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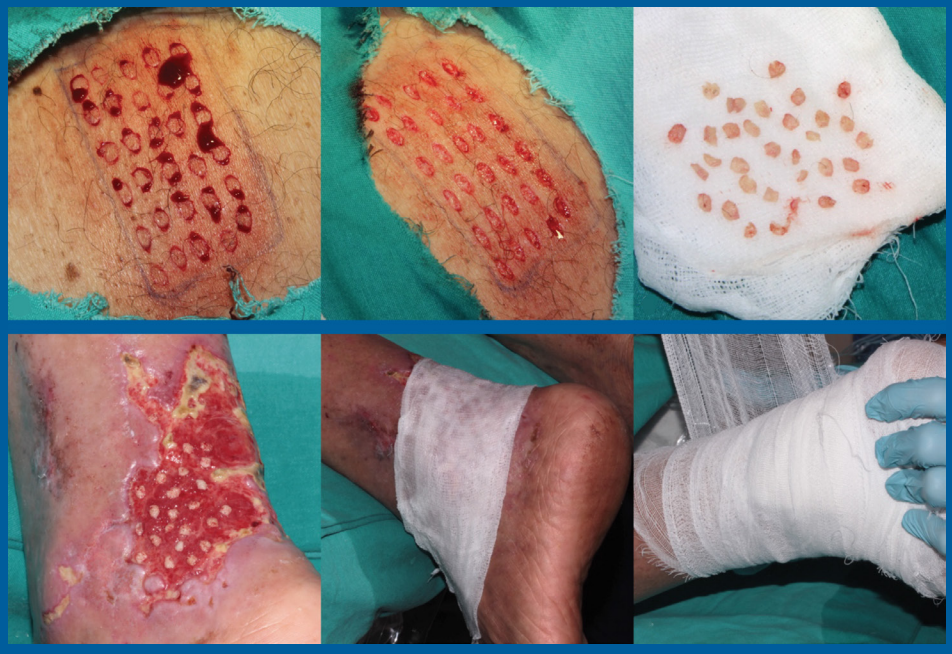

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Orginal Article

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).

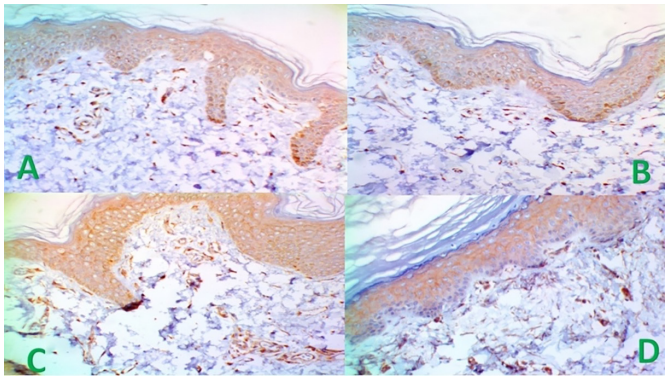

Figure 1. TWEAK immunohistochemical expression in the epidermis of the studied cases; TWEAK staining was higher in hypertrophic scar (C) and keloid (D) than in normal (A) and atrophic scar (B) cases [immunohistochemistry (x200)]

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

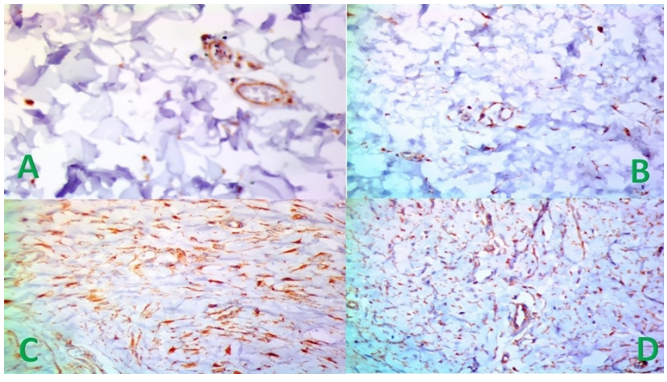

Figure 2. TWEAK immunohistochemical expression in the blood vessels and fibroblasts of the studied cases; TWEAK staining was higher in hypertrophic scar (C) and keloid (D) than in normal (A) and atrophic scar (B) cases [immunohistochemistry (x400)]

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

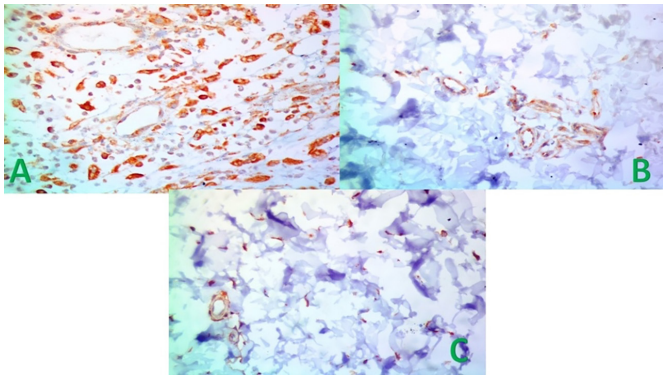

Figure 3. Elevated TWEAK expression in blood vessels and fibroblasts of granulation tissue (A) compared with normal (B) and atrophic scar (C) cases [immunohistochemistry (x400)]

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).

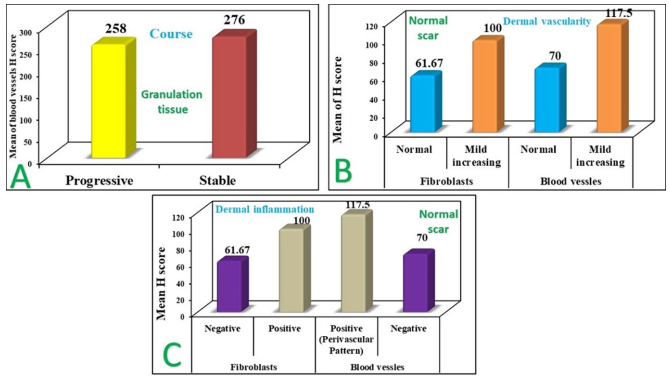

Figure 4. Relationships between mean H-score of TWEAK expression in (A)dermal blood vessels and disease course in granulation tissue, (B) dermal fibroblasts and blood vessels with dermal vascularity and inflammation (C) in normal scars

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

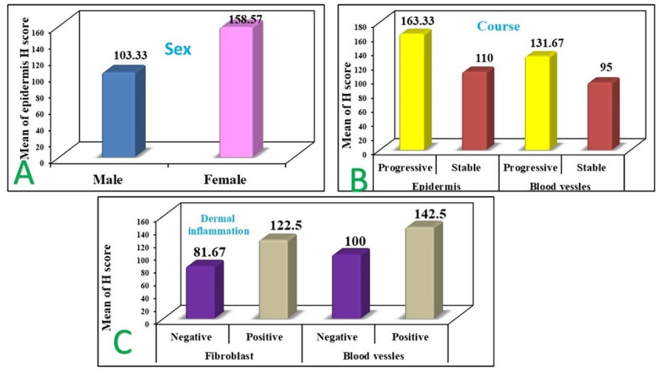

Figure 5. Relationships between mean H-score of TWEAK expression in atrophic scar cases; (A) in the epidermis according to sex, (B) in the epidermis and blood vessels according to the disease course (C) in dermal fibroblasts and blood vessels according to dermal inflammation

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).

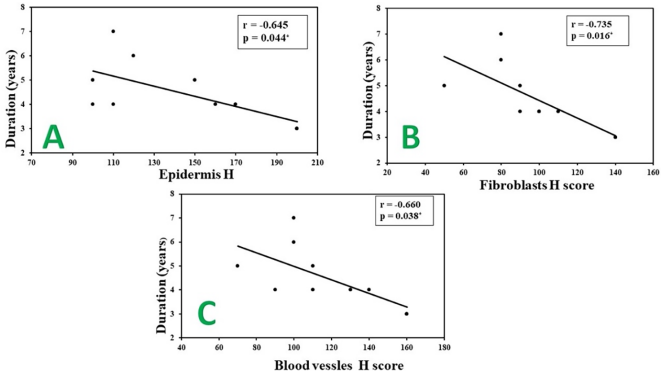

Figure 6. Correlations between mean H-scores of TWEAK expression in the epidermis (A), dermal fibroblasts (B), and blood vessels (C) with the duration of disease in cases with atrophic scars

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

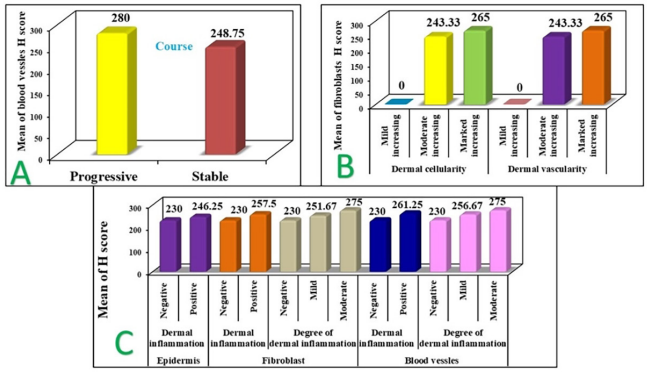

Figure 7. Relationships between mean H-score of TWEAK expression in hypertrophic scar cases; (A) dermal blood vessels and disease course, (B) dermal fibroblasts with dermal cellularity and vascularity, and (C) epidermis, fibroblasts, and blood vessels with dermal inflammation and degree of dermal inflammation

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

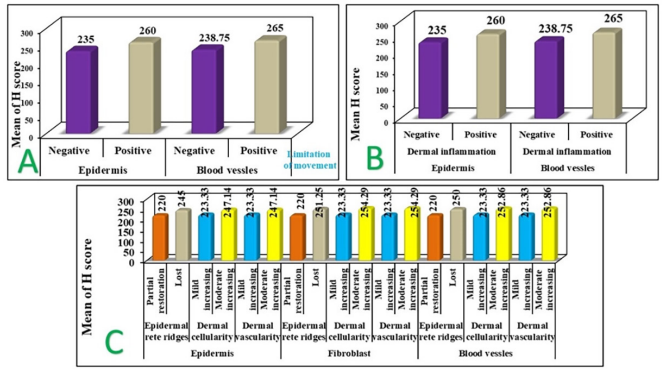

Figure 8. Relationships between mean H-score of TWEAK expression in keloid cases; (A) in the epidermis and blood vessels with limited movement, (B) in the epidermis and blood vessels with dermal inflammation, (C) in the epidermis, fibroblasts, and blood vessels with epidermal rete ridges, dermal cellularity,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

Is There an Association Between Male Alopecia Areata and the Ratio of Second to Fourth Finger Length?

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Abstract

Aim: The ratio between the lengths of the second and fourth fingers (2D to 4D) has been shown to indicate exposure to androgens prenatally. Although an association between alopecia areata (AA) and androgenic hormones has been recently suggested, the 2D to 4D ratio of AA has not yet been investigated. We aimed to investigate the lengths of the 2D to 4D in males with AA, as well as the potential links between 2D to 4D ratios and both AA and disease severity.

Materials and Methods: Male patients with AA and healthy volunteers participated in the study. A digital Vernier caliper was employed to measure the lengths of the fingers. The severity of alopecia tool and AA severity index were used to calculate the severity of AA.

Results: A total of 168 participants were recruited for the study. Compared with healthy controls, the 2D to 4D ratio was significantly lower in both hands of the patients ($P = 0.001$ for the right hand and $P < 0.05$ for the left hand). The right-hand 2D to 4D ratio showed better predictive capacity for AA development than the left hand (area under the curve: 0.952 vs. 0.638). The 2D to 4D ratio of patients and disease severity scores were not significantly correlated ($P > 0.05$).

Conclusion: To our knowledge, this is the first report to investigate the association between the 2D to 4D ratio and AA. The results of our research suggest that the 2D to 4D ratio of fingers is a possible predictor of AA development.

Keywords: Alopecia areata, androgens, fingers, sex hormones

Introduction

Alopecia areata (AA) is a chronic inflammatory disease characterized by non-scarring hair loss. Although the etiopathogenesis is not clearly known, the role of genetic predisposition and T cell-mediated autoimmunity in the development of the disease is emphasized. Emotional stress, psychiatric diseases, autoimmune thyroid diseases, atopy, vaccine use, viral infections, and anemia have been reported to trigger the development of this disease.[1] The prevalence of the disease is reported to be 0.58% and is slightly higher in females than in males.[2]

The ratio between the lengths of the second and fourth fingers (2D to 4D) has been shown to indicate exposure to androgens prenatally.[3,4] Males are known to have a lower 2D to 4D ratio compared to females.[5] 2D to 4D ratio has also been reported to be negatively correlated with testosterone concentration and sperm count in males.[6] There are numerous studies showing a link between the 2D to 4D ratio and many diseases, such as androgenetic alopecia (AGA), seborrheic dermatitis, vitiligo, acne vulgaris, polycystic ovary syndrome (PCOS), psychiatric diseases, and some malignancies.[7-13] The ratio of 2D to 4D in AA has not yet been investigated. Literature exists regarding the link between AA development and androgenic hormones.[14-16] We aimed to investigate the lengths of the 2D to 4D in males with AA, as well as the potential links between 2D to 4D ratios and both AA and disease severity.

