Orginal Article

Immunohistochemical Expression of Tumor Necrosis Factor Like Weak Inducer of Apoptosis (TWEAK) in Cutaneous Wound Healing

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Abstract

Aim: Wound healing is a physiological process vital for survival because it restores skin integrity. Pathological scarring is an erroneous consequence of wound healing that occurs as a result of either excessive collagen deposition or degradation. Tumor necrosis factor like weak inducer of apoptosis (TWEAK) is believed to be a cytokine with major contributing effects in angiogenesis, inflammation, and cell division. This study aimed to explore the expression of TWEAK in different groups of scar tissue using immunohistochemistry and to compare the results with clinical and histological data.

Materials and Methods: This study included 50 cases divided in 5 different wound healing groups. Detailed history taking and full physical examination were performed in addition to histopathological and immunohistochemical evaluation of TWEAK.

Results: In the epidermis, mean H-scores of TWEAK were higher in hypertrophic scars and keloids than in normal and atrophic scars. In dermal blood vessels and fibroblasts, the granulation tissue, hypertrophic, and keloid cases mean H-scores of TWEAK was higher than those of normal and atrophic cases.

Conclusion: Our findings highlighted the significance of TWEAK in angiogenesis, inflammation, and fibroblast proliferation pointing to helpful function in the normal healing process of wounds, but its higher level has been linked to the development of pathological scars.

Keywords: Wound healing, TWEAK, immunohistochemistry

Introduction

Tissue reconstitution emerges from an intensely programed succession of interconnected phases that define wound healing.[1] Keratinocyte, fibroblasts, vascular endothelial cells, and immune cells interact together to initiate hemostasis and inflammation, followed by cellular proliferation and finally matrix remodeling to restore tissue integrity.[2] However, as with any physiological procedure, abnormalities can occur as a result of system disruption, manifested as excessive healing with scar formation at one end or insufficient healing at the other.[3]

Despite being a common worldwide problem, estimating the number of people globally affected by scarring is considerably hard.[4] Skin scarring is a major source of discomfort that usually affects how patients feel and act physically.[5]

As an element of the tumor necrosis factor (TNF) family; tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a type II transmembrane protein that interacts with its receptor fibroblast growth factor-inducible 14 (Fn14) to exert its effects. Immune cells, notably macrophages and monocytes, are the primary producers of TWEAK.[6] TWEAK is considered a cytokine that controls a variety of tissue reactions, including proinflammatory activity, angiogenesis, and cell proliferation, highlighting its possible roles in both inflammation and

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cancer. Depending on this, there is mounting evidence that TWEAK/Fn14-induced tissue remodeling (either normal or pathologic) is a widely applicable mechanism in different organs and tissues.[7]

Despite the high occurrence and increased clinical burden of skin scars, it has proven difficult to manage them optimally.[5] Meanwhile, because of the role of TWEAK in regeneration and tissue remodeling, this finding encouraged us to investigate its role in cutaneous wound healing by examining its immunohistochemical (IHC) expression in different scar types.

Subjects and Methods

In this case-control study, 50 individuals were distributed into 5 groups: 10 cases had granulation tissue (4-30 days after injury), 10 cases had normal scars, 10 cases had atrophic scars, 10 cases had hypertrophic scars, and 10 cases had keloid scars. The Menoufia University Hospital outpatient clinic for dermatology, andrology, and sexually transmitted diseases identified cases with keloid, hypertrophic, and atrophic scars. Conversely, patients with granulation tissue and normal scars were drawn from the Menoufia University Hospital Plastic Surgery Department Outpatient Clinic between January and October of 2022.

Each enrolled subject gave written informed consent prior to the start of the study, which was approved by Menoufia University's Ethics Committee on Human Rights (IRB approval number and date: 6/2020 DERMA9).

A thorough history was taken from each participant, after which a general clinical and detailed dermatological examination was performed. The clinical evaluation of various scar types was conducted using the Manchester and Vancouver scales.[8]

Skin biopsy: An incisional skin biopsy was performed from the site of the lesion in each patient under local anesthesia and complete aseptic technique. The Pathology Department of Menoufia University Faculty of Medicine receives all biopsies where they undergo regular processing, ending with paraffin block embedding. Then two slices with a thickness of 5 microns (5 μm) were obtained from each block. One slide was stained with hematoxylin and eosin (H&E) for standard histopathological examination, and the other was mounted on positively charged slides and kept at room temperature (RT) for IHC staining.

Under a light microscope, sections stained with H&E were examined to validate the diagnosis of each type of scar according to the established histological criteria, and any alterations in the dermis and epidermis were assessed.

