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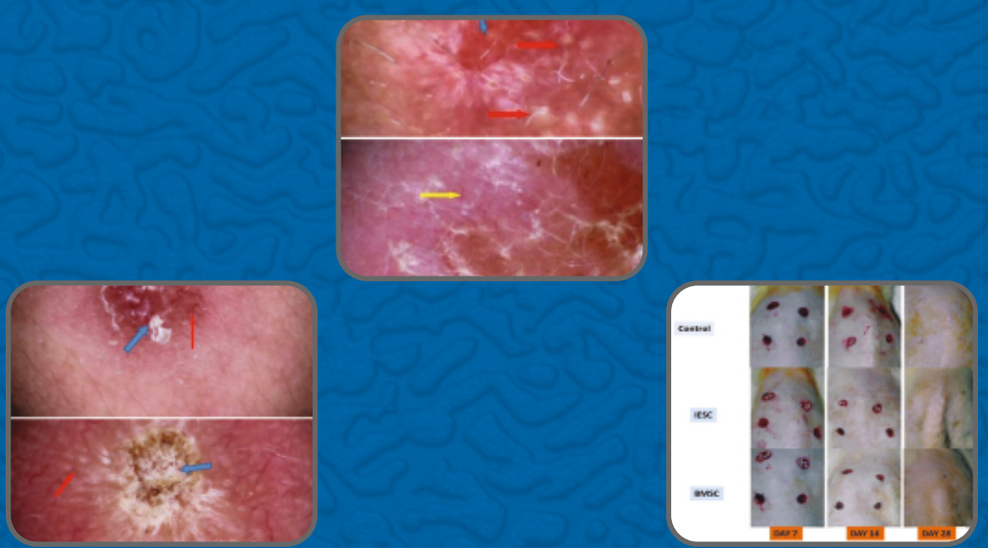

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Turkish Journal of Dermatology

Volume No. 13 Issue No. 3 July-September 2019

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Original Articles

Original Article

Where are We in Cosmetics and Esthetics Practices in Educational Clinics in Turkey?

Ezgi Özkur, Emre Kaynak[1], Mehmet Salih Gürel[2]

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Abstract

Objective: Cosmetic dermatology has recently gained importance with the recent increase in demand. The aim of this study is to identify cosmetic dermatology training in Turkey and to assess instructors' attitudes toward cosmetic dermatology training during residency and current cosmetic dermatology practices. **Methods:** This is a cross-sectional questionnaire study conducted with instructors to investigate the practice of cosmetics dermatology, the technical equipments, number of patients, and their ideas in the clinics that provide dermatology specialty education in Turkey. **Results:** At least one cosmetic procedure was found to be performed in 69% of the clinics that participated in the study (n = 55), and no cosmetic procedure was performed in 31% of the clinics. The mean number of application was 13.2 ± 12.3 weekly in the clinics that performed cosmetics procedure. The most common procedures were botulinum toxin injection with 63.6% (n = 35), chemical peeling with 60% (n = 33), and platelet-rich plasma with 60% (n = 33), respectively. The most common laser application was neodymium-doped yttrium aluminum garnet (28/55). The mean time spent for cosmetic procedures was 1–5 h weekly in the clinics which performed cosmetic procedures. Seventy-six percent (n = 42) of the participants felt inadequate for performing cosmetics procedures, and 95% (n = 52) reported that cosmetic dermatology education was required. **Conclusions:** Both theoretical and practical education must be given in educational clinics, and the infrastructure must be created, and the educational schedule must be standardized.

Keywords: Cosmetics, dermatology, education

Introduction

Specialty education is an organized education program with theoretical and practice studies of residents. Medical faculty graduates can study dermatology in Turkey after passing the medical specialty examination (MSE) which is held twice annually, and dermatology specialty education lasts 4 years in university hospitals, and training and research hospitals hospitals affiliated to the Turkish Ministry of Health. There has recently been an increasing interest on dermatology, and the mean MSE scores required for entering the dermatology specialty are higher than all other specialties. One of the reasons of this is the increased demand for esthetic and cosmetic procedures.

Dermatologists have contributed significantly to the evolution of cosmetic and esthetics dermatology including laser treatments, dermabrasion, botulinum toxin, chemical peeling, hair transplantation, and soft-tissue augmentation. Aspects of cosmetic dermatology include the maintenance of healthy skin, the prevention and treatment of skin aging and photodamage, and rejuvenation procedures. To continue as leaders in the safe performance of cosmetic dermatology procedures, future dermatologists must be properly trained. Moreover, dermatologists who received no cosmetic education are less preferred in private hospitals and clinics, and they may feel unqualified. Furthermore, if a training gap exists, this may adversely affect patient safety.

Moreover, there are many dermatological diseases which could be treated with cosmetic procedures. These indications are laser procedures for rosacea, infectious disorders (mostly

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papillomavirus lesions), the removal of hairs and tattoos, vitiligo, vascular lesions, cutaneous scarring, some skin tumors, postlesional hyperpigmentation and melasma, mesotherapy or platelet-rich plasma (PRP) for androgenetic alopecia, botulinum toxin injection for hyperhidrosis, as well as chemical peeling, Dermapen, and dermaroller treatments for acne scars and hyperpigmentation.[1-4] So that, cosmetic dermatology must not be apart from traditional dermatology education but should be a part of it. In the present study, we aimed to investigate the cosmetic dermatology training during residency and the current cosmetic dermatology practices in the clinics which provide dermatology specialty education in Turkey.

Methods

The present research is a cross-sectional descriptive questionnaire study. A questionnaire was prepared to investigate the cosmetic dermatology education and practices in dermatology education clinics including the detailed information about the cosmetic procedures performed in the clinics, the number of patients presenting for cosmetic procedures, technical equipments, the weekly time spent for cosmetic dermatology, cosmetic dermatology education, and the personal competency. The questionnaire was performed on February 2019 to the instructors who participated to general council meeting about education in dermatology of the Turkish Society of Dermatology. Participants who did not work in clinics which provide dermatology specialty education were excluded, and only one instructor was included from each clinic. The opinions of the participants were measured with their yes–no responses to the questions whether cosmetic procedures were performed (botulinum toxin, filler, etc.) in the clinic, personal competency, and whether the cosmetic education was required. The number of patients applying for these procedures and the monthly number of cosmetic procedures performed in the clinic were measured as the numerical data.

Data were analyzed using IBM SPSS 15.0 for Windows v.21.0. (IBM Corp., Armonk, NY). Number and percentage were given for descriptive statistics and categorical variables; and the mean, standard deviation, minimum, and maximum were given for numerical variables. The ethics board approval was granted.

Results

The study included the 75% ($n = 55$) of the total 73 clinics that provide dermatology specialty education in Turkey. We found that 76% ($n = 42$) of the participant clinics were from university hospitals, and 24% ($n = 13$) were from education and training hospitals. Sixty-nine percent ($n = 38$) of participants reported that cosmetic procedures were performed in their clinics; however, 31% ($n = 17$) of participants reported that no cosmetic procedure was performed in their clinics. The mean number of patients applying for cosmetic procedure was detected as 13.2 ± 12.3 in the cosmetic procedure performing clinics.

The percentages of cosmetic procedures performed among all participated training clinics are demonstrated in Figure 1. The most common procedures were botulinum toxin in 63.6% ($n = 35$), chemical peeling in 60% ($n = 33$), and PRP in 60% ($n = 33$), respectively. The least common procedures were the thread lift and pulsed dye laser procedures which were only performed in a total of 6 clinics (10.9%). The mean number of applications to these clinics is demonstrated in Table 1. Neodymium-doped yttrium aluminum garnet (Nd:YAG) laser device was detected in 28 clinics, carbon dioxide in 10 clinics, erbium YAG in 8 clinics, pulsed dye in 6 clinics, diode in 2 clinics, KTP in 2 clinics, alexandrite in 1 clinic, and Q-switched nd:YAG device was detected in 1 clinic.

We found that 90% ($n = 34$) of the cosmetic procedure performing clinics obtained the patient consent forms from their patients. Twenty-two percent ($n = 12$) of the participants claimed that they never participated to cosmetic education courses, meeting, or congress. A mean weekly time spent for these procedures was <5 h in 68% ($n = 26$) of cosmetic procedure performing education clinics. Seventy-six ($n = 42$) of the participants felt themselves unqualified for performing cosmetic procedures, and 95% ($n = 52$) of the participants suggested that cosmetic dermatology education was required in dermatology education [Table 2].

Discussion

In recent times, there has been an increasing demand for noninvasive cosmetic procedures worldwide. So-called noninvasive cosmetic procedures are botulinum toxin injection, filler, PRP, mesotherapy, dermaroller, dermapen, thread lift, and laser methods. A survey with 561 primary care physicians identified dermatologists as the most qualified specialists to inject botulinum toxin and fillers and to perform laser procedures.[5] Since the procedures are performed on the skin, and on subcutaneous tissues, dermatologists are the primary professionals in practice, also in the determination of the doses, monitoring of the effect, and in the management of the complications compared with the other branches. Şavk reported

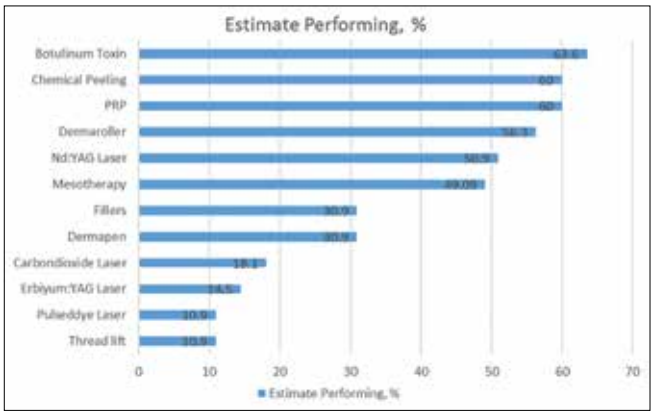

Figure 1: The percentages of cosmetic procedures performed among all participated training clinics in Turkey

the European Union of Medical Specialists (UEMS) as the most authorized and effective international politic organ for all medical practices including the medical specialty education in Europe, in her article in 2012.[6] UEMS evaluates the cosmetic dermatology within the scope of the dermatology branch. Furthermore, dermatocosmetology is described in the "important learning area" for the residents in the Core Learning Curriculum in Turkey.