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Materials and Methods

This cross-sectional research was approved by the KTO Karatay University Faculty of Medicine Ethics Committee (approval number: 2023/005, date: 26.09.2023). Between June and December 2023, 80 male participants with AA and 88 healthy volunteers were enrolled into this research. Patients were diagnosed based on clinical (patchy areas of hair loss) and dermoscopic findings consistent with AA. Healthy volunteers were selected from patients who presented to dermatology for medical screening and had no previous history of AA. Although the digit ratio is assumed to remain stable after puberty,[17] participants aged 16 years and younger were excluded to eliminate any possible puberty effect related to the digit ratio. We also excluded participants with a history of traumatic injury or disease that caused finger deformity. All participants provided informed consent. The severity of alopecia tool (SALT) was used to calculate the severity of AA with scalp involvement alone, while the alopecia areata severity index (AASI) was used to score the disease involving hairy areas of the head other than the scalp (with or without scalp involvement), such as the beard, eyebrows, and eyelashes. The SALT score was calculated according to the formula [(percent hair loss) right side x18] + [(percent hair loss) left side x18] + [(percent hair loss) top x40] + [(percent hair loss) back x24]. In AASI scoring, the formula [(percentage of hair loss) each eyebrow x15] + [(percentage of hair loss) eyelashes in each eye x10] was used for the upper face, [(percentage of hair loss) each cheek x20] + [(percentage of hair loss) each side of the neck x25] + [(percentage of hair loss) mustache x10] for the beard area.[18]

Second and 4th finger lengths in the palmar area from the intermetacarpophalangeal fold to the fingertip were measured using a digital Vernier caliper. If more than one fold was present at the interbase, the most proximal fold was used. Measurements were taken directly to avoid measurement errors caused by photocopying or scanning. Each measurement was confirmed by a second investigator. The 2D to 4D ratio was obtained by dividing the 2nd finger length by the 4th finger length. The 2D to 4D ratio was recorded for each hand of all participants.

The sample size was determined using the G*Power 3.1.94 software package (Franz Faul, Universität Kiel, Kiel, Germany). According to the t-test comparison of the 2D to 4D ratios of alopecia patients and control group in the study by by Bilgic et al.,[19] it was calculated that a total of 156 patients (78 in each group) should be included in the study for an effect size of 0.453, a margin of error of 0.05, and 80% power.

Statistical analysis

The data obtained from this research were examined using SPSS 22 (SPSS Inc., Chicago, IL, USA) software. The compatibility of the study parameters with normal distributed data was assessed using Kolmogorov-Smirnov test. In addition to descriptive statistical data (mean, frequency, standard deviation), Student's t-test was performed to compare numerical data between two groups for normally distributed variables. Pearson's test was used to assess correlations within normally distributed parameters. The most accurate cut-off point was determined by receiver operating characteristic (ROC) curve analysis. The level of significance was determined as $P < 0.05$.

Results

A total of 168 male participants, including patients (n = 80) aged between 18 and 75 years and healthy controls (n = 88) aged between 18 and 74 years, were recruited for the study. The average age of patients was 35.49±9.80, the average age of healthy controls was 35.86±12.37.

The duration of disease in the patient group ranged between 0.25 months and 48 months, with a mean of 5.40±7.62 months and a median duration of 3 months. SALT scores ranged between 0.2 and 28 with a mean of 5.73±5.64 and a median score of 4.4. AASI scores varied between 1 and 22.9, while the mean was 7.93±6.23 and that of the median was 6.6 (Table 1).

Compared with healthy controls, the 2D to 4D ratio was significantly lower in both hands of the patients ($P = 0.001$ for the right hand and $P < 0.05$ for the left hand) (Table 2, Figure 1).

The ROC curve was generated for the finger ratios of the right hand related to AA, with an area under the curve (AUC) of 0.952 and a standard error of 0.01. The ROC curve area was significantly greater than 0.5 ($P = 0.001$; $P < 0.05$). The cut-off value of the right hand 2D to 4D ratio for AA diagnosis was ≤ 0.99. This value had a sensitivity and specificity of 88.8% and 92.1%, respectively (Table 3, Figure 2a).

Table 1. Descriptive characteristics of variables in the patient group

	n	Minimum	Maximum	Mean ± SD	Median
Disease duration (months)	80	0.25	48	5.40±7.62	3.0
SALT score	56	0.2	28	5.73±5.64	4.4
AASI score	30	1	22.9	7.93±6.23	6.6

SALT: Severity of alopecia tool, AASI: Alopecia areata severity index, SD: Standard deviation

The ROC curve for the 2D to 4D left hand ratio in diagnosing AA was plotted as an AUC of 0.638 with a of 0.04. The ROC curve area was significantly greater than 0.5 ($P = 0.001$; $P < 0.05$). The cut-off value of the left-hand 2D to 4D ratio for AA diagnosis was ≤ 0.96. This value had a sensitivity and specificity of 55% and 63.6%, respectively (Figure 2b).

2D to 4D ratios did not significantly correlate with disease severity scores (SALT and AASI) ($P > 0.05$) (Table 4).

Discussion

We revealed a significantly reduced 2D to 4D ratio of both hands in male AA participants compared with healthy controls. The ROC analysis suggested that patients with a right hand 2D to 4D ratio smaller than 0.99 and left hand ratio smaller than 0.96 may have a relatively higher risk of developing AA.

2D to 4D ratio has been shown to indicate exposure to androgens prenatally.[3,20,21] Females affected by congenital adrenal hyperplasia have elevated levels of androgens as well as a lower 2D to 4D ratio than healthy females. Furthermore, because of the brain virilization effect of prenatal androgenic exposure, these patients are at risk of future gender confusion.[22,23] The Digit ratio is lower in many diseases thought to be related to androgens, suggesting a link between these diseases and prenatal androgen exposure.[7-13] For example, in a study investigating finger ratios in AGA, an androgen-dependent disease, the right hand 2D to 4D ratio revealed lower values than controls. Furthermore, in the patient group, the 2D to 4D ratio of the right hand had an inverse association with AGA grade.[12] In another study conducted in AGA, the

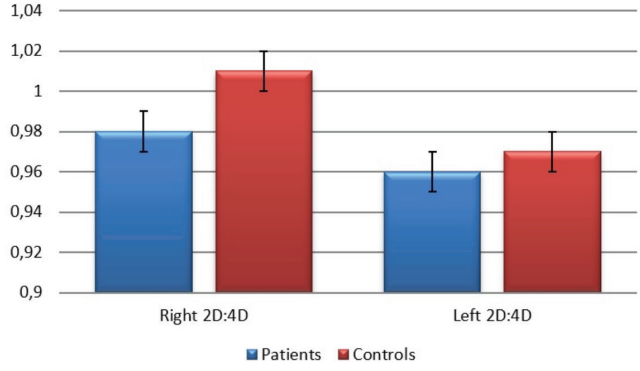

Figure 1: Comparison of right- and left-hand 2D to 4D ratios of the groups

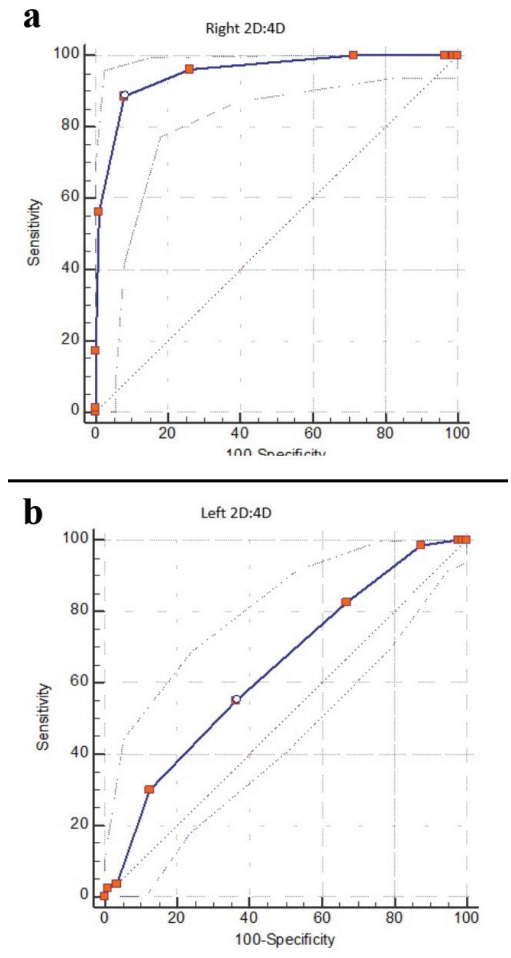

Figure 2. Receiver operating characteristic curves for right- and left-hand 2D to 4D ratios in the prediction of alopecia areata development

Table 2. Comparison of right- and left-hand 2D to 4D ratios among the groups

	Patients	Controls	*P* value
	Mean ± SD	Mean ± SD	
Right 2D to 4D	0.98±0.01	1.01±0.01	0.001*
Left 2D to 4D	0.96±0.01	0.97±0.01	0.001*

Student's t-test, *$P < 0.05$, SD: Standard deviation

Table 3. ROC analysis results for right and left-hand 2D to 4D ratios

	AUC	S.E.	95% CI	*P* value	Cut-off point	Sensitivity	Specificity
Right 2D to 4D	0.952	0.01	0.908-0.979	0.001*	≤ 0.99	88.8	92.1
Left 2D to 4D	0.638	0.04	0.561-0.711	0.001*	≤ 0.96	55.0	63.6

AUC: Area under the curve, CI: Confidence interval, S.E.: Standard error

Table 4. Correlation of SALT and AASI scores with right- and left-hand 2D to 4D ratios in the patient group

Patients		SALT score	AASI score
Right 2D to 4D	r	-0.172	0.087
	p	0.204	0.648
Left 2D to 4D	r	-0.070	0.057
	p	0.610	0.765

Pearson's correlation analysis. SALT: Severity of alopecia tool, AASI: Alopecia areata severity index

left-hand 2D to 4D ratio was reported to be lower than the control group. However, compared with controls, the 2D and 4D patterns of the right hand did not differ significantly among patients. There was no correlation between disease severity and left hand finger ratios.[24] According to a study by Bilgiç et al.[10] involving female patients diagnosed with acne vulgaris, 2D to 4D ratios were markedly decreased in both hands in comparison with the controls, whereas male patients showed no difference across the groups. In another study investigating the 2D to 4D ratios for seborrheic dermatitis, the finger ratio of the right hand was markedly reduced compared with that of the controls. Furthermore, the 2D to 4D ratio of the right hand had a negative association with the severity of the disease.[11] Demirbaş and Eker[9] found a significantly decreased right hand 2D to 4D ratio in patients with vitiligo compared with controls; however, they did not report an association with disease severity or finger ratios.

Hair follicles are protected from host immune defense mechanisms in the absence of MHC molecules and immune inhibitory secretions of membranous glycoproteins, perifollicular mast cells, and Treg cells. This is called the immune privilege. The main mechanism underlying the pathogenesis of AA is the disruption of the immune privilege area of hair follicles, resulting in cytotoxic T cell-mediated autoimmunity targeting the hair follicles leading to the recognition of hair follicle autoantigen by lymphocytes.[25] Androgenic hormones play an important role in terminal hair development through a complex mechanism via androgen receptors, androgen receptor coactivator, and 5-alpha reductase enzymes in hair follicles.[26] It has been suggested that androgen excess may disrupt the microenvironment of hair follicles consisting of dihydrotestosterone, growth factors, corticotropin-releasing hormone, cytokines, insulin, and vitamins.[16] There are several studies on the relationship between AA and sex hormone imbalance. In a study of female AA patients by Ranasinghe et al.,[16] it was reported that hyperandrogenism and clinical findings of hyperandrogenism, such as PCOS, adult acne and hirsutism were significantly increased compared with the normal population. It has been suggested that anti-androgen treatment agents should be investigated in the treatment of AA.[16] In another study by Hussein et al.,[15] compared to healthy controls, male participants with AA demonstrated a marked elevation in testosterone levels. Female AA patients did not have a marked difference in estrogen levels compared with controls. The previous study emphasized the possible role of hyperandrogenism in the pathogenesis of AA.[15] These findings may suggest a link between prenatal androgen exposure and AA, which is also thought to be related to androgens. The decreased 2D to 4D ratio in AA patients compared to the controls in this study revealed the potential involvement of androgenic exposure in the pathogenesis of the disease. The lack of correlation between AA severity scores (SALT and AASI) and digit ratios were not correlated may have been due to the small number of patients. It has been reported with a higher sensitivity of the 2D to 4D ratio of the right hand to prenatal androgen exposure compared with the 2D to 4D ratio of the left hand.[27] Features that differ between the sexes are most pronounced on the right part of the body in men. It has been reported that the finger ratios of the right hand are more sensitive to the effects of testosterone than the left hand.[28] Our study's 2D to 4D ratio cut-offs for both hands may be predictive factors for the development of AA. However, finger ratios were more sensitive and specific for AA development in the right hand than in the left hand. These findings suggest the potential prenatal involvement of androgen exposure in AA.

Study limitations

The results obtained from the present study should be interpreted within its limitations. The small number of participants is one of the limitations of the current study. This prevented us from making conclusive findings on the digit ratio and AA. Another limitation of this study is that it was a single-center study.

Conclusion

The current study is, to our knowledge, the first report to investigate the association between the 2D to 4D ratio and AA. According to our study, we have revealed two new points related to this issue. First, the 2D to 4D ratio is lower in male AA patients, and therefore, a low 2D to 4D ratio (cut-offs: smaller than 0.99 and 0.96 for right and left hand, respectively) may predict whether an individual will develop AA in the future because the finger ratio remains constant after birth. Second, the 2D to 4D ratio had higher sensitivity and specificity for the right hand with respect to the left hand as a predictor of AA development. Finally, the results of this study suggest that androgenic hormones may be associated with AA prenatally. To establish the role of androgenic exposure in AA development, larger patient populations are needed to be included in further studies.

Ethics

Ethics Committee Approval: This cross-sectional research was approved by the KTO Karatay University Faculty of Medicine Ethics Committee (approval number: 2023/005, date: 26.09.2023).

Informed Consent: All participants provided informed consent.

Authorship Contributions

Surgical and Medical Practices: M.Ö., Concept: M.Ö., T.A., Design: T.A., Data Collection or Processing: M.Ö., Analysis or Interpretation: M.Ö., Literature Search: M.Ö., T.A., Writing: M.Ö., T.A.

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Orginal Article

Association Between Acne Vulgaris and Face Mask Usage in Turkish Young Adults During the COVID-19 Pandemic: A Prospective Survey Study

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Abstract

Aim: Throughout the coronavirus disease-2019 (COVID-19) pandemic, the widespread use of personal protective equipment, including facial masks, was associated with an increased incidence of facial dermatoses, notably acne and dermatitis. The objective of this cross-sectional study was to ascertain the prevalence and clinical manifestations of acne vulgaris in young adults aged 17-24 years and to compare these characteristics before and during the COVID-19 pandemic, as well as to elucidate the association between facial mask use and acne exacerbation.