Immunohistochemical staining: The streptavidin-biotin amplified system was used in the immunostaining procedure. The primary antibody used was a rabbit polyclonal anti-TWEAK antibody (cat. no. YPA2246, Chongqing Biospes Co., Ltd, China) (100 μL concentrated and diluted by phosphate-buffered saline (PBS) in a dilution 1:100) with human lung tissue used as a positive control. First, tissue sections soaked in paraffin were rehydrated in a graduated sequence of ethanol, deparaffinised in xylene and treated with 3% hydrogen peroxide. Slides undergo heat-induced epitope retrieval in citrate buffer solution (Ph 6) for 20 min after washing with PBS. The slides were cooled before incubation with the primary antibody overnight at RT. The Universal Dakocytomation Labeled Streptavidin-Biotin-2 system (LSAB-2), horseradish peroxidase [(HRP kit, catalog no. k0679], was then applied to detect tissue immunoreactivity. Ultimately, a suitable substrate/chromogen (diaminobenzidine) reagent was used to observe the reaction, followed by counterstaining of the slides with Mayer's hematoxylin. Notably, a negative control for the staining process was created by exchanging PBS for the main antibody used.

Immunostaining interpretation of TWEAK expression: TWEAK immunoreactivity was separately assessed in the epidermis and dermal structures (fibroblasts and blood vessels). The percentage of positivity in addition to the intensity [mild (1), moderate (2), or strong (3) of staining were reported and then integrated together to determine the H-score as follows: H-score = (3% × of strong intensity) + (2% × of moderate intensity) + (1% × of mild intensity).[9]

Statistical analysis

Using a personal computer running the "Statistical Package for the Social Sciences (SPSS)" version 20.0 application, data were collected, tabulated, and statistically analyzed $P < 0.05$ was the level of significance.

Results

The clinical data describing patients' demographics (age and sex) as well as the clinical variables of the studied groups were summarized in Table 1 while histopathological data were listed in Table 2.

Immunohistochemical Expression of TWEAK in the Study Groups

All the studied cases (100%) showed positive expression of TWEAK in the epidermis and dermis (fibroblasts and blood vessels) with nucleocytoplasmic localization and diffuse distribution.

Table 1. Clinical data of the study groups

Variables	Granulation, (n=10)	Normal, (n=10)	Atrophic, (n=10)	Hypertrophic, (n=10)	Keloid, (n=10)
Age (years)					
Min.-max.	18.0-58.0	7.0-44.0	16.0-48.0	10.0-50.0	17.0-46.0
$\bar{X}$ ± SD	29.70±12.88	26.40±11.42	30.30±11.52	26.60±13.05	30.0±9.91
Median	25.0	27.50	27.50	26.0	29.0
Sex					
Male	8 (80%)	2 (20%)	3 (30%)	5 (50%)	4 (40%)
Female	2 (20%)	8 (80%)	7 (70%)	5 (50%)	6 (60%)
Onset					
Slow	1	10	7	6	6
Rapid	9	0	3	4	4
Course					
Progressive	5	2	4	2	2
Stable	5	8	6	8	8
Duration (years)					
Min.-max.	0.01-0.07	3.0-7.0	3.0-7.0	2.0-3.0	1.0-3.0
$\bar{X}$ ± SD	0.3±0.02	4.20±1.23	4.50±1.27	2.35±0.47	2.15±0.75
Median	0.03	4.0	4.0	2.0	2.0
Recurrence					
Yes	0	0	0	1	1
No	10	10	10	9	9
Family history					
Positive	0	0	0	1	0
Negative	10	10	10	9	10
Pain association					
Positive	4	1	0	0	0
Negative	6	9	10	10	10
Itching association					
Present	2	1	1	3	1
Absent	8	9	9	7	9
Limitation of movement					
Present	0	0	0	0	2
Absent	10	10	10	10	8
Manchester classification					
Min.-max.			11.0-14.0	12.0-15.0	11.0-17.0
$\bar{X}$ ± SD			12.30±1.6	13.50±1.27	14.0±1.76
Median			12.0	13.50	14.0
Vancouver classification					
Min.-max.			2.0-7.0	3.0-10.0	4.0-9.0
$\bar{X}$ ± SD			3.30±1.57	6.80±2.39	6.80±1.62
Median			3.0	7.0	7.0

