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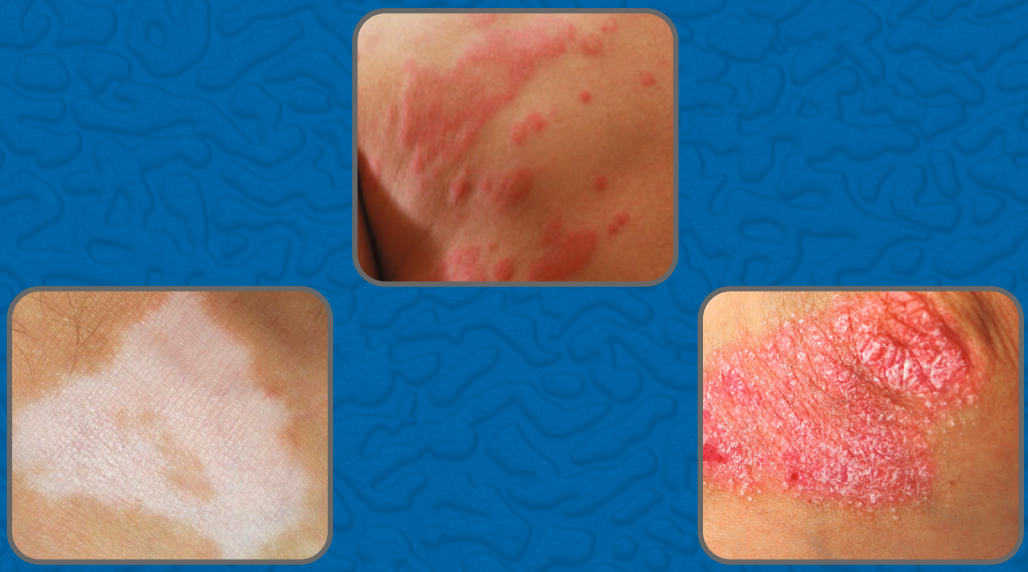

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Review Article

Propionibacterium acnes (*Cutibacterium acnes*) and Acne Vulgaris: The Latest Updates of Antimicrobial Activity

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Abstract

Propionibacterium acnes is commonly recognized for its acne pathogenesis. *P. acnes* produces chemotactic substances and activates the complement system. Resistant *P. acnes* strains were explained more than 40 years ago. For that reason, new antimicrobial agents for the topical treatment of skin infections have been researched, and it has been determined that plant extracts may be an alternative treatment for acne. In this review, antimicrobial studies of *P. acnes* have been reviewed.

Keywords: Acne, antimicrobial activity, *Propionibacterium acnes*

Introduction

The microbial community is mostly formed of bacteria, which include *Corynebacteria, Propionibacterium* and *Staphylocooci*.[1] *P.acnes* is a gram-positive bacteria and the anaerobic form exists on the surface of the human skin.[2] *P.acnes* colonises the sebaceous glands and the hair follicles of the human skin.[3] If the *Propionibacterium acnes* (*P.acnes*) becomes predominant in the sebaceous region, this prevents the colonisation of other harmful microorganisms.[4,5] Also, it can play an important role in acne vulgaris.[6] The pathogenesis of acne vulgaris is based on multiple factors, such as increased sebum production, *P. acnes* proliferation and inflammation.[7]

The main groups of therapeutic drugs are topical and systemic retinoids, antimicrobial agents, and systemic hormonal drugs.[8] Clindamycin, tetracyclines, erythromycin, metronidazole, nadifloxacin, and dapsone are used for anti-*Propionibacterium acnes* therapy.[9] A significant problem in the treatment is bacterial resistance. Currently, new retinoids are being used with antibiotics to decrease the risk of bacterial resistance.[7] Phytotherapy may be an alternative for acne treatment due to its low side effects, usage in local areas, and low costs.[10]

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New Data on *Propionibacterium acnes* Taxonomy

P. acnes was first isolated from patients with chronic skin diseases called "acne vulgaris."[11] The genus *Propionibacterium,* which was described by Orla-Jensen, belongs to the phylum of Actinobacteria and to the Propionibacteriales group.[12-14] The cutaneous group consists of *P. acnes*, *Propionibacterium avidum,* and *Propionibacterium granulosum*.[12]

High-resolution core genome studies have reported the new genus of *Cutibacterium* gen. nov. These specific genes were indicated in these cutaneous species; however, others disappeared by deletions of cutaneous *Propionibacterium* on the human skin. As a result of the 16S rRNA gene sequences, DNA G + C content, genome size, and gene content, *P. acnes* was renamed as *Cutibacterium acnes*.[15] *C. acnes* is predominant in the microbiota of pilosebaceous follicles of acne patients as opposed to unaffected skin.[16] As a result of genomic analysis, cutaneous *Propionibacterium* has now been changed to the new bacterial genus *Cutibacterium*. The names used for bacteria species are *C. acnes*, *Cutibacterium avidum*,

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Cutibacterium granulosum, *Cutibacterium namnetense,* and *Cutibacterium humerusii*.[17]

Cutibacterium acnes Feedback to Antibiotics

Systemic and topical antibiotics have been used for acne treatments.[18] The use of antibiotics may induce the spread of antibiotic resistance.[19]

Propionibacterium species are intrinsically resistant to metronidazole, tinidazole and ornidazole, aminoglycosides, sulfonamides, and mupirocin. On the other hand, *C. acnes* is susceptible to many antimicrobials. However, studies have reported that *C. acnes* has high rates of resistance to erythromycin and clindamycin.[18,20,21]

Bacterial biofilms also play an important role in antibiotic resistance and decrease the susceptibility to antibiotherapy.[16] The ability of biofilm formation of *C. acnes* was reported in 2007.[22] Studies have indicated that the development of *C. acnes* biofilms was higher in patients with acne than normal patients.[23]

In vitro Antimicrobial Effects of Natural Materials against *Propionibacterium acnes*

Resistant *P. acnes* strains were explained more than 40 years ago.[19] For that reason, new antimicrobial agents for the topical treatment of skin infections were researched, and it was found that plant extracts may be an alternative treatment for acne.[24] Weber *et al*. reported that hop extract has a high antimicrobial activity against *P. acnes* (minimum inhibitory concentration [MIC] of 3.1 μg/mL).[25] Furthermore, studies indicate that herbal ball extract with Kalmegh, rosmarinic acid, *Centella asiatica* extract, and *Rosa damascena* methanolic extract had antimicrobial activity against *P. acnes*.[8,10,24,26] It has been shown that *Boswellia serrata* extract is effective at low concentrations against *P. acnes* (MIC: 1 μg/mL).[27] Only a limited number of studies have studied the anti-*P. acnes* activities of herbal tea extracts. In terms of antimicrobial activity against *P. acnes,* duzhong extract showed the highest level, yerba mate extract showed a moderate level, and rose extract showed the least (Tsai *et al*., 2010). Eilami *et al*. found that hydroxytyrosol has an antibacterial effect against *P. acnes*.[28] *Angelica anomala* demonstrated effective activity against *P. acnes*.[29] Yamaguchi *et al*. reported that *Humulus lupulus*, which contains xanthohumol and lupulones, showed very effective inhibitory activity against *P. acnes*.[30]

Conclusion

Recently, cosmeceuticals and nutraceuticals are areas that are significantly increasing in popularity. The development of new botanical extracts and compounds against *P. acnes* has considerable potential.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Comparison of Serum Dehydroepiandrosterone Sulfate, Testosterone, and Dihydrotestosterone Levels in Males with Various Degrees of Acne Vulgaris Severity

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Abstract

Objective: This study compared the levels of dehydroepiandrosterone (DHEA) sulfate, testosterone, and dihydrotestosterone (DHT) in the serum of men with various degrees of severity grading of acne vulgaris. **Methods:** We conducted a cross-sectional analytic observational study and used the Combined Acne Severity Classification. Serum DHEA sulfate (DHEAS), testosterone, and DHT levels were measured by enzyme-linked immunosorbent assay. We recruited 63 males with acne vulgaris. **Results:** For mild, moderate, or severe acne, the mean serum level of DHEAS was 90.92, 153.54, and 166.67 ng/ml ($P = 0.000$); testosterone was 6.66, 8.11, and 8.97 ng/ml ($P = 0.445$); and DHT was 87.33, 111.72, and 124.71 ($P = 0.01$), respectively. *Post hoc* analysis showed significant differences for DHEAS and DHT serum levels. There were significant differences for DHEAS and DHT serum levels. **Conclusion:** There was no significant difference in serum testosterone levels between groups, although there was an increase in concentration by acne vulgaris severity.

Keywords: Acne vulgaris, degree of severity, dehydroepiandrosterone sulfate, dihydrotestosterone, testosterone

Introduction

Acne vulgaris is a chronic inflammatory skin condition that primarily occurs in the pilosebaceous unit, characterized by the appearance of comedones, papules, pustules, nodules, and cysts.[1,2] It is the most common skin problem of young people aged 12–24 years.[1,3,4] While the onset of acne vulgaris in women is faster than in men, the severity is higher in men than in women. The main reason for this phenomenon is probably due to higher levels of sebum and androgen hormones in males.[5-8] The prevalence of acne vulgaris, based on the previous study held in Palembang, Indonesia, was reported to be about 68.2%.[5] A hospital study in India obtained 309 acne vulgaris patients from 28,197 new patients who attended a dermatology outpatient unit between August 2006 and June 2008.[6] Further, the prevalence of acne vulgaris in a cross-sectional study in Yazd, Iran, was 85.9%.[8]

The pathogenesis of acne vulgaris is comprised of increased production of sebum, follicular hyperkeratinization, *Propionibacterium acnes* proliferation, and inflammation.[8,9] Two of these four factors, increased production of sebum and follicular hyperkeratinization, are highly correlated with androgen hormone stimulation.[7,10] Androgen hormones consist of the inactive precursor, such as dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androsterone. Testosterone and dihydrotestosterone (DHT) are the two most potent androgen hormones.[1,7,10] Evidence has shown a correlation between DHEAS and DHT levels with the number of acne vulgaris lesions in adult women.[11]

The determination of the severity level of acne vulgaris is varied and is based on the number and type of lesions; however, no single assessment criteria have ever been deemed the gold

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standard. The Combined Acne Severity Classification (CASC), one of the criteria used to determine the severity level of acne vulgaris, divides acne vulgaris into three levels: mild, moderate, and severe.[12,13]

Androgen hormones affect skin appendages, such as a sebaceous gland, that are involved in acne vulgaris pathogenesis and which appear to be dependent on biologically active androgens. DHEAS is considered the most important regulator of sebum secretion. Sebocytes will convert DHEAS into a more potent androgen, such as testosterone and DHT. The purpose of this study was to investigate which androgen hormones effect on acne vulgaris pathogenesis. The result of this study will provide an important contribution in which androgen hormones will be modified to affect acne vulgaris progression. The study aimed to compare the levels of DHEAS, testosterone, and DHT in serum from males with various degrees of severity grading of acne vulgaris.

Methods

Study design and subjects

This cross-sectional observational study involved males, aged 13–30 years, with various degrees of acne vulgaris severity, who were divided into mild, moderate, and severe groups. Each participant signed informed consent for medical treatment and any study approach. This study was approved by the Ethical Committee of Health Study of Regional General Hospital and Dr. Saiful Anwar Malang as written in the letter of ethical approval no. 400/97/K.3/302/2015, no. 400/66/K.3/302/2015, and no. 400/94/K.3/302/2015. Sample size calculation used the Lemeshow formula (n = [Zα2 p (1-p)]/d2) to determine a minimum sample size of 19 males for each severity group.

The exclusion criteria consisted of receiving topical therapy, such as antibiotics, benzoyl peroxide, tretinoin, adapalene, and other keratolytics (salicylic acid and sulfur). The topical treatment was given within 2 weeks. Subject with treatments that affect the activity of androgen hormone and pathogenesis of acne vulgaris, such as an oral retinoid, systemic antibiotic, spironolactone, corticosteroid and finasteride, and acneiform eruption-related drugs such as lithium, halogen, isoniazid, phenytoin, Vitamin B within 1 month before study must be excluded. Individuals were also excluded if their body mass index was >25.

Classification of acne vulgaris severity level

The severity level assessment was based on the CASC method and conducted by three examiners, in a subsequent order, on the same day. CASC divides acne vulgaris into three levels: mild, moderate, and severe. Criteria for mild acne were a Comedones count of <20, inflammatory lesion count of <15, or a total lesion count of <30. Criteria for moderate acne were a comedone count of 20–100, an inflammatory lesion count of 15–50, or a total lesion count between 30 and 125. Criteria for severe acne were a cyst count of >5, a comedone count of >100, an inflammatory lesion count of >50, or a total lesion count of >125.[12,13]

Hormone examination

Hormone concentration was measured from blood samples. The evaluations of serum DHEAS, testosterone, and DHT levels were conducted using an enzyme-linked immunosorbent assay (ELISA). Five milliliters of blood was taken from the middle cubital vein using Venoject and then put into a nonadditive Vacutainer and allowed to thicken. The blood was centrifuged, and the serum was collected and stored at −10°C. An ELISA was done following the accomplishment of all study participants. An Elabscience ELISA kit was used to measure serum DHEAS and DHT levels, and a Cusabio ELISA kit was used to measure serum testosterone levels. Hormone levels were obtained from the measurement of optical density at a 450-nm wavelength using a microplate reader.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 22, (IBM, 1 New Orchard Road Armonk, New York 10504-1722 United States). The Kolmogorov–Smirnov test was used to evaluate the normality of data distribution. Homogeneity data evaluation was performed using Levene's test. One-way ANOVA was performed to detect the differences of the mean DHEAS, testosterone, and DHT levels between each acne severity level in normally distributed data. *Post hoc* analysis was also done if there was a significant difference in the mean difference test.

Results

This study involved 63 men with acne vulgaris. This study evidenced a significant difference of average age in various severity levels of acne vulgaris [Table 1].

The average age of patients with mild acne vulgaris was 22.71 years, moderate acne vulgaris was 23.29 years, and severe acne vulgaris was 19.24 years. The Kolmogorov–Smirnov test showed a standard distribution of serum DHEAS testosterone and DHT levels ($P > 0.05$). Levene's test showed homogeneity variation in data for serum DHEAS, testosterone, and DHT levels ($P > 0.05$) [Table 1]. This study fulfilled all the criteria for one-way ANOVA.

Table 1: Age characteristics, serum hormone mean distribution, and variation

Characteristics	Categories	Value	*P*
Average age (years)	Mild acne vulgaris	22.71	0.000
	Moderate acne vulgaris	23.29	
	Severe acne vulgaris	19.24	
Kolmogorov-Smirnov test	DHEAS	1.140	0.149
	Testosterone	1.145	0.100
	DHT	1.034	0.235
Levene's test	DHEAS	2.827	0.067
	Testosterone	0.002	0.998
	DHT	2.839	0.091

DHEAS: Dehydroepiandrosterone sulfate, DHT: Dihydrotestosterone

Hormone level comparison for various severity levels of acne vulgaris

The average level of serum DHEAS, testosterone, and DHT was elevated in concordance with an increased severity level of acne vulgaris. The one-way ANOVA test showed a significant difference between serum DHEAS and DHT in various severity levels of acne vulgaris ($P < 0.05$). There was no significant difference in testosterone levels between each severity level of acne vulgaris [Table 2].

Post hoc analysis

Post hoc analysis displayed significant differences in serum DHEAS and DHT mean levels between mild and moderate acne vulgaris and between mild and severe acne vulgaris ($P < 0.05$). *Post hoc* testing did not demonstrate significant differences in the mean level of serum DHEAS and DHT between moderate acne vulgaris and severe acne vulgaris [Table 3].

Discussion

Acne vulgaris is a common skin disorder that affects teenagers and young adults. Epidemiological data show that 80% of acne vulgaris patients fall within the range of 11–30 years of age.[14] A descriptive study from the United States of America showed that the average age of acne vulgaris was 25 years.[15] On the other hand, a study in Hong Kong evidenced the age range for acne vulgaris as 15–25 years.[16] The average age of participants in this study [Table 1] was at the second decades and similar to previous studies.