Materials and Methods: This study was performed among 6,517 undergraduates studying at the university. A multiple-choice questionnaire was disseminated via email to all undergraduate students utilizing the electronic mailing addresses furnished by the university.

Results: 48.6% of the participants had acne vulgaris before the pandemic, and 18.8% had new-onset acne vulgaris during the pandemic. During the pandemic and before the pandemic, the frequency of acne was higher in females than males ($P < 0.001$, $P < 0.001$). The presence of papules-pustules, itching, pain, dryness, and flaking were more common in patients with newly developed acne in the pandemic ($P = 0.015$, $P < 0.001$, $P < 0.001$, $P = 0.001$, $P = 0.036$). The frequency of acne increased significantly in those who used masks, those who used masks every day, those who used masks for more than 4 hours during the day, those who used black masks, those who did not change their masks during the day, those who used facial cleansers every day, and those who used emollients ($P < 0.001$, $P = 0.001$, $P < 0.001$, $P = 0.001$, $P = 0.005$, $P < 0.001$, $P < 0.001$).

Conclusion: Wearing face masks results in the development of acne and causes considerable acne flare in acne patients before the pandemic. Mask-induced acne is a significant problem symptoms like itching, burning sensations, and dryness, which are more common than pre-pandemic acne.

Keywords: Acne vulgaris, masks, pandemics

Introduction

Acne vulgaris is a multifactorial chronic inflammatory disease with a complex pathogenesis, particularly in the pilosebaceous unit. Four key factors play vital roles in acne development: altered sebum production, altered keratinization of the pilosebaceous unit, *Cutibacterium acnes (C. acnes)* and inflammation.[1,2]

The onset of acne is correlated with sebum production. The prevalence increases with age, and the incidence is highest in adolescents and lowest in pre-pubertal children. After young adulthood, the prevalence of acne decreases with increasing age.[3]

During the coronavirus disease-2019 (COVID-19) pandemic, the increased utilization of personal protective equipment, such as masks, was associated with a heightened prevalence of facial dermatoses.[4,5] Prolonged use of surgical face masks has been linked to alterations in the epidermis, including decreased hydration levels, increased trans-epidermal water loss, altered pH, heightened erythema, and sebum production, all of which are associated with facial inflammatory

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dermatoses, particularly acne and irritant contact dermatitis.[6] Maskne, or mask-related acne, is a dermatological condition associated with the COVID-19 pandemic, predominantly observed in healthcare workers but also prevalent in the general population. This type of acne is a type of mechanical acne that arises from persistent occlusion and friction caused by the continuous use of face masks.[5]

In our study, we aimed to determine the frequency and clinical features of acne in young adult patients aged 17-24 and compare these characteristics before and during the pandemic. In addition, we wanted to reveal the relationship between mask use and the development of acne.

Materials and Methods

Study Design

This study was approved by the Karadeniz Technical University Faculty of Medicine Ethical Committee (approval number: 2024/80, date: 26.04.2024).

This cross-sectional analysis was conducted from September 2021 to May 2024 at Karadeniz Technical University Faculty of Medicine, which has a total of 25,000 actively enrolled undergraduates. A structured multiple-choice questionnaire was distributed electronically to the actively monitored e-mail addresses of all undergraduates, as furnished by the university. The questionnaire included a preamble detailing the study objectives and the responsible academic department. Prior to accessing the survey items, participants were presented with a consent form containing options to "agree to participate," "decline participation," or "ask for a reminder later." Non-consenters, indicated by selecting "decline participation," did not receive further correspondence. Conversely, individuals who chose the "ask for a reminder later" option were re-contacted. Incomplete responses were excluded from the analysis. The questionnaire incorporated visual aids to help identify acne vulgaris types and map the distribution of facial lesions. To validate the survey reliability, a control question was strategically embedded with varying answer choices. Inconsistency in responses to this repeated item resulted in the exclusion of the participant from the study dataset.

Instruments

The questionnaire developed by the investigators was specifically designed to assess the prevalence of acne vulgaris prior to the onset of the COVID-19 pandemic, to compare the incidence during the pandemic period, and to evaluate the exacerbation rates of acne vulgaris. We evaluated the types (surgical mask, cloth mask, etc.), frequency, and duration of use of face masks on the development of acne vulgaris.

Facial area involvement in acne (T-zone, O-zone, U-zone), acne symptoms (comedone, papule, pustule), pain, itching, dryness, and scale scores were assessed.

In the participants of the study; usage of face mask, preference of face mask (medical mask, fabric mask, N95 mask), color of mask (white/green, black, colored), frequency of usage (everyday, < 7 days/week), time of usage during the day (< 4 hours, 4-8 hours, > 8 hours), change of mask during the day, and whether there is an increase in acne after COVID-19 infection or COVID-19 vaccine were questioned.

In addition, the frequency of use of facial cleaners and emollients under face masks was questioned.

Statistical analysis

Data analysis was conducted utilizing SPSS software version 23.0 (Armonk, NY: IBM Corp.). Quantitative results are presented as frequencies (n) and percentages (%) for categorical variables. The chi-square test was used to compare categorical variables and the prevalence of distinct acne treatment modalities. Bonferroni's adjustments were made to the *P* values account for multiple comparisons. Logistic regression analysis was employed to investigate the correlations between the incidence of acne vulgaris and exacerbations during the pandemic and demographic factors. The independent variables included gender, face mask usage, smoking status, alcohol consumption, type of mask used, duration of mask wearing per day, frequency of mask replacement, history of COVID-19 infection and vaccination status, and use of facial skincare products such as cleansers and moisturizers. The Hosmer-Lemeshow test was used to assess the goodness of fit for the logistic regression model. Multicollinearity diagnostics were performed to identify and exclude interdependent variables. A *P* value 0.05 was considered statistically significant.

Results

Of the 25,000 undergraduates solicited, 6,517 (26%) completed the entire questionnaire. Among the respondents, 20.1% were enrolled in medical programs, whereas 79.9% were from other faculties, including engineering, science, economics and administrative sciences, and fine arts. The mean age of the participants was 21.17 years, with a standard deviation of ±3.12.

48.6% of the participants had acne vulgaris before the pandemic, and 18.8% had new-onset acne vulgaris during the pandemic. During the pandemic and before the pandemic, the frequency of acne was higher in women (69.6% and 61.5%) than males (69.6% and 30.1%). ($P < 0.001$, $P < 0.001$) Considering the facial location of acne lesions,

U-zone location was reported more frequently (51.7) and T-zone location (26.1%), and O-zone location (18.8%).

Comparison of the Clinical Features Between Patients with Pre-Pandemic and Newly Developed Acne During the Pandemic

The clinical features of patients with pre-pandemic and newly developed acne during the pandemic are summarized in Table 1.

Acne treatment intake was higher before the pandemic than during the pandemic ($P < 0.001$). When the facial location of acne lesions was evaluated, the most common U-zone, the second most common T-zone location, was found in those with acne before the pandemic, while the U-zone was the most common and the second most common O-zone location was found in those with new-onset acne during the pandemic period. The presence of papules-pustules, itching, pain, dryness, and flaking were more common in patients with newly developed acne in the pandemic ($P = 0.015$, $P < 0.001$, $P < 0.001$, $P = 0.001$, $P = 0.036$). In addition, flares were observed more frequently in lesions after COVID infection and after COVID vaccine ($P < 0.001$, $P < 0.001$).

Comparison of Face Mask Usage Habits Between Patients with and Without New-Onset Acne During the Pandemic

Comparison of face mask usage habits between patients with and without new-onset acne and acne flares during the pandemic is shown in Table 2. It has been observed that the frequency of acne increases significantly in those who use masks, those who use masks every day, those who use masks for more than 4 hours during the day, those who use black masks, those who do not change their masks during the day, those who use facial cleansers every day, and those who use emollients ($P < 0.001$, $P = 0.001$, $P < 0.001$, $P = 0.001$, $P = 0.005$, $P < 0.001$, $P < 0.001$).

Comparison of Face Mask Usage Habits Between Patients with and Without Acne Flares During the Pandemic

Acne flares were observed significantly more frequently during the pandemic period in women, those who wore masks, those who used masks every day, those who used daily emollients and cleaners, and those who did not receive the COVID vaccine ($P = 0.022$, $P = 0.029$, $P = 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.006$, Table 2).

Logistic Regression Analysis of the Association Between Acne Vulgaris Frequency and Acne Flares During the Pandemic and Demographic Characteristics

When we evaluated acne flares during the pandemic period in logistic regression analysis; it has been shown that having a COVID infection increases 1.48 times, using a mask every day 1.39 times, and washing the face every day 1.48 times (Table 3).

When we evaluated the factors affecting new acne development during pandemic; it has been shown that the female gender increases 1.42-fold, the use of alcohol 1.29-fold, wearing

Table 1. Clinical features of acne patients before and during the pandemic

	Acne before the pandemic	Acne during a pandemic	*P* value
Gender			
Female	1802 (60.6)	850 (69.6)	<0.001
Male	1174 (39.4)	372 (30.4)	
Medical student	733 (25.1)	223 (18.5)	<0.001
Acne treatment	1356 (45.6)	382 (31.3)	<0.001
Acne type			
O-zone	501 (16.3)	296 (24.5)	<0.001
U-zone	853 (27.8)	653 (54.1)	
T-zone	1604 (52.2)	232 (19.2)	
Comedon	1187 (39.9)	463 (37.9)	0.229
Papules/pustul	998 (33.5)	458 (37.5)	0.015
Itching	1142 (38.4)	617 (50.5)	<0.001
Dryness	1294 (43.5)	718 (58.8)	<0.001
Burning and tenderness	672 (22.6)	335 (27.4)	0.001
Smoking	157 (5.3)	73 (6.0)	0.665
Alcohol	62 (2.1)	31 (2.6)	0.036
Flare after COVID-19 infection	312 (10.8)	206 (17.8)	<0.001
Flare after vaccination	335 (11.3)	272 (22.3)	<0.001

COVID-19: Coronavirus disease-2019

Table 2. Comparison of face mask usage habits between patients with and without new-onset acne and acne flares during the pandemic

	Patients with new-onset acne	Patients without new-onset acne	*P* value	Patients with acne flare	Patients without flare	*P* value
Gender						
Female	850 (69.6)	2848 (53.8)	<0.001	1972 (66.5)	284 (61.1)	0.022
Male	372 (30.4)	2447 (46.2)		994 (33.5)	181 (38.9)	
Medical student	223 (18.5)	1067 (20.5)	0.119	699 (23.9)	108 (23.2)	0.741
Usage of the mask	1121 (91.7)	4637 (87.6)	<0.001	2712 (91.4)	439 (94.4)	0.029
Frequency of mask use						
< 7 days/week	526 (43.5)	2546 (48.7)	0.001	1320 (44.9)	246 (53.2)	0.001
7 days/week	684 (56.5)	2681 (51.3)		1619 (55.1)	216 (46.8)	
Time of daily mask use						
< 4 hours	547 (45.2)	2717 (51.9)		1332 (45.3)	211 (45.4)	
4-8 hours	528 (43.6)	2013 (38.4)	<0.001	1256 (42.7)	196 (42.2)	0.939
> 8 hours	135 (11.2)	510 (9.7)		351 (11.9)	58 (12.5)	
Mask type						
Medical mask	1108 (91.1)	4742 (90.6)		2702 (91.2)	430 (93.1)	
Fabric mask	61 (5.0)	305 (5.8)	0.431	144 (4.9)	18 (3.9)	0.414
N95 mask	49 (4.0)	188 (3.6)		116 (3.9)	14 (3.0)	
Colour of mask						
White/green	455 (37.5)	2042 (38.7)		1090 (36.9)	175 (37.6)	
Black	744 (61.3)	3076 (58.3)	0.001	1785 (60.4)	283 (60.9)	0.308
Colored	15 (1.2)	157 (3.0)		80 (2.7)	7 (1.5)	
Change in the mask	521 (42.7)	2017 (38.4)	0.005	1102 (37.4)	168 (36.1)	0.593
Confirmed COVID infection	467 (38.2)	1949 (36.8)	0.358	1137 (38.3)	172 (37.0)	0.579
COVID vaccine	1190 (97.7)	5114 (96.7)	0.077	2873 (97.0)	462 (99.4)	0.006
The type of vaccine						
Sinovac	140 (11.8)	635 (12.4)	0.531	337 (11.8)	51 (11.0)	0.656
Bionthec	1050 (88.2)	4475 (87.6)		2530 (88.2)	411 (89.0)	
Frequency of facial cleanser use						
< 7 days	495 (40.8)	2489 (48.1)	<0.001	1333 (45.3)	266 (57.2)	<0.001
7 days	717 (59.2)	2688 (51.9)		1609 (54.7)	199 (42.8)	
Usage of emollients	916 (75.0)	2890 (54.6)	<0.001	2058 (69.4)	284 (61.1)	<0.001

COVID: Coronavirus

Table 3. Logistic regression analysis of acne flare association according to demographic characteristics during the pandemic

Risk factor	OR (95% CI)	*P* value
Sex (female)	1,149 (0.921-1,433)	0.217
Not being a medical student	0.912 (0.715-1,165)	0.461
Smoker	1,187 (0.826-1,708)	0.354
Alcohol consumption	1,066 (0.741-1,532)	0.732
COVID vaccine	1,051 (0.855-1,292)	0.638
Confirmed COVID infection	1,487 (1,203-1,840)	0.012
Usage of the mask	0.656 (0.424-1,015)	0.058
Frequency of mask usage (7 days)	1,396 (1,127-1,729)	0.002
Time of daily mask usage (4-8 hours)	0.992 (0.789-1,246)	0.942
Time of daily mask use (> 8 hours)	0.899 (0.639-1,265)	0.541
Change in the mask	0.975 (0.788-1,206)	0.816
Frequency of facial cleanser usage (7 days)	1,487 (1,203-1,840)	<0.001
Usage of moisturizers	1,018 (0.824-1,257)	0.870

OR: Odds ratio, CI: Confidence interval, Omnibus test: $P < 0.001$, Nagelkerke's R-square: 2.9%, COVID: Coronavirus

the mask for 4-8 hours daily 1.26-fold, wearing the mask for more than 8 hours 1.29-fold, daily use of cleansers on the face 1.31-fold, and using moisturizers under the mask 1.72-fold (Table 4).

