factor-inducible 14 (Fn14), the main TWEAK receptor, is not expressed on normal melanocytes; however, it could be expressed in most of melanoma cell lines and targeting this receptor is considered a promising therapeutic option for melanoma patients.[16] Moreover, Fn14 is expressed on keratinocytes, and when it is activated by TWEAK, it induces keratinocyte apoptosis in coordination with tumor necrosis factor-α.[17] It is now well established that vitiligo is not a pure melanocyte defect, but keratinocytes also show structural and functional disturbances in vitiligo patients. In addition, keratinocytes undergo apoptosis in vitiliginous skin leading to deprivation of melanocytes of its keratinocyte-derived growth factors with subsequent passive death of melanocytes.[18,19] These facts together with the current findings suggest that TWEAK may play a role in the development of vitiligo.

Serum levels of TWEAK in our segmental vitiligo patients were significantly higher than in nonsegmental vitiligo patients. Vitiligo is classified into two major types, segmental vitiligo (SV), which may manifest as focal, acrofacial, generalized, or universal vitiligo; and nonsegmental vitiligo (NSV).[20] When compared to nonsegmental vitiligo, the segmental form is not associated with autoimmune diseases, has an early-onset and more rapid progression followed by a stable course, and responds poorly to medical treatment.[21,22]

Focal vitiligo appears as a hypopigmented patch that is not classically segmental in distribution. Focal vitiligo could be considered an initial stage of nonsegmental vitiligo or may be an abortive form of the segmental type. It may evolve into either SV or NSV.[23,24] The great difference in the course, associated autoimmune diseases and

TABLE 3 Diagnostic performance of TWEAK in vitiligo

No.=100	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC
Patients vs controls	226.2	80.0	56.67	75.47	62.96	71.25	0.7633
Segmental vs nonsegmental	463.5	100.0	80.95	76.92	100.0	84.0	0.8452

Abbreviations: AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; ROC analysis, receiver operating characteristic analysis.

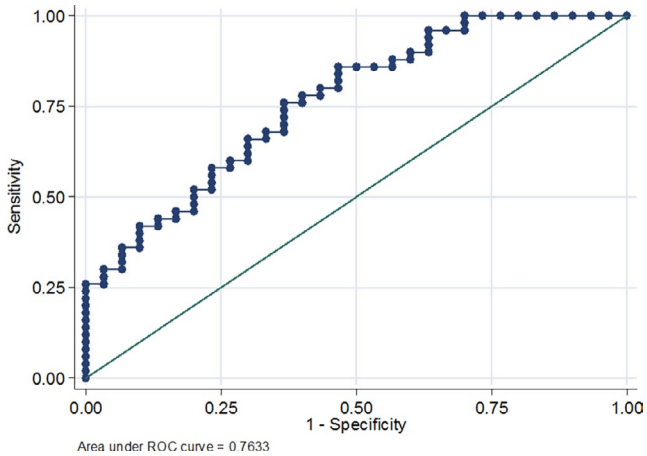

FIGURE 2 Cutoff point of TWEAK in diagnosing vitiligo

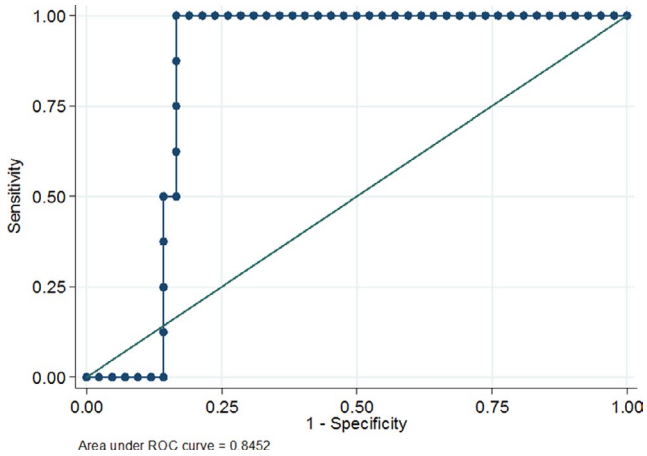

FIGURE 3 Cutoff point of TWEAK to differentiate between segmental vitiligo and nonsegmental vitiligo

the therapeutic outcomes between segmental vitiligo and nonsegmental vitiligo urges the search for a tool, which can predict the fate of these focal lesions.