There are 73 education clinics which provide dermatology specialty education in Turkey; yet, there is no standardized curriculum for cosmetic dermatology education, and there are differences between the clinics regarding the technical equipment, practitioner or auxiliary staff, and education hours. Residents evaluated the importance of cosmetics education as 4.33 in 5 score system in a cross-sectional questionnaire study of Yılmaz and Akkaya[7] conducted with 67 residents in 2006 in Turkey. In the same study, they reported the adequacy of cosmetic dermatology education as 1.83 (mean) out of 5 in their own clinics. Cengiz *et al*.[2] conducted a survey with 121 Turkish residents in 2014, and they found that 73 (60.3%) residents could not learn the cosmetic procedures in their clinics. In line with these studies, a study by Freiman *et al*. was conducted with 48 dermatology residents across Canada and reported that residents were least satisfied with cosmetic dermatology training in their residency program (2.7 of 5.0).[8]

The importance of cosmetic dermatology in dermatology specialty has also been increasing worldwide. Interestingly, Kiafar *et al*. reported that professors and program directors predicted residents' first priority would be practicing cosmetic dermatology and 41 (60.3%) of them agreed or strongly agreed that residents' desire to learn more about cosmetic procedures resulted in their decreased interest in learning medical procedures.[9] Although there is a concern in the questionnaire studies that cosmetic dermatology will be more popular than the medical dermatology, the requirement for adequate education was suggested in both foreign studies, and in our study.[10,11] Almost all studies conducted with education clinics and residents on this topic are reported from the USA. The most important reason is that cosmetic dermatology has a significant market share in the USA. A questionnaire study was conducted in 2013 with the administrators of the clinics providing dermatology specialty education in the USA and authors reported that 63% (20 participants of 32) of the administrators suggested that cosmetic dermatology education must be "compulsory."[12] The same study reported that theoretical education on liposuction, laser, filler, botulinum toxin injection, chemical peeling, dermabrasion, scar revision, and sclerotherapy was given in 67% of the clinics. Champlain *et al*. reported in their study conducted in the USA in 2018 that 90% of dermatology residents indicated that practice was more important than theoretical education in cosmetic dermatology education. The same study reported that 244 residents of 268 (91%) had the opportunity to perform hands-on cosmetic procedures by themselves.[13]

The most frequently performed cosmetic procedure was detected as the botulinum toxin injection (63.6%) among all clinics that participated in our study. The study of Kirby *et al*. reported 95% of 73 dermatology residents had the opportunity to perform botulinum toxin injection in their clinics in the USA. The practice rates for the laser procedures was reported as 97%, for filler was 85%, for chemical peeling was 85%, and the rate for sclerotherapy was 71% in the study.[12] In another study conducted with 3rd-year dermatology residents from the University of Texas Medical Faculty, authors reported that 75 (63.6%) trainees felt comfortable performing botulinum toxin injections, 55 (47%) were comfortable performing laser

Table 1: The weekly number of patients applying to education clinics which perform cosmetic procedure in Turkey in accordance with the cosmetic procedure type

	Median±SD	Minimum-maximum
Botulinum toxin	11.7±18.8	1-80
PRP	8.5±9.2	1-35
Nd:YAG laser	8.3±10.5	1-40
Dermaroller	7.3±12.1	1-50
Mesotherapy	7.2±10.0	1-50
Dermapen	5.6±7.5	1-25
Erbium YAG laser	5.4±12.0	1-40
Carbon dioxide laser	5.3±8.5	1-25
Filler	3.8±7.2	1-25
Pulsed dye laser	3.6±9.2	1-30
Thread lift	1.7±2.9	1-10

PRP: Platelet-rich plasma, SD: Standard deviation, Nd:YAG: Neodymium-doped yttrium aluminum garnet

Table 2: Opinions of the participants about the cosmetic dermatology practice, competency, and education

	n (%)
How frequently do you participate to cosmetic education courses (congress courses, company courses) in a year?	
Never	12 (22.2)
1-2	33 (61.1)
3-4	9 (16.7)
How many hours is spent for cosmetic patients in a week in the working order of your clinic?*	
1-5	26 (68.4)
6-9	5 (13.1)
10-25	5 (13.1)
25-40	2 (5.2)
I generally feel to have adequate competency for cosmetic procedures	
Yes	13 (23.7)
No	42 (76.3)
I generally feel to have adequate competency in coping with the possible complications after cosmetic procedures	
Yes	15 (27.3)
No	40 (72.7)
I think cosmetic practices/education are required in educational clinics and in the universities	
Yes	52 (94.6)
No	3 (5.4)

*For the clinics performing cosmetic procedures

surgery, 47 (39.8%) were comfortable performing chemical peels, and 37 (31.4%) were also comfortable performing fillers.[14] On the other hand, Plee *et al*. reported that only 3% of residents had a cosmetic dermatology education during residency, and authors underlined the need for greater focus cosmetic dermatology in France between 2005 and 2010.[15]

The most frequently used laser device in our study was the nd:YAG laser which was used in nearly half of the clinics. Bauer *et al*. reported that the most frequently used laser was pulsed dye (79%), second Q-switched nd:YAG (58%), and the third most frequently used was the fractional carbon dioxide (38%) in their study which they conducted with dermatology education clinics in the USA.[16] The comparison of the education clinics in Turkey with the education clinics in the USA showed that both the cosmetic procedures, and the diversity of the laser devices, and practices were inadequate in Turkey. This difference may attribute to the lack of financial resources, inadequate number of personnel, and problems in pricing. In addition, we found that 76% (n = 42) of the instructors felt themselves unqualified, and the number of patients applying to the clinics was low in general. In this step, "training of the instructor" must also be emphasized. Dermatology Post Specialty Education Board, that serves within the body of the Turkish Society of Dermatology, organized the first "Practical Cosmetics and Medical Esthetics Course on Anatomy and Fresh Cadaver for Dermatologists" this year. The increase of the number of such courses will also increase the number of the practices of the cosmetic procedures in the clinics.

The limitations of the study were that we could not reach all education clinics, and no face-to-face interview was performed with the participants during the questionnaire procedure. In addition, it is unclear whether the resident is included in the process even if the procedures are performed in the clinics.

Conclusions

The present study demonstrates the cosmetic dermatology practices, technical equipments, number of patients, and cosmetic dermatology education in the clinics providing dermatology specialty education in Turkey. Cosmetic dermatology seems to be a popular branch of dermatology which shows rapid progress and which needs to be a part of specialty education. Cosmetic dermatology education should not overshadow the medical dermatology education, however must be complementary. Cosmetic dermatology education must be so well-organized that residents must feel qualified and confident enough to perform the procedures in their professional life. Residents who cannot be provided such conditions may visit other clinics with external rotations for a limited time to provide this education or procedural competency can be achieved including with certain number of hands-on courses offered at local, regional, and national meetings for residents. There is a need for a cosmetic dermatology curriculum program, as well as a common curriculum program for traditional dermatology education, which could be practiced in each clinic that will standardize the cosmetic dermatology education and practice in specialty education.

Acknowledgments

This study was performed within the scope of the Turkish Society of Dermatology. We acknowledge the Turkish Society of Dermatology, to the president Emel Erdal Çalıkoğlu for her help and to the resident representatives Ozan Erdem and Çağdaş Akaslan in delivering and collecting the questionnaires.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Article

Histopathological Findings in Patients with Lipoid Proteinosis

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Abstract

Objective: Lipoid proteinosis (LP) is a rare autosomal recessive genodermatosis characterized by the accumulation of amorphous hyaline substance in the skin and mucous membranes. In this study, the histopathological findings of the patients who were admitted to our clinic and diagnosed with LP were examined. **Materials and Methods:** This prospective study included 18 patients who presented to our clinic between January 2014 and December 2018 and were confirmed by histopathological examination. A punch biopsy including epidermis, dermis, and subcutaneous tissues was obtained from the lesional skin of each patient evaluated clinically, and the material was stained with hematoxylin and eosin stain and periodic acid–Schiff (PAS) stain. These preparations were evaluated by a pathologist experienced in dermatopathology. **Results:** The most common histopathological findings in the epidermis were hyperkeratosis (88.8%) and pigmentary incontinence (83.3%) in the basal layer. The most common histopathological findings in the dermis were amorphous substance accumulation (100%), perivascular PAS positivity (33.3%), and PAS positivity around eccrine glands (11.1%). **Conclusion:** The findings of our study were similar to the histopathological findings of late-term skin lesions in LP patients previously described in the literature. In order to better understand the histopathological findings of skin lesions of LP patients, studies with a large number of patients including early skin lesions of LP are needed.

Keywords: Histopathology, hyaline substance, lipoid proteinosis

Introduction

Lipoid proteinosis (LP) is a rare autosomal recessive genodermatosis characterized by the accumulation of amorphous hyaline substance in the skin and mucous membranes. It affects both sexes equally. Although the incidence is not known exactly, about 350 patients have been reported in the literature. LP is more common in our country due to the frequent consanguineous marriages compared to the world countries.[1-6]

LP develops as a result of decreased expression in the ECM1 gene. ECM1 gene is located on 1. chromosome and plays an important role in angiogenesis, epidermal differentiation, and wound healing. The loss of normal function of ECM1 in LP results in infiltration with hyaline-like material in the skin, mucosa, and internal organs. On histopathological examination of the skin lesions of LP patients, diffuse dermal accumulation of hyaline material, basal membrane thickening of the dermoepidermal junction, and epidermal hyperkeratosis can be seen.[7-10] In this study, the histopathological findings of 18 patients who were admitted to our dermatology clinic and were diagnosed with LP and confirmed by histopathological examination were examined.

Materials and Methods

This prospective study included 18 patients who were admitted to our dermatology and venereal diseases clinic between January 2014 and December 2018, who were clinically diagnosed with LP and confirmed by histopathological examination. The sociodemographic characteristics (age and gender) and clinical findings (hoarseness and skin lesions) of the patients were recorded.

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A punch biopsy including epidermis, dermis, and subcutaneous tissue was obtained from the lesional skin of each patient evaluated clinically, and the material was stained with hematoxylin and eosin stain and periodic acid–Schiff (PAS) stain. These preparations were evaluated by a pathologist experienced in dermatopathology. Histopathological findings of the epidermis and dermis were recorded.

Statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA) package program. Continuous data were calculated as mean ± standard deviation and categorical data as frequency (%). The study was approved by the local ethics committee of Faculty of Medicine, Harran University (date: May 13, 2013, number: 5).

Results

Of the 18 patients included in the study, 13 (72.2%) were female and 5 (27.8%) were male. The ages of the patients ranged between 3 and 33 years, and the mean age was 15.6 ± 8.2 years. All patients included in the study had the complaint of hoarseness. Atrophic scars were seen on the skin of all patients, especially on the face. Six patients had hyperkeratotic verrucous plaques on their knees and elbows. Three patients had hair loss and nail anomalies, two had palmoplantar hyperkeratosis, one had tongue thickening, and one had pearly papules on the eyelids. No patients had vesicles, pustules, blisters, and hemorrhagic crusts.

Hyperkeratosis in epidermis in 16 (88.8%) patients, pigment incontinence in basal layer in 15 (83.3%) patients, and atrophy in epidermis in 1 (5.5%) patient were seen [Table 1].

In the dermis, 18 (100%) patients had amorphous accumulation, 6 (33.3%) patients had perivascular PAS positivity, 2 (11.1%) patients had PAS positivity around eccrine glands, 2 (11.1%) patients had fibrosis, 2 (11.1%) patients had inflammatory cells in papillary dermis, 1 (5.5%) patient had papillomatosis, 1 (5.5%) patient had the presence of melanophage, and 1 (5.5%) patient had flattening of the rete ridges [Table 1, Figures 1 and 2].