SD: Standard deviation, Min.: Minimum, max.: Maximum

Table 2. Histopathological data of the studied groups

Variables	Normal, (n=10)	Atrophic, (n=10)	Hypertrophic, (n=10)	Keloid, (n=10)
Epidermal thickening				
Decreased	9 (90%)	10 (100%)	6 (60%)	9 (90%)
Increased	1 (10%)	0 (0%)	4 (40%)	1 (10%)
Epidermal rete ridges				
Normal	0 (0%)	2 (20%)	0 (0%)	0 (0%)
Partial restoration	10 (100%)	7 (70%)	7 (70%)	2 (20%)
Lost	0 (0%)	1 (10%)	3 (30%)	8 (80%)
Dermal cellularity				
Mild increase	10 (100%)	0 (0%)	0 (0%)	3 (30%)
Moderate increase	0 (0%)	0 (0%)	6 (60%)	7 (70%)
Marked increase	0 (0%)	0 (0%)	4 (40%)	0 (0%)
Decreased	0 (0%)	10 (100%)	0 (0%)	0 (0%)
Dermal vascularity				
Normal	6 (60%)	9 (90%)	0 (0%)	0 (0%)
Mild increase	4 (40%)	0 (0%)	0 (0%)	3 (30%)
Moderate increase	0 (0%)	1 (10%)	6 (60%)	7 (70%)
Marked increase	0 (0%)	0 (0%)	4 (40%)	0 (0%)
Hair follicles				
Positive	2 (20%)	0 (0%)	0 (0%)	0 (0%)
Negative	8 (80%)	10 (100%)	10 (100%)	10 (100%)
Sweet and sebaceous glands				
Positive	2 (20%)	1 (10%)	0 (0%)	0 (0%)
Negative	8 (80%)	9 (90%)	10 (100%)	10 (100%)
Collagen fiber density				
Normal	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Abnormal	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Collagen fiber orientation				
Normal	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Abnormal	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Collagen fiber size				
Small	10 (100%)	9 (90%)	2 (20%)	0 (0%)
Mixed	0 (0%)	1 (10%)	8 (80%)	10 (100%)
Collagen fiber pattern arrangement				
Haphazard	10 (100%)	10 (100%)	10 (100%)	0 (0%)
Even	0 (0%)	0 (0%)	0 (0%)	10 (100%)
Dermal inflammation				
Positive (perivascular)	4 (40%)	4 (40%)	8 (80%)	2 (20%)
Negative	6 (60%)	6 (60%)	2 (20%)	8 (80%)
Degree of inflammation				
Mild	4 (40%)	4 (40%)	6 (60%)	1 (10%)
Moderate	0 (0%)	0 (0%)	2 (20%)	1 (10%)

Comparison Between the Studied Groups According to the H-score of TWEAK Expression in the Epidermis and Dermis

There were statistically significant differences between the studied groups regarding the mean H-score of TWEAK expression ($P < 0.001$) in the epidermis, dermal blood vessels, and dermal fibroblasts (Table 3). Detailed data considering the significance between the studied groups were demonstrated in (Table 3, Figures 1-3).

Relationship Between Mean H-score of TWEAK Expression in the Epidermis and Dermis (Fibroblasts and Blood Vessels) with Demographic and Clinical Data of Granulation Tissue

There was a significant relationship between the mean H-score of TWEAK in blood vessels and disease course, with a higher score in cases with a stable disease course ($P = 0.03$) (Figure 4).

Relationship Between Mean H-score of TWEAK Expression in the Epidermis and Dermis (Fibroblasts and Blood Vessels) with Demographic, Clinical, and Histopathological Data of Normal Scar Cases

Normal scar cases exhibiting a mild increase in dermal vascularity and positive dermal inflammation showed significantly increased TWEAK expression in both dermal fibroblasts ($p = 0.02$ for both) and blood vessels ($P = 0.03$ for both) (Figure 4).

Relationship Between Mean H-score of TWEAK Expression in the Epidermis and Dermis (Fibroblasts and Blood Vessels) with Demographic, Clinical, and Histopathological Data of Patients with Atrophic Scars

In atrophic scar cases; female cases showed a significantly higher TWEAK expression in the epidermis ($P = 0.03$). In addition, cases with a progressive course exhibited higher H-scores in both the epidermis and blood vessels, ($P = 0.02$; $P = 0.049$ respectively). Another significant relationship between atrophic scare cases with dermal inflammation and TWAEK expression in fibroblasts and blood vessels was observed ($P = 0.009$; $P = 0.02$ respectively). A negative correlation was

Table 3. Comparison between the different study groups according to the H-score of TWEAK expression in the epidermis and dermis (fibroblast and blood vessels)