Table 2: Comparison of serum dehydroepiandrosterone sulfate, testosterone, and dihydrotestosterone levels (mean±standard deviation)

Variables	Severity level of AV			*P*
	Mild	Moderate	Severe	
DHEAS	90.927±36.128	153.546±66.775	166.376±52.826	0.000
Testosterone	6.66±5.28	8.11±6.19	8.97±6.19	0.445
DHT	87.33±19.5	111.72±35.98	124.71±37.19	0.001

DHEAS: Dehydroepiandrosterone sulfate, DHT: Dihydrotestosterone, AV: Acne vulgaris

Table 3: *Post hoc* analysis of serum dehydroepiandrosterone sulfate and dihydrotestosterone mean comparison

Variables	Severity level	Mean	*P*
DHEAS	Mild	90.927±36.128	0.001
	Moderate	153.546±66.775	
	Mild	90.927±36.128	0.000
	Severe	166.376±52.826	
	Moderate	153.546±66.775	0.718
	Severe	166.376±52.826	
DHT	Mild	87.33±19.5	0.042
	Moderate	111.72±35.98	
	Mild	87.33±19.5	0.001
	Severe	124.71±37.19	
	Moderate	111.72±35.98	0.391
	Severe	124.71±37.19	

DHEAS: Dehydroepiandrosterone sulfate, DHT: Dihydrotestosterone

Severe acne vulgaris tends to occur in younger individuals. Acne vulgaris lesions are commonly said to act as predictors of the onset of puberty and are inclined to become more severe at a younger age than older.[6,7,15] Some studies showed that acne vulgaris would eventually regress in the patients' mid-20s or 30s and few will get acne vulgaris in their 40s.[6,15] Teenagers endure hormonal changes related to puberty and gonads; thus, they have increased production and secretion of androgen hormones.[17,18] Androgen hormones, such as DHEAS, testosterone, and DHT, are all known to be involved in gene arrangement, especially in genes responsible for the development of sebaceous glands and sebum production.[19,20] A prevalence study of acne vulgaris in Iran revealed that moderate and severe acne were more frequent in males than in females, although total prevalence was more frequent in females.[8] Estrogen has a protective effect against acne vulgaris in females. The effect of estrogen in acne vulgaris occurs through several mechanisms, such as a direct opposition effect on androgens, inhibition of androgen secretion, or modulating genes involved in sebaceous gland growth and function.[19,20]

The mechanism for increasing androgen hormone levels that result in the enlargement and overstimulation of sebaceous glands is still unknown.[17,19] As a precursor, DHEAS hormone will be altered into testosterone and then DHT, which subsequently binds the androgen receptor in sebocytes, follicular and epidermal keratinocytes, sweat glands, and probably, dermal papillary cells.[19,21] The androgen receptor is a nuclear receptor with a transcriptional propensity to reach its biologic effect.[21] DHT-androgen receptor binding interacts with deoxyribonucleic acid and arranges the genes involved in sebaceous cell proliferation and lipogenesis.[19] Androgen hormones play a critical role in follicular hyperkeratinization and affect sebum production of sebaceous glands.[17,20] The process above will result in an oversecretion of sebum, accompanied by cell accumulation on the skin's surface, thus blocking sebaceous gland estuaries. Clogged sebaceous gland estuaries will lead to dilation of the upper part of hair follicles and finally the formation of microcomedones.[7,17] These microcomedones will get bigger and, in addition to the increased proliferation of *Propionibacterium acnes*, causes the rupture of the follicular wall. It releases sebum, keratin, and bacteria into the dermis and stimulates an inflammatory response.[7,17]

The DHEAS hormone is a weak androgen precursor. Sebocytes and a small population of dermal papillary cells have the enzymatic capacity to transform DHEAS into another androgen with higher potency.[21] DHEAS hormone is majorly synthesized in the adrenal glands and reaches the skin through the blood vessels. This hormone is the most detected androgen in circulation, with relatively constant levels detected in both genders.[10,17,21,22] A study in Iraq successfully measured serum

DHEAS levels using the ELISA method in males with different levels of acne vulgaris severity. This study showed that the average level of serum DHEAS was significantly higher in the severe acne vulgaris group (4.05 ± 0.96 μg/ml) compared to the control (2.90 ± 0.27 μg/ml), mild (2.38 ± 0.46 μg/ml), and moderate (2.73 ± 0.63 μg/ml) groups.[23] Our study indicated that mean DHEAS levels increased by increasing acne vulgaris severity grade [Table 2]. There was a significant difference in mean serum DHEAS levels when comparing mild versus moderate acne vulgaris, as well as between mild and severe acne vulgaris [Table 3].

DHT is the most potent androgen-inducing keratinocyte hyperproliferation. The level of DHT hormone is lower than testosterone in both tissues and circulation. DHT binds androgen receptors with a higher affinity than testosterone. The bond between DHT and androgen receptors is more stable and thus more effective at increasing sebum production.[24] The elevation of DHT levels in infundibular keratinocytes leads to follicular hyperkeratinization.[7,25] The previous study showed a correlation coefficient of 0.75 with a significance level of 0.001 DHT level on female acne vulgaris group with either inflammatory or noninflammatory acne vulgaris lesion count.[11] Our study showed an increase in serum DHT levels that correlated with increasing acne vulgaris severity grade [Table 2]. There was a significant difference in mean serum DHT levels between mild and moderate acne vulgaris and between mild and severe acne vulgaris [Table 3].

The testosterone hormone induces the enlargement and secretion of sebaceous glands through binding with androgen receptors.[7,26] Testosterone also increases the proliferation of follicular keratinocytes.[3,7] Follicular hyperkeratinization causes pilosebaceous canal clogging and eventually development of microcomedones as an early lesion of acne vulgaris.[3,7,27] Our study showed no significant differences in mean serum testosterone levels between the three acne vulgaris severity groups, although increasing testosterone levels did trend with increasing the acne vulgaris severity grade [Table 2]. Blood testosterone concentrations are affected by various factors, which were not excluded from this study. Testosterone is often measured from human blood (serum), though some circulating testosterone fractions will be bound to albumin.[28,29] Blood testosterone concentrations are influenced by sleep duration and nicotine use.[29] Testosterone has low diurnal variation in Asian individuals.[30] In 2011, Ewadh demonstrated a significant difference in testosterone levels when comparing males with or without acne vulgaris.[17] A previous study by Miranti also showed that serum testosterone levels in females with severe acne vulgaris were higher than in those with mild acne vulgaris, though some of the results were not statistically significant.[31]

Conclusion

This study displayed significant differences of serum DHEAS and DHT levels between mild and moderate acne vulgaris, as well as between mild and severe acne vulgaris. Serum testosterone levels, though not significantly different, became elevated as the severity level of acne vulgaris increased. Therefore, our suggestion for future studies would be to investigate the correlation between each serum androgen concentration and counts of comedones, erythematous papules, pustules, or cyst nodule lesions.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Comparison of Serum Cyclooxygenase-2 Level between Melasma and Nonmelasma Patients in Dr. Saiful Anwar General Hospital, Malang, Indonesia

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Abstract

Background: Melasma is the hypermelanosis skin disease on the sun exposed area. Ultraviolet exposure leads to inflammation in the epidermis and dermis, one of which is marked by an increase in Cyclooxygenase-2 (COX-2). COX-2 expression involves in the production of prostaglandin-E2 (PGE2) that take part in tyrosinase activation and melanogenesis. **Aims and Objectives:** This study aimed to determine the differences in serum COX-2 levels in melasma and non-melasma patients in Dr. Saiful Anwar General Hospital Malang, Indonesia. **Materials and Methods:** A cross-sectional study using continuous sampling in melasma and non-melasma patients at the Dermatology and Venereology out-patient clinic from November to December 2017. The COX-2 serum levels examined by ELISA method. **Results:** From the 23 melasma and 23 non-melasma subjects, the mean value of serum COX-2 levels in the melasma and nonmelasma groups was not significantly different ($P > 0.05$) with value of 82.23±61.08U/L and 52.66±28.62 U/L, respectively. Those might be influenced by the other unknown variables who were not included in this study. Based on Melasma Severity Score (MSS), serum COX-2 levels differed significantly in moderate severity (49.55±14.26 U/L) and severe (112.1±72.32 U/L) ($P > 0.05$) might related to the capacity of the enzyme that induces epidermal hyperpigmentation. **Conclusion:** There were differences in COX-2 levels in melasma and non-melasma patients, but the difference was not statistically significant. However, there is a tendency that as the COX-2 level increases, so as the severity of melasma. Therefore, the severity of melasma possibly influenced by inflammation markers.

Keywords: Cyclooxygenase-2 serum, exposure, melasma, sun, ultraviolet

Introduction

Melasma is a hypermelanosis skin disease of the sun-exposed area, especially face. Melasma indicated by macules and patches of irregular shapes in light brown to dark brown.[1,2]

Melasma often occurs in Asian, Oriental, and Hispanic races as well as in Fitzpatrick Type III–VI skin.[3,4] In Indonesia, the female-to-male ratio of the disease is 24:1, especially in women of childbearing age with a history of direct exposure to ultraviolet (UV) light in prolonged intensity.[5]

The pathogenesis and causes of melasma remain unclear, and the therapy is still a challenge.[2] The development of melasma is influenced by many factors and depends on environmental interactions (UV exposure), hormones, and genetic predispositions.[4] Melasma is triggered by subclinical inflammation which is induced by UV radiation and regulated by genetic and hormonal factors.[2,6,7] UV exposure to skin can increase the expression of cyclooxygenase-2 (COX-2) and increase the production of prostaglandin E2 (PGE2).[8] PGE2 has an essential role in the activation of tyrosinase and melanogenesis.[9] This is according to Rodríguez-Arámbula *et al*. with histopathological results that significantly increased COX-2 and interleukin-17 (IL-17) in melasma lesions compared to normal skin, where COX-2 can play a role directly or indirectly

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in the pathogenesis of melasma through tyrosinase activation and melanogenesis. COX-2 and IL-17 act synergistically to prolong the inflammatory conditions in melasma.[10] Identification of factors associated with the pathogenesis of melasma is expected to show the new targets for more efficient melasma therapies and better prevention of recurrence.[11] Thus, the authors need to determine the role of inflammation in the pathogenesis of melasma by measuring COX-2 levels.

However, there is no previous study about the comparative assessment of serum COX-2 levels in patients with melasma and nonmelasma. While measurement of COX-2 serum levels is more acceptable to patients than measurements through the biopsy of melasma tissue. Thus, this study aimed to examine COX-2 levels in melasma and nonmelasma patients and determine their role in the pathogenesis of melasma and as an alternative therapeutic target in the future.

Methods

Research design

This was a cross-sectional analytic observational study conducted at Dr. Saiful Anwar General Hospital, Malang, Indonesia, on November–December 2017. The sample collection is conducted according to consecutive sampling method and was approved by the Ethics Committee of Dr. Saiful Anwar General Hospital (No. 400/168/K.3/302/2017).

Participants' enrollment

The participants of this study included women patients in the age group of 21-55 years old who attending the Cosmetodermatology Division of Outpatient Department of Dermatology and Venereology. All patients were informed about the study procedures, risks, and benefits. The participants who opted to take part were included in the study after signing an informed consent form. The patients who met the inclusion criteria were the research participants. Patients were excluded from this study if they met the exclusion criteria. All participants were examined for their demography characteristics including age, gender, skin type, pattern of melasma, sun exposure duration, sunblock usage, and duration.

The inclusion criteria for melasma patients were women aged 21–55 years with a diagnosis of melasma based on medical history and clinical features and score of the Modified Melasma Area and Severity Index (mMASI) above 5.8. The inclusion criteria for nonmelasma participants were women aged 21–55 years, without skin disorders (i.e., melasma or other mild skin disorders).

The exclusion criteria were other patient conditions based on history taking and medical records. Those conditions include (1) systemic diseases that can affect COX-2 levels (malignancy, autoimmune diseases, diabetes mellitus, and kidney failure); (2) suffering from other skin diseases that affect serum COX-2 levels (skin malignancy, vitiligo, lichen planus, and psoriasis); (3) currently using topical corticosteroid melasma therapy and topical bleaching agent for the past 1 week (pregnant, breastfeeding, or menopause); (4) currently using hormonal contraception or estrogen/progesterone hormone replacement therapy for the past 2 months; (5) taking drugs (antimalarials, tetracycline, minocycline, doxycycline, anticonvulsants, amiodarone, antipsychotics, angiotensin-converting enzyme inhibitors, diuretics, and sulfonylureas which can affect the appearance of melasma) for the past 1 week; and (6) currently receiving oral treatment of corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) (salicylates, propionic acid derivatives, acetic acid derivatives, enolic acid derivatives, fenamic acid derivatives, and COX-2 selective inhibitor) in the past 1 month.

Scoring of the Modified Melasma Area and Severity Index

All melasma patients were measured for determining mMASI score which was expressed in Equation 1 according to Pandya *et al*. Scores of mMASI were grouped according to the Melasma Severity Score (MSS), which include mild (2.7–4.9), moderate (5.8–7.2), and severe (8.0–9.8).[12]

$$mMASI = \left(0.3 \times A(f) \times \left(D(f) + H(f)\right)\right) + \left(0.3 \times A(lm) \times \left(D(lm) + H(lm)\right)\right) + \left(0.3 \times A(c) \times \left(D(c) + H(c)\right)\right)$$

Where A: Area of involvement, D: Darkness, H: Homogeneity of: (f): Forehead, (lm): Left malar, (c): Chin.

However, mMASI score would be used in this study above 5.8. Thus, the patients with moderate and severe levels were chosen as the research participants, but the mild had to be eliminated/excluded.

Collection of blood samples

Five milliliters of venous blood was taken from the cubital vein of melasma and nonmelasma (control) patients and allowed it to clot in the plain tube at room temperature. The serum was aspirated after centrifugation at 3000 rpm for 20 min. It was divided into few aliquots in plastic tubes and then stored (−80°C) until the time of estimation.

Determination of cyclooxygenase-2 serum level using enzyme-linked immunosorbent assay

Examination of blood serum COX-2 levels was made using the RayBio® Human COX-2 (Cyclooxygenase 2) ELISA kit. This assay employs an antibody specific for human COX-2 coated on a well plate. Standards and samples were pipetted into the wells, and COX-2 presented in a sample was bound to the wells by the immobilized antibody. The wells were washed, and biotinylated anti-human COX-2 antibody was added. After washing the unbound biotinylated antibody, horseradish peroxidase-conjugated streptavidin was pipetted to the wells. The wells were washed again, and 3,3',5,5'-tetramethylbenzidine chromogenic substrate solution was added to the wells and the color developed which indicated the amount of COX-2 bound. The solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm using ELISA reader board 550.

Statistical analysis

Data collection sheet was processed using the Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp, United States). Comparison test was carried out using the unpaired *t*-test formula if the distribution was normal, whereas nonparametric test using the Mann–Whitney test if the data distribution was not normal ($P < 0.05$).

Results

Demographic conditions of research participants

From a total of 1593 (11.61%) melasma patients attending the Cosmetodermatology Division of Outpatient Department of Dermatology and Venereology, Dr. Saiful Anwar General Hospital, Malang, Indonesia, in 2016, 185 patients were enrolled as the study participants. The research has been conducted on 23 patients of melasma and 23 nonmelasma participants. The mean age of melasma patients was 47.60 years (standard deviation [SD] ± 6.27) and nonmelasma participants was 42.35 (SD ± 7.64) ($P = 0.019$). Fitzpatrick's skin types, demographic data, family history, sun exposure duration, sun exposure duration time, sunblock usage, sunblock usage duration, and sunblock usage duration time are shown in Table 1. Melasma patients had dominantly positive melasma family history. Sun exposure duration of melasma patients was below 6 h at the time between 09.00 a.m. and 03.00 p.m. By the same duration, the nonmelasma patients were exposed at the time below 09.00 a.m. Most of the melasma patients had used sunblock after melasma happened with usage duration below 6 h/day at 09.00 a.m. until 03.00 p.m. However, nonmelasma had a good prevention using sunblock before melasma symptoms.