Table 1: Histopathological findings in patients with lipoid proteinosis

Histopathological findings	*n* (%)
Epidermis	
Hyperkeratosis	16 (88.8)
Pigmentary incontinence in basal layer	15 (83.3)
Atrophy	1 (5.5)
Dermis	
Amorphous substance accumulation	18 (100)
Perivascular PAS positivity	6 (33.3)
PAS positivity around eccrine glands	2 (11.1)
Fibrosis	2 (11.1)
Inflammatory cells in papillary dermis	2 (11.1)
Papillomatosis	1 (5.5)
Presence of melanophage	1 (5.5)
Flattening of the rete ridges	1 (5.5)

PAS: Periodic acid-Schiff

Discussion

LP is a rare autosomal recessive genodermatosis characterized by amorphous hyaline material accumulation in the skin, mucosa, and visceral organs.[11] Hoarseness caused by infiltration of the larynx mucosa in LP is a characteristic finding. This symptom is often mistaken by clinicians for more common diseases such as chronic laryngitis. The cause of hoarseness is the deterioration of wave formation due to accumulation of subepithelial hyaline material and incomplete closure of vocal cords with air leakage during vocalization. In the following years, the skin and mucosal changes become clinically evident. Tongue thickening, epiglottis and vocal cord thickening, and frenulum sclerosis result in limited tongue movements. Infiltration of the gums and salivary glands can lead to tooth loss and recurrent parotid attacks. Dental anomalies are hyperplasia or aplasia of upper incisors, premolars, and molars.[12] Nearly, in half of the cases, intracranial calcifications and epilepsy have been reported. Memory problems, behavioral disorders, and mental retardation are among the other reported neurological and psychiatric symptoms. On computed tomography, sickle-shaped calcifications in the bilateral temporal lobe or hippocampus–amygdala complex are pathognomonic and are responsible for accompanying behavioral disorders.[13,14] The disease typically has a stable or slow progression and has a normal life span, unless there is airway obstruction or deadly epileptic seizures.[4,5]

Cutaneous findings in LP usually occur in the first 2 years of life. At first, recurrent vesicles in variable size, pustules, bullae, and hemorrhagic crusts are seen. Lesions are often seen in traumatic areas such as face and distal extremities, and they heal with atrophic scar. Then, a thickened skin is formed which often creates a wax appearance due to the dermal accumulation of amorphous hyaline substance in the facial skin, eyelids, axils, and scrotum. Finally, hyperkeratotic, verrucose papules and plaques are seen on the surfaces exposed to friction such as elbows, knees, and hands. Thickened scalp, hair loss, and nail anomalies can be seen. The yellowish, wax-colored papules, called "Moniliform blepharosis," are lined up along the eyelids.[6,8,10,11,14]

Diseases such as erythropoietic protoporphyria, papular mucinosis, amyloidosis, and xanthoma disseminatum should be considered in the differential diagnosis of LP. The treatment approach in LP is symptomatic. Despite the presence of various treatment alternatives including dimethyl sulfoxide, acitretin, D-penicillamine, and surgical intervention, there is no single effective treatment for LP.[2,4-8,10]

The exact pathogenesis of LP is not understood. The overproduction of basal membrane type IV collagen by epithelial or endothelial cells and the increased synthesis of noncollagen glycoproteins by fibroblasts appear to be important

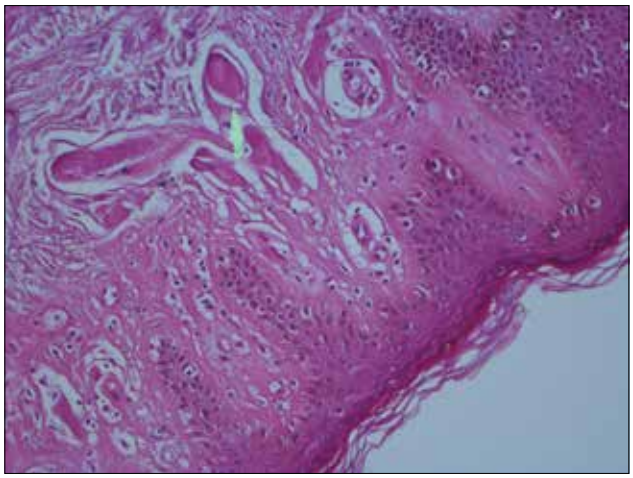

Figure 1: Eosinophilic hyaline material (indicated by arrow) is observed in the papillary dermis and around the blood vessels (H and E, ×200)

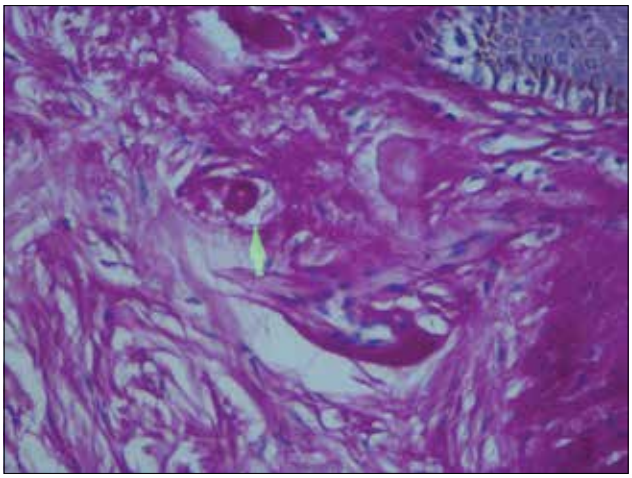

Figure 2: Periodic acid–Schiff-positive eosinophilic hyaline material observed around the papillary dermis and blood vessels (indicated by arrow) (PAS, ×400)

in pathogenesis.[15,16] The major clinical manifestations of LP are associated with the accumulation of amorphous material around blood vessels and connective tissue. Concentric layers of basement membrane-like material contain collagen and laminin, whereas amorphous deposits contain mainly noncollagen proteins. In addition, accumulations are PAS positive, indicating the presence of neutral mucosaccharides.[3]

Hematoxylin and eosin-stained sections of early skin lesions of LP show pink hyaline-like thickening of capillaries in the papillary dermis. Ko and Barr found that intra-epidermal bullae formation with large nondiskeratotic acantholysis in the early lesions of LP and possibly LP was an acantholytic dermatosis.[17] Rao *et al*. found intra-epidermal bullae without acantholysis in the early lesions of LP.[18] Gutte *et al*. detected subepidermal bulla consisting of fibrin and extracellular erythrocytes in the early lesions of LP.[19]

Kaya *et al*. found atrophy of the epidermis and a homogeneous pink material in the papillary dermis in a patient with an early erosive vesicular stage of LP. The amorphous material is deposited in thick bundles around the blood vessels, perpendicular to the skin surface. The ends of the dermal papilla are extremely narrowed and are mostly eroded by the amorphous material. A possible sudden increase in amorphous mass may be the cause of epidermal atrophy. In addition, as a result of perivascular deposits, erosions and ulceration may occur due to obstruction of the capillary circulation in the papillary dermis.[20]

The old skin lesions of LP show hyperkeratosis, sometimes papillomatosis, and a thickened dermis with pink hyaline bundles in a diffuse pattern. These bundles are often aligned vertically to the dermoepidermal junction. There is less scattered hyaline accumulation in the lower dermis. Hyaline mantle may encircle hair follicles, sebaceous glands, and rarely erectile pili muscle.

Elmas *et al*. found accumulation of eosinophilic material around small vessels in superficial dermis and slightly papillomatous changes in histopathological examination of skin lesions of a patient with LP.[21] Akoglu *et al*. found epidermal hyperkeratosis and eosinophilic material accumulation around blood vessels in the papillary dermis, adnexal epithelium, and eccrine glands in the skin biopsies of three LP patients. The material was PAS positive, diastase resistant, and amyloid negative.[22]

In a study conducted by Dertlioğlu *et al*. on nine LP patients, minimal hyperkeratosis and acanthosis; eosinophilic PAS positivity in the papillary dermis; and diastase-resistant, amyloid-negative material accumulation around blood vessel, adnex epithelium, and eccrine glands were seen.[4]

Conclusion

In our study, hyperkeratosis, epidermal atrophy, papillomatosis, pigmentary incontinence in the basal layer, presence of melanophage and flattening of rete ridges, inflammatory cells in the papillary dermis, amorphous substance accumulation in the dermis, perivascular PAS positivity, PAS positivity around eccrine glands, and fibrosis were observed. Because the skin lesions of our patients were not early lesions, histopathological examination of the lesions did not reveal any findings such as acantholysis and intra-epidermal or subepidermal blisters. The findings of our study were similar to the histopathological findings of late-term skin lesions in LP patients, previously described in the literature. In order to better understand the histopathological findings of skin lesions of LP patients, studies with a large number of patients including early skin lesions of LP are needed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Article

Dermoscopic Features of Cutaneous Leishmaniasis Lesions

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Abstract

Objective: This study aimed at determining the dermoscopic characteristics of cutaneous leishmaniasis (CL) and comparing these characteristics to the previous results. **Materials and Methods:** The prospective study included a total of 225 lesions from 69 patients with the ages between 1 and 70 years who were admitted to our dermatology clinic between March 1, 2016, and August 1, 2016, diagnosed with CL using smears of skin lesions, and did not receive any previous antileishmanial treatment. **Results:** When the lesions were dermoscopically examined for their general characteristics, the most common findings were erythema (100%), teardrop-like structures (59.1%), and hyperkeratosis (53.3%). The most common vascular structures in the lesions were linear vessels (50.2%), dotted vessels (39.6%), and hairpin-like vessels (32.9%). **Conclusion:** Our data were comparable to those of the previous studies in literature. Although there is no specific dermoscopic feature specific to CL, we presume that dermoscopic findings may contribute to differential diagnosis in the presence of clinically similar cutaneous lesions.

Keywords: Cutaneous leishmaniasis, dermoscopy, linear vessels, teardrop-like structures

Introduction

Leishmaniasis is a vector-borne, common infection affecting 12 millions of individuals in 98 countries including Turkey, with >350 million people being at risk. Cutaneous leishmaniasis (CL) is the most common form of the disease.[1-4] The definitive diagnosis of CL is made by laboratory methods including microscopic examination of the Giemsa-stained smears, histopathologic examination, culture, or polymerase chain reaction.[5-9] Dermoscopy is a noninvasive *in vivo* diagnostic technique that facilitates the examination of epidermis and superficial dermis. Today, dermoscopy is highly important for the diagnosis and treatment monitorization in almost all fields of dermatology including infectious and inflammatory dermatoses, mainly early diagnosis of melanoma.[10-14] This study aimed at determining the dermoscopic characteristics of CL, comparing these characteristics to the previously published studies, and demonstrating whether dermoscopy is a valuable technique for the diagnosis of CL.

Materials and Methods

The prospective study included a total of 225 lesions from 69 patients with the ages between 1 and 70 years who were admitted to our dermatology clinic between March 1, 2016, and August 1, 2016, diagnosed with CL using smears of skin lesions, and did not receive any previous antileishmanial treatment.

Dermoscopic findings and clinical characteristics of the lesion were noted before initiating antileishmanial treatment in all patients. All the included lesions were individually examined and assessed. Macroscopic and dermoscopic images were taken. DermLite photo equipment (3Gen Inc. 31521 Rancho Viejo Road, Suite 104 San Juan Capistrano, CA 92675 USA) was used as the dermoscope, (Canon inc, shimomaruko, 3-chumo, Ohta-ku, Tokyo, Japan) was used as the camera, and Canon EF 50 mm f/1.8 II was used as the lens. All information was noted. At least three dermoscopic images were taken from each lesion. While taking images by the dermoscope, lesions were wiped using 60% alcohol to obtain more quality images, to view deeper regions, and to prevent light reflection. Minimal pressure was applied using the dermoscope to protect the main vascular structure and prevent vascular collapse. The examination was performed after all patients and lesions were collected.

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Based on the dermoscopic appearance of the lesions, features including erythema, hyperkeratosis, crust, teardrop-like structures, milia-like cyst, white-starburst pattern, ulceration, scar, and orange areas were assessed. Vascular structures in the lesions were assessed based on morphology including dotted vessels, hairpin-like vessels, linear vessels, comma-like vessels, and arborizing vessels.

Written consent was taken from the patients. The study was approved by the local ethics committee of Faculty of Medicine, Harran University (date: March 01, 2016, number: 8).

Statistical analysis

Statistical analyses were performed using the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) package software. Percentage was used for descriptive statistics, and Chi-square test was used for pair-wise analyses. $P < 0.05$ was considered to be statistically significant.

Results

Out of 69 patients included in the study, 32 (46.4%) were female and 37 (53.6%) were male, with a total of 225 lesions being examined. The patients' ages were between 1 and 70 years. Thirty-four (49.2%) patients were symptomatic. The number of patients with itching was 27 (39.1%), with pain was 6 (8.7%), and with burning sensation was 2 (3%). Ninety-five lesions (42.2%) were on the face, 7 (3.1%) were on the neck, 98 (42.7%) were on the upper extremity, and 25 (11.1%) were on the lower extremity [Table 1]. One hundred and forty lesions (62.2%) were papules, 54 (24%) were plaques, 23 (10.2%) were nodules, and 8 (3.6%) were noduloulcerative.