H-score	Granulation tissue, (n=10)	Normal scar, (n=10)	Atrophic scar, (n=10)	Hypertrophic scar, (n=10)	Keloid, (n=10)	F	*P* value
Epidermis H-score							
Min.-max.	-	60.0-150.0	100.0-200.0	230.0-260.0	220.0-260.0	55,306*	<0.001*
$\bar{X} \pm SD$	-	123.0±31.99	142.0±39.38	243.0±10.59	240.0±14.91		
Median	-	135.0	135.0	245.0	240.0		
p_1			0.405	<0.001*	<0.001*		
Sig. bet. grps.			p_2<0.001*; p_3<0.001*; p_4=0.994				
Fibroblast H-score							
Min.-max.	250.0-290.0	30.0-120.0	50.0-140.0	230.0-280.0	220.0-270.0	194.24*	<0.001*
$\bar{X} \pm SD$	271.0±12.87	77.0±27.51	98.0±27.41	252.0±16.19	245.0±17.16		
Median	270.0	80.0	95.0	250.0	250.0		
p_0		<0.001*	<0.001*	0.277	0.062		
p_1			0.190	<0.001*	<0.001*		
Sig. bet. grps.			p_2<0.001*; p_3<0.001*; p_4=0.946				
Blood vessel H-score							
Min.-max.	250.0-290.0	30.0-130.0	70.0-160.0	230.0-290.0	220.0-270.0	119,650*	<0.001*
$\bar{X} \pm SD$	267.0±14.18	89.0±35.73	117.0±29.83	255.0±18.41	244.0±16.47		
Median	270.0	100.0	110.0	260.0	250.0		
p_0		<0.001*	<0.001*	0.806	0.235		
p_1			0.094	<0.001*	<0.001*		
Sig. bet. grps.			p_2<0.001*; p_3<0.001*; p_4=0.850				

*: Statistically significant at $P \leq 0.05$, F: F for One-way ANOVA test, pairwise comparison between each 2 groups was performed using post-hoc test (Tukey), p: *P* value for comparing between the different study groups, p_0: *P* value for comparing the granulation tissue groups, p_1: *P* value for comparing the normal scar group and the groups, p_2: *P* value for comparing atrophic and hypertrophic groups, p_3: *P* value for comparing atrophic and keloid groups, p_4: *P* value for comparing hypertrophic and keloid groups, Min.: Minimum, max.: Maximum, SD: Standard deviation, Sig. bet. grps.: Significance between groups, TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

found between the mean H-scores of TWAEK expression in the epidermis, dermal fibroblasts, and blood vessels with disease duration (P = 0.04; P = 0.02; P = 0.04 respectively), (Figures 5, 6).

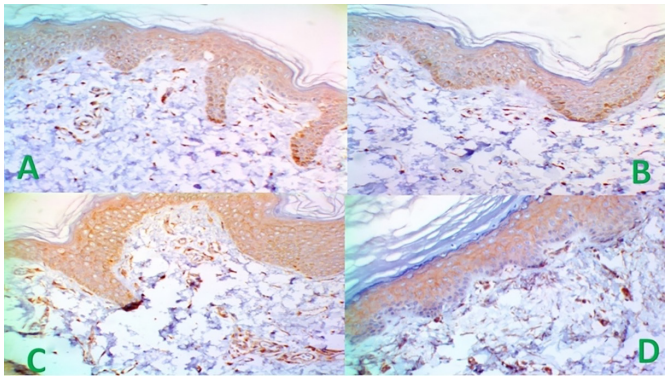

Figure 1. TWEAK immunohistochemical expression in the epidermis of the studied cases; TWEAK staining was higher in hypertrophic scar (C) and keloid (D) than in normal (A) and atrophic scar (B) cases [immunohistochemistry (x200)]

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

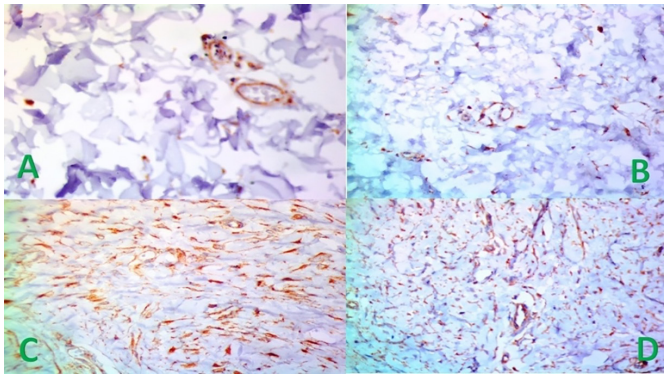

Figure 2. TWEAK immunohistochemical expression in the blood vessels and fibroblasts of the studied cases; TWEAK staining was higher in hypertrophic scar (C) and keloid (D) than in normal (A) and atrophic scar (B) cases [immunohistochemistry (x400)]