Serum cyclooxygenase-2 levels of melasma and nonmelasma

The levels of COX-2 between melasma and nonmelasma patients are shown in Table 2. The ranges of serum COX-2 levels of the melasma and nonmelasma groups were 35.67–238.89 U/L and 23.56–150.11 U/L, respectively. The mean of COX-2 level of melasma and nonmelasma patients was not significantly different ($P > 0.05$). Figure 1 shows a melasma patient with centrofacial- and epidermal-type melasma and had mMASI score about 9.1 (severe melasma).

Severity of melasma based on the Modified Melasma Area and Severity Index score

The total mean value mMASI score of serum COX-2 levels is presented in Table 3, whereas Table 4 presents the comparison of serum COX-2 levels based on the MSS. Based on the mMASI score grouped according to MSS, there were 11 people in the moderate group (mMASI score: 5.8–7.2) and 14 people in the severe group (mMASI score: 8.0–9.8).

Type of melasma and effect of sunblock and ultraviolet exposure against serum cyclooxygenase-2 levels

Based on the type of melasma, the groups of melasma patients were categorized as follows: 5 – epidermal type, 1 – dermal type, and 17 – mixed type. The mean serum COX-2 levels in the melasma group based on melasma type are presented in Table 5. Serum COX-2 levels did not show significantly

Table 1: Demographic condition of research participants (*n*=23)

Condition	Category	Melasma, *n* (%)	Nonmelasma, *n* (%)	*P*
Age	-	47.60±6.27	42.35±7.64	0.019*
Fitzpatrick's skin type	III	2 (8.6)	2 (8.6)	-
	IV	19 (82.6)	17 (73.9)	
	V	2 (8.6)	4 (17.3)	
Melasma family history	Negative	8 (34.8)	11 (47.8)	0.369
	Positive	15 (65.2)	12 (52.2)	
Sun exposure duration	<6 h	19 (82.6)	20 (86.9)	-
	>6 h	4 (17.4)	3 (13.0)	
Sun exposure duration time	<09.00 A.M	2 (8.7)	8 (34.8)	-
	09.00 A.M-03.00 P.M	16 (69.6)	6 (26.1)	
	<09.00 A.M-15.00 P.M	4 (17.4)	1 (4.4)	
	<09.00 A.M, >15.00 P.M	2 (8.7)	9 (39.1)	
Sunblock usage	Never	10 (43.5)	13 (56.5)	-
	Before melasma	3 (13.0)	10 (43.5)	
	After melasma	10 (43.5)	0 (0.0)	
Sunblock usage duration	Never	10 (43.5)	12 (52.1)	-
	<6 h	8 (34.8)	10 (43.5)	
	>6 h	5 (21.7)	1 (4.4)	
Sunblock usage duration time	Never	10 (43.5)	13 (56.5)	-
	<09.00 A.M	8 (34.8)	10 (43.5)	
	09.00 A.M-03.00 P.M	5 (21.7)	0 (0.0)	
	<09.00 A.M, >03.00 P.M	0 (0.0)	0 (0.0)	

*There were different significantly among melasma and nonmelasma category condition

different ($P > 0.05$) according to melasma type (epidermal, dermal, and mixed).

The differences in serum COX-2 levels in melasma and nonmelasma patients in some UV exposure and the use of sunscreen are shown in Table 6. Most of the conditions which may cause dynamics of COX-2 levels did not show the significant difference although most of the conditions showed slightly higher in melasma patients than nonmelasma participants. However, the usage of sunblock in melasma patients will significantly produce COX-2 level higher than nonmelasma patients.

Table 2: Value of serum cyclooxygenase-2 levels of melasma and nonmelasma patients

Patients	Serum COX-2 level (U/L)			
	Range	Median	Mean±SD	*P* value of mean
Melasma	35.67-238.89	52.66	82.23±61.08	0.063*
Nonmelasma	23.56-150.11	44.00	52.66±28.62	

*Serum COX-2 levels between melasma and nonmelasma patients showed different significantly. COX: Cyclooxygenase-2, SD: Standard deviation

Table 3: Comparison of Modified Melasma Area and Severity Index value and serum cyclooxygenase-2 levels in melasma patients

	Lowest	Highest	Mean±SD
mMASI score	6.80	15.30	9.63±2.73
COX-2 melasma level	35.67	238.89	82.23±61.08

mMASI: Modified Melasma Area and Severity Index, COX-2: Cyclooxygenase-2, SD: Standard deviation

Table 4: Comparison of serum cyclooxygenase-2 levels according to Melasma Severity Score

MSS category	Serum COX-2 level		
	Mean±SD	Median	*P* value of mean
Moderate	49.55±14.26	44.89	0.051
Severe	112.19±72.32	104.72	

SD: Standard deviation, MSS: Melasma Severity Score, COX-2: Cyclooxygenase-2

Discussion

The exact of melasma cause remained unknown despite many factors involved in this disease pathogenesis.[13] The study about melasma is complex yet focused on the examination of the basic biochemistry, pharmacology, and physiology of the melanocortin system, the development of melanosomes, genetics, diseases associated with abnormal pigment, and environmental exposure to chemical materials.[14] To improve the understanding of the pathogenesis, scientists have to master the genomic first and basic proteomic melasma, including hundreds of proteins involved in pigmentation.[15]

A study by Passeron and Picardo (2018) suggested the latest evidence on the pathophysiology of melasma and suggested that melasma might be a photoaging skin disorder affecting genetically predisposed individuals.[16] Various factors including UV light exposure and melasma history family have a possible impact on the development of melasma in almost all patients.[17] UVA, UVB, and sunlight can affect the process of melanogenesis, but the involvement of hormones is also essential in the difficulty of melasma.[3,6,18-20] Solar irradiation with UVB (280–315 nm) and UVA (315–400) and the shorter wavelengths of visible light stimulate these cells to promote melanogenesis and melanocyte proliferation. The main effects of acute and chronic exposure to UV radiation are DNA damage, inflammation, and immunosuppression.[21] The whole array of changes caused by UV radiation in exposed skin is termed as photoaging. A primary cause of aging is the imbalance between reactive oxygen species production and their neutralization by natural antioxidant systems, which generates oxidative stress leading to the progressive deterioration of the organs and its resultant clinical and histological changes.[21-23] Acute exposure is known to trigger worsening or relapses of melasma lesions. Chronic exposure, especially of UVA1 and visible light that penetrate deeper into the skin, might chronically affect the basal membrane and the dermis component to induce, in genetically predisposed patients, the melasma lesions.[16]

In this study, the mean age for the melasma and nonmelasma groups was 47.60 ± 6.27 and 42.35 ± 7.64 years, respectively.

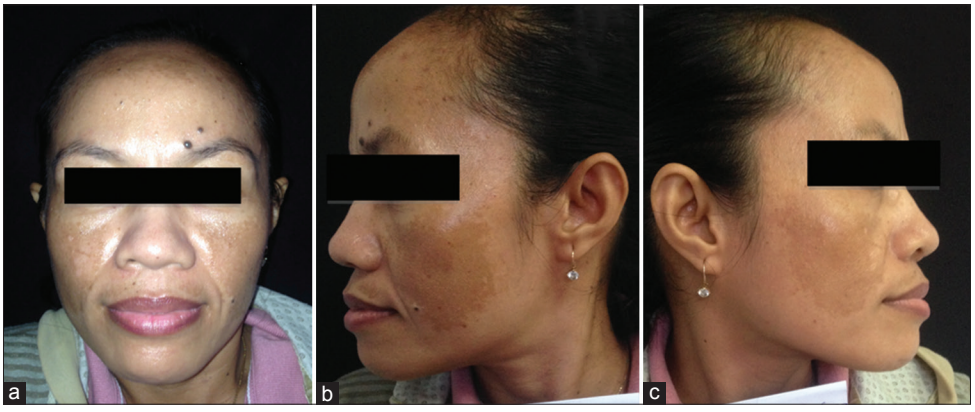

Figure 1: One of the melasma patients' appearance in this study from (a) front, (b) left, and (c) right

Similar with Ortonne *et al*., in nine clinics spread across the world obtained a mean age of 42.9 ± 9; in the United States 45.0 ± 10.7, in France 41.0 ± 7.46, in Germany 35.1 ± 7.18, in the Netherlands 40.7 ± 8.86, in Mexico 39.5 ± 7.77, in Italy 41.3 ± 5.91, in Singapore 48.7 ± 6.71, in South Korea 37.5 9.33 and in Hong Kong 48.7 7.83.[17]

The beginning of melasma lesions is indicated by the impaired integrity of the stratum corneum, slower repairability, and an increase of inflammatory cells in the development of melasma lesions in Asian skin.[24,25] Histologically, a positive correlation was found between COX-2 immunohistochemical staining with solar elastosis and melanin in the epidermis.[10] COX-2 expression induced by UV exposure involves inflammation due to UV exposure, edema, keratinocyte proliferation, and epidermal hyperplasia.[26] COX-2 will affect the local inflammatory response through action on immune cells.[27]

Melasma patients in this study were women aged 21–55 years with a diagnosis of melasma based on medical history and clinical features and mMASI score >5.8. A study by Pandya *et al.* sought to stratify the mMASI into ranges correlating with mild, moderate, and severe melasma, so that clinicians can better interpret melasma studies and investigators can identify patients with moderate-to-severe melasma by correlating MSS categories to mMASI scores.[12] In this study, the mMASI score was used above 5.8 because we need to evaluate patients with moderate-to-severe melasma.[12]

The serum COX-2 levels in the melasma and nonmelasma groups were 35.67–238.89 and 23.56–150.11 U/L, respectively. The mean value of serum COX-2 levels of the melasma and nonmelasma groups was 82.23 ± 61.08 and 52.66 ± 28.62 U/L, respectively ($P = 0.063$). This is not by the study by Rodríguez-Arámbula *et al*. that COX-2 expression using immunohistochemistry on histopathology of melasma and nonmelasma lesions was significantly different ($P < 0.001$).

Table 5: Differences in serum COX-2 levels in melasma type

Melasma type	Mean±SD	Median	*P*
Epidermal	77.67±50.37	58.67	0.746
Dermal	140.45±135.14	72.72	
Mixed	76.38±55.53	52.67	

*P<0.05: there are significant differences based on Kruskal Wallis

In melasma and nonmelasma lesions, the mean value of COX-2 expression was 8.3 ± 2 and 6.2 ± 0.6, respectively.[10] This was probably due to an examination of COX-2 levels taken from serum that might have been influenced by other variables outside of the research. The COX-2 enzyme is in the smooth endoplasmic reticulum and bound to the cell's core membrane.[27] The measurement of COX-2 levels in serum and tissue certainly has a different result, whereas the measurement of COX-2 serum levels is more acceptable to patients than the measurements through the biopsy of melasma tissue. The biopsy of melasma lesion can be a cosmetical problem. Late "scarring" with or without hypo- or hyperpigmentation is a common complication seen after healing of the skin biopsy site. Hypopigmented scars are common when biopsies are taken for hyperpigmented lesions. Scars can be atrophic scar or hypertrophic. Occasionally, patients may develop a keloid over the biopsy site.[28]

The previous reported that the most common cause of melasma is a combination of UV exposure, genetic tendency, and hormonal influences. Moreover, there are still many systemic factors that can affect both melasma and serum COX-2 levels that have not been included in the exclusion criteria in this study. Systemic disease factors that can affect melasma are endocrine disorders, liver disease, and nutritional deficiency.[1]

COX-2 is regulated by growth factors, light, and cytokines and is likely to be involved in the inflammatory process due to UV, photoaging, and photocarcinogenesis.[29] Repeated UV exposure is known to cause a chronic increase in expression of PGE2 which is induced by COX-2.[9] IL-17 induces COX-2 synergistically to prolong the inflammatory state in melasma. High levels of IL-17 in the epidermis in melasma lesions can be the main key to the persistence of melasma.[10]

The COX-2 enzyme is usually not present in basal conditions or may be in deficient amounts. The COX-2 enzyme is rapidly induced by various stimuli, including proinflammatory cytokines, such as IL-1, tumor necrosis factor-alpha, and growth factors, to produce prostaglandin synthesis associated with inflammation and carcinogenesis. Substantial evidence suggests that irregular COX-2 expression and prostaglandin synthesis affect chronic inflammatory conditions.[30] Systemic diseases that can affect serum COX-2 levels are a malignancy, systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, atherosclerosis, diabetes mellitus, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's

Table 6: Differences in serum COX-2 levels of melasma and non-melasma patients in some conditions

Condition	COX-2 Level (U/L)		Significancy test (*P*)
	Melasma	Non Melasma	
UV Exposure <6 h (*n*=39)	83.88±63.17 (*n*=19)	54.07±30.01 (*n*=20)	0.101
UV Exposure >6 h (*n*=7)	74.39±57.63 (*n*=4)	43.26±17.31 (*n*=3)	0.400
Exposure time 09.00-15.00 (*n*=22)	63.49±51.89 (*n*=16)	39.53±12.79 (*n*=6)	0.275
Did not using sunblock (*n*=23)	119.23±73.04 (*n*=10)	51.52±31.20 (*n*=13)	0.005*
Use sunblock before melasma (*n*=14)	42.33±6.01 (*n*=3)	61.40±34.77 (*n*=11)	0.573

*P<0.05: there are significant differences based on the Mann Whitney test

disease, inflammatory bowel disease, chronic hepatitis, liver cirrhosis, osteoarthritis, and failure of galaxies.[31-33] Some of these systemic diseases have not been included in the exclusion criteria in this study.

The average total score of mMASI in this study was 9.63 ± 2.73, with the lowest and highest scores being 6.8 and 15.3, respectively. Based on MSS, serum COX-2 levels were found at a moderate level of 49.55 ± 14.26 U/L and a severe level of 112.19 ± 72.32 U/L ($P = 0.05$). According to Rodríguez-Arámbula *et al*. investigation, COX-2 was thought to have a direct relationship with the pathogenesis of melasma based on the result that the mMasi score was positively related to T-cells and COX-2 expression. The expression of COX-2 and the severity of melasma may be related to the capacity of the enzyme that induces epidermal hyperpigmentation through prostaglandin production in the photoaging state played primarily by chronic inflammatory cells and mediators.[10]

Some limitations in this study that possibly cause bias include COX-2 examination from blood serum, where COX-2 levels in blood serum are influenced by several factors. The results of COX-2 levels in this study do not necessarily describe COX-2 levels derived from melasma but also can be possible from other factors in the body. In this study, exclusion was carried out with various conditions that could lead to an increase in serum COX-2 but only based on medical history. The method to measure melasma severity uses the mMASI method that is measured subjectively so that it needs more objective examination such as Mexameter or Chromameter. It is necessary to research with histopathological examination on biopsy results of melasma skin lesions and normal skin. In order to obtain more accurate results, it is necessary not only to review the medical history but also physical and laboratory examinations to rule out systemic diseases that can affect serum COX-2 levels.