In daily clinical practice, with the appearance of the first vitiligo patch, the patient and his/her parents come in a panic attack. They start asking several questions about its fate and whether it will spread or not. We think that finding an objective method to predict the behavior of this "scary patch" would reduce the patient's stress and help the physician to tailor the suitable treatment plan. The significant elevation of TWEAK in segmental vitiligo when compared to nonsegmental vitiligo together with its high sensitivity (100%) and specificity (80.95%) in differentiating between segmental and nonsegmental vitiligo at a cutoff point of 463.5 ng/mL also can help better understand segmental vitiligo pathogenesis and may suggest that TWEAK is a promising marker to predict the fate of focal vitiligo and subsequent prediction of its progress. In fact, there are no data to show whether TWEAK differences between SV and NSV would be distinguishable at that point or not. However, the current results encourage more research and follow-up for patients with focal vitiligo to determine their prognosis and correlate the fate of the focal lesions with serum TWEAK levels.

The significant serum TWEAK level elevation in vitiligo patients might put the therapeutic value of targeting TWEAK in vitiligo under question. The TWEAK/Fn14 pathway has become a potential therapeutic target in different autoimmune and inflammatory diseases, such as chronic autoimmune arthritis, systemic lupus erythematosus, and experimental autoimmune encephalomyelitis.[25-27] Targeting TWEAK/Fn14 pathway may be mediated by anti-TWEAK antibodies, anti-Fn14 antibodies, Fn14-Fc (a fusion protein of the ectodomain of Fn14 with the Fc domain of IgG), soluble TWEAK, and Fc-TWEAK.[28]

5 | CONCLUSION

TWEAK may play a role in vitiligo pathogenesis, differentiate between segmental and nonsegmental vitiligo, and represent a promising therapeutic target in vitiligo. Although the authors suggest that TWEAK may be a potential predictor for the fate of focal vitiligo, there were only 50 patients enrolled in this work. Larger scale investigations are recommended.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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REFERENCES

1. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: A comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. *J Am Acad Dermatol*. 2011;65:473-491.
2. Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. A mouse model of vitiligo with focused epidermal depigmentation