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

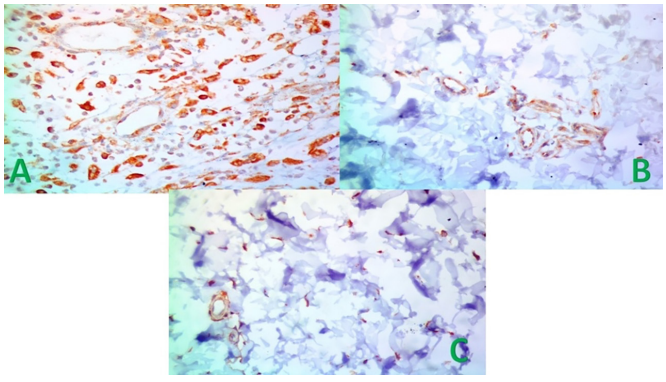

Figure 3. Elevated TWEAK expression in blood vessels and fibroblasts of granulation tissue (A) compared with normal (B) and atrophic scar (C) cases [immunohistochemistry (x400)]

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

Relationship Between Mean H-score of TWEAK Expression in the Epidermis and Dermis (Fibroblasts and Blood Vessels) with Demographic, Clinical, and Histopathological Data of Hypertrophic Scar Cases

In hypertrophic scar; the mean H-score of TWEAK expression in dermal blood vessels appeared more elevated in patients with progressive disease (P = 0.02). Moreover, there were significant relationships regarding mean H-scores of TWEAK expression in dermal fibroblasts with dermal cellularity and vascularity (P = 0.03 for both). Patients with positive dermal inflammation, especially those with moderate degree of dermal inflammation, showed higher TWEAK expression in the epidermis (P = 0.04), fibroblasts (P = 0.02; P = 0.001 respectively) and blood vessels (P = 0.02; P = 0.019 respectively) (Figure 7).

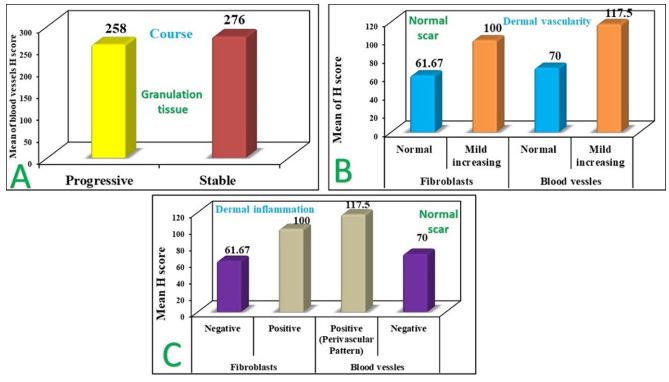

Figure 4. Relationships between mean H-score of TWEAK expression in (A)dermal blood vessels and disease course in granulation tissue, (B) dermal fibroblasts and blood vessels with dermal vascularity and inflammation (C) in normal scars

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

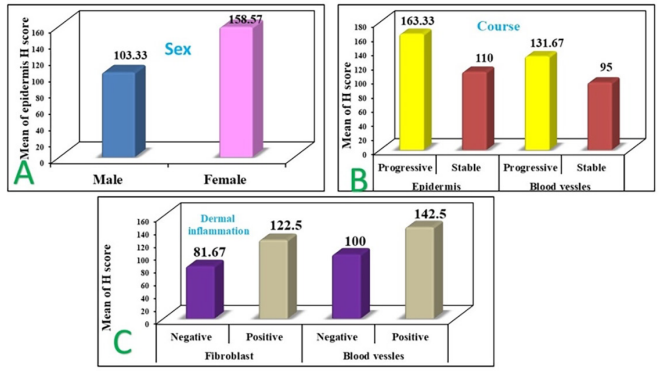

Figure 5. Relationships between mean H-score of TWEAK expression in atrophic scar cases; (A) in the epidermis according to sex, (B) in the epidermis and blood vessels according to the disease course (C) in dermal fibroblasts and blood vessels according to dermal inflammation

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

Relationship Between Mean H-score of TWEAK Expression in the Epidermis and Dermis (Fibroblasts and Blood Vessels) with Demographic, Clinical, and Histopathological Data of Patients with Keloid Scars

Patients who experienced limitation of movement showed higher H-score of TWEAK in the epidermis and dermal blood vessels ($P = 0.001$; $P = 0.03$ respectively). In addition, cases exhibiting lost epidermal rete ridges, moderate increase in both dermal cellularity and vascularity together with patients exhibiting positive dermal inflammation, had increased TWEAK expression in the epidermis ($P = 0.001$; $P = 0.009$; $P = 0.009$; $P = 0.001$ respectively), fibroblasts ($P = 0.009$; $P = 0.001$; $P = 0.001$ respectively) and blood vessels ($P = 0.009$; $P = 0.001$; $P = 0.001$; $P = 0.03$ respectively) (Figure 8).