According to a research by Rodríguez-Arámbula *et al*., the presence of COX-2 involvement in melasma may explain the good response to the treatment of topical anti-inflammatory drugs.[10] In the study of Jung *et al*., the clinical efficacy of madecassoside (the main triterpene glycoside isolated from *Centella asiatica*) significantly reduced melanin index due to UV exposure in the 8th week after topical application.[34] However, there has been no research on the use of systemic COX-2 inhibitors in melasma. Another study by Kim *et al*. explains that COX-2 is suspected to be the target candidate for the development of therapeutic antimelanogenic agents or lightening agents for hyperpigmentation disorders such as melasma, postinflammatory hyperpigmentation, and solar lentigo.[9]

There has been no study of COX-2 systemic drug application in melasma, but there are studies in other diseases, namely aspirin (acetylsalicylic acid) and other NSAIDs (indomethacin, piroxicam, sulindac, diclofenac, and celecoxib) which are useful for reducing skin cancer incidence and as therapy actinic keratosis.[35,36] The molecule decreases prostaglandin production by inhibiting COX-1 and COX-2, whereas celecoxib is a specific COX-2 inhibitor.[37,38] Therefore, further research is needed on COX-2 inhibitors as candidates for melasma therapy agents.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

An Effective and Practical Diagnostic Clinical Method in Primary Scarring Alopecia: Dermoscopy

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Abstract

Objective: There are several studies on the dermoscopy of the cicatricial alopecia. When the national literature is reviewed, however, only one original study focusing on the subject exists. Here, we aimed to investigate the dermoscopic features of the patients with primary cicatricial alopecia. **Materials and Methods:** In this study, dermoscopic findings of 40 patients with clinical and histopathological diagnosis of primary cicatricial alopecia were retrospectively reviewed. Dermoscopic examination was performed by a handheld dermoscope with 10-fold magnification. Photographing was performed using a dermoscope attached to a cell phone camera with 2-fold digital zoom. **Results:** Tubular perifollicular scale in lichen planopilaris (n = 12), cutaneous clefts with emerging hairs and three-dimensional yellow dots in dissecting cellulitis (n = 6), tufted hairs in folliculitis decalvans (n = 6), and follicular plugs and branching vessels in discoid lupus erythematosus (n = 6) were the main findings. No characteristic finding was found for pseudopelade of Brocq (n = 8) and frontal fibrosing alopecia (n = 2). **Conclusion:** Dermoscopy is a noninvasive, effective, and practical diagnostic tool for the differential diagnosis of primary cicatricial alopecia. The retrospective nature, lack of a control group, and relatively small number of the patients are the main limitations of our study.

Keywords: Alopecia, dermoscopy, handheld dermoscope, primary cicatricial alopecia, trichoscopy

Introduction

Hair has an important role in personal appearance and self-perception. In this context, hair diseases and hair loss not only may affect physical and mental health, but also can cause important problems in psychosocial sense.[1] So that, early diagnosis and treatment of hair diseases is crucial.

Cicatricial alopecia refers to a form of alopecia that results in an irreversible damage in hair follicle. In primary cicatricial alopecia, target of the inflammatory process is hair follicle and interfollicular area is relatively preserved. This inflammatory process results fibrosis in the hair follicle corresponding permanent hair loss clinically. Lichen planopilaris (LPP), pseudopelade of Brocq (PB), folliculitis decalvans (FD), dissecting cellulitis (DS), frontal fibrosing alopecia (FFA), and discoid lupus erythematosus (DLE) are the most common causes of primary cicatricial alopecia.[2,3]

The permanent nature of hair loss rises the importance of early diagnosis and differential diagnosis of primary cicatricial alopecia. Although physical examination provides important clues to diagnosis, in some cases, biopsy and histopathological examination may be needed.

There are several studies in the relevant literature regarding the dermoscopic diagnosis of cicatricial alopecia. However, to the best of our knowledge, there is only one original research focusing on the subject in the relevant Turkish literature.[4] In this study, we aimed to investigate the dermoscopic findings of primary cicatricial alopecia cases.

Materials and Methods

In this study, age, sex, symptoms, disease durations, and dermoscopic images of the cases having clinical and histopathological diagnosis of primary cicatricial alopecia were retrospectively reviewed. Patients who admitted to

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the outpatient dermatology clinic of Ahi Evran University Training and Research Hospital between December 2017 and September 2018 were included in the study. Dermoscopic examination was performed using a Dermlite II ProHR polarized handheld dermoscope with ×10 (Dermlite, San Juan Capistrano, California, USA). The lesions were photographed with a dermoscope attached to a high-resolution mobile camera phone using ×2 digital zoom. Thus, ×20 fold magnification was obtained. The entire lesional skin was examined and photographed for all the cases. All the images obtained were examined, and the findings detected were recorded. Diseases causing noncicatricial alopecia such as psoriasis and seborrheic dermatitis and the causes of secondary cicatricial alopecia such as tinea capitis profunda and physical trauma were excluded. All the procedures followed the Helsinki declaration, and the study was approved by the local clinical research ethics committee.

Statistical analysis

Statistical analysis was performed using SPSS 24.0 software (SPSS Inc., Chicago, Illinois, USA). Arithmetic mean, standard deviation, and ratio were used in the analysis of the demographic parameters (age and sex). The diagnostic value of the dermoscopic data in each disease group was calculated using diagnostic sensitivity and specificity tests.

Results

The study included a total of 40 patients (14 males and 26 females). The mean age of the patients was 35.9 ± 6.6 years. The demographic data and mean disease durations are detailed in Table 1. The most common presenting symptom was hair loss. Itching and pain were the other presenting symptoms. Twelve patients had LPP, 8 had PB, 6 had DS, 6 had FD, 6 had DLE, and 2 had FFA. All the

Table 1: The demographic features and the mean disease durations

Diseases	Number of cases (n=40)	Mean age	Female/male ratio	Mean disease duration (year)
DS	6	31.5±7.5	0:6	1.5±0.89
FD	6	38.6±7.4	1:5	1.05±0.62
LPP	12	30.5±7.1	5:7	1.3±1
DLE	6	31.3±6	4:2	2±1.3
FFA	2	48±5.6	0:2	3.5±0.7
PB	8	36.6±5.8	4:4	4±2

PB: Pseudopelade of Brocq, LPP: Lichen planopilaris, FD: Folliculitis decalvans, DS: Dissecting cellulitis, DLE: Discoid lupus erythematosus, FFA: Frontal fibrosing alopecia

Table 2: The dermoscopic findings and their frequencies

Finding	DS (n=6), n (%)	FD (n=6), n (%)	LPP (n=12), n (%)	DLE (n=6), n (%)	FFA (n=2), n (%)	PB (n=8), n (%)
Epidermal scale	3 (50)	4 (66.6)	6 (50)	2 (33.3)	-	3 (37.8)
Perifollicular extending scale	3 (50)	6 (100)	9 (75)	1 (16.6)	-	-
Perifollicular tubular scale	2 (33.3)	-	10 (83.3)	-	-	-
Follicular plug	1 (16.6)	-	-	6 (100)	-	-
Epidermal erosion/ulceration	2 (33.3)	2 (33.3)	3 (25)	-	-	-
Cutaneous cleft with emerging hair	5 (83.3)	-	-	-	-	-
Honeycomb pigmentation	2 (33.3)	-	-	2 (33.3)	2 (100)	4 (50)
Cicatricial white structureless areas	6 (100)	6 (100)	10 (83.3)	5 (83.3)	2 (100)	8 (100)
Red structureless areas	6 (100)	6 (100)	10 (83.3)	2 (33.3)	-	-
Yellow structureless areas	-	-	2 (16.6)	1 (16.6)	-	-
Irregular linear vessels	4 (66.6)	5 (83.3)	3 (25)	4 (66.6)	-	3 (37.8)
Branched vessels	-	2 (33.3)	-	3 (50)	-	-
Three dimensional yellow dots	2 (33.3)	-	-	-	-	-
Dotted vessels	5 (83.3)	5 (83.3)	4 (33.3)	3 (50)	-	2 (25)
Coiled vessels	-		2 (16.6)	2 (33.3)	-	-
Broken hairs	6 (100)	-	3 (25)	1 (16.6)	-	-
Black dots	6 (100)	-	3 (25)	-	-	-
Yellow dots	2 (33.3)	-	-	-	-	-
White dots	1 (16.6)	-	-	-	-	-
Scattered dotted pigmentation	-	-	-	3 (50)	-	-
Pili torti	1 (16.6)	-	2 (16.6)	-	-	-
Tufted hairs	-	6 (100)	2 (16.6)	2 (33.3)	-	-

PB: Pseudopelade of Brocq, LPP: Lichen planopilaris, FD: Folliculitis decalvans, DS: Dissecting cellulitis, DLE: Discoid lupus erythematosus, FFA: Frontal fibrosing alopecia

cases were evaluated for a total of 24 different dermoscopic findings [Table 2].

Perifollicular tubular scale was present in 10 (83.3%) LPP cases [Figure 1]. This finding was detected in only 2 of the remaining 28 patients and both of them had DS. The sensitivity and specificity of this finding in the diagnosis of LPP were 83.3% and 92.8%, respectively.

All of the patients with PB (n = 8) had cicatricial white structureless areas whereas 4 (50%) patients showed honeycomb pigmentation pattern [Figure 2].

Cutaneous cleft with emerging hair [Figure 3a] was detected in 5 (83.3%) DS cases and none of the remaining 35 cases showed this finding. The three-dimensional yellow dots [Figure 3b] were another remarkable finding for DS. This finding was also not detected in any of the remaining cases. Cicatricial white and red structureless areas, broken hairs, and black spots were the other findings observed in all of the DS cases. The sensitivity and specificity of the cutaneous cleft with emerging hair finding for the diagnosis of DS were 83.3% and 100%, respectively. All of the FD cases (100%) showed tufted hairs [Figure 4]. Two (16.6%) LPP and 2 DLE (33.3%) cases also demonstrated tufted hairs. Cicatricial white and red structureless areas were observed in all of the FD cases [Figure 4]. The sensitivity and specificity of the tufted hairs finding for the diagnosis of FD were 100% and 88.24%, respectively. Follicular plugs were detected in all of the DLE cases (100%) [Figure 5a]. Among the remaining 34 cases, only one DS case had this finding. Scattered dotted pigmentation [Figure 5b] was detected in half (50%) of the DLE cases, and this finding was not observed in any of the remaining 34 cases. The sensitivity and specificity of the follicular keratotic plug for DLE were 100% and 97%, respectively. The sensitivity and specificity of the scattered dotted pigmentation were 50% and 100%, respectively. Two patients had FFA and both showed two dermoscopic findings: cicatricial white areas and honeycomb pigment pattern [Figure 6]. All the dermoscopic findings observed and their frequencies are detailed in Table 2.

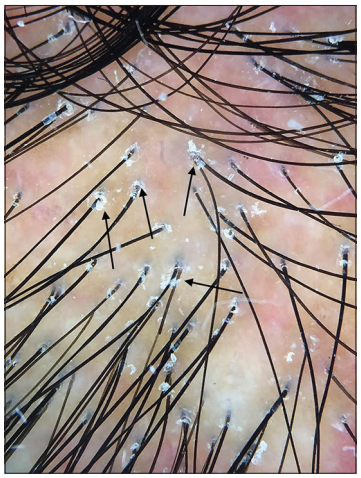

Figure 1: Perifollicular tubular scale in lichen planopilaris (black arrow)

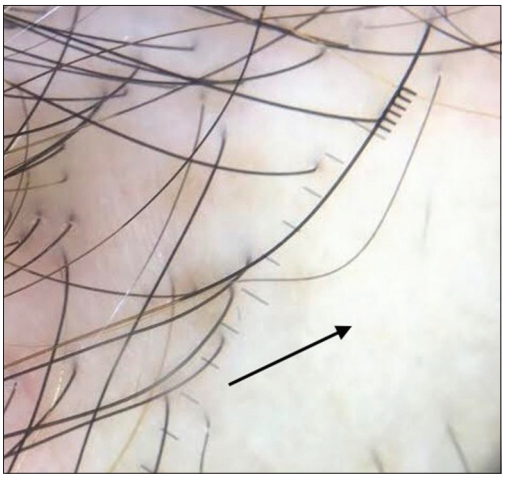

Figure 2: White cicatricial structureless areas in pseudopelade of Brocq (black arrow)

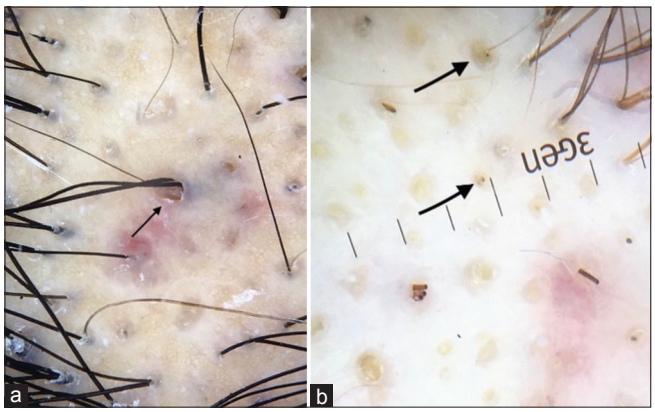

Figure 3: (a) Cutaneous cleft with emerging hair in dissecting cellulitis (black arrow) and (b) three-dimensional yellow dots in dissecting cellulitis (black arrow)

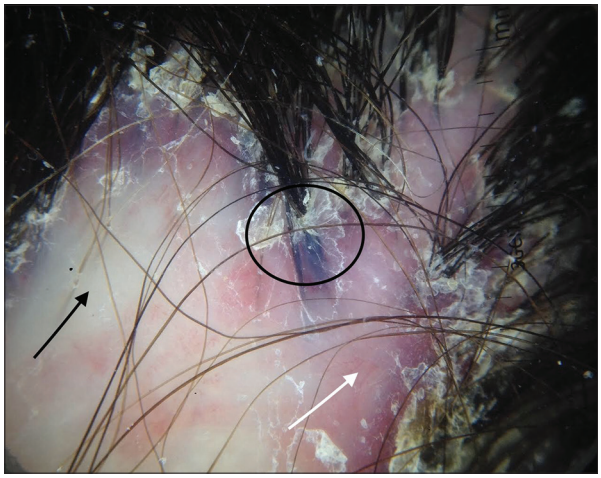

Figure 4: Tufted hairs (black circle), cicatricial white structureless areas (black arrow), and red structureless areas (white arrow) in folliculitis decalvans

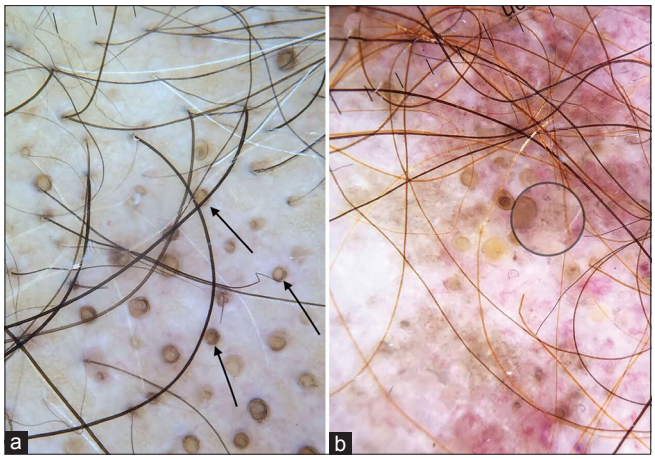

Figure 5: (a) Follicular plugs in discoid lupus erythematosus (black arrow). (b) Scattered dotted pigmentation in discoid lupus erythematosus (circle)

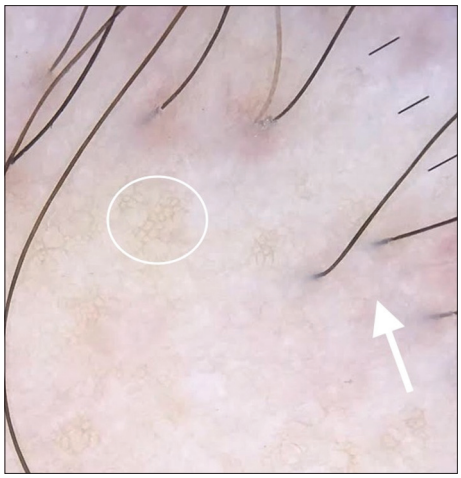

Figure 6: Cicatricial white structureless areas (white arrow) and honeycomb pigmentation pattern (circle) in frontal fibrosing alopecia

Discussion

Hair diseases constitute a remarkable part of daily dermatology practice. Although it is usually possible to diagnose alopecia with a thorough clinical history and physical examination, it becomes difficult to make a definitive diagnosis in cases showing no characteristic clinical features. Histopathological examination, as an invasive diagnostic method, can be used in such cases. However, it usually does not provide specific findings. All these difficulties in the diagnostic process, particularly in cases of cicatricial alopecia, raise the search for new diagnostic methods.[5,6]

Recently, dermoscopy has emerged as a noninvasive diagnostic tool in the diagnosis of alopecia and various researches describing dermoscopic findings of cicatricial and noncicatricial alopecias have been published.[7,8] To the best our knowledge, however, the most comprehensive study reported from Turkey so far is a research, in which 29 cases including 24 primary and 5 secondary cases of cicatricial alopecia were analyzed.[4]

In another study reported from Turkey, handheld dermoscopic findings of 21 primary cicatricial alopecia cases were investigated.[9] In our study, a total of 40 cases of primary cicatricial alopecia were analyzed in terms of the handheld dermoscopic findings.