Table 1: Anatomic locations of cutaneous leishmaniasis lesions (n=225)

Location	Lesions, *n* (%)
Face	95 (42.2)
Forehead	12 (5.3)
Periorbital region	18 (8)
Nose	9 (4)
Cheek	42 (18.7)
Perioral region	10 (4.4)
Ear	2 (0.9)
Chin	2 (0.9)
Neck	7 (3.1)
Anterior side	5 (2.2)
Posterior side	2 (0.9)
Upper extremities	98 (42.7)
Arm	11 (4.9)
Forearm	61 (27.1)
Hand	26 (11.6)
Lower extremities	25 (11.1)
Thigh	2 (0.9)
Leg	11 (4.9)
Foot	12 (5.3)
Total	225

When the lesions were dermoscopically examined for their general characteristics, the most common findings were erythema (100%), teardrop-like structures (59.1%), and hyperkeratosis (53.3%), and the least common findings were scar (8.9%) and ulceration (3.5%). The most common vascular structures in the lesions were linear vessels (50.2%), dotted vessels (39.6%), and hairpin-like vessels (32.9%) [Table 2 and Figures 1-5].

When dermoscopic characteristics were assessed by body regions, the most common characteristics were teardrop-like structures (71.7%), linear vessels (58.7), and arborizing vessels (48.9%) on the face; teardrop-like structures (85.6%) and dotted vessels (71.4%) on the neck; dotted vessels (51.0%), white-starburst pattern (46.9%), and teardrop-like structures (44.8%) on the upper extremities; and teardrop-like structures (62.5%) and dotted vessels (58.3%) on the lower extremities [Table 3 and Figures 1-5].

When dermoscopic characteristics were assessed by lesion types, erythema was the most common finding for all lesion types. Other most common findings were teardrop-like structures and linear vessels in papular lesions; hyperkeratosis and teardrop-like structures in plaque lesions; crust, hyperkeratosis, teardrop-like structures, and pustules in nodular lesions; and teardrop-like structures and linear vessels in noduloulcerative lesions [Table 4 and Figures 1-5].

Discussion

There are four previous studies investigating the dermoscopic characteristics of CL.[15-18] First, Llambrich *et al*. assessed

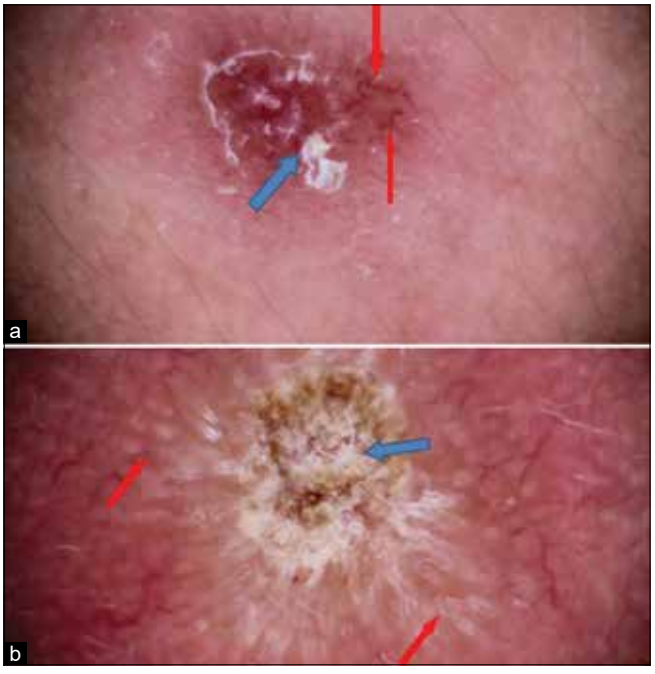

Figure 1: (a) At the center of the lesion, there are crusts and hyperkeratosis (blue arrow), and in the periphery, there are reticular branching and linear vessels (red arrow). (b) Crust and hyperkeratosis (blue arrow) at the center of the lesion; in the periphery, tear-shaped structures (red arrow) with reticular branching and some with targetoid appearance are seen

Table 2: Dermoscopic features of cutaneous leishmaniasis

Dermoscopic features	Study				
	Our study (Turkey) (*n*=225), *n* (%)	Yücel *et al*.[15] (Turkey) (*n*=145), *n* (%)	Ayhan *et al*.[16] (Turkey) (*n*=144), *n* (%)	Taheri *et al*.[17] (Iran) (*n*=144), *n* (%)	Llambrich *et al*.[18] (Spain) (*n*=26), *n* (%)
General features					
Erythema	225 (100)	145 (100)	127 (100)	118 (81.9)	26 (100)
Hyperkeratosis	120 (53.3)	NR	19 (15.0)	48 (33.3)	13 (50)
Crust	79 (35.1)	51 (35.2)	89 (70.1)	NR	NR
Ulceration	8 (3.5)	51 (35.2)	56 (44.1)	85 (59)	12 (46)
Teardrop-like structures	133 (59.1)	58 (40)	54 (42.5)	60 (41.7)	14 (53)
Milia-like cyst	60 (26.7)	NR	20 (15.7)	7 (4.9)	NR
Orange areas	76 (33.8)	19 (%13.1)	20 (15.7)	63 (43.8)	NR
White-starburst pattern	76 (33.8)	27 (18.6)	11 (8.6)	87 (60.4)	10 (38)
Pustules	25 (11.1)	NR	11 (8.6)	NR	NR
Scar	20 (8.9)	NR	22 (17.3)	NR	NR
Perilesional hypopigmented halo	NE	4 (2.8)	NR	NR	NR
Vascular features					
Hairpin-like vessels	74 (32.9)	25 (17.2)	50 (39.4)	54 (37.5)	5 (19)
Arborizing vessels	70 (31.1)	53 (36.6)	49 (38.6)	15 (10.4)	3 (11)
Linear vessels	113 (50.2)	78 (53.8)	33 (26.0)	44 (30.6)	15 (57)
Dotted vessels	89 (39.6)	23 (15.9)	31 (24.4)	88 (61.1)	14 (53)
Comma-like vessels	49 (21.8)	6 (4.1)	25 (19.7)	43 (29.9)	19 (73)
Crown-like vessels	NE	NR	2 (1.6)	NR	NR
Strawberry pattern	NE	NR	2 (1.6)	NR	NR

NE: Not evaluated, NR: Not reported

Table 3: Results of dermoscopic features by localization in cutaneous leishmaniasis

	Face		Neck		Upper extremities		Lower extremities	
	n (%)	*P*	*n* (%)	*P*	*n* (%)	*P*	*n* (%)	*P*
Teardrop-like structures	66 (71.7)	0.001	6 (85.6)	0.146	43 (44.8)	0.000	15 (62.5)	0.721
Milia-like cyst	27 (29.3)	0.449	3 (42.9)	0.325	24 (25)	0.626	5 (20.8)	0.494
White-starburst pattern	19 (20.7)	0.001	4 (57.1)	0.184	45 (46.9)	0.000	6 (25)	0.336
Hairpin-like vessels	16 (17.4)	0.000	4 (57.1)	0.165	42 (43.8)	0.003	9 (37.5)	0.611
Arborizing vessels	45 (48.9)	0.000	3 (42.9)	0.789	12 (12.5)	0.000	8 (33.3)	0.804
Linear vessels	54 (58.7)	0.034	3 (42.9)	0.990	40 (41.7)	0.027	10 (41.7)	0.375
Dotted vessels	19 (20.7)	0.000	5 (71.4)	0.174	49 (51.0)	0.002	14 (58.3)	0.047
Comma-like vessels	22 (23.9)	0.519	4 (57.1)	0.021	13 (13.5)	0.010	8 (33.3)	0.147

26 lesions of 25 CL patients using dermoscopy and observed teardrop-like structures in 53%.[18] In their study, Ayhan *et al*. detected teardrop-like structures in 42.5% of the lesions, and teardrop-like structures were detected only in lesions located on the face and the posterior and lateral sides of the neck, however, nowhere else on the body.[16] In their study, Yücel *et al*. observed teardrop-like structures in 40% of the lesions, and these structures were most frequently observed in nodular lesions.[15] Teardrop-like structures were observed in 59.1% of the lesions in our study. These structures were particularly observed in nonchronic, crustless, new papular lesions (60%, $P = 0.728$).

Histopathologically, teardrop-like structures were considered to be follicular fillings plugged with keratin formed due to pressure on the hair follicle from the sides.[15] Keratin-plugged follicular fillings can also be seen in nonpigmented actinic keratosis, which lead to a strawberry pattern.[19] In the strawberry pattern in nonpigmented actinic keratosis, keratin plugs are not inside the structure but around the pseudoweb with a large erythema. No ulcer or crust is found at the center.[19,20] Differences like this may help with the dermoscopic differential diagnosis of CL.

White starburst-like patterns were first described by Llambrich *et al*. in CL lesions and were thought to be caused by parakeratotic hyperkeratosis. This finding was found in 38% of the lesions diagnosed with CL.[18] In their study, Taheri *et al*. detected this finding in 60.4% of the lesions, mainly in nodular lesions on the upper extremities.[17] In their study, Yücel *et al*. detected this finding in 19% of the lesions, especially in noduloulcerative lesions.[15] In our

study, white starburst-like patterns were detected in 33.8% of the lesions, most commonly in papular lesions on the neck. As white starburst-like patterns were not detected in dermatological lesions previously, it can be considered an important criterion for the dermoscopic assessment of CL lesions.

Arborizing vessels were first found to be useful for the diagnosis of basal-cell carcinoma (BCC) in 1990.[21] In their study, Ayhan *et al*. observed arborizing vessels in 38.6% of the

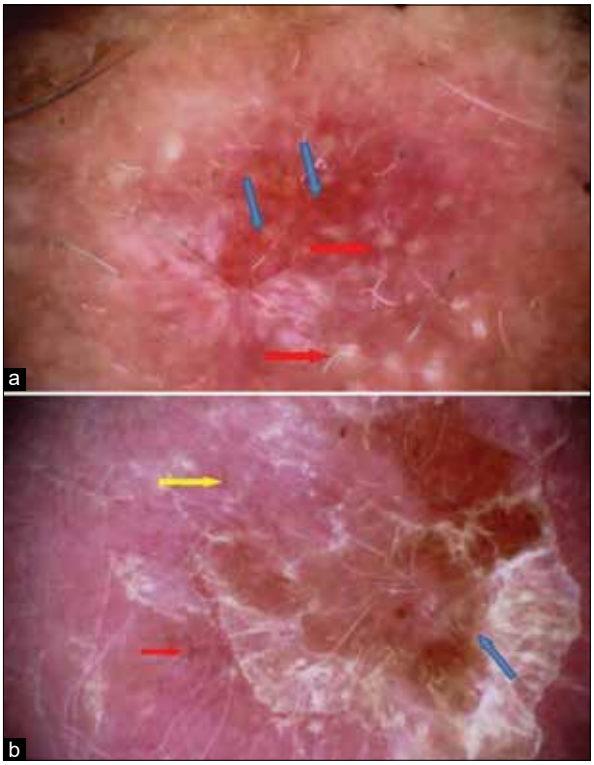

Figure 2: (a) At the center of the lesion, there are ulcer, scar areas, and dotted vessels (blue arrow); in the periphery, there are some ellipse or round tear structures (red arrow). (b) At the center of the lesion, there are crusts and hyperkeratosis (blue arrow), and in the periphery, there are reticular branching (red arrow) and linear vessels (yellow arrow)