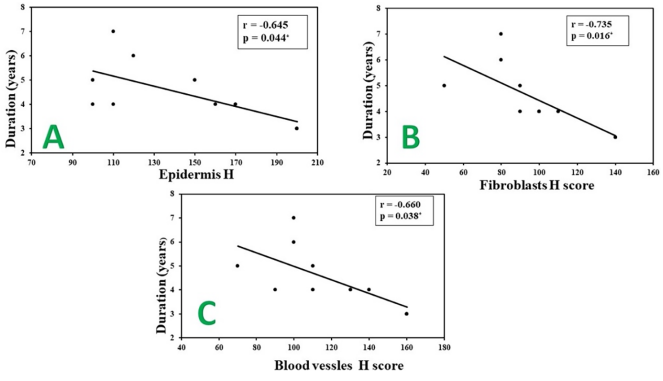

Figure 6. Correlations between mean H-scores of TWEAK expression in the epidermis (A), dermal fibroblasts (B), and blood vessels (C) with the duration of disease in cases with atrophic scars

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

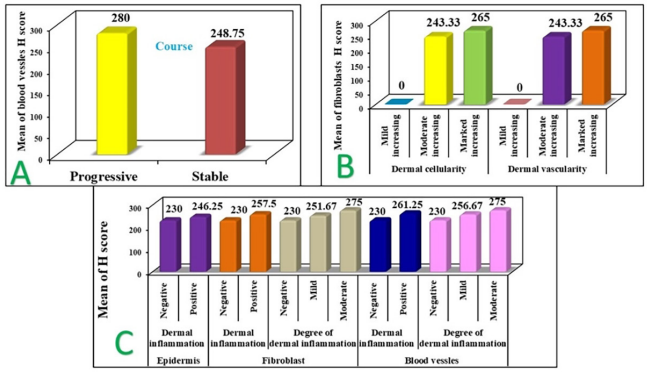

Figure 7. Relationships between mean H-score of TWEAK expression in hypertrophic scar cases; (A) dermal blood vessels and disease course, (B) dermal fibroblasts with dermal cellularity and vascularity, and (C) epidermis, fibroblasts, and blood vessels with dermal inflammation and degree of dermal inflammation

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

Discussion

Skin-wound healing is a complex process involving interrelated and overlapping mechanisms of cell migration and proliferation, the synthesis of extracellular matrix, growth factors, and cytokines that coordinate the healing process. Due to its complexity, the wound healing process can be divided into three phases: inflammatory, proliferative, and remodeling phases.[10]

This study aimed to evaluate TWEAK in n cutaneous wound healing by examining its IHC expression in different types of scars and to correlate the obtainable results with available clinical and pathological data.

The current study exhibited positive TWEAK expression in different layers of the epidermis, dermal blood vessel endothelium, and fibroblasts in all patients. In line with these results, Liu et al.,[11] showed that Fn14 (TWEAK sole receptor) was expressed in normal epidermal keratinocyte, in addition to dermal components such as endothelial cells and fibroblasts. Moreover, TWEAK and its receptor showed Fn14 showed relatively low levels in healthy tissues in contrast to their elevated expression in cases of tissue damage.[12]

In the present study, TWEAK was predominantly expressed in the cytoplasm, with concurrent coloration of nuclei observed mostly in dermal fibroblasts. Agreed with us, TWEAK cytoplasmic localization was observed in studies applied on skin and other tissues.[13,14] Notably, cells can express TWEAK in two different forms: as a full-length, membrane-bound protein in addition to soluble protein (sTWEAK), which is produced through TWEAK's proteolysis; however, it is unknown what mechanism regulates how much of each form is produced.[15] It is interesting to note that full-length TWEAK showed both nuclear and cytoplasmic expression, in contrast