In the present study, we detected white cicatricial areas reflecting permanent follicle loss in 37 cases. In 2 LPP and 1 DLE cases where this finding was not detected, the presence of red structureless areas indicating active stage of the disease along with follicle loss was remarkable. Based on this finding, we suggest that initial dermoscopic examination of the lesions should be focused on the presence or absence of white and red structureless areas making possible the differential diagnosis of cicatricial and noncicatricial alopecia.

When reviewing the relevant literature, it seems that there are few studies on the dermoscopic features of DS.[7,10,11] Rakowska *et al*. described the three-dimensional yellow dots (a yellow dot resembling a soap bubble along with an emerging dystrophic hair) in 8 DS cases in a study including 84 cicatricial alopecia cases.[7] In another study, again Rokawska *et al*. described the "cutaneous cleft with emerging hairs" for DS.[11] In the study of Abedini *et al*. investigating the validity of trichoscopic findings in primary cicatricial alopecia, however, none of the six cases with DS showed the above-mentioned two findings.[12] We detected three-dimensional yellow dots and cutaneous cleft with emerging hair in two and five DS cases, respectively. The fact that the cutaneous cleft finding was not detected in any of the remaining cases of cicatricial alopecia suggests that this finding can be considered quite characteristic for DS.

In spite of absence of the studies including a large series focused on dermoscopic findings of FD, tufted hairs are considered to be a characteristic finding for FD.[4,7] In our study, the presence of tufted hairs in all of six FD cases supported this view. We observed tufted hairs also in two LPP and two DLE cases. In the study of Abedini *et al*., tufted hairs were detected in 40% and 7.1% of the FD and LPP cases, respectively.[12]

LPP is known as a form of lichen planus affecting hairy skin. Tubular perifollicular scale has usually been considered a characteristic dermoscopic finding for LPP.[4,7,13,14] In our study, perifollicular tubular scale was detected in 10 out of 12 cases with LPP. We detected this finding also in two DS cases. The presence of this finding in only 2 out of the remaining 28 cases supports the opinion that the finding is very suggestive of LPP. The sensitivity and specificity of perifollicular tubular scale finding for the diagnosis of LPP were 83.3% and 92.8%, respectively. In the study of Abedini *et al*., the sensitivity and specificity of this finding for LPP were 91.4% and 88.2%, respectively.[12]

Loss of follicular openings, follicular plugs, branching vessels, honeycomb pigment pattern, and follicular red dots are the dermoscopic findings described for scalp localized DLE.[15-17] In our study, follicular keratotic plugs were observed in all six DLE cases. This finding was found in only one DS case

out of the remaining 34 cases. On the other hand, DLE cases were found to be rich in vascular structures. Irregular linear, branching, dotted, and coiled vessels were detected in 4, 3, 3, and 2 cases, respectively. The sensitivity and specificity of follicular keratotic plugs in the diagnosis of DLE were 100% and 97%, respectively. In the study of Abedini *et al*., the sensitivity and specificity of the same finding were 57.1% and 89.8%, respectively.[12] In our study, the sensitivity and specificity of scattered dotted pigmentation in the diagnosis of DLE were 50% and 100%, respectively. In the study of Abedini *et al*., the sensitivity and specificity of this finding were 7.1% and 96.8%, respectively.[12]

FFA is considered a subtype of LPP resulting in cicatricial alopecia in the frontal region, especially in women. The absence of follicular openings, perifollicular scale, and perifollicular erythema are the dermoscopic findings described for FFA.[18,19] We detected brown reticular pigmentation (which was thought to be related to sun exposure due to long-term hair loss) and white structureless areas reflecting follicular loss in both FFA cases.

PB is classified as a specific type of primary cicatricial alopecia by some authors, while some authors argue that it is the end stage of many types of cicatricial alopecia.[20] Trichoscopic findings of the entity are not specific. None of the eight PB cases included in the present study had a history of erythema and inflammation, indicating that the condition may have developed secondary. In this context, all the PB cases included were considered as primary cicatricial alopecia. No specific dermoscopic findings have been reported in BPP cases in the relevant literature.[21] We also did not observe a specific clue to PB. Cicatricial white structureless areas, epidermal scales, and honeycomb pigmentation pattern were the dermoscopic findings detected for PB in the present study.

Conclusion

Making easy to evaluate follicular loss, dermoscopy can be used as a firstline ancillary diagnostic method in the diagnosis of cicatricial and noncicatricial alopecia. On the other hand, cutaneous cleft and tufted hairs seem to be quite characteristic findings for DS and FD, respectively. We think that these findings may serve as useful clues to differential diagnosis of the two entities. Characteristic follicular plugs of DLE and tubular perifollicular scales of LPP may also provide useful clues to the differential diagnosis. When it comes to PB, dermoscopy may be valuable regarding the exclusion of the other causes of cicatricial alopecia. The retrospective nature, lack of a control group, and relatively small number of the patients are the main limitations of our study.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Depression in Patients with Functional Itch Disorder

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Abstract

Objective: Patients with pruritus sine materia (PSM) are often misdiagnosed as idiopathic pruritus (pruritus of unknown origin) when the cause of pruritus is not found. Some of these patients may be diagnosed with functional itch disorder (FID) which is also known as psychogenic pruritus (pruritus of psychological origin). Since antidepressants can be used in the treatment of psychogenic pruritus, the differentiation of FID from idiopathic pruritus is important. The aim of this study was to investigate the prevalence of depression in patients with FID. **Materials and Methods:** A total of 117 patients with FID who were diagnosed as idiopathic pruritus or PSM in their previous assessments and 117 controls took part in the research. The psychiatric assessment for depression was conducted using the Beck Depression Inventory (BDI) and the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) major depression criteria. The study employed a descriptive and cross-sectional method. **Results:** Forty-four patients with FID (37.6%) had major depression (DSM-5) and 74 patients with FID (63.2%) had clinically significant depression (BDI). The frequency of depression among FID patients was significantly higher than controls. **Conclusions:** Our findings highlight the importance of diagnosing FID in patients with PSM. The presence of FID diagnostic criteria in a patient should prompt dermatologists for a psychiatry consultation.

Keywords: Depression, functional itch disorder, pruritus sine materia, psychogenic pruritus, somatoform pruritus

Introduction

The International Forum for the Study of Itch considers the terms "itch" and "pruritus" synonymous and defines six etiological categories of pruritus: dermatological, systemic, neurological, psychogenic, mixed, and "others.[1]" Psychogenic pruritus is the kind of itch related to psychological disorders.[2] Psychogenic pruritus is known also as functional itch disorder (FID), psychogenic itch, somatoform pruritus,[3] or functional pruritus.

Psychogenic itch is not an idiopathic pruritus (pruritus of unknown origin), and it is not an elimination diagnosis. The disorder is poorly known by both psychiatrists and dermatologists. When there are no other diagnoses to propose, psychogenic itch is often mislabeled as idiopathic pruritus. The French Psychodermatology Group (FPDG) is a group of experts in dermatology, psychology, and psychiatry. This group has proposed a definition of psychogenic pruritus as "an itch disorder where itch is at the center of the symptomatology and where psychological factors play an evident role in the triggering, intensity, aggravation, or persistence of the pruritus" and has suggested calling it "functional itch disorder."[4]

To assess the diagnosis of FID, it is necessary to exclude possible internal diseases and skin diseases with both clinical and laboratory evaluations and to determine clinical characteristics, association of itch with psychological disorders.[4] According to a study, patients who scored high on depression measures reported higher degrees of pruritus compared with patients who reported not being depressive.[5] Antidepressants were found effective in the treatment of psychogenic pruritus in another study.[6] In this study, we tried to evaluate depression in patients who are diagnosed as FID according to diagnostic criteria of FPDG.

Materials and Methods

Overview

In outpatient departments, questionnaires are generally considered as the convenient tools to screen the candidates for

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psychodermatology illnesses. Our preference in choosing the convenient questionnaire to screen for depression was based on their diagnostic accuracy in screening and the feasibility of their administration. We chose a short screening instrument for depression, Beck Depression Inventory (BDI). This could be self-administered by patients before their meeting with the clinician or in the waiting area. Another screening test utilized was the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) major depression criteria which implement short interviews to screen for major depression which is practical for dermatology outpatient clinics.[7-10]

The study was designed to evaluate, within 6 months, 117 consecutive patients and 117 controls who were admitted to the department of dermatology in our hospital. The patients had pruritus >6 months with a normal laboratory examination and a negative skin prick test. None of the FID patients had any prediagnosed psychiatric, internal, or dermatological illness. None of the controls had any prediagnosed internal or psychiatric illness.

The patients with pruritus sine materia (PSM) were classified as having psychogenic pruritus using suggested diagnostic criteria from the FPDG.[4] These included three compulsory criteria: localized or generalized PSM, chronic pruritus (>6 weeks), and the absence of a somatic cause. Three additional criteria from the following seven items were present moreover: a chronological relationship of pruritus with one or several life events that could have psychological repercussions, variations in intensity associated with stress, nocturnal variations, predominance during rest or activity, associated psychological disorders, pruritus that is improved by psychotropic drugs, and pruritus that is improved by psychotherapies [Table 1].

Participants and measures

The study was carried out after obtaining the approval of the local ethical committee in our institution, and patients signed an informed consent form before participating in the study (Kecioren Training and Research Hospital, Institutional Review Board #1050/13-01-2016). All the patients in this study were diagnosed as idiopathic pruritus or PSM in their previous assessments. Eligibility criteria for patients were as follows: the presence of general pruritus with a negative skin prick test, no systemic drug treatment, age of 16 years or older, and disease duration longer than 6 months. The reasons for exclusion were relevant skin disease, internal diseases such as diabetes, thyroid disease, and/or active hepatitis, and positive skin prick test.

Table 1: Diagnostic criteria for functional itch disorder from the French Psychodermatology Group (4)

Three compulsory criteria
Localized or generalized pruritus sine material (without primary skin lesion)
Chronic pruritus (>6 weeks)
No somatic cause
Three of seven optional criteria
A chronological relationship between the occurrence of pruritus and one or several life events that could have psychological repercussions
Variations in intensity associated with stress
Nycthemeral variations
Predominance during rest or inaction
Associated psychological disorder
Pruritus that could be improved by psychotropic drugs

In the absence of primary skin findings, the physical examination focused on looking for evidence of a systemic disease and findings of conjunctival pallor, thyromegaly, splenomegaly, or stigmata of liver disease. Lymph nodes were palpated for signs of lymphadenopathy. In both patient and control groups, blood and urine analyses were performed during previous visits for exposing possible etiology of pruritus. In addition, all the patients and controls had skin prick test composed of common 13 skin allergens (tree mix, Betulaceae, grass mixtures, pine and grain pollens, cereal mix, *Dermatophagoides farinae, Dermatophagoides pteronyssinus, Alternaria, Aspergillus* mix, cockroach, mosquito, cat hair, and dog hair).

To compare the collected data of patients with FID with the group of patients with a diagnosed skin condition, 117 participants with a chronic (longer than 6 months) skin disease without any complaint of pruritus were enrolled as the comparison group.

Questionnaires

Both psychiatric and demographic-clinical questionnaires were used for each of the FID patients and controls. The demographic-clinical questionnaire was composed of two questions on demographic data (age and sex) and two questions on clinical data (type of additional diseases and duration of diseases). The psychiatric assessment for depression was conducted using a validated questionnaire, BDI, and interrogated DSM-5 major depression criteria.

Beck Depression Inventory and Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition major depression criteria

The BDI, created by Beck *et al.* in 1961, is a 21-question multiple-choice self-report inventory, one of the most widely used psychometric tests for measuring the severity of depression.[8,11] Each question has a set of at least four possible responses, ranging in intensity. When the test is scored, a value of 0–3 is assigned for each answer and the total score is compared to a key to determine the severity of depression. The standard cutoff scores are as follow: 10–18 indicates mild depression (MID), 19–29 indicates moderate depression (MOD), and 30–63 indicates severe depression (SED). Higher total scores indicate more severe depressive symptoms.[11] In addition to this, to observe more severe depressive symptoms, we used DSM-5 major depression criteria. It is the most widely used criteria for diagnosing major depression, and it is found in the American Psychiatric Association's DSM-5.[10]

Statistical analysis

In investigating data compatibility to normal range, Shapiro–Wilk test was used. In comparison of groups not in normal range, Mann–Whitney U-test was used for two groups. Univariate analyses of the categorical variables were summarized as percentages and compared with the Chi-square test. Pearson's Chi-square test, Pearson's exact Chi-square test, and Fisher's exact Chi-square test were used in cross-analysis. The odds ratios were calculated by cross-tabulation. Spearman's correlation coefficients were calculated for the variables that were not commodious to normal distribution and determined direction and magnitude of correlation between variables. For all the comparisons, a two-tailed value of $P < 0.05$ was considered statistically significant. Calculations were performed using SPSS 21 for Windows (IBM Corp., Armonk, NY, USA).

Results

Sample characteristics

All the consecutive FID patients who met the inclusion criteria were enrolled in the study. A study group of 117 patients with FID and a control group of 117 patients with other skin disorders that lack pruritus complaint were included in the study. In the control group, the prevalence rates of the specific dermatological conditions in the sample were as follows: acne (n = 26), melasma (n = 22), verruca plantaris (n = 24), tinea unguium (n = 25), and vitiligo (n = 20). The questionnaires were completed by all the patients in the study and control groups.

Eighty (68.4%) of patients were female and 37 (31.6%) of patients were male. The mean age of the patients was 43.4, with a standard deviation of ±16.1 (ranged between 16 and 82). The mean duration of pruritus was 27.96 months, with a standard deviation of ±20.42 (ranged between 6 and 120). Eighty-one (69.2%) of controls were female and 36 (30.8%) of patients were male. The mean age of the controls was 37.64, with a standard deviation of ±17.03 (ranged between 16 and 79).

Beck Depression Inventory

Seventy-four patients with FID (63.2%) and 59 controls (50.4%) had a total score ≥10, and the difference between the two groups was statistically significant (P = 0.047). It included mild (32.4%), moderate (33%), and severe (17%) depression in patients with FID according to the standard cutoff scores. On the other hand, there was no difference in disease severity (MID, MOD, and SED) between the two groups (χ^2 = 3.689; P = 0.158) [Table 2]. When the responses to questions were compared between controls and FID patients, patients with FID revealed higher scores to some questions. The responses that are statistically significant were summarized in Table 3. The most common symptom was feeling sad. Seventy-eight patients with FID (66.7%) described themselves as feeling sad (χ^2 =10.182; P = 0.037) [Table 3].