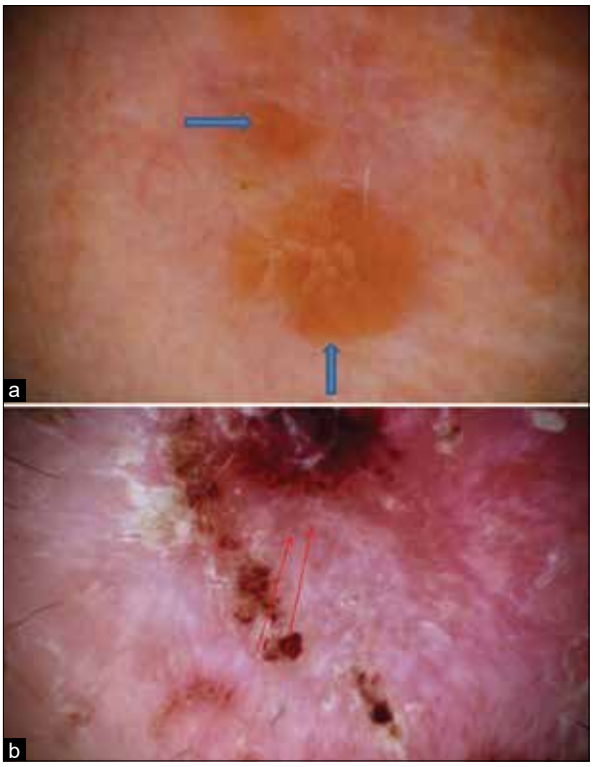

Figure 3: (a) The dermoscopic examination of the lesion shows orange areas (blue arrow). (b) At the center of the lesion, dotted vessels (red arrow) are seen

Table 4: Results of dermoscopic features by type of lesion in cutaneous leishmaniasis

Dermoscopic features	Papules		Plaques		Nodules	
	n (%)	*P*	*n* (%)	*P*	*n* (%)	*P*
General features						
Erythema	140 (100)	0.000	54 (100)	0.000	23 (100)	0.000
Hyperkeratosis	59 (42.1)	0.000	45 (83.3)	0.000	12 (52.2)	0.936
Crust	42 (30.0)	0.039	10 (18.5)	0.003	22 (95.7)	0.000
Ulceration	1 (0.7)	0.001	NE	NE	1 (4.3)	0.928
Teardrop-like structures	84 (60.0)	0.728	33 (61.1)	0.732	11 (47.8)	0.245
Milia-like cyst	29 (20.7)	0.010	18 (33.3)	0.204	9 (39.1)	0.154
Orange areas	39 (27.9)	0.016	23 (42.6)	0.116	8 (34.8)	0.914
White-starburst pattern	57 (40.7)	0.005	10 (18.5)	0.007	7 (30.4)	0.721
Pustules	6 (4.3)	0.000	5 (9.3)	0.804	11 (47.8)	0.000
Scar	2 (1.4)	0.000	10 (18.5)	0.004	5 (21.7)	0.058
Vascular features						
Hairpin-like vessels	48 (34.3)	0.567	12 (22.2)	0.056	11 (47.8)	0.108
Arborizing vessels	38 (27.1)	0.099	20 (37.0)	0.281	9 (39.1)	0.381
Linear vessels	71 (50.7)	0.850	28 (51.9)	0.784	10 (43.5)	0.495
Dotted vessels	51 (36.4)	0.218	25 (46.3)	0.245	9 (39.1)	0.965
Comma-like vessels	32 (22.9)	0.615	14 (25.9)	0.397	2 (8.7)	0.109

NE: Not evaluated

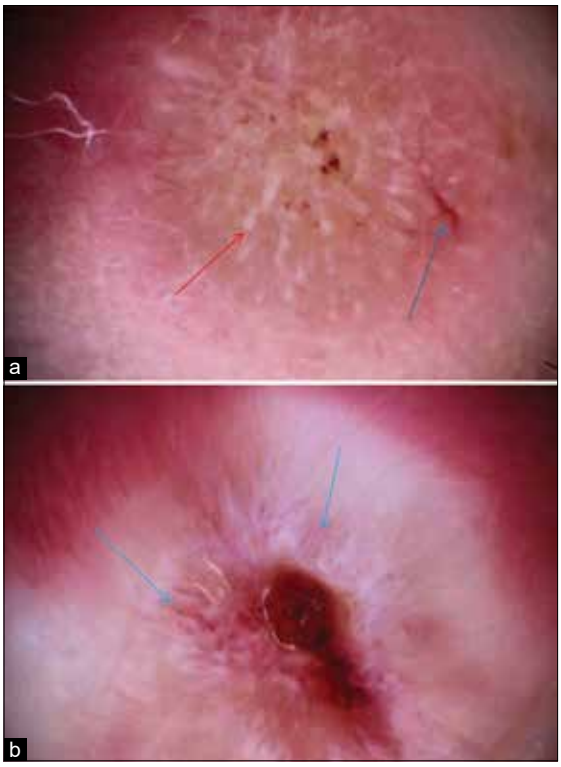

Figure 4: (a) The dermoscopic examination of the lesion shows arborizing vessels (blue arrow) and milia-like cysts (red arrow). (b) The dermoscopic examination of the lesion shows hairpin-like vessels (blue arrow)

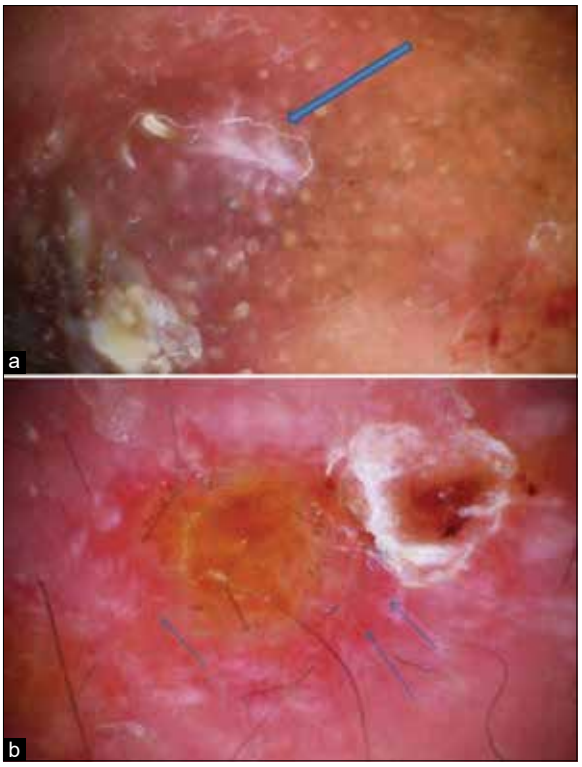

Figure 5: (a) The dermoscopic examination of the lesion shows starburst pattern (blue arrow). (b) The dermoscopic examination of the lesion shows dotted vessels (blue arrow)

patients diagnosed with CL, most commonly in lesions on the face.[16] In their study, Yücel *et al*. observed arborizing vessels in 36.6% of the lesions, most commonly in papular lesions.[15] In our study, arborizing vessels were observed in 31.1% of all patients and in 27.1% of papular lesions ($P = 0.099$), with most of these lesions (48.9%) being placed on the face ($P = 0.000$). The presence of arborizing vessels and follicular fillings indicates CL diagnosis.

Hairpin-like vessels are mostly seen in seborrheic keratosis, keratoacanthoma (KA), and nonmelanocytic tumors such as squamous cell carcinoma (SCC), usually accompanied by a white halo surrounding the lesion.[22-24] Hairpin-like vessels were detected in 19% of CL patients in the study by Llambrich *et al*.[18] and in 17.2% of the cases in the study by Yücel *et al*.[15] These vessels were commonly observed in lesions on the lower extremities in both studies.[15,18] Hairpin-like vessels were observed in 37.5% and 39.4% of the lesions in the studies by Taheri *et al*. and Ayhan *et al*., respectively. These vessels were commonly detected in lesions on the upper extremities.[16,17] Hairpin-like vessels were detected in 32.9% of the lesions in our study. In our study, hairpin-like vessels were more common in nodular lesions. Teardrop-like structures existing in the absence of white irregular area, white halo, and central keratin plug were more commonly observed at the center; being observed less commonly and found in larger ulcers, hairpin-like vessels were detected more in the lesions on the neck. These might help in the differential diagnosis of KA and SCC from CL.

Linear vessels are in various sizes with a single fold.[25] Zalaudek *et al*. detected linear vessels in 70.8% of the CL lesions on the face and in 17.9% of the SCC lesions.[20] Yücel *et al*. detected linear vessels in 53.4% of the CL patients,[15] Ayhan *et al*.[16] in 26%, and Taheri *et al*.[17] in 30.6%. These vessels were frequently observed on the face.[15-17]

In our study, linear vessels were observed in 50.2% of the lesions, and these lesions were more commonly detected in noduloulcerative lesions (52.2%, $P = 0.843$) and on the face (58.7%, $P = 0.034$). As linear vessels may also be observed in KA and SCC, a careful differential diagnosis should be made.

Dotted vessels are observed due to placement of short and vertical capillaries around the lesion.[26] They can be seen in verruca vulgaris, actinic keratosis, seborrheic keratosis, Bowen's disease, and also in many small keratinized tumors such as SCC.[27] In their study, Ayhan *et al*. observed dotted vessels in 24.4% of the CL lesions. These vessels were mostly observed in the lesions on the upper extremities.[16] In their study, Taheri *et al*. observed dotted vessels in 61.1% of the CL lesions, and these vessels were mostly observed in ulcerative plaques on the lower extremities.[17] Dotted vessels were observed in 39.6% of the lesions in our study. Dotted vessels were mostly observed on the neck area (71.4%, $P = 0.174$) and in noduloulcerative lesions (47.8%, $P = 0.392$).

Comma-like vessels are slightly curved vessels with a diameter of ≥1 mm. They can be observed in lesions including melanoma,[27] dermal nevus,[28] dermatofibroma,[29] and

BCC.[23,24] Studies on CL patients have found comma-like vessels in 73%,[18] 4.1%,[15] 19.7%,[16] and 29.9%[17] of the lesions. While these vessels were most commonly observed on the face in the study by Ayhan *et al*.,[16] they were most commonly observed on the lower extremities in the study by Taheri *et al*.[17] In their study, Yücel *et al*. observed comma-like vessels most commonly in nodules and ulcerative lesions.[15] Comma-like vessels were observed in 21.8% of the lesions in our study. Comma-like vessels were most commonly observed in plaque lesions (25.9%, $P = 0.394$) and on the neck (57.1%, $P = 0.021$).

Lupus vulgaris is one of the granulomatous diseases which can be confused with CL. Orange areas are also known as salmon-colored ovoids.[15] Arborizing vessels and orange areas mostly accompany each other, and these structures are mostly observed in lesions of leishmaniasis recidivans. Therefore, orange areas are considered to be a sign of chronification.[16] In a study by Yücel *et al*., orange areas were observed in 13.1% of the lesions.[15] They were observed in 15.7% of the lesions in the study by Ayhan *et al*.[16] and in 43.8% in the study by Taheri *et al*.[17] In our study, the combination of arborizing vessels and orange areas was observed in 34.2% of the lesions.

Conclusion

In our study, the vascular and nonvascular structures of the CL lesions were assessed using dermoscopy. The most common morphology of the vascular structures was linear vessels and dotted vessels and of the nonvascular structures were erythema, hyperkeratosis, crust, and teardrop-like structures. Although there is no specific dermoscopic feature specific to CL, we presume that dermoscopic findings may contribute to the differential diagnosis in the presence of clinically similar cutaneous lesions.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Internalized Stigma in Patients with Acne Vulgaris, Vitiligo, and Alopecia Areata

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Abstract

Background: Internalized stigma, another aspect of stigma, is the adoption of negative attitudes and stereotypes of the society regarding people's illness. **Aims and Objectives:** The primary aim of this study was to investigate the internalized stigma state of acne vulgaris (AV), vitiligo, and alopecia areata (AA) patients and to identify the factors influencing internalized stigma. **Materials and Methods:** A total of 150 patients (50 AV, 50 vitiligo, and 50 AA) who applied to the outpatient clinic were consecutively enrolled in this study. The sociodemographic characteristics of the patients were recorded. In addition, patients answered the Internalized Stigma Scale (ISS), the Dermatology Life Quality Index, the Perceived Health Status, the General Health Questionnaire, and the Acne Quality of Life Scale. **Results:** In this study, the Cronbach's alpha coefficient for the whole ISS scale was calculated as 0.91 for AV, 0.91 for vitiligo, and 0.93 for AA. **Conclusion:** The present study indicates that patients with AV, AA, and vitiligo internalize the negative stereotype judgment of the society for themselves. High levels of internalized stigma in the studied patients presented a parallel trend to the negative quality of life (QoL). Therefore, internalized stigma may be one of the major factors affecting the QoL in these diseases.