requires IFN-gamma for autoreactive CD8(1) T-cell accumulation in the skin. *J Invest Dermatol*. 2012;132:1869-1876.
3. Rodrigues M, Ezzedine K, Hamzavi I, Pandya AG, Harris JE. Vitiligo Working Group. New discoveries in the pathogenesis and classification of vitiligo. *J Am Acad Dermatol*. 2017;77(1):1-13.
4. Wiley SR, Winkles JA. TWEAK, a member of the TNF superfamily, is a multifunctional cytokine that binds the TweakR/Fn14 receptor. *Cytokine Growth Factor Rev*. 2003;14(3–4):241-249.
5. Burkly LC, Michaelson JS, Hahm K, Jakubowski A, Zheng TS. TWEAKing tissue remodeling by a multifunctional cytokine: role of TWEAK/Fn14 pathway in health and disease. *Cytokine*. 2007;40(1):1-16.
6. Winkles JA. The TWEAK-Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. *Nat Rev Drug Discov*. 2008;7:411-425.
7. Liu Q, Xiao S, Xia Y. TWEAK/Fn14 Activation Participates in Skin Inflammation. *Mediators Inflamm*. 2017;2017:1-12.
8. Bilgiç Ö, Sivrikaya A, Toker A, Ünlü A, Altınyazar C. Serum levels of TWEAK in patients with psoriasis vulgaris. *Cytokine*. 2016;77:10-13.
9. Sidler D, Wu P, Herro R, et al. TWEAK mediates inflammation in experimental atopic dermatitis and psoriasis. *Nat Commun*. 2017;8:15395.
10. Li M, Chen T, Guo Z, Li J, Cao N. Tumor necrosis factor-like weak inducer of apoptosis and its receptor fibroblast growth factor-inducible 14 are expressed in urticarial vasculitis. *J Dermatol*. 2013;40(11):891-895.
11. Hamzavi I, Jain H, McLean D, et al. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. *Arch Dermatol*. 2004;140:677-683.
12. England JM, Rowan RM, van Assendelft OW, et al. Protocol for evaluation of automated blood cell counters. International Committee for Standardization in Haematology (ICSH). *Clin Lab Haematol*. 1984;6(1):69-84.
13. Shang J, Eberle J, Geilen CC, et al. The role of nuclear factor-kappa B and melanogenesis in tumor necrosis factor-alpha-induced apoptosis of normal human melanocytes. *Skin Pharmacol Appl Skin Physiol*. 2002;15(5):321-329.
14. Larribere L, Khaled M, Tartare-Deckert S, et al. PI3K mediates protection against TRAIL-induced apoptosis in primary human melanocytes. *Cell Death Differ*. 2004;11(10):1084-1091.
15. Parsad D, Kumar R. Melanocytorrhagy and apoptosis in vitiligo: connecting jigsaw pieces. *Indian J Dermatol Venereol Leprol*. 2012;78(1):19-23.
16. Zhou H, Ekmekcioglu S, Marks JW, et al. The TWEAK receptor Fn14 is a therapeutic target in melanoma: immunotoxins targeting Fn14 receptor for malignant melanoma treatment. *J Invest Dermatol*. 2012;133(4):1052-1062.
17. Zimmermann M, Koreck A, Meyer N, et al. TNF-like weak inducer of apoptosis (TWEAK) and TNF-α cooperate in the induction of keratinocyte apoptosis. *J Allergy Clin Immunol*. 2011;127(1):200-207.e10.
18. Lee AY. Role of keratinocytes in the development of vitiligo. *Ann Dermatol*. 2012;24(2):115-125.
19. Bakry OA, Shoeib MAEM, El Kady N, Attalla S. Re-appraisal of Keratinocytes' Role in Vitiligo Pathogenesis. *Indian J Dermatol*. 2018;63(3):231-240.
20. Taïeb A, Picardo M, Members VETF. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. *Pigment Cell Res*. 2007;20(1):27-35.
21. Hann SK, Lee HJ. Segmental vitiligo: clinical findings in 208 patients. *J Am Acad Dermatol*. 1996;35(5 Pt 1):671-674.
22. Mulekar SV. Long-term follow-up study of segmental and focal vitiligo treated by autologous, noncultured melanocyte-keratinocyte cell transplantation. *Arch Dermatol*. 2004;140(10):1211-1215.
23. El-Mofty AM, el-Mofty M. Vitiligo. A symptom complex. *Int J Dermatol*. 1980;19(5):237-244.
24. Ezzedine K, Lim HW, Suzuki T, et al. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res*. 2012;25(3):E1-E13.
25. Zhao Z, Burkly LC, Campbell S, et al. TWEAK/Fn14 interactions are instrumental in the pathogenesis of nephritis in the chronic graft-versus-host model of systemic lupus erythematosus. *J Immunol*. 2007;179(11):7949-7958.
26. Desplat-Jégo S, Creidy R, Varriale S, et al. Anti-TWEAK monoclonal antibodies reduce immune cell infiltration in the central nervous system and severity of experimental autoimmune encephalomyelitis. *Clin Immunol*. 2005;117(1):15-23.
27. Park JS, Kim SM, Jung KA, et al. Inhibition of the TWEAK/Fn14 pathway attenuates autoimmune arthritis in a SKG mouse model. *Histol Histopathol*. 2017;32(5):481-490.
28. Wajant H. The TWEAK-Fn14 system as a potential drug target. *Br J Pharmacol*. 2013;170(4):748-764.

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