Table 2: The frequency of positively and/or negatively diagnosed patients and controls according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition and Beck Depression Inventory questionnaires, *n* (%); *Pearson's Chi-square test; and odds ratio

Inventory Questionnaires	Severity	Patients, *n* (%)	Controls, *n* (%)	χ^2, *P**	OR
DSM-5	−	73 (62.4)	90 (76.9)	5.84	2.04
	+	44 (37.6)	27 (23.1)	0.016	
BDI	MID	24 (32.4)	28 (47.5)	3.689	
	MOD	33 (44.6)	23 (39.0)	0.158	
	SED	17 (23.0)	8 (13.5)		

*According to DSM-5 major depression criteria, +: In table refers to the patients who fulfilled at least five major depression criteria including at least one depressed mood and loss of interest or pleasure in the same 2-week period, According to BDI questionnaire scores, MID refers to 10-18 points, MOD refers to 19-29 points, and 30-63 points refers to SED. OR: Odds ratio, MID: Mild depression, MOD: Moderate depression, SED: Severe depression, BDI: Beck Depression Inventory, DSM-5: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

Table 3: The percentage, frequency, and χ^2 and *P* values of the high-score responses to questions of the Beck Depression Inventory in functional itch disorder patients in comparison with controls

Questions	Frequency (%) (*n*=117)	χ^2, *P**
Feeling sad	78 (66.7)	10.182, 0.037
Discourage about the future	67 (57.3)	9.241, 0.026
Feeling failure as a person	61 (52.1)	18.565, 0.001
Crying	66 (56.4)	14.120, 0.003
Losing interest in other people	65 (55.6)	10.703, 0.013
Difficulty in making decisions	54 (46.2)	10.980, 0.012
Feeling tired	50 (42.7)	14.305, 0.003
Losing weight	62 (53)	9.749, 0.021
Losing interest in sex	42 (35.9)	8.325, 0.040

*Pearson Chi-square test

Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition major depression criteria

Forty-four patients with FID (37.6%) and 27 patients from the control group (23.1%) fulfilled at least five major depression criteria including at least one depressed mood and loss of interest or pleasure in the same 2-week period (χ^2 = 5.84; P = 0.016; odds ratio [OR] = 2.04) [Table 2]. Fifty-nine patients with FID (50.4%) expressed a more depressed mood than controls (χ^2 = 9.374; P = 0.003; OR = 2.3). Ninety-two patients with FID (78.6%) described themselves as having a loss of interest or pleasure (χ^2 = 20.332; P = 0.001; OR = 3.7) while 53% of patients with FID (n = 62) had insomnia or hypersomnia (χ^2 = 4.402; P = 0.049; OR = 1.7).

Discussion

Psychodermatology describes an interaction between dermatology and psychiatry; the incidence of psychiatric

disorders among dermatological patients is estimated between 30% and 60% in the literature.[12-15] The presence of a concomitant psychiatric illness is predominantly observed in patients with various dermatological diseases, and PSM is among them.[15] Pruritus without any skin lesion is named as PSM. A detailed patient history is particularly important in patients with generalized pruritus who lack primary skin lesions because the differential diagnosis for this presentation is broad and includes organic and psychiatric disorders that are associated with significant morbidity.[16] In chronic PSM without any somatic cause, one must consider FID.[3] In this study, it was well understood that most of the patients who were followed as idiopathic PSM met the diagnostic criteria of FPDG. Thus, these conditions might not be idiopathic and may have a psychological background.

FID is also known as psychogenic pruritus.[3] In psychogenic pruritus, the reason of pruritus becomes psychogenic; there are cycles of stress leading to pruritus as well as pruritus contributing to stress.[17] Although there are some studies which report that psychogenic pruritus is noted in patients with psychiatric conditions such as depression, anxiety, aggression, obsessive–compulsive disorders, psychoses, and substance use,[18-22] there exists no study evaluating depression frequency in patients who are diagnosed as FID. In this regard, untreated patients with chronic PSM in this study were classified as having FID using suggested ten diagnostic criteria (3 – compulsory and 7 – optional) from the FPDG[4] [Table 1]. We preferred to use these criteria which were validated by an international study for a more objective and standard diagnosis.[3]

Instead of the term "psychogenic pruritus," the FPDG[4] proposes the use of other terms such as "functional itch disorder" and "somatoform pruritus." In addition to this, in underlying FID where no somatic or psychiatric diagnosis coexists, FPDG proposes the use of "functional disorders" rather than "somatoform disorders." The onset of an associated psychological symptom or a psychiatric disorder should not necessarily be found when FID is diagnosed but may be revealed later in case of an associated mental disorder.[3] Parallel with this, we found that forty-four patients with FID (37.6%) had major depression and seventy-four patients with FID (63.2%) had clinically significant depression. Regarding international classifications of psychiatric diseases, psychogenic pruritus is not cited in the Tenth Revision of the International Classification of the Diseases;[23] however, pruritus is reported under the diagnosis "other somatoform disorders" (F45.8). The term "psychogenic pruritus" was not used also in the DSM-4,[24] but it could be recognized among the following four diagnoses listed in the DSM-4: conversion disorder, undifferentiated somatoform disorders (300.81), unspecified somatoform disorder (300.82), and pain disorder associated with psychological factors (307.80).

It is not easy to determine whether it is a psychogenic pruritus, neuropathic pruritus, idiopathic pruritus, or somatoform pruritus. Neuropathic itch refers to pruritus caused by neuronal or glial damage,[25] whereas psychogenic itch is related to psychological disorders.[2] Neuropathic pruritus is caused by lesions of the afferent neural pathways. There are some clues to differentiate neuropathic itch; the distribution often corresponds to a particular spinal segment, often a sensory deficit or an aberration in sensory perception such as allodynia (nonpainful stimuli evoke pain), allokinesis (sensation of itch produced by innocuous stimuli that would not ordinarily induce itch), or hyperpathia (evoked pain grossly out of proportion to painful stimuli) present.[25,26] In this study, a thorough physical examination was conducted, and patients' history was taken in detail in order to rule out neuropathic pruritus. Other differential diagnoses of psychogenic pruritus are psychogenic urticaria and psychogenic dermographism; however, there are temporary and recurrent visible urticarial lesions in those cases. Another important differential diagnosis is self-inflicted skin lesions (SISLs)[27] such as psychogenic excoriations[28] and dermatitis artefacta. The psychopathology in SISLs is impulsive and compulsive; the main symptom is not pruritus but scratching. In contrast, psychogenic pruritus is related to an illusion of pruritus where pruritus is the main complaint. Another differential diagnosis is abusive skin excoriations observed in the pediatric population.

In a study of 100 psychiatric inpatients, the prevalence of generalized pruritus was 42%.[29] Psychogenic pruritus is encountered in patients with primary psychiatric disorders. It is known as a clinical pattern of the somatoform disorders that have subjective complaints by the patients.[26,30,31] One study reports that 6.5% of outpatients at a clinic specializing in psychodermatology suffered from "somatoform pruritus" (using a definition close to those in DSM-4).[32] On the other hand, the frequency of FID is not known because the differential diagnosis of FID is difficult and FID is often mislabeled as idiopathic pruritus.[3]

In our study, 44 patients with FID (37.6%) and 27 patients from the control group (23.1%) fulfilled at least five major depression criteria in this study. Seventy-four patients with FID (63.2%) had a total score ≥10 and were diagnosed as having clinically significant depression. The main psychiatric disorders encountered in dermatology patients are anxiety, depression (mood disorders), and body dysmorphic disorder.[33] Hughes *et al.* reported that 30% of dermatology outpatients and 60% of dermatology inpatients suffered from a psychiatric disorder.[34] Ludwig *et al.* found that in a public health outpatients' service of a dermatology clinic, the frequency of anxiety was 40.3% and the frequency of depression was 43.7%.[35] Al Shahwan *et al.* reported that the frequency of mood disorders in Arab dermatology outpatients was 29% for anxiety and 14% for depression.[36] In our study, the frequency of depression was statistically higher in patients with FID than controls, while the frequency of depression was also high in controls. Depression is noted in patients with acne (n = 26) and vitiligo (n = 20) in the control group because the main dermatological disorders with concomitant psychiatric illnesses are known as dermatitis, acne, pruritus,

eczema, atopic dermatitis, urticaria, alopecia, psychocutaneous disorders, psoriasis, and vitiligo.[15,32-44]

Disfiguring skin disorders with chronic pruritus (>6 weeks) such as atopic dermatitis and prurigo nodularis are often associated with social problems and with psychic disorders such as depression or anxiety.[1,45] One study mentions that persistent skin diseases had a higher psychiatric comorbidity in comparison to the intermittent and incidental skin diseases.[46] In our study, the frequency of depression was 63.24% in patients with FID. When DSM-5 major depression criteria were taken into account, 37.6% of patients were noted to have major depression. This is in line with the findings in a study on patients with psychogenic pruritus (consisting of lichen simplex chronicus, neurotic excoriation, prurigo nodularis, and pruritus that is intermittent, short term, and severe and without physical signs) where all the patients were found to have affective disorders (depressions, anxieties, and mixed anxiety and depressive disorders) and 18% (12/65) also had associated personality disorders.[47]

There is little knowledge about the influence of chronic pruritus itself on comorbid symptoms of depression. In a study with 284 participants[48] with chronic pruritus (atopic dermatitis, prurigo nodularis [pruritus with multiple scratch lesions], and chronic pruritus of other origins [chronic pruritus with little or no scratch lesions]), patients with chronic pruritus had a more negative body concept than healthy individuals. Higher levels of depression and anxiety were related to a more negative body image. Patients with chronic pruritus of other origins had higher scores in terms of grooming, daily activities, and acceptance of one's body by others than patients with atopic dermatitis. On the other hand, there is also a little knowledge about the influence of depression on chronic pruritus. In a study, patients who scored high on depression measures reported higher degrees of pruritus compared with patients who reported not being depressive.[5]

Contrary to our findings, in another study[37] with 114 adult males with dermatological disorders, the percentage of depression was 66.6% in patients with pruritus while no depression was observed in chronic fungal infections. The depression is commonly associated with psychogenic pruritus, and these patients with psychogenic pruritus secondary to depression may also present with prominent anxiety and agitation supporting our study.[49] The prevalence of major depression in our study was higher when DSM-5 was utilized as a screening test instead of BDI. It could be a result of the fact that BDI is a self-reported questionnaire while DSM-5 criteria are clinician-administered and thus might give a more accurate evaluation.

We found a high frequency of depression in patients with FID. Although there has been no clinical trial of pharmacological treatment for psychogenic itch,[50] antidepressant drugs such as tricyclic antidepressants (mainly doxepin) and selective serotonin reuptake inhibitors (fluoxetine, sertraline, paroxetine, citalopram, fluvoxamine, and escitalopram)[6] are recommended and have an acceptable risk of adverse effects. Results of our study, with a high prevalence of depression in FID patients, might be supportive in favor of antidepressant use in treatment of this peculiar patient group.

There are some limitations in our study. We preferred to use a screening test such as BDI questionnaire to screen for the psychiatric conditions accompanying the itch in a group of dermatology outpatients because they are readily available, convenient, and time-saving when compared with a psychiatry consultation. Nevertheless, including a professional specializing in psychiatry would have been optimal in terms of making accurate diagnosis of the psychiatric conditions. Furthermore, addition of a third group of healthy patients could make the comparison of FID patients with normal population available while increasing the statistical power of the study; however, we were unable to include a third group because of our methodology and the institutional review board decisions. Although this study provides some more scientific data on the relationship between the psychological disorders and FID, more studies are needed to conclude on this matter, given the scant information available in the literature.[12-15]

Conclusion

Our findings highlight the importance of diagnosing psychogenic pruritus (FID) in patients with PSM. The presence of FID diagnostic criteria in a patient should prompt dermatologists for a psychiatry consultation for evaluation of a psychological comorbidity.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Quality of Life in Turkish Patients with Autoimmune Blistering Diseases: Reliability and Validity of the Autoimmune Bullous Disease Quality of Life and the Treatment of Autoimmune Bullous Disease Quality of Life Questionnaires

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Abstract

Background: The Autoimmune Bullous Disease Quality of Life (ABQOL) and the Treatment of Autoimmune Bullous Disease Quality of Life (TABQOL) questionnaires, which are specific for autoimmune blistering diseases (AIBDs), were developed in Australia. **Aims and Objectives:** The aim of this study was to validate the Turkish version of the ABQOL and TABQOL questionnaires and to assess the reliability of them in the Turkish population. **Materials and Methods:** The Turkish versions of the ABQOL and TABQOL questionnaires were produced by forward–backward translation of the original English version. The patients were requested to complete ABQOL and TABQOL questionnaires on day 0 and after 7 days for a 2nd time sent by post. Furthermore, patients also completed other health-related quality of life scales on day 0. **Results:** A total of 68 patients with AIBDs were recruited. A subset of 20 (29.4%) patients completed the day 7 questionnaire. Both the Turkish versions of the ABQOL and TABQOL questionnaires had a high internal consistency (0.86 and 0.88, respectively) and test–retest reliability (0.87 and 0.87, respectively). The correlation between ABQOL and TABQOL scores was moderate (Pearson's $R = 0.609$). **Conclusion:** We have shown that the Turkish versions of ABQOL and TABQOL questionnaires are valid and reliable instruments. They can be used to measure treatment burden in Turkish AIBD patients.

Keywords: Autoimmune blistering diseases, Autoimmune Bullous Disease Quality of Life, health-related quality of life, pemphigoid, pemphigus, Treatment of Autoimmune Bullous Disease Quality of life

Introduction

Autoimmune blistering diseases (AIBDs) cover a variety of diseases such as pemphigus vulgaris (PV), pemphigus foliaceus (PF), bullous pemphigoid (BP), and epidermolysis bullosa acquisita (EBA). They are all characterized by mucosal and/or cutaneous blistering caused by autoantibodies targeting specific adhesion molecules of the skin/mucosa. PV and BP are the most frequently reported AIBDs in Turkey.[1] The mean incidence of pemphigus was 4.7 new cases per million people per year (95% confidence interval: 4.1–5.4) in the latest prospective research,[2] similar to that of other South-Eastern European countries.[3-5] On the other hand, BP and other subepidermal bullous diseases are thought to have a lower incidence in Turkey, although there are no epidemiological studies of their incidence in Turkey.[1]

Similar to other dermatological diseases, health-related quality of life (HQoL) information is seen as increasingly important in determining therapeutic outcomes of AIBD. This information could help to get a better understanding of AIBD and to

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develop a successful method of treatment. Furthermore, the main therapies used to control AIBDs, such as steroids and immunosuppressive agents, may cause serious adverse effects. One of the main reasons for mortality in patients with AIBDs is therapy-related complications.[6] Therefore, it is important to pay attention to the patients' HQoL and treatment-related quality of life, psychological states, as well as clinical status.

The Autoimmune Bullous Disease Quality of Life (ABQOL) questionnaire was developed in Australia to document the quality of life in patients with AIBD.[7] The Treatment of Autoimmune Bullous Disease Quality of Life (TABQOL) questionnaire represents a quantifiable instrument developed to determine the HQoL impacts of treatments specific for AIBD.[8] These patient-reported outcomes (PROs) are being used in sponsored clinical trials in AIBD. Hence, for Turkish patients to be included in future trials in AIBD, it is important to validate these PROs in Turkish.

The aim of this study was to assess the reliability and validity of Turkish ABQOL and TABQOL questionnaires and document the HQoL in Turkish AIBD patients using the ABQOL and TABQOL questionnaires.

Materials and Methods

Autoimmune Bullous Disease Quality of Life–Treatment of Autoimmune Bullous Disease Quality of Life translation

Forward translation of the original versions from English to Turkish was performed by an independent qualified translator. Content validity was obtained by back translation to English by another independent qualified translator with no access to the original English questionnaire. To make sure the translated Turkish questionnaires contained the same meaning as the English questionnaires, the back translation to English was assessed by the Australian investigator and no revision was needed.

To pilot test the questionnaire, we recruited ten AIBD patients to complete the questionnaire. An experienced interviewer pretested patients by asking them what they thought the question was asking, what the answers were, and to explain how they decided their answers. There were no misunderstood points. Subsequently, the final Turkish versions of the ABQOL and TABQOL questionnaires were administered for the study. The 17-item ABQOL and TABQOL questionnaires have four optional answers (each scored from 0 to 3 points), in which a higher score represented a lower HQoL (ranging from 0 to 51 points).[7,8]

Patient recruitment

We enrolled patients with AIBD who attended the Department of Dermatology and Venereology of a tertiary referral center for AIBD in Turkey, fulfilled the criteria and were willing to participate in the study by signing the consent form. The patients were interviewed during routine medical appointments at the outpatient clinic or on admission to the hospital. The time of recruitment was 12 months between February 2017 and February 2018. The inclusion criteria were diagnosis of AIBD, the age of >18 years, Turkish as native language, and being able to read and understand scales. The medical history regarding the subset of AIBD, disease status, duration of disease, disease severity, and applied treatment was collected. Sociodemographic characteristics of the patients which may influence the quality of life (age, sex, level of income, educational level, and marital status) were also recorded.