Keywords: Acne, alopecia areata, internalized stigma, vitiligo

Introduction

Dermatological diseases discriminate an individual from others; in other words, they stigmatize the patient due to the visibility of the skin lesions. The skin is the most important part of our body interacting with the outside world. Diseases located on the areas of the body that can be easily seen during this interaction cause significant psychosocial influences, especially stigmatization. Acne vulgaris (AV), alopecia areata (AA), and vitiligo are among the most important target diseases because their individual lesions are frequently localized on visible areas. These diseases may lead to significant psychosocial influences in the patient: deterioration in the body perception, decrease in self-esteem, and social withdrawal. Furthermore, anxiety and depression are more commonly reported in these patients.[1-8]

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Internalized stigma, another aspect of stigma, is the adoption of negative attitudes and stereotypes of the society regarding peoples' illness.[9] The patient presumes that other people have a reaction toward his/her illness and eventually withdraws from the social life, ending up with decreased self-esteem and life satisfaction, increased depression and suicidality, and difficulty in coping with the illness.[10,11] Consequently, patients may face social cohesion problems and unemployment. Furthermore, compliance with treatment may become affected, increasing the risk of treatment failure and poor disease control.[10,12] According to the available current literature, the Internalized Stigma Scale (ISS) has not been applied to patients with vitiligo and AA. The ISS has been first used by our group on

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patients with AV and a high degree of internalized stigma was found.[13] The primary aim of this study was to investigate the internalized stigma state of patients with AV, AA, and vitiligo and to identify the factors influencing internalized stigma state in these three diseases.

Materials and Methods

A total of 150 patients (50 AV, 50 vitiligo, and 50 AA) who applied to our dermatology and venereology outpatient clinic have been consecutively enrolled in this study. Informed consent was obtained from all participants, and the study was conducted according to the principles of the Declaration of Helsinki. Local ethics committee's approval for the study was granted (70904504/548). The sociodemographic characteristics of the patients and other parameters which may influence internalized stigma and quality of life (QoL) (age, sex, duration of disease and the age of onset, history of psoriasis in the family, accompanying diseases, level of income, educational level, marital status, smoking, and alcohol consumption) were recorded. Disease severity scores such as Vitiligo European Task Force (VETF) score, Vitiligo Disease Activity (VIDA) score, Severity of Alopecia Tool (SALT) score, and Food and Drug Administration (FDA) Acne Global Grade were used to assess disease severities. In addition, the patients answered the ISS, the Dermatology Life Quality Index (DLQI), the Perceived Health Status (PHS) Questionnaire, the General Health Questionnaire (GHQ), and Acne Quality of Life Scale (AQLS) on the day of enrollment.

Questionnaires

Internalized Stigma Scale

The ISS was developed by Ritsher *et al*.[9] for mental illnesses and has been adapted for many diseases other than psychological disorders. The ISS is a Likert-type scale composed of 29 items measuring the internalization of stigma experienced by the patients. It has five dimensions, namely, alienation (6 items), stereotype endorsements (7 items), perceived discrimination (5 items), social withdrawal (6 items), and stigma resistance (5 items). ISS values range from 4 to 91. High ISS scores mean more severe internalized stigma. Those who score 0–25 points on the ISS are considered as having low stigmatization, those who score 26–39 points are considered as having moderate stigmatization, and those who score 40 are rated as having high stigmatization.[9]

The Dermatology Life Quality Index

The DLQI is the first quality-of-life scale developed for dermatological disorders. It is composed of ten questions in total and the scores range from 0–30. High values indicate that the disease has a significant influence on daily life, job, school life, leisure time activities, and interpersonal relationships. The Turkish version has been validated by Oztürkcan *et al*.[14]

Perceived Health Status

The PHS is a Likert-type scale examining general health using a single question. In analyses, Likert scorings are classified as 1, 2, and 3 ("worse than good") and 4 and 5 ("good").[15,16]

The General Health Questionnaire-12 scale

The GHQ-12 has been developed by Goldberg and Hillier in order to define mental status in public and primary health-care establishments.[17] Although the GHQ-12 has been developed to distinguish general mental disorders, it contains items questioning basic symptoms of depression including enjoyment, sense of calm, distractibility, and sleeplessness.[18] The validity and reliability of the Turkish version have been evaluated by Kilic *et al*. (Cronbach's alpha = 0.78).[19]

Acne Quality of Life Scale

It is a measure of nine questions developed by Gupta *et al*. It aims to determine the relationship between acne severity and psychological morbidity due to acne. The answers to the questions are scored 1, 2, 3, and 4 according to the degree that the patients have suffered due to their acne. The total score is calculated by summing the points of each problem. A high score indicates more deterioration in the QoL.[20] The questions refer to feelings of restlessness besides others, decrease in socialization, difficulty with girlfriend or boyfriend, difficulties with close friends, difficulty with family relations, feeling isolated, ridiculing, romantic reluctance, and rejection by friends.[20,21] The reliability study of the Turkish version of this scale was performed by Demirçay *et al*.[21]

Disease Severity Scores

Acne vulgaris

Acne severity is assessed in four stages with the FDA Global Score.[22] The four stages are as follows: mild: the closed or

Table 1: Evaluation of Cronbach's alpha value and subscales of the Internalized Stigma Scale in patients with acne vulgaris, vitiligo, and alopecia areata

The ISS subscales	AV (n=50)	AA (n=50)	Vitiligo (n=50)	Psoriasis research (n=100)	Turkish form (n=203)	Original form (n=127)
Alienation	0.88	0.88	0.88	0.83	0.84	0.79
Stereotype endorsement	0.14	0.66	0.48	0.70	0.71	0.72
Perceived discrimination	0.69	0.82	0.65	0.70	0.87	0.75
Social withdrawal	0.90	0.93	0.83	0.84	0.85	0.80
Stigma resistance	0.07	0.64	0.46	0.68	0.63	0.58
Total	0.91	0.93	0.91	0.89	0.93	0.90

AV: Acne vulgaris, AA: Alopecia areata, ISS: Internalized Stigma Scale

Table 2: Main demographic characteristics of patients with acne vulgaris

	n (%)
Sex	
Male	23 (46)
Female	27 (54)
Marital status	
Single	46 (92)
Married	4 (8)
Income level	
High: Income exceeds expenses	7 (14)
Moderate: Income is equal to expenses	37 (74)
Low: Income is less than expenses	6 (12)
Educational status	
Secondary school	4 (8)
High school	22 (44)
Postgraduate	11 (22)
Faculty	13 (26)
Concomitant diseases	
No	50 (100)
Yes	0
Severity of AV (according to the FDA Global Score)	
Mild	18 (36)
Moderate	17 (34)
Severe	14 (28)
Very severe	1 (2)
Progress of disease	
Acute	3 (6)
Chronic intermittent	37 (74)
Chronic persistent	10 (20)
Distribution area	
Face and neck	48 (96)
Mostly on body	1 (2)
Mostly on extremities	1 (2)
PHS	
Very good	7 (14)
Good	27 (54)
Moderate	14 (28)
Bad	2 (4)
Very bad	0

FDA: Food and Drug Administration, AV: Acne vulgaris, PHS: Perceived Health Status Questionnaire

open comedonal lesions and few papules and pustules are visible (usually < 10) and no nodules are found; moderate: inflammatory lesions such as papules and pustules are also evident (10–40 lesions). More comedones are also available. There may be a small number of nodules; severe: there are numerous comedones (40–100) and deep, large inflammatory nodules (up to 5) with common papules and pustules (40–100). In general, widespread involvement such as the face, trunk, and back is present; very severe: severe lesions such as nodulocystic acne or acne conglobata are present. There are numerous large painful pustules and nodular lesions, as well as numerous papules, pustules, and comedones.

Vitiligo

Vitiligo Disease Activity Score

The VIDA is a scoring system that measures vitiligo activity, rated at a maximum of + 4 and a minimum of − 1. The last year of patients' vitiligo assessment is based on the subjective evaluation of the disease activity status. Low VIDA scores indicate that the disease activity is low.[23]

Vitiligo European Task Force Scale

In recent years, the use of the VETF scale has been recommended in assessing the severity of vitiligo and treatment response.[24,25] The VETF scale is a scoring system that assesses the extent of involvement of vitiligo, disease progression, and spread of lesions. The area of occupation is calculated according to the nine rules used in burns. Staging is scored between 0 and 4 according to skin and hair pigmentation status in vitiligo patches. The spread of the lesions is classified as + 1: progressive, 0: constant, and − 1: regression.[24]

Alopecia areata

Disease severity is determined by using the SALT.[26] The SALT scoring system is based on the involvement percentages of AA.

Statistical analysis

In the present study, the reliability of ISS and all subscales of the ISS was examined by calculating the Cronbach's alpha internal consistency coefficients. It was accepted that the minimum acceptable level of these values was 0.70. It was observed that some of the internal consistency coefficients of the ISS were <0.70 in this study. For this reason, to ensure the

Table 3: Correlation between the Internalized Stigma Scale, the Acne Quality of Life Scale, the Dermatology Life Quality Index, the General Health Questionnaire, and the perceived health status

The ISS subscales	Acne FDA Global Score	AQLS	DLQI	GHQ	PHS
Alienation	0.058	<0.001	<0.001	<0.001	0.467
Stereotype endorsement	0.094	0.001	0.002	<0.001	0.534
Perceived discrimination	0.005	0.001	0.002	<0.001	0.269
Social withdrawal	0.552	<0.001	<0.001	<0.001	0.479
Stigma resistance	0.107	0.434	0.716	0.788	0.021
Total	0.059	<0.001	<0.001	<0.001	0.268

AQLS: Acne Quality of Life Scale, DLQI: The Dermatology Life Quality Index, GHQ: The General Health Questionnaire, PHS: Perceived Health Status Questionnaire, FDA: Food and Drug Administration, ISS: Internalized Stigma Scale

Table 4: Main demographic characteristics of patients with vitiligo

	n (%)
Sex	
Male	26 (52)
Female	24 (48)
Marital status	
Single	26 (52)
Married	21 (42)
Divorced	3 (6)
Income level	
High: Income exceeds expenses	5 (10)
Moderate: Income is equal to expenses	30 (60)
Low: Income is less than expenses	15 (30)
Education	
Primary school	9 (18)
Secondary school	10 (20)
High school	18 (36)
Postgraduate	6 (12)
Faculty	4 (8)
Master's degree	3 (6)
Concomitant diseases*	
No	45 (90)
Yes	5 (10)
Clinical condition	
Localized	12 (24)
Generalized	38 (76)
Nail changes**	
Yes	9 (18)
No	41 (82)
Hair changes (poliosis)	
Yes	3 (6)
No	47 (94)
Progress of vitiligo	
Acute	3 (6)
Chronic intermittent	17 (34)
Chronic persistent	23 (46)
Stable	7 (14)
Family history of vitiligo***	
No	35 (70)
Yes	15 (30)
First-degree relative	8 (16)
Second-degree relative	7 (14)
Concomitant diseases	
Atopy	3 (6)
AA	3 (6)
Connective tissue disease	1 (2)
Thyroid disease	6 (12)
Psychiatric disease	1 (2)
Vitiligo treatment	
No	2 (4)
Yes	48 (96)
PHS	
Very good	4 (8)
Good	29 (58)
Moderate	12 (24)
Bad	3 (6)
Very bad	2 (4)

*Concomitant diseases: diseases such as diabetes, hypertension, etc. **Nail findings: Pitting, thickening, discoloration, red lunula, etc. ***First degree relatives: mother, father, brother; 2nd degree relatives: members of the parent family (uncle, uncle, aunt, etc.). AA: Alopecia areata, PHS: Perceived Health Status Questionnaire

validity and reliability of the ISS, further studies are needed to be examined in detail. Data were analyzed using PASW 22 (SPSS/IBM, Chicago, IL, USA). Descriptive statistics such as frequency distribution, mean, and standard deviation were used to describe the sample. The assumption of normal distribution suitability was examined by the Shapiro–Wilk test. In the cases where the parametric test assumptions were used, the "Student's *t*-test" was used to determine the difference between the two independent groups. The "Mann–Whitney U-" test was used for the two groups when the parametric test assumptions were not met. Categorical data were analyzed using "Chi-square significance test" or "Fisher's exact test." A 95% significance level (or $\alpha = 0.05$ error margin) was used to determine the differences in the analyses.