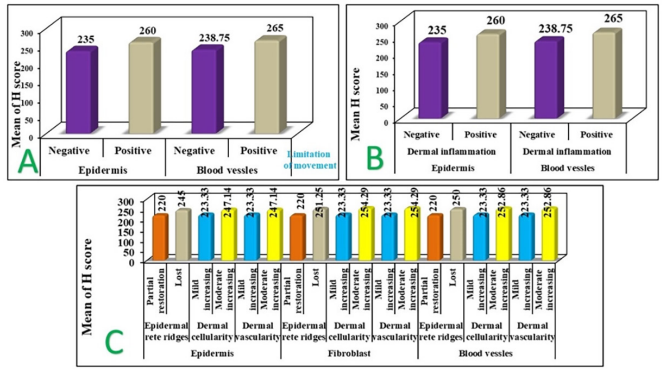

Figure 8. Relationships between mean H-score of TWEAK expression in keloid cases; (A) in the epidermis and blood vessels with limited movement, (B) in the epidermis and blood vessels with dermal inflammation, (C) in the epidermis, fibroblasts, and blood vessels with epidermal rete ridges, dermal cellularity, and dermal vascularity

TWEAK: Tumor necrosis factor-like *weak* inducer of apoptosis

to the cleaved form, which is localized only in the cytoplasm.[16] Nevertheless, it is still unclear what function TWEAK serves in the nucleus.[17]

Regarding epidermal expression of TWEAK in the studied groups; cases with hypertrophic and keloid scars exhibited higher H-scores than those with normal and atrophic scars. It was noted that upon exposure to injury, TWEAK released by immune cells combines with Fn14 receptors inhabitant in tissue-related epithelial, endothelial, and stromal cells, triggering tissue repair.[18] Zhang et al.,[19] assumed that TWEAK expression in epithelial keratinocyte of keloid tissue has a role in keloid development. This occurs via the ability of keratinocyte to secrete cytokines activating keloid fibroblasts or by undergoing epithelial mesenchymal transformation.[20,21] The latter could be an explanation for higher expression of TWEAK in our hypertrophic scar and keloid epidermis, as both are fibroproliferative disorders.

In the current study, dermal blood vessels and fibroblasts in granulation tissue and hypertrophic and keloid scars had higher mean H-scores of TWEAK than that of normal and atrophic scars. Studies have highlighted the favorable role of the TWEAK/Fn14 pathway in acute tissue injury. This relies on the capacity of TWEAK/Fn14 to be temporarily activated after acute injury to coordinate the response of inflammatory, endothelial cells, and fibroblasts with subsequent initiation of healing.[7,18] This provides an explanation for higher TWEAK expression in blood vessels and fibroblasts of the studied granulation tissue samples as an acute tissue response to injury. On the other side; persistent activation of the TWEAK/Fn14 axis was noted in tissues with chronic inflammation and fibrosis, causing pathological tissue remodeling.[7] This pathway exerts a proinflammatory effect by inducing the expression of numerous cytokines, chemokine, and matrix metalloproteinases, thereby amplifying chronic inflammation and tissue damage, resulting in fibrosis.[22] Thus, TWEAK appeared to be upregulated in blood vessels and fibroblasts of the studied cases with hypertrophic scars and keloids, as pathological scars exhibit more dermal chronic inflammation, angiogenesis, and fibrosis.

The granulation tissue cases under study exhibited a decrease in TWEAK H-score values in the blood vessels in those with progressive disease, denoting persistent chronic granulation tissue. Notably, persistent unhealthy granulation tissue is a feature of chronic wounds that is commonly observed in cases exhibiting wound infection or poor blood supply, as in diabetic patients.[23] In the same way, reductions in sTWEAK concentrations were observed in patients with diabetes and atherosclerosis.[24,25]

In this study, higher TWEAK expression by fibroblasts and the vascular endothelium was observed in normal scars with increased dermal vascularity and inflammation. This could be related to the critical role of dermal endothelial cells and fibroblasts in wound regeneration.[26] In addition to the effect of TWEAK, it enhances angiogenesis via the proliferation and migration of endothelial cells.[27] Moreover, TWEAK can aggravate skin inflammation by upregulating the intercellular adhesion molecule, thus improving polymorphonuclear leukocyte adherence to vascular endothelial cells.[28]

In the current work; female cases with atrophic scars showed significant elevation of TWEAK expression in epidermal keratinocyte. This finding could be related to the considerable role of the female sex hormone; estrogen in regulation of cutaneous wound healing. Estrogen can activate extracellular signal-regulated kinase and phosphatidylinositol 3-kinase pathways, which in turn initiate the proliferation of epidermal keratinocyte that encourage wound re- epithelization.[29] At the same instant, some studies noted higher levels of TWEAK expression in female patients with myocardial infarction[30] which point to the role of female sex hormones in the induction of TWEAK levels.