Discussion

In our study, we observed that nearly one of two students had developed acne starting before the pandemic, and new acne developed in 18.8% of the participants during the pandemic period. In both periods, acne frequency was higher in females.

This observation aligns with the results reported by Altun and Topaloglu Demir[7] and Techasatian et al.,[8] who both identified females as having a higher propensity for the development of maskae, whereas Falodun et al.[2] did not show any sex predilection for maskne.

In the literature, Kiely et al.[9] reported new-onset acne in 53% of 337 participants and acne lesion flares in 46.6% during the pandemic, whereas Villani et al.[10] Reported rates of 76.3% and 23.7% in another study. In a study conducted by Tuncer Vural[11] it was observed that 40.5% of the participants experienced the onset of new acne, whereas 20.5% reported exacerbations of pre-existing acne. Contrary to the literature, new-onset acne was less common, but the incidence of acne flares was quite high at 86.4% in our study. Unlike our study, Kiely et al.[9] conducted a study on healthcare professionals. However, in our study, no increase was found between medical students from other faculties regarding new-onset acne development and acne flares. We believe that this is due to the fact that all faculty students received online distance education during the pandemic period.

In our study, we found that new acne development was higher in those who used masks every day, those who wore masks for more than 4 hours a day, those who preferred black masks, and those who did not change their masks during the pandemic period.

In the existing literature that examined the variables of mask type, color, and usage duration, the results have been mixed. Yaqoob et al.[12] no significant correlation was observed between the incidence of acne and the daily use of face masks. Conversely, Tuncer Vural[11] reported that increasing the frequency of mask changes, a finding corroborated by our study, reduced the incidence of acne. Nonetheless, the relationship between acne development and the number of masks worn per day was not established in the study by Yaqoob et al.[12] We think that as the duration of mask use is prolonged and when the same mask is used during the day, the increased humidity and occlusion increase the clogging of the pores, and the irritation causes acne and increases flares. Our study is the first to evaluate the effect of mask color on acne, and we believe that the increased incidence of acne in black mask users may be due to the dyes in these masks.

Hua et al.[6] assessed the dermatological impacts of surgical masks and N95 respirators, noting that these masks create microenvironmental changes in the skin, such as dehydration, increased sebum production, and elevated pH levels, which collectively foster conditions conducive to the proliferation of *C. acnes* and the activation of inflammatory lesions. The microbial contamination of masks and the anaerobic environment created by prolonged mask use can enhance bacterial virulence and promote the proliferation of opportunistic pathogens. This can lead to microbiome imbalance, increasing susceptibility to acne development.[13-15]

Table 4. Logistic regression analysis of association of acne vulgaris frequency during pandemic

Risk factor	OR (95% CI)	*P* value
Sex (female)	1,416 (1,220-1,643)	< 0.001
Not Being a medical student	1,239 (1,043-1,472)	0.015
Smoker	0.843 (0.672-1,059)	0.142
Alcohol consumption	1,293 (1,040-1,606)	0.021
Confirmed COVID infection	1,076 (0.533-1,133)	0.283
COVID vaccine	1,505 (0.993-2,282)	0.054
Usage of the mask	1,224 (0.958-1,563)	0.106
Frequency of mask usage (7 days)	1,052 (0.915-1,209)	0.477
Time of daily mask usage (4-8 hours)	1,258 (1,002-1,580)	0.048
Time of daily mask use (> 8 hours)	1,291 (1,115-1,494)	0.001
Change in the mask	1,017 (0.889-1,164)	0.802
Frequency of facial cleanser usage (7 days)	1,310 (1,143-1,502)	<0.001
Usage of moisturizers	1,719 (1,496-1,975)	<0.001

OR: Odds ratio, CI: Confidence interval, Omnibus test: $P < 0.001$, Nagelkerke's R-square: 5.6%, COVID: Coronavirus

However, the literature presents divergent results concerning the link between mask type and acne development. Techasatian et al.[8] observed that approximately half of the participants experienced adverse skin reactions to face masks, with acne constituting 40% of these responses. Studies by Chaiyabutr et al.[16] and Techasatian et al.[8] indicated a higher prevalence of acne with surgical masks compared to cloth masks.[19,22,24] In contrast, Yaqoob et al.[12] and Foo et al.[17] identified a positive correlation between the use of N95 masks and acne development, whereas Choi et al.[18] Reported that cotton face masks intensified acne flares. Roy et al.[19] found that surgical masks increased the risk of acne by 2.40 times and N95 masks by 3 times. Han et al.[20] suggested that cloth masks were more likely to cause acne than surgical and FFP2/KN95 masks, potentially due to prolonged use without proper washing and the accumulation of sweat and environmental dirt. In contrast to these findings, our study did not reveal a significant difference in the onset of new acne or the exacerbation of existing acne across different mask types during the pandemic. Supporting our results, İnan Doğan and Kaya[21] and Tuncer Vural[11] reported no association between mask type and acne. We hypothesized that the minimal use of N95 and fabric masks in our cohort may have limited our ability to discern a clear relationship between acne development and flares. Daye et al.[22] and Choi et al.[18] both concluded that extended mask-wearing durations are associated with increased acne flares. Similarly, Techasatian et al.[8] demonstrated that the risk of adverse skin reactions, including acne, escalates with mask wearing periods of 4-8 hours per day and further increases beyond 8 hours of use per day. These findings are in concordance with our study, in which regression analysis revealed that wearing a face mask for more than 4-8 hours, and again beyond 8 hours augmented the risk of acne development by factors of 1.29 and 1.25, respectively. Mask-related acne has been observed to manifest predominantly on the chin, particularly within the "O"-zone of the face, presenting primarily as mild papular eruptions that are often accompanied by comedones and seborrhea. Kiely et al.[9] reported that among individuals who developed Maskne following the onset of the COVID-19 pandemic, most (85.5%) experienced papulopustular eruptions, 46% experienced comedonal breakouts, and 22.5% suffered from nodulocystic lesions. Notably, a small fraction (12.8%) of these individuals sought medical advice for their acne. In our study, we found that papules/pustules develop more frequently in those with acne during the pandemic compared with those with acne before the pandemic; we did not detect any difference in the rate of comedones. In addition, the U- and O-zone localization of acne lesions increased significantly in those with new-onset acne during the pandemic. We believe that the increase in the O- percent and U-zone locations of new-onset acne during the pandemic is due to the areas where the mask is placed on the face.

Szepietowski et al.[23] reported that approximately 20% of young individuals wearing face masks experienced episodes of itch, corroborating the findings of Zuo et al.,[24] who documented a 14.9% incidence of itch attributed to face mask use. In a study by Tuncer Vural[11] showed that 35.5% of participants with new-onset acne experienced itching, 20.5% had dryness, and 19.5% had burning and tenderness. In our study, these symptoms were examined in more detail (rates were 50.5%, 58.8%, 27.4%, respectively), and we showed that the incidence of these symptoms was significantly higher than that of acne before the pandemic.

Factors associated with increased rates of maskne included female sex, younger age, history of acne, family history of acne, working in a "hot and sweaty" environment, use of emollients under the masks, and use of face shields and goggles.[8] In our study, acne patients with COVID-19 who used a mask every day and washing their face every day were found to have an increased risk of acne flares. Further analysis revealed that female gender, use of alcohol-based products, prolonged mask wearing for periods of 4-8 hours and over 8 hours daily, daily application of facial cleaners, and regular use of emollients beneath the mask were associated with increased rates of new acne development during the pandemic. We believe that the acne flare caused by the use of facial cleanser may also be due to the use of an inappropriate cleanser. Our study was also a cross-sectional study, and the properties of the cleanser were not examined. These limitations may have made it difficult to assess the relationship between cleanser use and acne. This should be confirmed by studies that examined the properties of cleansers. In addition, we think that regular use of a moisturizer under the mask leads to acne development by increasing occlusion and humidity.

Study limitations

The primary limitation of this study was its reliance on self-reported data on acne occurrence. Furthermore, the participants were not subjected to prospective follow-up evaluations, which could have provided more objective and consistent assessments of acne development.

Conclusion

The results of this study indicate that the use of face masks is associated with the onset of acne and significantly exacerbates acne flares in individuals with a pre-pandemic history of acne. The phenomenon of mask-induced acne is a notable dermatological issue, with symptoms such as itching, burning sensations, and dryness being more prevalent than acne cases prior to the pandemic. Strategies to mitigate such conditions include minimizing the duration of mask use. However, the

application of emollients underneath masks could potentially contribute to the development of acne. Additionally, data suggest that women are at an increased risk of developing acne during the pandemic compared with their male counterparts.

Ethics

Ethics Committee Approval: This study was approved by the Karadeniz Technical University Faculty of Medicine Ethical Committee (approval number: 2024/80, date: 26.04.2024).

Informed Consent: It was obtained.

Authorship Contributions

Concept: D.A.A., L.B.S., Design: D.A.A., L.B.S., İ.E.A., Supervision: D.A.A., L.B.S., İ.E.A., Data Collection or Processing: D.A.A., L.B.S., Analysis or Interpretation: D.A.A., L.B.S., İ.E.A., Literature Search: D.A.A., L.B.S., İ.E.A., Writing: D.A.A., L.B.S., Critical Review: D.A.A., L.B.S.

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Orginal Article

Significance of Fucosyltransferase 8 and Transforming Growth Factor-β1 Expression in Plaque Psoriasis: A Clinical and Immunohistochemical Study

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Abstract

Aim: Psoriasis is a chronic disease characterized by epidermal hyperproliferation and dermal inflammation. Fucosyltransferase 8 (FUT8) is a single-core fucosylation enzyme in mammalian cells. Transforming growth factor-beta (TGF-β), a multipotent cytokine, was identified in epidermal keratinocytes. This study aimed to assess the expression of FUT8 and TGF-β1 in psoriatic skin compared with control skin using immunohistochemistry and to explore the link between these expressions with accessible clinical and pathological information.

Materials and Methods: This was a case-control study that included 60 participants: thirty cases with chronic psoriasis vulgaris and 30 gender- and age-matched normal controls. A comprehensive medical history and examination were conducted, and the severity of psoriasis was evaluated using the psoriasis area and severity index score. Immunohistochemical analysis of FUT8 and TGF-β1 was conducted.

Results: FUT8 expression in keratinocytes gradually increased from control skin to perilesional and lesional skin. Regarding the dermis, FUT8 exhibited significant differences between lesional and perilesional skin and controls in terms of the expression of inflammatory cells (χ^2=40.0, $P < 0.001$), percentage of positive cells, and H-score of inflammatory cells ($P = 0.002$, $P < 0.001$), respectively. Significant positive correlations were observed among TGF-β1 and FUT8 in terms of the percentage of positive cells and H-score in the lesional epidermis ($P = 0.013$, $P < 0.001$, respectively).

Conclusion: FUT8 and TGF-β1 were shown to be overexpressed in psoriasis, showing correlations with severity and with each other, suggesting their potential involvement in the development of psoriasis.

Keywords: Fucosyltransferases, immunohistochemistry, psoriasis, transforming growth factor beta 1

Introduction

Psoriasis is a multisystem immune-related disorder that generally affects the skin, joints, or both. The prevalence rate of this condition in the western population is approximately 2-3%, whereas that in Egypt varies from 0.19% to 3%.[1]

Psoriasis can manifest in several clinical forms, including pustular, erythrodermic, and guttate psoriasis. The chronic plaque variant, which affects approximately 90% of patients, typically presents as scaly erythematous lesions involving the extensor areas, trunk, and scalp.[2]

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Psoriasis is a multifactorial disorder with multifaceted pathogenesis that involves an abnormal immune reaction in the skin, genetic predisposition, and exposure to several environmental agents, such as trauma, infections, and drugs.[3]

The injurious inflammatory processes associated with psoriasis extend beyond the skin and are responsible for a growing list of coexisting comorbidities, such as chronic

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kidney disorder, gastrointestinal disorders, mood disorders, cancer, and cardiovascular and metabolic diseases (obesity, hypertension, dyslipidemia, and diabetes).[4]

Fucosylation is a frequent posttranslational alteration of glycolipids and glycoproteins.[5] The human genome encodes 13 different fucosyltransferase (FUTs), with α-(1,6)-FUT8 being the only one that catalyzes α-(1,6)-linked core fucosylation in the Golgi apparatus.[6,7] Fucose is added to asparagine-linked N-acetylglucosamine (GlcNAc) moieties by FUT8, which is a characteristic shared by N-linked glycan core structures.[8]

Conserved throughout evolution, a family of secreted polypeptide factors called transforming growth factor-beta (TGF-β) regulates several aspects of physiological embryogenesis, adult tissue homeostasis, and cell growth and differentiation. Additionally, its members are involved in the pathophysiological processes that lead to different diseases.[9,10]

In human tissue, TGF-β1, TGF-β2, and TGF-β3 are the three isoforms, and skin is a significant target for TGF-β1, playing a vital role in etiopathogenesis of psoriasis, and epidermal keratinocytes have been shown to express its receptors.[9,11]

Kim et al.[12] claimed that core fucosylation only affects the way TGF-β receptors move across their cell surfaces. The pharmacological or genetic suppression of TGF-β RII's N-linked glycosylation significantly decreased the receptor's ability to move over its cell surface and hampered its interaction with the TGF-β1 ligand. Furthermore, Gao et al.[13] discovered that FUT8 controls the proliferation, migration, and fibrosis of human embryonic lung fibroblasts that are stimulated by TGF-β1.