Complete remission off therapy, partial remission off therapy, complete remission on minimal therapy, partial remission on

Table 1: Main demographic characteristics of patients with autoimmune blistering diseases

Variable	*n* (%)
Patients enrolled (*n*)	68
Age (years)	51.14±13.48
Sex, *n* (%)	
Male	24 (35.2)
Female	44 (64.7)
Marital status, *n* (%)	
Single	6 (8.8)
Married	53 (77.9)
Divorced	3 (4.4)
Widow/widower	6 (8.8)
Income level, *n* (%)	
High: Income exceeds expenses	4 (5.8)
Moderate: Income is equal to expenses	41 (60.2)
Low: Income is less than expenses	23 (33.8)
Educational status, *n* (%)	
Primary school	26 (38.2)
Secondary school	10 (14.7)
High school	18 (26.4)
Collage	5 (7.35)
Faculty	8 (11.7)
Postgraduate	1 (1.4)
Concomitant diseases, *n* (%)	
Yes	19 (27.9)
No	49 (72.1)
Current therapies, *n* (%)	
Off therapy	9 (27.9)
Systemic steroids	49 (72)
Topical Steroids	10 (14.7)
Topical antibiotics	1 (1.4)
Doxycycline	1 (1.4)
Dapsone	5 (7.3)
Rituximab	4 (5.8)
Therapies used in disease history, *n* (%)	
Systemic steroids	57 (83.8)
Topical Steroids	20 (29.4)
Topical antibiotics	3 (4.4)
Azathioprine	0
Mycophenolate mofetil	1 (1.4)
Methotrexate	1 (1.4)
Doxycycline	2 (2.9)
Dapsone	4 (5.8)
Rituximab	24 (35.2)

minimal therapy, and relapse were evaluated according to the consensus statement on the definitions of disease, endpoints, and the therapeutic response of the pemphigus.[9] Other outcome definitions used in this study are described below:

Complete remission during tapering is defined as the absence of new or established lesions while the patient was tapering therapy at that particular time point.

Partial remission during tapering is defined as the presence of transient new lesions that heal within 1 week while the patient was tapering therapy at that particular time point.

The patients were requested to complete the ABQOL and TABQOL questionnaires on day 0 and after 5–7 days for a 2nd time sent by post. Furthermore, patients also filled out other HQoL scales (the Dermatology Life Quality Index [DLQI], the Short Form-36 [SF-36], the Perceived Health Status [PHS], and the General Health Questionnaire [GHQ]-12), which are commonly used in dermatological diseases and have previously been validated in Turkish patients, on day 0 to evaluate their correlation with the ABQOL and TABQOL.[10-16]

The Dermatology Life Quality Index

The DLQI is the first quality of life scale developed for dermatological diseases. It contains ten questions in total and the scores range 0–30. High values show that the disease has significant influence on daily life regarding job, school life, leisure activities, and interpersonal relationships. The Turkish version was validated by Ozturkcan *et al*.[10]

The General Health Questionnaire-12 scale

The GHQ-12 has been developed by Goldberg and Hillier to define mental status in public and in primary health-care services.[11] Although the GHQ-12 was developed to detect general mental disorders, it contains questions evaluating basic symptoms of depression concerning enjoyment, sense of calm, distractibility, and sleeplessness.[12] The validity and reliability of the Turkish version was performed by Kilic *et al*. (Cronbach's alpha = 0.78).[13]

The Short Form-36

The SF-36 assesses HQoL and composed of 36 items in eight areas as follows: (1) limitations in physical activities, (2) limitations in social activities, (3) limitations in usual role activities, (4) bodily pain, (5) general mental health, (6) limitations in usual role activities, (7) vitality (energy and fatigue), and (8) general health perception. These scales are scored from 0 to 100 following a standard evaluation system.[14] The SF-36 questionnaire was translated into Turkish and validated by Kocyigit *et al*.[15] High scores suggest a better HQoL.[14,15]

Perceived Health Status

PHS is a Likert-type scale examining general health using a single question. In analyses, Likert scores are classified as 1, 2, and 3 ("worse than good") and 4 and 5 ("good").[16]

Objective disease severity was measured using the validated scores: Pemphigus Disease Area Index (PDAI) for pemphigus, Bullous Pemphigoid Disease Area Index (BPDAI) and BPDAI-pruritus for BP, Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) for pemphigus and pemphigoid, the visual analog scale-pruritus score for DH, Epidermolysis Bullosa Acquisita Disease Area Index for EBA.[9,17-22]

Table 2: Patient characteristics of autoimmune blistering diseases

AIBD	*n* (%)
PV	49 (72)
PF	3 (4.4)
BP	8 (11.7)
EBA	3 (4.4)
Dermatitis herpetiformis	5 (7.3)
Clinical stages, *n* (%)	
Complete remission during tapering	13 (19.1)
Complete remission on minimal therapy	14 (20.5)
Complete remission off therapy	11 (16.1)
Partial remission during tapering	2 (2.9)
Partial remission on minimal therapy	3 (4.4)
Partial remission off therapy	2 (2.9)
Relapse/flare	23 (33.8)
Total course of disease (months)	45.44±70.04
Duration of last clinical situation (weeks)	21.77±47.07
PDAI (*n*=52)	3.26±9.40
BPDAI (*n*=8)	15.42±10.16
BPDAI- *P* (*n*=8)	9.85±9.87
ABSIS (*n*=60)	4.88±8.49
VAS- pruritus (*n*=5)	1.20±2.68
EBADAI (*n*=3)	8.33±6.02
DLQI	0.41±0.69
PHS	3.44±0.92
GHQ-12	4.57±4.47
SF-36 physical functioning	57.19±25.93
SF-36 role-physical	48.07±43.37
SF-36 bodily pain	70.5±29.73
SF-36 general health	50.70±12.16
SF-36 vitality	54.07±15.17
SF-36 social functioning	57.92±25.92
SF-36 role-emotional	50.76±30.67
SF-36 mental health	55.32±12.50
ABQOL	17.70±8.94
TABQOL	18.78±9.08

ABQOL: Autoimmune Bullous Disease Quality of Life Questionnaire, TABQOL: Treatment of Autoimmune Bullous Disease Quality of Life Questionnaire, DLQI: Dermatology Life Quality Index, SF-36: Medical Outcome Study 36-item short-form questionnaire, GHQ-12: General Health Questionnaire, PHS: Perceived Health Status, PDAI: Pemphigus Disease Area Index, ABSIS: Autoimmune Bullous Skin Disorder Intensity Score, BPDAI: Bullous Pemphigoid Disease Area Index, BPDAI-p: Bullous Pemphigoid Disease Area Index-pruritus score, EBADAI: Epidermolysis Bullosa Acquisita Disease Area Index, VAS-pruritus: Visual Analog Scale-pruritus, AIBD: Autoimmune blistering diseases, PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, BP: Bullous pemphigoid, EBA: Epidermolysis bullosa acquisita

Statistics

The statistical analysis was carried out using R-3.5.1 and R-Studios 1.1.456.[23] $P < 0.05$ was used to assess the

significance for all statistical analyses. To define the sample, variables were expressed as mean ± standard deviation and categorical variables as the number and percentage. To determine the relationship between the two variables, the Pearson's correlation coefficient was used when the assumption of normality was provided and Spearman's ρ correlation coefficient was used when not. Intraclass correlation (ICC) was used to calculate internal consistency, and Cronbach's alpha was used to calculate test–retest reliability. The convergent validity of ABQOL and TABQOL was calculated using Pearson's correlation.

Table 3: Mean Autoimmune Bullous Disease Quality of Life Questionnaire and Treatment of Autoimmune Bullous Disease Quality of Life Questionnaire scores according to gender, clinical condition, and disease type

Variables	ABQOL	TABQOL
Sex		
Female	19.20±9.31	20.63±8.20
Male	14.82±7.57	15±9.81
Clinical condition		
Complete remission during tapering	16.76±8.32	20.36±7.17
Complete remission on minimal therapy	14.5±7.98	15.91±9.14
Complete remission off therapy	14.36±7.65	14.90±7.17
Partial remission during tapering	20.5±6.36	21±2.82
Partial remission on minimal therapy	14.33±9.01	18.66±6.65
Partial remission off therapy	34±14.14	22.5±2.12
Relapse/flare	20.68±8.67	21.2±11.36
Autoimmune blistering disease types		
Pemphigus vulgaris	17.16±8.97	18.25±8.78
Pemphigus foliaceus	12.66±3.05	17.5±7.7
Bullous pemphigoid	19.14±10.41	19.5±12.62
EBA	21.33±2.08	24±2.64
Dermatitis Herpetiformis	21.8±11.32	21.33±14.29

EBA: Epidermolysis bullosa acquisita, ABQOL: Autoimmune Bullous Disease Quality of Life Questionnaire, TABQOL: Treatment of Autoimmune Bullous Disease Quality of Life Questionnaire

Results

A total of 68 patients with AIBDs were recruited between February 2017 and February 2018. A subset of 20 (29.4%) patients completed the day 7 questionnaire. Of the 68 patients recruited, 24 were men and 44 were women. Patients' ages ranged from 23 to 83 years, with a mean age of 51.15 ± 13.48 years. Other patient characteristics are shown in Table 1.

Most of the patients had PV (n = 49, 72%), followed by BP (n = 8, 11.7%), DH (n = 5, 7.3%), PF (n = 3, 4.4%), and EBA (n = 3, 4.4%). The mean disease duration of all patients was 45.44 ± 70.04 months. Most of the patients were in complete or partial remission (PDAI and BPDAI <5) and even patients with relapses were mild as they had minor relapses. The mean ABQOL score and TABQOL score for all patients were 17.70 ± 8.94 and 18.78 ± 9.08, respectively. Other AIBD characteristics of patients are shown in Table 2. The mean ABQOL and TABQOL scores according to gender, clinical

Table 4: Mean values of quality of life questionnaires and patients' characteristics according to different blistering disease types

	A: Suprabasal blistering diseases (PV, PF)	B: Subepidermal blistering diseases (BP, EBA)	C: Others (DH)	*P*, A-B-C
Age	50.1	55.2	52.2	0.796
Sex	0.3	0.4	0.6	0.322
Income level	2.2	2.1	2.6	0.379
Educational level	2.4	2.5	1.8	0.648
Marital status	2.1	2.1	1.6	0.086
Concomitant diseases	0.2	0.4	0.6	0.069
DLQI	0.2	0.9	0.7	0.062
PHS	3.5	3.0	3.4	0.465
GHQ-12	4.5	4.2	5.2	0.966
SF-36 physical functioning	58.4	58.6	37.5	0.508
SF-36 role-physical	49	45.4	43.7	0.931
SF-36 bodily pain	71.2	69.3	65	0.973
SF-36 general health	51.9	47.7	43.7	0.671
SF-36 vitality	54.4	50.4	60	0.533
SF-36 social functioning	56.6	65.4	53.1	0.563
SF-36 role-emotional	52	48.4	41.6	0.730
SF-36 mental health	53.6	60.3	62	0.240
ABQOL	16.9	19.8	21.8	0.456
TABQOL	18.2	21	21.3	0.591

ABQOL: Autoimmune Bullous Disease Quality of Life Questionnaire, TABQOL: Treatment of Autoimmune Bullous Disease Quality of Life Questionnaire, DLQI: Dermatology Life Quality Index, SF-36: Medical Outcome Study 36-item short-form questionnaire, GHQ-12: General Health Questionnaire, PHS: Perceived Health Status, PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, BP: Bullous Pemphigoid, EBA: Epidermolysis bullosa acquisita, DH: Dermatitis herpetiformis

Table 5: Correlation between Autoimmune Bullous Disease Quality of Life Questionnaire and Treatment of Autoimmune Bullous Disease Quality of Life Questionnaire L and clinical parameters of patients

Variables	ABQOL		TABQOL	
	P	*r*	*P*	*r*
Age	0.099	−0.203	0.692	−0.052
Sex[&]	0.057	−0.235	0.022	−0.029
Total course of disease	0.555	0.073	0.953	−0.008
Clinical stage	0.110	0.197	0.037	0.268
Duration of last clinical condition[¥]	0.032	−0.263	0.019	−0.299
Marital Status	0.764	−0.037	0.440	0.101
Education level	0.923	0.012	0.686	0.052
Concomitant diseases	0.484	−0.087	0.803	−0.033
Income level	0.069	0.224	0.470	−0.094
PDAI	0.143	0.204	0.0004	0.482
ABSIS	0.052	0.238	0.002	0.387
BPDAI*	0.823	−0.115	0.925	0.059
BPDAI-p*	0.965	0.023	0.844	0.123
VAS-Pruritus*	0.104	0.799	0.204	0.949
EBADAI*	0.118	0.983	0.273	−0.909

*There were limited number of patients in these groups. Thus, analysis of them is not very valid, [&]Female patients had higher ABQOL and TABQOL scores, [¥]Recent changes in clinical condition of patients has significant effect to TABQOL scores. Short duration is correlated with higher scores. Pearson's correlation was used to get the *P* value and correlation value. ABQOL: Autoimmune Bullous Disease Quality of Life Questionnaire, TABQOL: Treatment of Autoimmune Bullous Disease Quality of Life Questionnaire, PDAI: Pemphigus Disease Area Index., ABSIS: Autoimmune Bullous Skin Disorder Intensity Score, BPDAI: Bullous Pemphigoid Disease Area Index, BPDAI-p: Bullous Pemphigoid Disease Area Index-pruritus score, EBADAI: Epidermolysis Bullosa Acquisita Disease Area Index, VAS-pruritus: Visual analog scale-pruritus

condition (outcome), and disease type are shown in detail in Table 3.

Both the Turkish versions of the ABQOL and TABQOL questionnaire have a high internal consistency (Cronbach's alpha coefficient 0.88 for TABQOL and 0.86 for ABQOL) and test–retest reliability (the ICC coefficient 0.872 for ABQOL and 0.879 for TABQOL). The correlation between ABQOL and TABQOL (total scores) is Pearson's $R = 0.609$.

When we examined the mean values of quality of life questionnaires and patients' characteristics according to different blistering disease types, there was no significant difference among the parameters shown in Table 4.

In terms of a correlation between ABQOL and TABQOL and clinical parameters of the patients, it was shown that ABQOL and TABQOL scores were reversely correlated with the duration of that clinical stage. On the other hand, TABQOL scores were directly correlated with PDAI and ABSIS. However, it was also shown that increased TABQOL scores were found in women and patients with partial remission and relapse [Table 5].

When we evaluated the mean values of quality of life questionnaires and patients' characteristics according to different stages of disease, only DLQI was shown to be significantly different among groups (0.017) [Table 6]. On the other hand, evaluation of the mean values of quality of life questionnaires and patients' characteristics according to different stages of therapy showed that DLQI and PHS were significantly changed among groups (both $P = 0.02$) [Table 7].

Discussion

In this study, we validated the Turkish version of the disease-specific HQoL instruments, namely ABQOL and TABQOL, and assessed them in the Turkish population. Our results showed high internal consistencies of ABQOL and TABQOL with a Cronbach's alpha of 0.86 and 0.88, respectively. Cronbach's alpha of above 0.70 is ideal to examine the reliability of patient-reported measures for internal consistency of a questionnaire.[24] Our results were not only above the ideal 0.70 but also similar to previous research results, showing high internal consistencies.[25-28] In terms of test–retest reliability, the intraclass correlation coefficient was 0.872 for ABQOL and 0.879 for TABQOL. The correlation between ABQOL and TABQOL (total scores) was Pearson's $R = 0.609$. Thus, the Turkish versions of ABQOL and TABQOL questionnaires have been shown to be valid and reliable.

The highest ABQOL and TABQOL scores belonged to patients with EBA and DH. This was followed by patients with BP and then patients with PV. The lowest ABQOL and TABQOL scores belonged to patients with PF [Table 3]. These results could be related to severe itch symptoms, especially seen with EBA and DH, and a chronic course of these two diseases without good therapeutic options as recently PV, PF, and BP can be under control more effectively.

In terms of clinical condition and ABQOL-TABQOL scores, it was shown that patients with partial remission off therapy had the highest ABQOL and TABQOL scores [Table 3]. This was followed by patients with relapse and then patients with partial remission during tapering. Although patients with relapses were expected to have the highest scores, our result could be due to the anxiety and fear in patients who experience new lesions when they are off therapy, described as partial remission off therapy. As expected, patients with complete remission had lower ABQOL and TABQOL scores [Table 3].