TWEAK expression in the studied atrophic scar cases appeared to be elevated in those with progressive course and dermal inflammation. Interestingly, acne is the most important reason for atrophic scars, where development of scar in those cases depends upon the severity of inflammation with more influx of adaptive immune cells (T and B lymphocytes) in addition to angiogenesis.[31,32] Such excessive inflammation, in turn, activates the destruction of collagen and elastic fibers. [32] On the same line, TWEAK is an important trigger of adaptive immune response through activating B cell proliferation and differentiation.[33]

It is worth to say that Lee et al.,[34] demonstrated that despite the true presence of inflammatory cells in acne scars, they disappeared with full fibrosis. Owing to the role of TWEAK in the augmentation of inflammation, the previous observation could explain the significant negative correlation between TWEAK expression in atrophic scar tissue and disease duration.

A significant association was observed between the mean H-score of TWEAK expression in dermal vasculature and the disease course of hypertrophic scars; individuals who had a progressive disease course had increased TWAEK expression. This finding could be related to the substantial role of TWEAK in the augmentation of inflammation, as the progression of all fibroproliferative disease is influenced mainly by inflammation.[35]

Indeed; TWEAK level in dermal fibroblasts demonstrated an important association with dermal cellularity. Crucially, the TWEAK/Fn14 pathway may directly influence a

fibrogenic response by boosting fibroblast growth, through its collaboration with transforming growth factor β1 and interleukin-13 (IL-13).[36] Additionally, TWEAK directly encouraged the maturation of fibroblasts into myofibroblast.[37] As both TWEAK and fibroblasts could enhance wound angiogenesis by the release of angiogenic growth factors like fibroblast growth factor and vascular endothelial growth factors,[38,39] a significant association of TWEAK level in dermal fibroblasts with dermal vascularity was observed in the enrolled hypertrophic scar patients.

An outstanding significant relationship was observed between the H-score of TWEAK in the epidermis, fibroblasts, and blood vessels with dermal inflammation in cases with hypertrophic scars. This may be related to the nature of hypertrophic scars as pathologic scars with persistent inflammation.[40] Numerous studies have highlighted that epithelial keratinocytes along with other resident skin cells (dermal fibroblasts and microvascular endothelial cells) have a fundamental role in the establishment of skin inflammation. This occurs through the emission of multiple cytokines like TNF-alpha (α), IL-6, and 8.[41-43] TWEAK/Fn14 axis engagement improves the attraction of inflammatory cells and skin local cell-mediated generation of cytokines, all of which in turn lead to chronic inflammation.[11] TWEAK also fosters fibrosis as a result of its ubiquitous proinflammatory activity.[22]

During keloid formation; an imbalance occurs in the released cytokines during wound-healing, where an ongoing autocrine positive feedback loop takes place, intensifying a cycle of fibroblast proliferation that eventually leads to keloids.[44] TWEAK; is among these cytokines regulating the interaction of keratinocyte with dermal resident cells.[18,19] Activation of TWEAK/Fn14 pathway could enhance fibrosis through its proinflammatory action causing fibroblast expansion and matrix deposition aside its role in the enhancement of dermal vascularity.[38,45] Furthermore, myofibroblast become refractory to programed cell death upon TWEAK/Fn14 binding by promoting the recruitment of TNFR-related factors and cellular inhibitors of apoptosis proteins.[45,46] Wound contract due to the myofibroblasts' contractile power.[47] This runs in parallel with our results, in which the TWEAK H-score level in the epidermis and blood vessels appeared higher in patients with movement limitation in keloid scars. The above details could also explain the elevated H-score in keloid cases exhibiting more dermal cellularity, vascularity, and inflammation.

Of note, histologic abnormalities in the keloid epidermis have been reported in several studies. This includes increased epidermal thickness and hyperproliferation in addition to the reduction or absence of rete ridges.[48,49] Increased dermal vascularity has been also reported and was associated with epidermal thickness.[49] Moreover, the TWEAK/Fn14 interaction has also the ability to enhance keratinocyte proliferation;[50] thus, its level in the studied keloid cases was elevated in association with rete ridge abnormality.

Study limitations

The small sample size in each group together with the introduction of the study at one center were the main limitations.

Conclusion

TWEAK plays a beneficial role in the normal wound healing but its elevated level was also associated with pathological scar formation.

Ethics

Ethics Committee Approval: It was approved by Menoufia University's Ethics Committee on Human Rights (IRB approval number and date: 6/2020 DERMA9).

Informed Consent: Each enrolled subject gave written informed consent prior to the start of the study.

Authorship Contributions

Concept: W.A.S., R.A.H., I.S., Design: W.A.S., R.A.H., I.S., Data Collection or Processing: W.A.S., R.A.H., A.S.A., Analysis or Interpretation: W.A.S., R.A.H., A.S.A., Literature Search: W.A.S., R.A.H., A.S.A., I.S., Writing: W.A.S., R.A.H., A.S.A., I.S.

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