Therefore, this study aimed to investigate the immunostaining of FUT8 and TGF-β1 in skin biopsies of lesional and perilesional skin biopsies from psoriatic skin compared with normal control skin.

Materials and Methods

Study Cohort

This designed case-control study was conducted on 30 cases of psoriasis vulgaris diagnosed using clinical and histopathological methods, in addition to 30 age- and sex-matched normal subjects who were designated as controls.

Every participant signed a written informed consent form before initiating the research work, and approval for the study was obtained from the Menoufia University Faculty of Medicine Research Ethics Committee, which agreed with the Declaration of Helsinki 1975 (reviewed in 2000) (approval number and date: 8/2021 DERMA 13). Selected cases were asked to discontinue local anti-psoriatic (15 days) or systemic (1 month) anti-psoriatic therapy before the beginning of the study.

Exclusion criteria: Any case with one or more of the following was excluded from the study:

- Dermatological diseases except plaque psoriasis.

- Any systemic autoimmune or inflammatory disease.

Full history taking with an emphasis on disease duration in years, course, and onset of psoriasis was obtained from every case where early-onset psoriasis was defined as psoriasis started before the age of 40 years or late-onset psoriasis as psoriasis started after the age of 40 years old.[1]

Full physical and dermatological examinations were performed to evaluate the lesion site and involvement of the palm, sole, and scalp. In addition, evidence of Koebner's phenomenon was also assessed. The severity of psoriasis was assessed using the psoriasis area and severity index (PASI) score. Mild cases were defined as PASI < 7, moderate cases as PASI 7-12, and severe cases as PASI > 12.[14]

Skin biopsies: First, 3 mm punch skin biopsies were taken from the involved skin (lesional skin), perilesional skin (2 cm away from the lesion),[15] and coordinated skin sites of control subjects. Then, 10% neutral formalin was used for fixation, and paraffin blocks were formed. Furthermore, 4 μm thick sections were cut as follows: one section for regular hematoxylin and eosin stain histological examination and an additional two sections for immunostaining using FUT8 and TGF-β1 as primary antibodies.

Immunohistochemical Staining

The streptavidin-biotin-amplified system was employed for immunostaining. Anti-FUT8 immunoglobulin G Rabbit Polyclonal Antibody (0.1 mL concentrated and diluted 1:100) (Abbexa Ltd, Cambridge, UK, catalog no. abx338636) and Polyclonal anti-TGF-β1 antibody (Chongqing Biospes Co., Ltd, China; 100 μL, catalog no. YPA1196) were used as primary antibodies. Heat retrieval was performed using citrate buffer for the two primary antibodies. Human breast cancer tissue and human bone marrow tissue slides were prepared as positive controls for FUT8 and TGF-β1 individually. In contrast, negative control slides were checked each time.

Analysis of FUT8 and TGF-β1 Expressions

FUT8 and TGF-β1 immunostaining were evaluated semiquantitatively. The positivity of expression was recognized as cytoplasmic or nucleocytoplasmic brownish

staining for FUT8[16] and cytoplasmic staining for TGF-β1[17] by 3,3'-diaminobenzidine reaction. The percentage of positive cells was then evaluated. In terms of stain intensity, there were three categories for stain intensity: mild, moderate, and strong. The H-score (histo-score) was calculated using the following formula: H-score= 1 x percentage of cells with mild intensity + 2 x percentage of cells with moderate intensity + 3 x percentage of cells with strong intensity.[18] Furthermore, FUT8 and TGF-β1 expressions in epidermal and dermal inflammatory cells were evaluated, and H-scores were recorded.

Statistical analysis

Revision of the collected data was done for accuracy, and then the data were coded and analyzed using the Statistical Package for Social Sciences (SPSS version 23) program. A suitable evaluation agreed with the type of data obtained for each parameter. Numbers and percentages were presented using qualitative data, whereas quantitative data were presented as mean and standard deviation. Correlations were detected using appropriate statistical tests. *P* value ≤ 0.05 was the level of significance.[19]

Results

Demographic Data of the Study Groups

Cases of plaque psoriasis were 18 (60%) male and 12 (40%) female patients. The age range was 18-75 years with 44.83±15.64 years as $\bar{X}$ ± standard deviation (SD) value. The control group comprised 20 (66.7%) males and 10 (33.3%) females. The age range was 20-70 years with 39.67±14.63 years as $\bar{X}$ ± SD value. Age and sex differences between cases and controls were not statistically significant (*P* > 0.05 for both). The clinical data of the cases are presented in Table 1.

Histopathological data of the studied cases: Ten cases (33.3%) patients had mild acanthosis, seven cases (23.3%) had moderate acanthosis, and 13 cases (43.3%) had notable acanthosis. Additionally, 15 cases (50%) patients were characterized by mild hyperkeratosis, 9 cases (30%) had moderate hyperkeratosis, and 6 cases (20%) had marked hyperkeratosis. In 9 cases (30%), parakeratosis was mild; in 10 cases (33.3%), it was moderate; and in 11 cases (36.7%), it was significant. Suprapapillary thinking was found in all (100%) patients with psoriasis, whereas Munro's microabscesses and spongiform pustules were found in 4 (13.3%) and 3 (10%) cases, respectively. All patients (100%) exhibited blood vessel dilation in the papillary dermis. Inflammation was mild in 11 (36.7%) cases, moderate in 11 (36.7%), cases, and marked in 8 (26.7%) cases.

Table 1. Clinical information of the studied patients (n = 30)

Variables	n	%
Onset		
Early	17	56.7
Late	13	43.3
Course		
Stationary	18	60.0
Progressive	12	40.0
Duration (years)		
Minimum-maximum	1.0-10.0	
$\bar{X}$ ± SD	5.70±3.58	
Median (IQR)	6.0 (2.0-9.0)	
Family history		
Positive	10	33.3
Negative	20	66.7
Risk factors		
Yes	**10**	**33.3**
HTN	1	3.3
Smoking	6	20.0
T2DM	3	10.0
No	20	66.7
Site of affection		
Extremities	9	30.0
Axial and extremities	14	46.7
Axial	7	23.3
Scalp affection		
Positive	21	70.0
Negative	9	30.0
Nail affection		
Positive	8	26.7
Negative	22	73.3
Joint affection		
Yes	7	23.3
No	23	76.7
Palm and sole affection		
Yes	9	30.0
No	21	70.0
Itching		
Yes	19	63.3
No	11	36.7
Koebnerization		
Yes	11	36.7
No	19	63.3
PASI score		
Minimum-maximum	3.0-15.0	
$\bar{X}$ ± SD	9.28±3.74	
Median (IQR)	8.60 (6.0-13.0)	
Severity		
Mild	10	33.3
Moderate	9	30.0
Severe	11	36.7

$\bar{X}$: Mean, SD: standard deviation, IQR: Interquartile range, T2DM: Type 2 diabetes mellitus, PASI: Psoriasis area and severity index, HTN: Hypertension

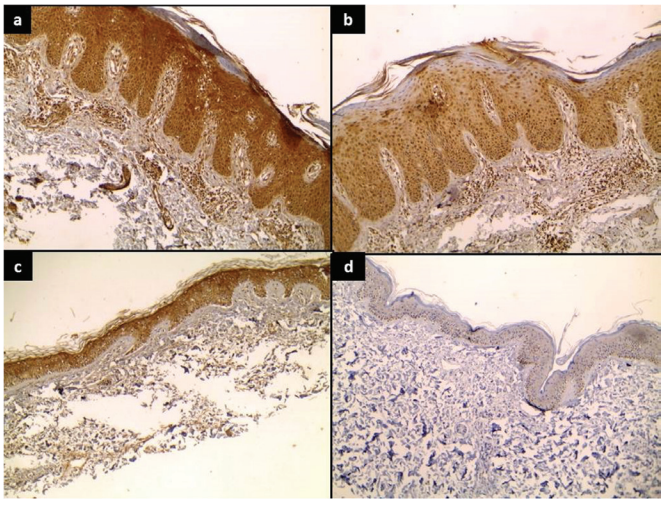

Figure 1. Immunohistochemical staining of FUT8 showed (a) strong expression in lesional skin (IHC, x100); (b) moderate expression in lesional skin (IHC staining, x100); (c) moderate expression in perilesional skin (IHC staining, x40); (d) negative expression in control skin (IHC staining, x40)

FUT8: Fucosyltransferase 8, IHC: Immunohistochemistry

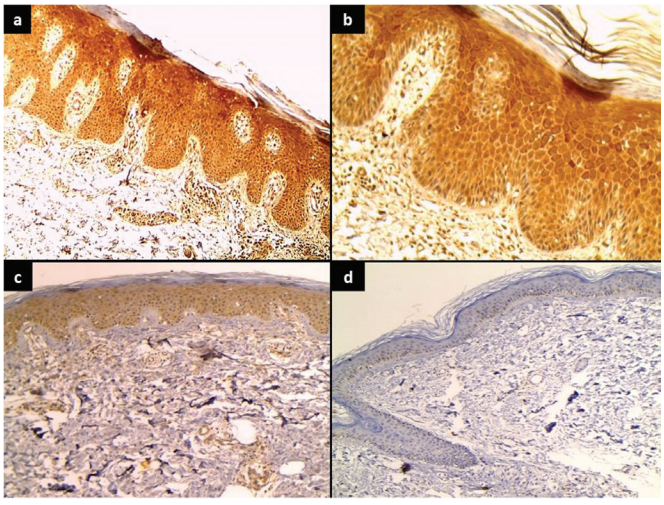

Figure 2. Immunohistochemical staining of TGF-β1 showed (a) strong expression in lesional skin (IHC, x100); (b) high-power view showed strong expression in lesional skin (IHC staining, x400); (c) mild expression in perilesional skin (IHC staining, x40); (d) negative expression in control skin (IHC staining, x40)

TGF-β1: Transforming growth factor-beta, IHC: Immunohistochemistry

Immunohistochemical staining of FUT8 and TGF-β1 in lesional, perilesional, and control skin is was demonstrated in Figure 1 for FUT8 and Figure 2 for TGF-β1.

Evaluation of FUT8 Immunohistochemical Expression in Control and Lesional and Perilesional Skin of the Studied Cases

In terms of the epidermis, statistically significant differences were found among the lesional and perilesional skin of the studied cases and controls regarding the FUT8 status (χ^2=12.306, P = 0.001), intensity (χ^2=16.610, P = 0.002), percentage of positive cells, and H-score (H=36,697, 47,455, respectively, P < 0.001 for both) with an ongoing, progressive higher expression of FUT8 in keratinocytes from the control skin than in the perilesional and lesional skin (Table 2).

Concerning the dermis, there were statistically significant differences between the lesional abd perilesional skin of the studied cases and controls regarding the FUT8 status in inflammatory cells (χ^2=40.0, P < 0.001), percentage of positive cells (inflammatory) (H=12,037, P = 0.002), and H-score (inflammatory) (H=16,224, P < 0.001) Table 2.

Evaluation of TGF-β1 Immunohistochemical Expression Between Control Skin and Lesional and Perilesional Skin of the Studied Cases

Regarding the epidermis, statistically significant differences were detected between the lesional and perilesional skin of the studied cases and control skin regarding status (χ^2=29,515, P < 0.001), percentage of positive cells, and H-score of TGF-β1 (H=7,462 and 11,008; P = 0.024 and 0.004, respectively) Table 3.

Regarding the dermis, a statistically significant difference was observed in the TGF-β1 status between the control skin and the lesional and perilesional skin of the studied cases (χ^2=47,312, P < 0.001), as shown in Table 3.

There was an ongoing progressive higher expression of TGF-β1 in keratinocytes and dermal inflammatory cells from control skin in comparison with perilesional and lesional skin, as presented in Table 3.

Correlation Between FUT8 and TGF-β1 Regarding Percentage of Positive Cells and H-score of Expression in the Epidermis of Lesional Skin

There were significant positive correlations between TGF-β1 and FUT8 regarding the percentage of positive cells (r=0.448, P = 0.013) and H-score in the epidermis of lesional skin (r=0.694, P < 0.001) (Figure 3a, b).

Moreover, there were significant positive correlations between TGF-β1 and FUT8 in the dermis of lesional skin regarding the percentage of positive cells (r=0.448, P = 0.013) and H-score (r=0.694, P < 0.001) (Figure 3c, d).