There was no significant difference among the mean values of the quality of life questionnaires and the patients' characteristics. This result suggests the idea that the existence of AIBD is the main burden on one's quality of life, and this does not significantly change due to social and environmental factors, such as income level or educational level. However, TABQOL and ABQOL scores were found to be higher in women than in men [Table 5].

In terms of any correlation between ABQOL and TABQOL and the clinical parameters of patients, it was shown that ABQOL and TABQOL scores were reversely correlated with the duration of the last clinical stage. This could be due to psychological disturbance of patients regarding disease activity

Table 6: Mean values of quality of life questionnaires according to different stages of disease

	Patients within tapering of therapy	Patient without any therapy or with minimal therapy	Patient with relapses	*P*
Age	48.47	52.76	52	0.33
Sex	0.43	0.33	0.26	0.54
Income level	2.34	2.20	2.33	0.73
Educational level	2.60	2.13	2.80	0.37
Marital status	1.91	2.33	2.06	0.07
Concomitant diseases	0.26	0.33	0.20	0.62
DLQI	0.81	0.20	0.24	0.02
PHS	3.08	3.80	3.26	0.02
GHQ-12, mean	6.30	3.66	3.73	0.20
SF-36 physical functioning	57.60	55.17	60.71	0.76
SF-36 role-physical	50	57.75	25	0.08
SF-36 bodily pain	58.18	78.18	73.92	0.06
SF-36 general health	45.83	53.16	53.27	0.10
SF-36 vitality	54.77	52.24	56.78	0.55
SF-36 social functioning	57.27	56.98	60.89	0.90
SF-36 role-emotional	43.93	56.32	50	0.33
SF-36 mental health	57.81	54.06	54	0.55
ABQOL	20.68	15.73	17.26	0.18
TABQOL	21.20	16.28	20.46	0.22

ABQOL: Autoimmune Bullous Disease Quality of Life Questionnaire, TABQOL: Treatment of Autoimmune Bullous Disease Quality of Life Questionnaire, DLQI: Dermatology Life Quality Index, SF-36: Medical Outcome Study 36-item short-form questionnaire, GHQ-12: General Health Questionnaire, PHS: Perceived Health Status

Table 7: Mean values of quality of life questionnaires according to different stages of therapy

Variables	Group 1: Complete remission (off therapy/minimal therapy or during tapering)	Group 2: Partial remission (off therapy/minimal therapy or during tapering)	Group 3: Patient with relapses	*P*, Group 1-2-3
Age	52.39	53.14	48.47	0.32
Sex	0.34	0.14	0.43	0.36
Income level	2.26	2.14	2.34	0.67
Educational level	2.42	2	2.60	0.64
Marital status	2.26	2.14	1.91	0.09
Concomitant diseases	0.31	0.14	0.26	0.63
DLQI	0.17	0.46	0.81	0.017
PHS	3.65	3.42	3.08	0.09
GHQ-12	3.55	4.42	6.30	0.18
SF-36 physical functioning	57.22	55.71	57.60	0.86
SF-36 role-physical	48.61	39.28	50	0.79
SF-36 bodily pain	77.15	75	58.18	0.08
SF-36 general health	53.93	49.40	45.83	0.07
SF-36 vitality	53.33	55.71	54.77	0.92
SF-36 social functioning	58.26	58.21	57.27	0.98
SF-36 role-emotional	54.62	52.38	43.93	0.36
SF-36 mental health	54.33	52.57	57.81	0.56
ABQOL	15.23	21.71	20.68	0.57
TABQOL	17.02	20.42	21.20	0.37

ABQOL: Autoimmune Bullous Disease Quality of Life Questionnaire, TABQOL: Treatment of Autoimmune Bullous Disease Quality of Life Questionnaire, DLQI: Dermatology Life Quality Index, SF-36: Medical Outcome Study 36-item short-form questionnaire, GHQ-12: General Health Questionnaire, PHS: Perceived Health Status

changes causing decrease in patients' quality of life in the early stages of the change [Table 5].

The cutoff values described by Boulard *et al*. suggested for PDAI are 15 and 45 and 17 and 53 for ABSIS, distinguishing moderate, significant, and extensive pemphigus forms.[29] The mean PDAI in our patient group was 3.26 ± 9.40 and the mean ABSIS was 4.88 ± 8.49, showing that our patient group was mainly consistent with moderate disease activity. Therefore, the mean values of disease severity scores were smaller than

the previous studies examining the same topic.[25-28] This could be the reason why we could not find a correlation between ABQOL and disease severity scores although TABQOL scores were directly correlated with PDAI and ABSIS [Table 5]. In the Greek study, it was shown that ABQOL is significantly correlated with PDAI, ABSIS, and BPDAI. This could be due to high disease activity of their patient group (mean PDAI was 35.8 ± 32.3 and mean ABSIS was 19.4 ± 10.92).[27] Similar results were also found in a Polish study.[26]

Until recent years, dermatology-specific HQoL instruments were used for monitoring disease activity and evaluating the effectiveness of care in AIBDs. The SF-36 and DLQI have shown a significant decrease in quality of life of patients with AIBDs. Paradisi *et al*. found that patients with pemphigus had a significantly impaired overall quality of life compared with healthy subjects.[30] A high prevalence of psychiatric comorbidity was also observed in pemphigus patients.[31] The SF-36, DLQI, and GHQs have been used to monitor the HQoL and psychological status of patients with PV.[32-34] The patients in this study cohort had a range of AIBD across a range of disease stages. However, most of the patients had low disease activity scores as most of them were followed for a long time in our clinic. Only the DLQI was shown to be significantly different among groups ($P = 0.017$) when we evaluated the mean values of quality of life questionnaires and patients' characteristics according to different stages of disease [Table 6]. Moreover, evaluation of the mean values of quality of life questionnaires and patients' characteristics according to different stages of therapy showed that DLQI and PHS were significantly changed among groups (both $P = 0.02$) [Table 6]. The reason that we have not found significant differences in the ABQOL and TABQOL between different stages of disease and different stages of therapy could be due to a lack of significant difference between disease activity scores in these subgroups. Furthermore, the HQoL burden is often thought to be independent of objective disease burden and clinical severity.

ABQOL was shown to have advantages in AIBD patients over the generic HQoL instruments (DLQI, SF-36, and GHQ) and can be a promising patient-based measure for evaluating disease burden, monitoring disease activity, and examining the response to therapeutic intervention.[25]

The reason for finding a significant correlation between TABQOL and PDAI and ABSIS but not with ABQOL in our study could be due to the fact that HQoL depends on the effects of treatment (often long-term and with the risk of serious adverse events). AIBD treatments have an adverse impact on HQoL by causing a greater morbidity, complications arising from these treatments, and low compliance with medical recommendations. These correlations suggest that the impact of AIBD and AIBD treatment presents a similar level of impairment in QOL.[35]

Limitations

The limitation of our study is the small numbers of patients with BP, PF, EBA, and DH and most of our patients had low disease activity scores making hard to evaluate the correlation of ABQOL and TABQOL scores with disease activity and different stages of diseases. This could be the case because the study was conducted by a single university center. However, the incidence of these disorders, especially for BP, is also low in the Turkish population compared with Western countries such as USA and European.

Conclusions

The creation of a standardized disease-specific outcome measure, such as the ABQOL and TABQOL, is important to allow comparisons between different research studies.[36] Turkish ABQOL and TABQOL questionnaires can be used as clinical evaluation tools in daily routine and/or outcome measures for clinical trials to establish better analysis of treatments for AIBD in Turkey.

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Conflicts of interest

There are no conflicts of interest.

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Bleomycin Therapy Using Multipuncture Technique for Resistant Warts

Sir,

The case report titled "Successful Treatment of a Resistant Periungual Wart Case with Bleopuncture Method" by Yalçın *et al*. in the Turkish Journal of Dermatology (Turk J Dermatol 2018; 12: 191–3) recalled an often-ignored bleomycin therapy in the treatment of resistant warts.[1]

Cutaneous warts sometimes fail to respond to routine treatments (cryotherapy, electrosurgery, topical salicylic acid, fluorouracil, potassium hydroxide, and retinoid treatments and combinations). In these cases, bleomycin therapy has been a treatment option which has been in the literature for a long time, but is not preferred too frequently.

Bleomycin is considered as a third-line therapy for resistant warts and has been regarded as level 1 strength of evidence for the treatment of warts.[2] It is obtained from "*Streptomyces verticillus*" and shows an inhibitory effect on the virus and the host cell by inhibiting DNA and protein synthesis. In the treatment of warts, two application methods were noteworthy until recent years. First, intralesional bleomycin (IL Bleo) therapy has been shown to be a very effective treatment method (success rates vary between 14% and 99%), but it is not often preferred because of its rare however scary side effects (pain, chemical cellulitis, postinflammatory hyperpigmentation, Raynaud's phenomenon, tissue necrosis, onychodystrophy, and flagellate hyperpigmentation).[3]

These side effects can be largely preserved by the application of bleomycin utilizing "multipuncture" technique. In our case report and literature review published in 2016, we reviewed the efficacy of bleomycin using "multipuncture" technique in the treatment of warts.[4] According to our literature review, 74%–100% cure rates were obtained for palmoplantar and periungual warts which are resistant to other topical therapies. It was applied in the range of 0.1–3 U/ml in 2–4-week intervals. No significant adverse effects were observed during and after the procedure except for the local pain.[4-9] In our case report, a 56-year-old female with a 3-year history of plantar warts (previously treated with salicylic acid, fluorouracil, and cryotherapy without any success) was treated with multipuncture bleomycin therapy.[4] A concentration of 3 U/mL bleomycin was applied with occlusion after prickling the warts, and it was kept on the warts with a stretch film for 12 h. The procedure was repeated 4 times with 2-week intervals. At the end of 2 months, complete resolution was achieved.[4]

Bleomycin can be found in 15 and 30 mg vials in our country and its cost varies between 30 and 80 TL. The most preferred procedure for multipuncture technique was to prick the wart through the intradermal area with a sterile needle followed by preparing a 1 U/mL bleomycin in saline solution (a vial containing 15 units of bleomycin and 15 mL of normal saline solution is mixed to achieve a concentration of 1 U/mL). One unit is equivalent to the activity of 1 mg of bleomycin. After that, this bleomycin solution dropped to the area or on the gauze and then closed on the area for a few hours with sterile gauze.[1,4-9] A small number of recurrences were observed in 6–18-month follow-up periods in the treated warts[6,8,9] [Table 1].

Furthermore, in recent years, there have been small studies evaluating the efficacy and safety of topical bleomycin therapy applied by coated microneedles or combined with microneedling, electroporation, or ablative laser therapy.[10-15] In some of them, researchers even compared it to IL Bleo, intralesional saline, or cryotherapy.[13-15] First, Konicke and Olasz presented three patients with warts who were cured with microneedling combined with topical application of 1 U/mL bleomycin (MN + Bleo). All patients were cured after an average of four treatments performed in every 2–4 weeks, and no recurrence was seen in 3–5 months' time.[10]

Moreover, in an innovative work, Lee *et al*. created bleomycin-coated microneedles and studied the mechanical properties and drug delivery properties of them. They demonstrated that microneedles delivered bleomycin successfully into the subepidermal skin layer of warts and more than 80% of the bleomycin dissolved into the skin *in vitro* within 15 min. To conclude, they suggested that bleomycin-tip-coated microneedles are an effective, easy, and painless way for wart treatment.[11] Suh *et al*. used a different method and they treated warts with bleomycin application (1 U/mL) after ablative carbon dioxide fractional laser (three passes of laser with single-pulse treatment parameters of 180 mJ pulse energy and 100 spots/cm^2 density in the static mode). Seventeen patients with a total of 38 warts were successfully treated at every 2 weeks with six consecutive sessions of this protocol.[12] Ryu *et al*. conducted a comparative study to evaluate the therapeutic effects of newly developed bleomycin microneedle patch regards to cryotherapy. They recruited 42 patients with more than two wart lesions in each and the lesions were treated by one of the above-mentioned methods randomly. Their study demonstrated 76.2% clearance rate for cryotherapy and 61.9% clearance rate for the bleomycin microneedle patch at week 16. This new therapeutic method was found to be an effective, convenient, and painless treatment modality when compared with cryotherapy.[13] In another study, Al-Naggar *et al*. compared IL Bleo (with a single injection of 1 U/mL bleomycin) and microneedling with topical spraying of bleomycin (MN + Bleo) performed every

Table 1: Multipuncture bleomycin therapy for warts are summarized

Study-case report, years	Method	Number of patients/ warts	Location of warts	Range of application (interval/number)	Concentration of bleomycin (amount used)	Duration of follow-up (months)	Complete clearance (%)	Side effects
Shelley and Shelley, 1991[5]	A bifurcated vaccination needle, 40 times per 5 mm^2, dry dressing for 24 h	66/258	Palms, soles, dorsal aspect of hands and feet, forearms, face, penis, knees, paronychial areas	-/1 once	1 U/ml	6	92	Local pain
Munn *et al.*, 1996[6]	Topical lidocaine for 1 h, monolete needle	62/not given	Palmar, plantar, periungual	4 weeks/4 times	1 U/ml	12	92	Local pain
Sardana *et al.*, 2010[7]	26-gauge hypodermic needle, occlusion for 2 h	1/1	Periungual-subungual	4 weeks/5 times	1U/ml (2 ml)	18	100	Local pain, inflammation, eschar formation
AlGhamdi and Khurram, 2011[8]	Intralesional lidocaine 2% for 1 h, 27-gauge needle, topical antibiotic, applied simple dressing	15/15	Periungual	4 weeks/one or two times	0.1 U/ml (1 ml)	6	86.6	Local pain, mild hyperpigmentation
AlGhamdi and Khurram, 2012[9]	Intralesional lidocaine 2% for 1 h, 27-gauge needle, topical antibiotic, applied simple dressing	23/23	Plantar	4 weeks/one or two times	0.1 U/ml (1 ml)	6	74	Local pain
Temel and Akman-Karakas, 2016[4]	26-gauge needle, occlusion for 12 h	1/3	Plantar	2 weeks/4 times	3 U/ml (1 ml)	18	100	Local pain
Yalçın *et al.*, 2018[1]	Topical lidocaine-prilocaine occlusion for 2 h, multiple pricks with lancet, occlusion for 2 h after bleomycin	1/5	Periungual	-/1	1 U/ml	1	100	None

Concentration of bleomycin: 1 U/ml concentration of bleomycin describes that a vial containing 15 units of bleomycin and 15 mL of normal saline solution is mixed to achieve a concentration of 1 U/mL

2 weeks for a maximum of four sessions. Sixty patients were recruited to the study and divided into two groups equally. They presented 83.3% complete clearance rate of warts in the MN + Bleo group compared to 70% in the IL Bleo group.[14] Gamil *et al*. recently published another comparative study where 54 patients were divided into three groups (18 patients each). The first group was treated by dermapen with topical bleomycin (1 mg/1 mL) for a maximum of four sessions at 2-week intervals. On the other hand, the second group received IL Bleo (1 unit/mL) for a maximum of four sessions at 3-week intervals, and the control group was intralesional saline for a maximum of four sessions. Complete clearance was found to be highest in Group 1 (88.9%) as opposed to 83.3% in Group 2 and 5.6% in the control group.[15]

In the recent case of Yalçın *et al*., a single-session multipuncture method was used to successfully treat periungual warts.[1] As is seen in these case reports and the literature, multipuncture technique is a safe, effective, and well-tolerated treatment. Thus, it should be included in our preferences as its use is easy to apply, it has good results and comfortable for patients, and it keeps the cost low for both the patient and the health system. In the following years, multipuncture bleomycin therapy can take part in the higher step in the treatment algorithm of recalcitrant warts.

However, there is a need for prospective controlled studies to determine the most appropriate frequency and dosage of the method, standardization of the application, and the side effect profile.

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Conflicts of interest

There are no conflicts of interest.

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