Results

The Cronbach's alpha coefficient for the whole scale was calculated as 0.91 for AV, 0.91 for vitiligo, and 0.93 for AA. Similar results were also observed when the reliability coefficients regarding the subscales of ISS were compared with Cronbach's alpha values obtained from the original reliability validity study[9] and the reliability validity studies of the Turkish version for psychiatric disorders[27] and psoriasis[28] which is the first dermatological disease studied by this scale [Table 1].

Acne vulgaris

The mean age of the patients with AV was 20.58 ± 3.71 years. The major sociodemographic and clinical features of patients with AV participating in the study are summarized in Table 2. The lowest value of the ISS in the five subscales belongs to the subscale of resistance to stigmatization, and the highest value belongs to the subscale of social withdrawal [Table 1]. The mean AQLS of patients with AV was 14.40 ± 6.19, the mean DLQI was 6.78 ± 5.58, the mean ISS was 59.48 ± 15.40, the mean GHQ was 23.60 ± 4.47, and the mean PHS was 3.78 ± 0.73. A statistically significant correlation was found between the ISS and DLQI ($r = 0.596$, $P < 0.001$), the GHQ ($r = 0.594$, $P < 0.001$), and the AQLS ($r = 0.587$, $P < 0.001$). The relationship between ISS and AQLS, DLQI, GHQ, and PHS of patients with AV is summarized in Table 3. To examine the criterion validity of the ISS, the Acne FDA Global Score was used. There was a positive correlation between acne severity and DLQI ($P = 0.024$). The ISS scores ($P = 0.005$) showed a statistically significant increase with the increase of education level. The AQLS scores were statistically significantly higher in males than in females ($P = 0.012$).

Table 5: Correlation between the Internalized Stigma Scale; the Vitiligo European Task Force-area, Vitiligo European Task Force-staging, and Vitiligo European Task Force-spread scales; the Dermatology Life Quality Index; the General Health Questionnaire; and Perceived Health Status Questionnaire

The ISS subscales	VETF-area	VETF-staging	VETF-spread	DLQI	GHQ	PHS	VIDA score
Alienation	0.049	0.469	0.315	<0.001	0.966	0.770	0.566
Stereotype endorsement	0.591	0.661	0.791	0.085	0.894	0.321	0.971
Perceived discrimination	0.671	0.392	0.612	<0.001	0.790	0.774	0.356
Social withdrawal	0.509	0.128	0.726	<0.001	0.842	0.994	0.655
Stigma resistance	0.917	0.436	0.523	0.333	0.277	0.260	0.487
Total	0.243	0.231	0.108	<0.001	0.463	0.368	0.566

DLQI: The Dermatology Life Quality Index, GHQ: The General Health Questionnaire, PHS: Perceived Health Status Questionnaire, VETF: Vitiligo European Task Force, VIDA: Vitiligo Disease Activity Score, ISS: Internalized Stigma Scale

The mean AQLS for married individuals was statistically significantly lower ($P = 0.017$). In married individuals, DLQI and GHQ scores were lower, and the ISS score was higher, although not statistically significant ($P = 0.243$, $P = 0.957$, and $P = 0.680$, respectively).

Vitiligo

The mean age of the patients with vitiligo was 34.28 ± 13.02 years [Table 4]. The mean age at onset of the disease ($P = 0.02$) was lower, and the duration of illness ($P = 0.04$) was longer in women. The mean DLQI of the patients with vitiligo was 4.70 ± 5.33, the mean ISS value was 51.68 ± 14.34, the mean GHQ was 23.18 ± 5.38, and the mean PHS was 3.92 ± 3.03. In patients with vitiligo, the lowest value of the ISS in the five subscales belongs to the subscale of resistance to stigmatization, and the highest value belongs to the subscale of alienation [Table 1]. The mean VETF area-score of the patients was 9.68 ± 8.30, the VETF staging-score was 6.02 ± 3.22, the VETF-spreading score was 0.28 ± 3.01, and the VIDA score was 1.04 ± 1.26. No correlation between disease severity and ISS was observed. A significant correlation was found between vitiligo type and VETF area ($P = 0.001$) and staging score ($P = 0.006$). Patients with generalized vitiligo were found to have higher area and staging scores. This finding confirms the association of patients with generalized body involvement with more severe disease. There was a statistically significant correlation between the ISS and DLQI ($r = 0.540$, $P < 0.001$) in patients with vitiligo. Married individuals had lower GHQ scores ($P = 0.024$). The correlation between the ISS and VETF area–staging–spreading scores, VIDA score, GHQ score, DLQI score, and PHS score is summarized in Table 5.

Alopecia areata

The mean age of the alopecia patients was 30.92 ± 10.92 years [Table 6]. The age of first application to the hospital was statistically significantly higher ($P = 0.025$), and the duration of illness was longer in women ($P = 0.016$). In patients with AA, the lowest value among the five subscales of the ISS belonged to the subscale of resistance to stigmatization and the highest value belonged to the social withdrawal subscale [Table 1]. The mean ISS was determined as 59.46 ± 15.82, the mean DLQI of the patients was 6.64 ± 6.13, the mean GHQ was 24.04 ± 5.73, and the mean PHS was 3.80 ± 0.80. GHQ scores were statistically significantly higher in women ($P = 0.003$). The mean SALT score was 1.96 ± 1.41. A statistically significant correlation was also found between the ISS and DLQI ($r = 0.508$, $P < 0.001$) and GHQ ($r = 0.329$, $P = 0.024$) in patients with AA [Table 7].

Comparison of all diseases

The mean ISS scores of AV (59.48 ± 15.40) and AA (59.46 ± 15.82) were higher than that of vitiligo patients (51.68 ± 14.34). There was no significant correlation between disease severity scores (SALT, FDA Global Acne, VETF, and VIDA scores) and the mean values of the ISS in patients with AV, AA, and vitiligo [Table 8].

Discussion

The mean total ISS scores of AV, vitiligo, and AA in our study were high (>40). They were comparable with those obtained from psychiatric and dermatologic patient populations.[28-33] The ISS subscale with the lowest value among all the three diseases was the subscale of resistance to stigmatization. This result alerts us as it shows how vulnerable the patients are to stigma. When dermatological diseases locate on visible body parts, they cause distress and psychological effects on individuals. Thus, high ISS scores may be explained by this effect. Moreover, some behaviors of health professionals (doctors, nurses, and technicians) may also cause an increase in patients' distress. All practitioners and other health-care providers should be aware of this aspect of dermatological diseases and practitioners should add psychiatric examination or evaluation into their routine practices to improve patients' health. Furthermore, health-care providers need to strengthen patients' stigma resistance abilities.

The mean ISS and DLQI scores of AV and AA were higher than those of vitiligo. This could be due to the disease course of vitiligo as it is usually different from that of AV and AA. Patients with AA and AV often have effective treatment options for short-term therapy. However, these diseases usually relapse and cause more anxiety because of quick changes (improvement and worsening). On the other hand, vitiligo is usually more stable, and changes can only be seen in the long term which can result in patients' acceptance of the situation and coping mechanisms. Furthermore, vitiligo

Table 6: Main demographic characteristics of patients with alopecia areata

	n (%)
Sex	
Male	23 (46)
Female	27 (54)
Marital status	
Single	23 (46)
Married	25 (50)
Divorced	2 (4)
Income level	
High: Income exceeds expenses	13 (26)
Moderate: Income is equal to expenses	28 (56)
Low: Income is less than expenses	9 (18)
Education	
Primary school	2 (4)
Secondary school	6 (12)
High school	21 (42)
Postgraduate	9 (18)
Faculty	9 (18)
Master's degree	3 (6)
Concomitant diseases*	
No	49 (98)
Yes	1 (2)
Clinical condition	
Patchy alopecia	42 (84)
Alopecia totalis	3 (6)
Alopecia universalis	5 (10)
Nail changes**	
Yes	16 (32)
No	34 (68)
Progress of AA	
Acute	13 (26)
Chronic intermittent	28 (56)
Chronic persistent	8 (16)
Stable	1 (2)
Family history of AA***	
No	43 (86)
Yes	7 (14)
First-degree relative	5 (10)
Second-degree relative	2 (4)
Concomitant diseases	
Atopy	4 (8)
Connective tissue diseases	3 (6)
Thyroid diseases	9 (18)
Psychiatric diseases	1 (2)
SALT scale****	
S1	31 (62)
S2	5 (10)
S3	3 (6)
S4	7 (14)
S5	4 (8)
PHS*****	
Bad	16 (32)
Good	34 (68)

*Concomitant diseases: diseases such as diabetes, hypertension, etc. **Nail findings: Pitting, thickening, discoloration, red lunula, etc. ***First degree relatives: mother, father, brother; 2nd degree relatives: members of the parent family (uncle, uncle, aunt, etc.). ****SALT scoring; S1: Hair loss <25%, S2: Hair loss 25-49%, S3: Hair loss 50-74%, S4: Hair loss 75-99%, S5: Hair loss 100% of the scalp. *****Perceived health question; 1, 2, 3 are classified as 1, 2 and 3 ("worse than good"), and 4 and 5 ("good"). AA: Alopecia areata, SALT: Severity of Alopecia Tool, PHS: Perceived Health Status Questionnaire

Table 7: Correlation between the Internalized Stigma Scale, the Severity of Alopecia Tool score, the Dermatology Life Quality Index, the General Health Questionnaire, and Perceived Health Status Questionnaire

The ISS subscales	SALT score	DLQI	GHQ	PHS
Alienation	0.198	0.004	0.005	0.025
Stereotype endorsement	0.706	0.005	0.052	0.001
Perceived discrimination	0.874	0.001	0.022	0.099
Social withdrawal	0.272	0.001	0.039	0.634
Stigma resistance	0.272	0.665	0.159	0.515
Total	0.332	<0.001	0.024	0.057

DLQI: The Dermatology Life Quality Index, GHQ: The General Health Questionnaire, PHS: Perceived Health Status Questionnaire, SALT: Severity of Alopecia Tool, ISS: Internalized Stigma Scale

Table 8: Comparison of Cronbach's alpha values of acne vulgaris, vitiligo, and alopecia areata diseases in terms of the Internalized Stigma Scale, the Dermatology Life Quality Index, and the General Health Questionnaire

Dermatological diseases	Cronbach's alpha values		
	ISS	DLQI	GHQ
AV	0.911	0.812	0.644
Vitiligo	0.919	0.329	0.586
AA	0.933	0.915	0.654

ISS: Internalized Stigma Scale, DLQI: The Dermatology Life Quality Index, GHQ: The General Health Questionnaire, AV: Acne vulgaris, AA: Alopecia areata

can be located on nonvisible body sites. However, AV and AA are almost always located on visible body parts. All these factors could affect the increased ISS levels in AV and AA. We also observed a positive correlation between the mean values of ISS and QoL scores for all the three diseases. This result demonstrates that the significance of internalized stigma is correlated with negative life quality. The dermatological diseases studied in our study are shown to affect the QoL of patients. Thus, by acknowledging this, practitioners may improve the general health of these individuals. Moreover, AV and AA were shown to affect the GHQ in our study, which suggests the detrimental effects of these diseases on general health.