Relationship between FUT8 and TGF-β1 and clinical information of the studied cases: A statistically significant association was found between FUT8's strong intensity of expression in the epidermis of lesional skin and the presence of itching (χ^2=8,585, P = 0.015) (Figure 4a). There was a

Table 2. Comparison of FUT8 immunohistochemical expression in patients' lesional and perilesional skin and control skin

	Lesional, (n = 30)		Perilesional, (n = 30)		Control, (n = 30)			
FUT8	**n**	**%**	**n**	**%**	**n**	**%**	**Test of sig. (p)**	**Sig. bet. grps.**
Epidermis								
Status								
Positive	**30**	**100.0**	**30**	**100.0**	**23**	**76.7**	χ^2=12,306* (^{MC}p=0.001)*	p_1= - $^{FE}p_2$=0.011* $^{FE}p_3$=0.011*
Negative	0	0.0	0	0.0	7	23.3		
Localization								
Cytoplasmic	30	100.0	30	100.0	23	100.0	-	-
Intensity								
Mild	11	36.7	20	66.7	19	82.6	χ^2=16,610* (^{MC}p=0.002)*	p_1=0.018* p_2=0.001* $^{MC}p_3$=0.449,
Moderate	9	30.0	8	26.7	4	17.4		
Severe	10	33.3	2	6.7	0	0.0		
Percentage of positive cells								
Min.-max.	40.0-90.0		40.0-90.0		10.0-70.0		H=36,697* (<0.001)*	p_1=0.511 p_2<0.001* p_3<0.001*
$\bar{X}$ ± SD	74.67±16.97		71.67±16.21		37.83±15.94			
Median (IQR)	80.0 (70.0-90.0)		70.0 (60.0-90.0)		40.0 (30.0-50.0)			
H-score								
Min.-max.	70.0-270.0		40.0-270.0		10.0-80.0		H=47,455* (<0.001)*	p_1=0.016* p_2<0.001* p_3<0.001*
$\bar{X}$ ± SD	145.3±64.69		99.0±48.52		43.04±18.20			
Median (IQR)	120.0 (90.0-180.0)		90.0 (70.0-120.0)		40.0 (30.0-55.0)			
Dermis								
Status (inflammatory)								
Positive	30	**100.0**	**18**	**60.0**	**6**	**20.0**	χ^2=40.0* (<0.001)*	p_1<0.001* p_2<0.001* p_3=0.002*
Negative	0	0.0	12	40.0	24	80.0		
Intensity (inflammatory)								
Mild	11	36.7	9	50.0	4	66.7	χ^2=4,926 (^{MC}p=0.290)	p_1=0.227, $^{MC}p_2$=0.276 $^{MC}p_3$=1,000,
Moderate	9	30.0	7	38.9	2	33.3		
Severe	10	33.3	**2**	**11.1**	**0**	**0.0**		
Percentage of positive cells (inflammatory)								
Min.-max.	40.0-90.0		60.0-90.0		30.0-70.0		H=12,037* (0.002)*	p_1=0.845 p_2=0.001* p_3=0.001*
$\bar{X}$ ± SD	74.67±16.97		77.22±12.27		41.67±16.02			
Median (IQR)	80.0 (70.0-90.0)		80.0 (70.0-90.0)		35.0 (30.0-50.0)			
H-score (inflammatory)								
Min.-max.	70.0-270.0		70.0-270.0		30.0-80.0		H=16,224* (<0.001)*	p_1=0.127 p_2<0.001* p_3=0.005*
$\bar{X}$ ± SD	145.3±64.69		112.2±51.51		58.33±17.22			
Median (IQR)	120.0 (90.0-180.0)		90.0 (80.0-120.0)		60.0 (50.0-70.0)			

IQR: Interquartile range, SD: Standard deviationi χ^2: Chi-square test, H: Kruskal-Wallis test. Pairwise contrast between each two groups was done using post-hoc test (Dunn's for multiple comparisons test), FE: Fisher's exact test, MC: Monte Carlo, *: $P \leq 0.05$ is the level of significance. p: Comparison between the three studied groups, p_1: Comparison between lesional and perilesional, p_2: Comparison between lesional and control; p_3: Comparison between perilesional and control

statistically significant relationship between a higher FUT8 percentage of positive cells in the epidermis of lesional skin and smoking (U=33.0, $P = 0.044$) and higher disease severity (H=8,674, $P = 0.013$) (Figure 4b, c, respectively).

There was a statistically significant relationship between high FUT8 mean H-scores and the progressive course of psoriasis (U=46.50, $P = 0.008$), concomitant axial and extremity involvement (H=9,216, $P = 0.010$), and higher disease severity (H=11,113, $P = 0.004$) (Figure 4d-f, respectively).

Regarding TGF-β1, there was a statistically significant relationship between TGF-β1's strong intensity of expression and progressive course (χ^2=6,941, $P = 0.017$), PASI score of the studied cases (H=13,710, $P = 0.001$), and severe disease (χ^2=12,285, $P = 0.006$) (Figure 5a-c, respectively).

Table 3. Comparing TGF-β1 immunohistochemical expression in patients' lesional and perilesional skin and control skin

	Lesional, (n = 30)		Perilesional, (n = 30)		Control, (n = 30)			
	n	%	n	%	n	%	Test of sig. (p)	Sig. bet. grps.
Epidermis								
Status								
Positive	**30**	**100.0**	**22**	**73.3**	**12**	**40.0**	χ^2=29,515* (<0.001)*	$^{FE}p_1$=0.005* p_2<0.001* p_3=0.009*
Negative	0	0.0	8	26.7	18	60.0		
Localization								
Cytoplasmic	30	100.0	22	100.0	12	100.0	-	-
Intensity								
Mild	15	50.0	17	77.3	9	75.0	χ^2=5,258 (^{MC}p=0.217)	$^{MC}p_1$=0.091, $^{MC}p_2$=0.371, $^{FE}p_3$=1,000,
Moderate	12	40.0	5	22.7	3	25.0		
Severe	3	10.0	0	0.0	0	0.0		
Percentage of positive cells								
Min.-max.	30.0-90.0		0.0-90.0		30.0-70.0		H=7,462* (0.024)*	p_1=0.185. p_2=0.007* p_3=0.087,
$\bar{X} \pm SD$	68.67±19.95		54.0±35.58		48.33±13.37			
Median (IQR)	70.0 (50.0-90.0)		70.0 (0.0-80.0)		50.0 (40.0-55.0)			
H-score								
Min.-max.	30.0-240.0		0.0-140.0		30.0-100.0		H=11,008* (0.004)*	p_1=0.008* p_2=0.004* p_3=0.387
$\bar{X} \pm SD$	114.3±63.66		64.67±45.69		58.33±19.46			
Median (IQR)	95.0 (50.0-180.0)		75.0 (0.0-90.0)		55.0 (45.0-70.0)			
Dermis								
Status (inflammatory)								
Positive	30	100.0	**12**	**40.0**	**4**	**13.3**	χ^2=47,312* (<0.001)*	p_1<0.001* p_2<0.001* p_3=0.020*
Negative	0	0.0	18	60.0	26	86.7		
Intensity (inflammatory)								
Mild	15	50.0	9	75.0	3	75.0	χ^2=2,742 (^{MC}p=0.670)	$^{MC}p_1$=0.364, $^{MC}p_2$=0.742, $^{FE}p_3$=1,000,
Moderate	12	40.0	3	25.0	1	25.0		
Severe	3	10.0	0	0.0	**0**	**0.0**		
Percentage of positive cells (inflammatory)								
Min.-max.	30.0-90.0		50.0-90.0		50.0-70.0		H=2,385 (0.303)	p_1>0.05, p_2>0.05, p_3>0.05,
$\bar{X} \pm SD$	68.67±19.95		75.0±12.43		60.0±11.55			
Median (IQR)	70.0 (50.0-90.0)		75.0 (70.0-85.0)		60.0 (50.0-70.0)			
H-score (inflammatory)								
Min.-max.	30.0-240.0		60.0-140.0		50.0-100.0		H=1,931 (0.381)	p_1>0.05 p_2>0.05 p_3>0.05
$\bar{X} \pm SD$	114.3±63.66		90.83±25.39		72.50±20.62			
Median (IQR)	95.0 (50.0-180.0)		85.0 (75.0-95.0)		70.0 (60.0-85.0)			

IQR: Interquartile range, SD: Standard deviation, χ^2: Chi-square test, FE: Fisher's exact, MC: Monte Carlo, H: Kruskal-Wallis test. Pairwise contrast between each two groups were performed using the post hoc test (Dunn's for multiple comparisons test); *: $P \leq 0.05$ is the level of significance. p: Comparison between the three studied groups; p_1: Comparison between the lesional and perilesional groups; p_2: Comparison between the lesional and control groups; p_3: Comparison between the perilesional and control

The percentage of FUT8-positive cells in the epidermis of lesional skin and the PASI score of the studied cases showed a statistically significant positive correlation (r=0.512, P = 0.004). Indeed, there was a positive correlation between the H-score of FUT8 in the lesional skin's epidermis and the PASI score of the cases under study (r=0.626, $P < 0.001$). Similarly, a substantially positive correlation was found between the H-score of TGF-β1 in the lesional skin's epidermis and the PASI score (r=0.854, $P < 0.001$) (Figure 5d-f, respectively).

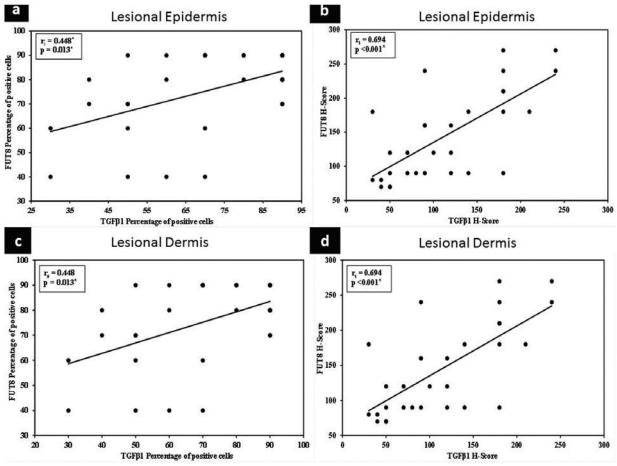

Figure 3. Statistically significant positive correlations between TGF-β1 and FUT8 regarding (a) the percentage of positive cells (r=0.448, P = 0.013) and (b) the H-score in the epidermis of lesional skin (r=0.694, $P < 0.001$). Statistically significant positive correlations between TGF-β1 and FUT8 in the dermis of lesional skin regarding (c) percentage of positive cells (r=0.448, P = 0.013) and (d) H-score (r=0.694, $P <$ 0.001)

TGF-β1: Transforming growth factor-beta, FUT8: Fucosyltransferase 8

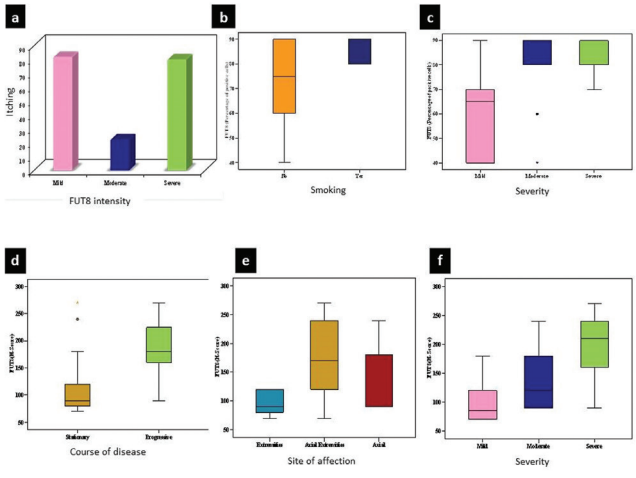

Figure 4. Statistically significant relationship between strong FUT8 expression in the epidermis of lesional skin and (a) presence of itching (χ^2=8,585, P = 0.015). Statistically significant relationship between higher FUT8 percentage of FUT8-positive cells in the epidermis of lesional skin and (b) smoking (U=33.0, P = 0.044) and (c) higher disease severity (H=8,674, P = 0.013). A statistically significant relationship between high FUT8 mean H-score and (d) progressive course of psoriasis (U=46.50, P = 0.008), (e) concomitant axial and extremity affection (H=9,216, P = 0.010) and (f) higher disease severity (H=11,113, P = 0.004)

FUT8: Fucosyltransferase 8

Relationship between FUT8 and TGF-β1 and histopathological data of the studied cases: There were significant relationships regarding the mean H-score of FUT8 lesional skin epidermis and hyperkeratosis (H=7,643, P = 0.022) and there were statistically significant relationships regarding the mean percentage of positive cells of TGF-β1 in epidermis of lesional skin and acanthosis (H=6,453, P = 0.040), hyperkeratosis (H=14,286, P = 0.001), parakeratosis (H=7,473, P = 0.024), and inflammation (H=8,108, P = 0.017) (Figure 6).

Discussion

This study reported that increased expression of FUT8 and TGF-β1 in psoriasis was associated with disease severity and also correlated with each other, suggesting their potential involvement in the development and progression of psoriasis.

Psoriasis is a prolonged, immune-mediated, inflammatory dermatosis. It is a lifelong disorder that has a negative impact on patients' quality of life.[20] The etiology of this condition is multifactorial and includes genetic aberrations, environmental causes, and abnormal immune responses.[3]

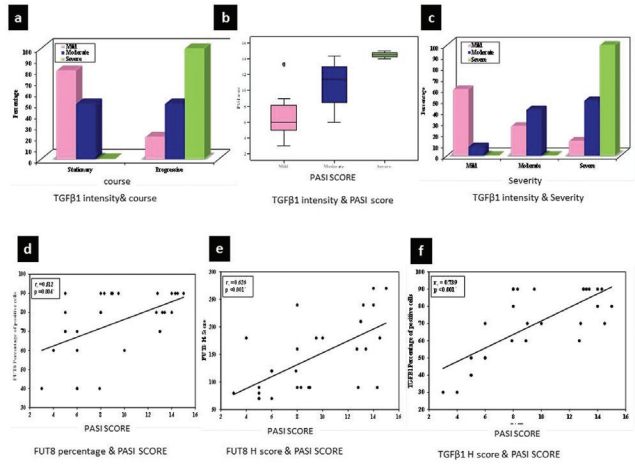

Figure 5. Statistically significant relationship between TGF-β1 strong intensity of expression and (a) progressive course (χ^2=6,941, P = 0.017), (b) PASI score of the studied cases (H=13,710, P = 0.001) and (c) severe disease (χ^2 = 12,285, P = 0.006). Statistically significant positive correlation between the percentage of FUT8-positive cells in the epidermis of lesional skin and the PASI score of the studied cases (r=0.512, P = 0.004) (d). Significant positive correlation between the H-score of FUT8 in the epidermis of lesional skin and the PASI score of the studied cases (r=0.626, $P < 0.001$) (e) and a significant positive correlation between the H-score of TGF-β1 in the epidermis of lesional skin and the PASI score (r=0.854, $P < 0.001$) (f)

TGF-β1: Transforming growth factor-beta, PASI: Psoriasis area and severity index, FUT8: Fucosyltransferase 8

FUT8 upregulation has been observed in several malignancies. The epidermis of psoriatic skin is distinguished by higher expression of FUT8.[21]

TGF-β is a multipotent cytokine responsible for regulating cellular growth and differentiation, maintaining and enhancing the inflammatory response, and producing proinflammatory mediators such as interleukin-17 (IL-17) and IL-22 in psoriasis.[3]

TGF-β1 promotes angiogenesis, vasodilatation, and fibroblast growth, all of which are observed in the early stages of psoriasis. There is compelling evidence that the overexpression of latent TGF-β1 in the epidermis is closely linked to skin inflammation resembling psoriasis.[9]

FUT8 was expressed in 76.7% of normal skin biopsies in the epidermis, and according to reports, FUT8 has a physiological role in healthy skin and significantly affects cellular proliferation and differentiation.[22] In mammals, the fucosylation of glycoconjugates is associated with numerous biological activities, such as blood antigens and cell adhesion.[23]

Comparable findings were demonstrated by Blander et al.,[24] who reported that certain FUT in T lymphocytes in normal skin were markedly elevated. Kelel et al.[21] also reported that in human cells, FUT8 facilitates the transfer of the guanosine diphosphate-L-fucose moiety to the innermost GlcNAc structure of an N-linked glycan via an alpha-1,6 linkage, including skin. Additionally, other studies have demonstrated that FUT8 is widely expressed in mammalian tissues, including skin.[22]

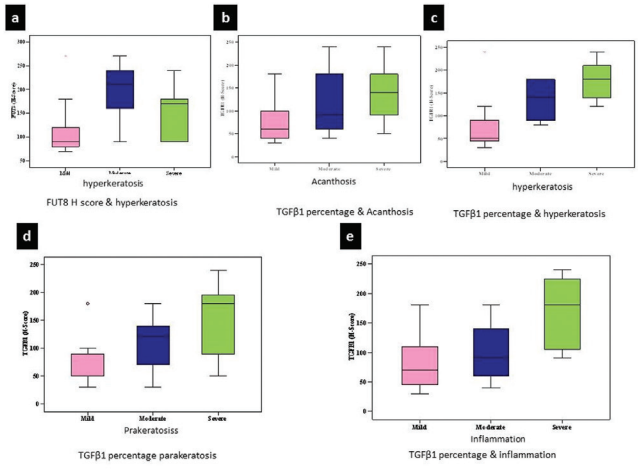

Figure 6. Significant relationship between mean H-score of FUT8 in the epidermis of lesional skin and hyperkeratosis (H=7,643, $P = 0.022$). (a) Statistically significant relationship between mean percentage of positive cells of TGF-β1 in epidermis of lesional skin and acanthosis (H=6,453, $P = 0.040$) (b), hyperkeratosis (H=14,286, $P = 0.001$) (c), parakeratosis (H=7,473, $P = 0.024$) (d), and inflammation (H=8,108, $P = 0.017$) (e)

TGF-β1: Transforming growth factor-beta: FUT8: Fucosyltransferase 8

Upon comparing lesional psoriatic skin (epidermis) to both normal and perilesional epidermis, FUT8 expression was considerably higher, and this overexpression could provide proof of its involvement in the etiology of illness. Similar to these results, Kelel et al.[21] documented the upregulation of FUT8 expression in the epidermis of individuals with psoriasis. Furthermore, the authors reported that the proliferation of cells in the lesional epidermis was linked to FUT8 overexpression. FUT8 expression was detected in the epidermis of all perilesional skin biopsies in the current study and was significantly higher than that in control skin. The evidence indicated that pathological changes in psoriasis were also seen in perilesional skin, as reported by Micali et al.[25] who stated that although the capillaries in lesional skin appear to be more dilated, tortuous, and elongated, their density is comparable to that of uninvolved skin. Slight epidermal hyperplasia, punctiform spongiotic areas with involvement of the stratum basale and around the subpapillary blood vessels, and mild inflammatory reaction with an increase in the number of macrophages, mast cells, and lymphocytes were also observed in the perilesional skin of psoriasis patients.[26]

The findings of this study revealed a significant positive correlation between PASI scores and FUT8 expression in lesional skin (epidermis). Kelel et al.[21] also reported that FUT-8 regulated cyclin expression significantly in cells of the epidermis of patients with psoriasis and was correlated with the severity of the disease in order that the epidermal growth factor receptor signaling pathway upsurges keratinocyte proliferation.

The results of this investigation revealed a statistically significant correlation between hyperkeratosis and FUT8 expression. Similar results were reported by Ito et al.[27] who found that skin with ichthyosis showed altered N-glycan profiles compared with normal skin, and these findings indicated reduced activities of N-acetylglucosaminyltransferase II and FUT8. The biological function of N-glycans in keratinization was proposed based on altered N-glycan structures in hyperkeratotic skin and the fucose-labeled glycoproteins from keratinocytes in psoriasis lesions may be linked to abnormal development of the psoriatic epidermis.[28]

Regarding the dermis of the studied psoriatic cases, FUT8 was also found to be significantly expressed in the dermis of the studied cases, which may be attributed to the role of FUT8 in inflammation. Liu et al.[29] reported that during a diversity of pathological procedures, like a response to inflammation, altered fucosylated structures frequently appeared. Fujii et al.[30] discovered that T-cell-mediated intestinal inflammation was prevented in FUT8-deficient mice, and under colitic conditions, FUT8-/- mice produced fewer inflammatory

cytokines. Additionally, because T-cell antigen receptor-related glycoproteins in the lipid raft fraction did not accumulate as well in FUT8-deficient T-cells, these cells responded to inflammatory stimuli with reduced sensitivity.

The present investigation identified a statistically significant correlation between itchiness and FUT8 expression. Puan et al.[31] reported that FUT6 (another member of the FUT family) deficiency exhibits a reduced itch sensitivity due to basophil affection. Indeed, Zou et al.[28] reported a strong correlation between N-glycan indicators and psoriasis clinical indices.

Moreover, our investigation of the correlation between FUT8 expression in the epidermis and smoking revealed a significant association. This correlation can be elucidated by evidence that supports the pivotal role of smoking in stimulating the generation of reactive oxygen species, leading to inflammation and the development of cancer.[32] As a cytoprotective measure against excessive reactive oxygen species, the body's antioxidant response is believed to enhance the synthesis of FUT8 mRNA, perhaps leading to an increase in core fucose.[33] However, FUT8 activity was reduced in mice exposed to cigarette smoke, indicating that a long smoking duration is key to such changes.[34]

According to this study, 40% of normal skin biopsies (epidermis) exhibited TGF-β1. TGF-β1 was reported to play a physiological role in normal skin. Fibroblasts and epithelial cells are responsible for tissue secretion in a specific, function-, and context-dependent manner.[35] TGF-β1 is involved in skin formation. It promotes the production of extracellular matrix components and/or proliferation in cultures of skin-derived fibroblasts.[36]

Similar findings were demonstrated by Ghosh et al.[37] who stated that TGF-β was present in normal dermal fibroblasts. Differential expression of TGF-β is observed in almost all skin component cells. In the context of a healthy human epidermis, basal cellular layer expression of TGF-β3 predominates; however, TGF-β1 expression is also present. Therefore, it has been proposed that this is both required and constitutive for epithelial homeostasis.[11]

As per the current study, lesional psoriatic skin (epidermis) significantly expressed more TGF-β1 than either normal or perilesional skin (epidermis). This overexpression could provide proof of TGF-β1 involvement in the pathogenesis of psoriasis. Likewise, Li et al.[38] found that in transgenic mice, keratinocyte-targeted overexpression of TGF-β1 in cell type induces pathological changes in which keratinocytes show phenotypical and molecular alterations similar to psoriasis.

Furthermore, cases with psoriasis and normal control subjects had a statistically significant difference in genetic polymorphism of the *TGF-β1* gene at codon 10 with susceptibility to psoriasis in a section of Egyptian cases.[9]

Ahmed et al.[39] established that the *TGF-β1* gene's codon 10 and 25 polymorphisms may enhance a person's vulnerability to psoriasis. However, they do not appear to affect the disease's severity or its serum level. Moreover, Doi et al.[40] reported that the psoriatic epidermis exhibits a significant decrease in TGF receptors, and this reduction in signaling of TGF-β is explained by the TGF-β's role in enhancing epidermal proliferation of keratinocytes as TGF-β1 is a strong inhibitor of keratinocyte growth. Additionally, these results confirm the distinct functions of various TGF-β1 codons, which should be considered in experimental studies of targeted therapy.

Upon studying TGF-β1 expression in perilesional skin, the current research exhibited its expression in 73.3% of perilesional skin biopsies (in the epidermis), and its expression was significantly higher than that in normal skin. Similar to these results, Owczarczyk-Saczonek et al.[41] reported the high expression of TGF-β in perilesional psoriatic patients. This can be explained by the pathological changes detected in perilesional skin, which are also caused by psoriasis.[25]

Regarding the expression of TGF-β1 in dermal inflammatory cells, the expression was statistically significant. Abdou et al.[42] reported similar results and stated that this may be due to the role of TGF-β1 in inflammation.

Regarding the histopathological data of lesional skin, the present study reported a statistically significant relationship between TGF-β1 expression and hyperkeratosis and parakeratosis. Similarly, Sellheyer et al.[43] reported that the skin of mice expressing different TGF-β1 constructs revealed hyperkeratosis. Liarte et al.[11] reported that on intensified TGF-β expression, reactive hyperkeratosis and parakeratosis develop.

Moreover, TGF-β1 and FUT8 levels in the lesional and perilesional skin of the studied psoriatic cases were significantly positively correlated. The association between the TGF-β receptor complex pathway and FUTs was aberrantly expressed in non-small-cell lung cancer, and human peroxisome assembly factor 2 inhibited TGF-β signaling and cell migration.[44]

The biological processes mediated by TGF-β receptors may be significantly impacted by core fucosylation. FUT8 showed higher regulation throughout TGF-β induced epithelial-mesenchymal transition (EMT) in breast cancer cells, and the overexpression of FUT8 changed the core-fucosylated N-glycans on targets on the surface of the cell, namely, TGF-β RI and RII complexes trying to advance binding of ligand and stimulating downstream of signaling.[45]

These processes facilitate the transformation of epithelial phenotypes into mesenchymal ones with enhanced migratory and invasive capability in breast cancer cells, which may result in distal lung metastasis. The transcriptional level of FUT8 may be partially regulated by elevated mRNA levels of FUT8 in TGF-β-induced EMT.[45] Therefore, the collaboration between TGF-β1 and FUT8 could play a role in psoriasis.

Study limitations

This study had a small sample size and was conducted at a single center; thus, further multicentric longitudinal studies are warranted for further assessment of the role of FUT8 and TGF-β1 in psoriasis.

Conclusion

Increased levels of FUT8 and TGF-β1 in psoriasis are directly related to disease severity and to each other, indicating their roles in the development and advancement of psoriasis. Additionally, implementing targeted therapy for FUT8 and TGF-β1 may prove beneficial in the management of psoriasis. The key strengths of this work lie in its uniqueness as one of the few studies that examined the expression of FUT8 and TGF-β1 in psoriasis and compared their levels in lesional and perilesional skin with normal skin.

Ethics

Ethics Committee Approval: This study was approved by the Menoufia University Faculty of Medicine Research Ethics Committee (approval number and date: 8/2021 DERMA 13).

Informed Consent: Every participant signed a written informed consent form before initiating the research work.

Authorship Contributions

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

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Comment on "Association Between Serum Zinc Levels and Multiple Cutaneous Warts: A Cross-Sectional Study"

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Dear Editor,

It is valuable to comment on the article titled "Association between serum zinc levels and multiple cutaneous warts: A cross-sectional study" which is published by Mani et al.[1] in the latest issue of your fabulous journal. Mani et al.[1] assessed in a case-control study the correlation between serum zinc levels (SZL) and multiple cutaneous warts. They found that compared with controls, patients had a significantly higher mean SZL (P = 0.0001). The duration or the number of warts was not significantly correlated with SZL.[1] Due to the following methodological limitations, the study findings have to be questioned. In the study methodology, Mani et al.[1] estimated the SZL was estimated using Sigma-Aldrich Kit (Bangalore), and the normal range of SZL was regarded as 60-180 µg/dL. It is important to mention that serum zinc concentrations are influenced by numerous determinants, such as age, gender, time of venipuncture, fasting status, race, health status, anemia, and serum albumin concentration.[2] The tool employed in Mani et al.'s[1] study was not based on the aforementioned determinants of serum zinc estimation. Hopefully, reliable age and gender reference intervals (RI) for serum zinc were derived based on the US National Health and Nutrition Examination Surveys data. The calculated RI of SZL is 9.5-16.0 µmol/L (62.1-104.6 µg/dL) for children, 9.5-18.0 µmol/L (62.1-117.6 µg/dL) for adult males, and 9.5-16.5 µmol/L (62.1-107.8 µg/dL) for adult females. Following optimum sample collection protocols and assuring analytical precision and accuracy, these RIs can be confidently shifted for routine use in other biochemistry laboratories with accepted analytical achievement in external quality assurance plans.[3] This indicates that these RIs perform accurately, produce comparable and reproducible results, and identify and correct errors to prevent negative outcomes or incorrect diagnoses. In fact, there are notable differences in the SZL between the aforementioned calculated RI of SZL[3] and that used in Mani et al.'s[1] study. To better disclose the association between SZL and cutaneous warts, we believe that referring to the estimated RI of SZL[3] in the study methodology is a sound option.

Ethics

Financial Disclosure: The author declared that this study received no financial support.

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Punch Grafting Technique for the Treatment of Chronic Venous Leg Ulcers

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Dear Editor,

Venous leg ulcers are the most common cause of chronic wounds in the lower extremities.[1] Venous insufficiency and increased venous pressure are the main contributors to ulcer development.[2] The primary treatment step is restoration of venous function, along with general wound care principles. Surgical interventions, such as skin grafting, can be used to promote wound healing in selected cases.[3] In this letter, we describe a patient treated with the punch grafting technique, a simple procedure that requires little experience and can be performed using basic instruments available in a dermatologic surgery room.

A 63-year-old male patient presented with a six-month history of an oozing, painful, non-healing ulcer with irregular edges and purulent base (Figure 1A, B). The patient had previously used various topical treatments but without any benefit. Pretibial edema, varicose veins, and eczematous dermatitis around the surrounding skin were also noted. Doppler ultrasonography confirmed venous insufficiency. Compression therapy, together with intravenous antibiotics, topical corticosteroids, oral pentoxifylline, and oral diosmin/hesperidin, were initiated. Intermittent mechanical debridement was performed as necessary. In the 2nd week of follow-up, the purulence regressed and the ulcer became vividly red. At this stage, skin grafting was decided to improve recovery. Before the procedure, written informed consent was obtained from the patient.

The donor site was selected from the lumbosacral area, and infiltration anesthesia was administered. After anesthesia, approximately thirty incisions were made using a 4 mm punch instrument (Figure 2A). The incisions were superficial enough not to reach the subcutaneous fat. Skin grafts were harvested using forceps and a #15 blade and were collected on gauze soaked in saline (Figure 2B, C). The donor area was then covered with antibiotic ointment and left for secondary healing. The collected grafts were transplanted onto the wound base at 1 cm intervals (Figure 3A). The wound was then covered with petroleum-soaked gauze, and a compression bandage was applied (Figure 3B, C). The patient was instructed to rest and elevate his legs. The first dressing change was done on the 5th day, and it was observed that most of the punch grafts were

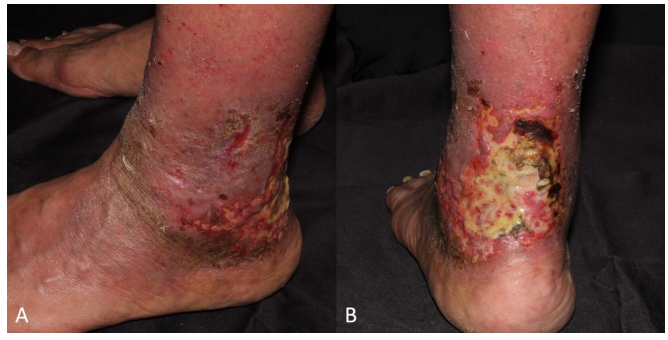

Figure 1. Clinical appearance of the ulcer at the time of admission. Lateral (A) and posterior (B) aspects of the left ankle

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attached to the wound base (Figure 4A). Ten days after the procedure, the same procedure was repeated for the rest of the wound (Figure 4B). Most grafts survived except those located on the mobile ankle crease (Figure 4C). Compression therapy was continued, and the ulcer healed within 1 month without the necessity of any additional procedures (Figure 4D). No complications occurred in the donor area.

The implantation of small pieces of skin into chronic wounds to accelerate healing was first described by Reverdin in the late 19th century.[4] Reverdin's original technique involves pinching a piece of skin and then removing it superficially using a surgical blade. Because the grafts obtained in this way have different shapes and thicknesses, they may cause cobblestoneing when healed. Using a punch tool for harvesting produces grafts of uniform shape and thickness. However, if grafts are taken full thickness, including adipose tissue, it is necessary to drill holes in the recipient site for graft survival, which makes the operation more complicated.[5] These difficulties can be overcome by harvesting grafts superficially, as in pinch grafting, after punch incisions are made in the donor area. In this way, many grafts of similar shape and thickness can be quickly taken and placed directly on the wound. As a simple and practical technique, punch grafting can help shorten healing times and reduce pain in patients with leg ulcers.

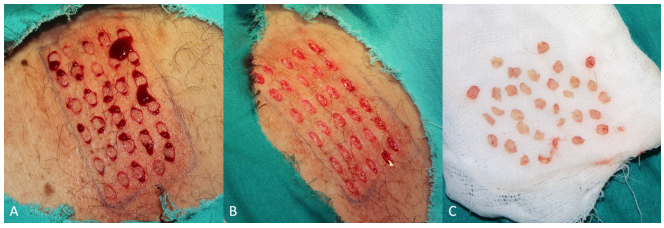

Figure 2. Harvest the punch grafts. Incisions were made a few mm apart using a 4 mm punch tool (A), and the grafts were removed superficially using a #15 blade, note that adipose tissue is not visible (B). Punch grafts with uniform shape and thickness were collected on saline-soaked gauze (C)

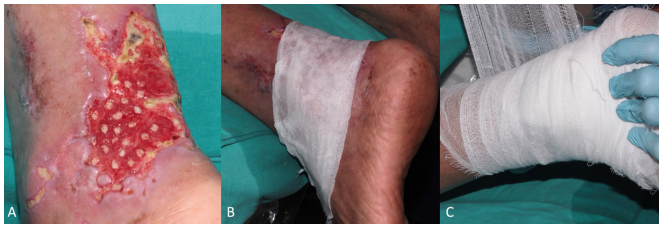

Figure 3. Punch grafts were placed directly on the wound base at approximately 1 cm intervals, ensuring that the dermal sites were in contact with the wound surface (A). The wound was covered with petrolatum-soaked gauze (B), and compression bandages were then applied (C)

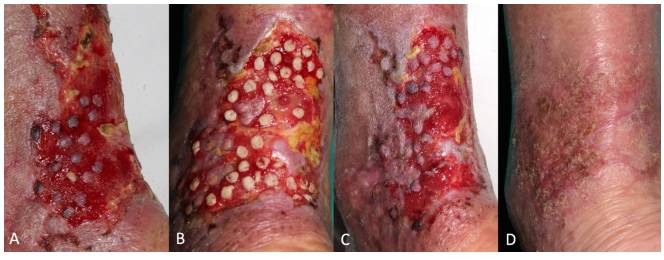

Figure 4. Follow-up of the patient. On the 5th day of the first procedure, most of the implanted grafts survived (A). The second punch grafting procedure was performed 10 days after the first operation (B). Grafts located on the lower parts of the wound were eliminated due to the mobility of the area, but the remaining grafts survived (C). The ulcer completely healed within one month without the need for a third operation (D)

Ethics

Informed Consent: Written informed consent was obtained from the patient.

Authorship Contributions

Surgical and Medical Practices: O.E., A.S.Ş., F.A., G.A., V.A.E., Concept: O.E., A.S.Ş., Design: O.E., Data Collection or Processing: O.E., A.S.Ş., Analysis or Interpretation: O.E., Literature Search: O.E., A.S.Ş., Writing: O.E., A.S.Ş., F.A., G.A., V.A.E.

Conflict of Interest: The authors declared that they have no conflict of interest.

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Flare-Up Phenomenon Triggered by Patch Testing of Topical Ointments Containing Nitrofurazone and Polyethylene Glycol

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Dear Editor,

Nitrofurazone-containing ointments are commonly prescribed as topical agents treatment of skin-related diseases by non-dermatology specialties, particularly surgical departments. However, the active ingredient nitrofurazone and the vehicle polyethylene glycol (PEG) are significant contact sensitizers that can result in allergic contact dermatitis.[1] Herein, we report a patient who had an allergic contact dermatitis due to nitrofurazone-containing ointment and a flare-up reaction after patch testing.

A 51-year-old male presented with erythema, edema, and yellow crusts on the left cheek as well as erythematous patches with multiple tiny pustules on the left neck to the back (Figure 1a, b). About 10 days before presentation, he had a soft tissue infection on his cheek and was administered oral amoxicillin-clavulanic acid and topical nitrofurazone-containing ointment. Nitrofurazone was the active ingredient in the topical ointment used by the patient, and the vehicles were PEG 300, PEG 1000, and PEG 4000. The patient did not have fever or lymphadenopathy. Laboratory tests were within normal limits, including the total blood count with differentials, erythrocyte sedimentation rate, and C-reactive protein level. Microbial cultures did not contain pathogenic microorganisms. Histopathological examination of the inflamed neck area revealed predominantly perivascular and interstitial infiltration of lymphocytes and eosinophils in the upper and mid dermis. The patient was diagnosed with allergic contact dermatitis caused by nitrofurazone-containing ointment. All lesions were cleared with systemic prednisolone at a dose of 0.8 mg/kg/day along with topical 0.05% clobetasol propionate cream within 6 days.

One month after ceasing treatment, the patient underwent patch testing using the TRUE test and a topical commercial ointment containing nitrofurazone and PEG, which are considered the causative agents of allergic contact dermatitis. Patch tests were evaluated according to the morphological criteria recommended by the International Contact Dermatitis Research Group.[2] The TRUE test was negative at 48th and 72th hours. However, the nitrofurazone-containing ointment (commercial product, applied directly) produced a weak positive reaction, 25% ointment (mixed with white petrolatum) produced a questionable reaction, and petrolatum (control area) produced no reaction (Figure 2). Additionally, itchy, irregularly bordered, erythematous, mildly edematous patches emerged on the neck, nape, and shoulders where

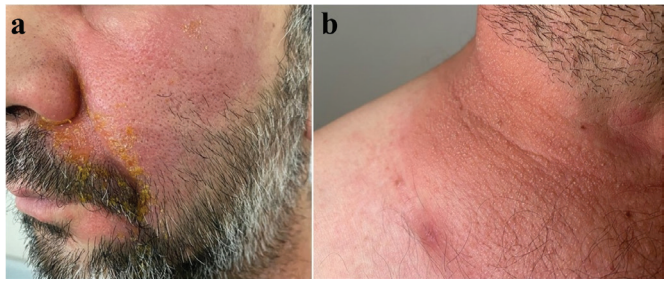

Figure 1. (a) Images showing erythema, edema, and yellow crust on the left cheek. (b) Erythematous patch with multiple tiny pustules on the left side of the neck

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previous allergic contact dermatitis had been observed (Figure 3a, b). The reaction was considered a flare-up phenomenon triggered by the skin patch test carried out with nitrofurazone-containing ointment. Within a few days, the response subsided with treatment with topical corticosteroids and systemic antihistamine.

A strong sensitizer, nitrofurazone, also known as nitrofural (5-nitro-2-furaldehyde semicarbazone), frequently causes severe allergic contact dermatitis in patients suffering from burns, stasis eczema, or other chronic dermatitis. Topical nitrofurazone sensitivity rates ranged from 3.3% to 36.2%.[1,3,4] Özkaya and Kılıç[1] reported reactions to PEG in 42% of 836 patients who underwent patch testing, and in 80% of these patients, nitrofurazone sensitivity was also detected. The strong correlation between nitrofurazone sensitivity and PEG sensitivity points out that PEG enhances the penetration of nitrofurazone, especially in regions of disrupted skin barrier.

The flare-up phenomenon refers to a drug-induced acute inflammatory skin reaction that emerges in areas of the skin where dermatitis has previously taken.[5,6] Twelve hours after exposure to contact allergens, skin-resident CD8-positive tissue resident memory cells trigger a sudden and strong neutrophil infiltration in the epidermis, resulting in a flare-up reaction.[6] Although nitrofurazone-containing topical are widely used, no flare-up phenomena have been reported in the literature after patch testing with nitrofurazone or PEG. The diagnosis of allergic contact dermatitis due to nitrofurazone-containing ointments is quite simple by taking a detailed medical history from a patient living in countries where these agents are prescribed frequently. Therefore, patch testing is not typically required. Commercially available tests lack nitrofurazone and PEG, so physicians should prepare test samples using these agents in the test area. In summary, if the frequency of patch testing is low, the flare-up phenomenon will be less frequent. Detailed dermatological examination of the whole body may be needed to notice a flare-up phenomenon to observe subtle yet newly developed phenomena that are not yet symptomatic. Mild reactions can be easily underdiagnosed and unreported.

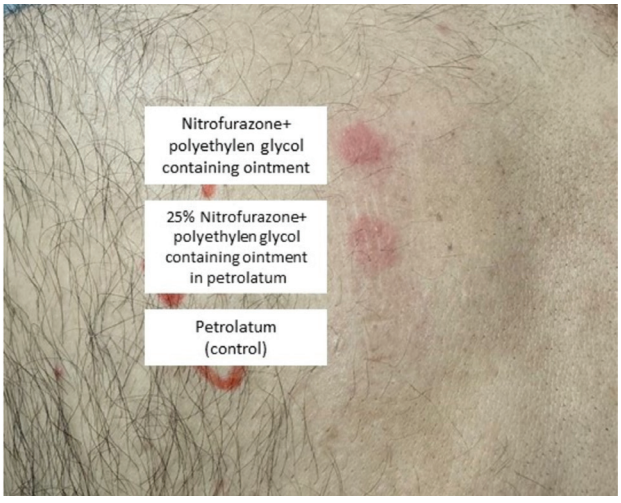

Figure 2. Patch test results at 48th hour: weak positive reaction with nitrofuzarone and polyethylene glycol ointment, questionable reaction with 25% ointment in petrolatum, and negative reaction with petrolatum

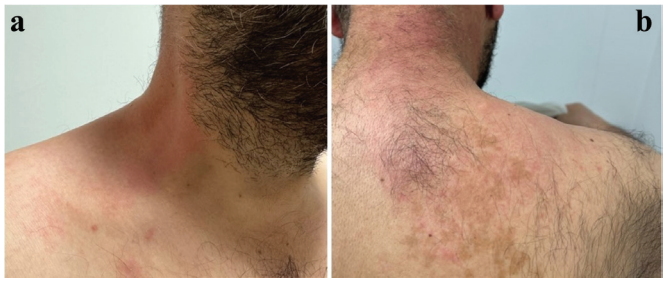

Figure 3. (a) Flare-up phenomenon on the left side of the neck at the 48th hour of the skin patch test. (b) Flare-up phenomenon on the nape and back during the 48th hour of the skin patch test

The limitation of our case report was the lack of pure test samples, including only nitrofurazone and PEG products, due to unavailability. Testing had to be performed using the commercial ointment that the patient had used. Consequently, we could not determine whether nitrofurazone or PEG is responsible for dermatitis. Therefore, the patient was advised not to use medications and cosmetics, including either agents along with PEG-containing foods.

In conclusion, nitrofurazone and PEG may induce allergic contact dermatitis, and patch testing including these agents may cause flare-up phenomenon in skin areas where dermatitis has previously existed.

Ethics

Informed Consent: Informed consent to publish their photographs and related clinical information was obtained from the patient. The patient signed a consent form allowing the use of their images and details for scientific and educational purposes and ensuring anonymity.

Authorship Contributions

Surgical and Medical Practices: G.A., B.Y.İ., Concept: G.A., F.D., F.K., Design: G.A., Data Collection or Processing: B.Y.İ., F.K., Analysis or Interpretation: G.A., B.Y.İ., F.D., F.K., Literature Search: G.A., B.Y.İ., F.D., F.K., Writing: G.A., B.Y.İ.

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