However, there was no significant correlation between disease severity scores (SALT score, Acne FDA Global Score, or VETF and VIDA scores) and the mean values of the ISS in our study. These results show that the presence of the disease affects the ISS independently from the severity of these diseases. On the other hand, it is difficult to fully evaluate the relationship between the severity of the disease and the ISS, as a significant proportion of our patients were under treatment and were using systemic therapeutic options, especially those with severe illnesses. For vitiligo, we used both VETF (assessing disease activity, stage, and spreading) and VIDA (patients assessing the progression of the disease by themselves) scores as this could help us to evaluate both physicians' and patients' perspectives.

Married individuals were shown to have lower AQLS, DLQI, and GHQ scores and higher ISS scores. This finding was consistent with that of the study of Alpsoy *et al*.[10] They suggested that family members and their social environment and responsibilities may cause internalized stigma to be more pronounced in married individuals. Moreover, they put forward the idea of having a regular sexual life in married individuals, which might increase the perception of stigmatization. Education level was another significant factor in terms of ISS and DLQI scores. The ISS and DLQI scores were getting higher directly with the higher educational level. This suggests that patients with higher expectations due to understanding of the disease and/or higher status in the society are usually affected more severely. Although we could not find any relation with internalized stigma and gender, Kötekoğlu *et al*. found that the level of internalized stigma is more pronounced in male patients.[13] They suggested that the appearance of acne lesions may have adversely affected the perception that the opposite sex does not find them attractive enough, which can increase the likelihood of romantic rejection. They also suggested that the decreased internalized stigma in women could be due to the ability of easy camouflage with makeup, which can prevent the negative image of the acne.[13]

In women with vitiligo, scores of ISS and DLQI were found to be high even though they did not reach statistical significance, compared to males. Overall, the results showed that female patients are affected psychologically more severely by the disease, that their general health perceptions are more influenced, and that they experience stigmatization of the society and internalized stigmatization more often.

The strength of our study was comparing internalized stigma, with validated disease severity scores and accepted QoL scales, associated with three types of dermatological diseases in patients who frequently visit dermatology outpatient clinics. However, there were some limitations of our work. The cross-sectional study design led to limitations in revealing causality between illnesses and stigmatization. Furthermore, limited number of patients in each subtype of disease made it difficult for our outcomes to generalize to all individuals. Furthermore, QoL scales are based on individual responses and they only assess the level of impact of individuals accepting participation in the study. Moreover, the low number of samples in each disease group may have caused the inability to show a significant relationship/s between some variables.

Conclusion

The present study indicates that patients with AV, AA, and vitiligo internalize the negative stereotype judgments of the society for themselves. High levels of internalized stigma in the studied patients presented a parallel trend to the negative QoL. Therefore, internalized stigma may be one of the major factors affecting the QoL in these diseases. A successful psychoeducation program is essential to reduce stigmatization and to raise awareness of the disease. This issue is also crucial in terms of establishing compliance with treatment.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

The Efficacy of Interfollicular Epidermal Stem Cells versus Bone Marrow-Derived Mesenchymal Stem Cells in Cutaneous Wound Healing in Diabetic Rats

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Abstract

Objective: Many sources of stem cells, such as bone marrow stem cells, embriyonic stem cells, adipose-derived stem cells, epidermal stem cells were used extensively for diabetic wound healing. This study investigated whether interfollicular epidermal stem cell versus bone marrow-derived mesenchymal stem cell effectiveness in the enhancement of diabetic wound healing. **Methods:** The streptozotocin-induced Spraque-Dowley rats with 5 mm punch biopsy were used. Rats were divided into three groups: group I diabetic controls receiving no stem cells; group II, rats receiving bone marrow-derived mesenchymal stem cells; group III, rats receiving interfollicular epidermal stem cells. Wound healing was assessed clinically that regarding healing time and wound size. **Results:** Clinical results showed that wound size was significantly reduced in mesenchymal and interfollicular epidermal stem cell-treated groups as compared with controls. Complete wound-healing times were 19.4±2.85 days in bone marrow-derived mesenchymal stem cells group versus 20.3±3.45 days in interfollicular epidermal stem cells group and 24.7±4.17 in the control group. In the measurement of the wound area, there were no significant differences between the bone marrow-derived mesenchymal stem cells group (P=0.115) and interfollicular epidermal stem cells group (P=0.085). **Conclusions:** Interfollicular epidermal stem cells were found as effective as bone marrow-derived mesenchymal cells in the treatment of the diabetic wound.

Keywords: Bone marrow, diabetes, stem cells, wound

Introduction

Diabetes is a common major health problem in the world. Poor wound healing process in foot ulcers extremely serious condition in diabetic patients.[1-3] Wound healing requires a well-orchestrated integration of the complex biological and molecular events. Cell proliferation and migration, extracellular matrix deposition, and remodeling are the main steps in normal wound healing.[4-6] In diabetic models, altered expression of many molecular factors contributes to wound healing process. Reduced chemotactic ability influences inflammatory cells into the diabetic wound tissue.[1,2] New therapeutic modalities for chronic wounds are currently being used in clinical studies.[6,7] Recently, topical or systemic adult stem cell-based therapy has been widely used for diabetic wound repair and tissue regeneration.[8-10] Several preclinical and clinical studies showed that different types of stem cells, such as bone marrow-derived mesenchymal stem cells (BMSCs), endothelial progenitor cells, adipose-derived stromal cells, and epidermal stem cells (ESCs), have been used for wound healing.[10-13] BMSCs which are pluripotent stem cells are capable of differentiation into different types of cells, such as fibroblasts, adipocytes, vascular endothelial cells, and epithelial cells.[11-15] BMSCs promote that ability to migrate to the wound area, regeneration of damaged tissue, and stimulate proliferation and differentiation promote for wound healing.[12,13,15-18] On the other hand, ESCs are primitive, unique,

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multipotent stem cells that located in interfollicular epidermis and around the hair follicles. Interfollicular ESCs (IESCs) are crucial for wound coverage and restoring epidermal maintenance.[19-21] IESCs and their progenitors induce the proliferative phase in wound healing process and help to restore the barrier function of the skin.

In this present study, considering these previous clinical studies, we try to compare the beneficial effects of bone marrow mesenchymal stem cell and IESC populations in the healing of the wounds in diabetic rats.

Materials and Methods

Forty streptozotocin-induced diabetes Sprague-Dawley (SD) rats were selected; these rats were divided into three groups (n = 10). Group I – diabetic controls received 0.5 ml phosphate-buffered saline (PBS) with no stem cells; Group II – rats receiving BMSCs; and Group III – rats receiving IESCs. Group IV was used tissue sample source for the bone marrow mesenchymal stem cells and IESCs.

Animals

Adult male, SD rats, the average weight of 250–300 g, were used for the experimental process. The rats consumed standard rat chow and water *ad libitum*. The protocol for this study was approved by the Gulhane Investigation and Development Center (AR-2005/49).

Diabetic rat models

Diabetes was induced, after 24-h starvation, by intravenously injecting streptozocin (60 mg/kg in 0.9% NaCl, adjusted to a pH 4.0 with 0.2 Mol/l sodium citrate). The rats were anesthetized with methoxyflurane before injection. Diabetes was verified 7 days later by evaluating blood glucose levels with the use of glucose oxidase reagent strips Glikolyzer (GA-100- Kyoto Daiichi Kagaku Co Ltd. Kyoto Japan). Rats with a blood glucose level 300 mg/dl were considered to be diabetic and also after 1 week of having this level of blood glucose, 5 mm punch biopsy obtained for wounds. Glucose levels were measured every 3 days regularly. Age-matched normal SD rats served as controls.

Isolation, culture, and labeling of the stem cells

Bone marrow mesenchymal stem cells

BMSCs were obtained from the long bones (tibia and fibula) of 12 weeks of SD rats by surgical operation. Sterilization for long bones was done with alcohol for 4 h. After the distraction head of the bone, mesenchymal stem cells were collected by aspiration. Aspiration material resuspended in complete culture medium supplemented with 1% penicillin-streptomycin (Sigma, USA, P4333). Cells were incubated at 37°C, in 5% humidified CO_2 for 14 days. Media were changed every 5 days. When cell population reaches to the 80%–90% confluence, cultures were washed with PBS (P5493, Sigma, USA) and cells were trypsinized with 0.25% trypsin (Sigma, USA, T1246) in 1 ml ethylenediaminetetraacetate (EDTA) (Sigma, USA, E6758) for 5 min at 37°C. After centrifugation (at 2,400 RPM for 20 min at room temperature), cell pellets were resuspended and incubated in culture flasks. After then, 100 ml of BMSCs suspension were used for the flow cytometer. According to criteria proposed by the Mesenchymal and Tissue Cell Committee, BMSC must express CD29, and lack expression of CD45. The BMSC positive for CD29 (Sigma, USA, SAB) and negative for CD45 (Sigma, USA, Ox-1) were used in this study. Flow cytometer showed the presence of marker CD29 on more than 80% and absence of CD45 (<1% of cells).

Interfollicular epidermal stem cells

The 5 mm punch skin biopsy was taken from the dorsal part of the rats. A sample of the skin was maintained for 6 h in DMEM, containing penicillin and streptomycin (50 μg/ml each), gentamicin (10 μg/ml), and an antimycotic agent (amphotericin B, 10 μg/ml). Then, the sample of the skin was kept in a plastic dish with 0.5% dispase II (Boehringer; Mannheim, Germany); for 18 h in a refrigerator (12°C). Each enzyme-treated piece was dissected horizontally it into two halves. The dermal surface was removed. Cells were incubated in a solution of 0.05% trypsin and 1% EDTA (both from Sigma) for 15 min at room temperature and the enzyme activity was then blocked with 2 ml of medium containing 10% FCS. After centrifugation, the supernatant was removed, and keratinocytes were gently resuspended in serum-free growth medium and cultured on six-well culture plates (Falcon), at a final seeding, the cell density of approximately 20×10^6 viable cells per well. After 12-h incubation at 37°C, unattached cells were removed by aspiration, and attached keratinocytes were then maintained in culture. Primary keratinocytes that formed colonies were left to grow until they reached about 70% subconfluence in the culture plate. Cultures were kept at 37°C in a 5% CO_2-in-air atmosphere in a humidified incubator.

Separation of the interfollicular epidermal stem cells

ESCs, especially located at basal layer which are called IESCs express different stem cell marker such as α_6 integrin (CD 49f) and β_1 integrin (CD 29). ESCs were tried to found using by fluorescence-activated cell sorting (FACS) analyzing according to their cell surface markers.[22,23] We analyzed only freshly isolated cells. ESCs were examined using two-color fluorescence dot plots for α_6 integrin (CD 49f-Sigma USA SAB) and β_1 integrin (CD 29-Sigma USA SAB). Dual staining was performed using fluorescein isothiocyanate (FITC)-conjugated (CD 49f) monoclonal antibody and FITC-conjugated (CD 29) monoclonal antibody from Serotec (Raleigh, N. C). FITC/Mouse Ig G1: RPE Ab from Serotec was used as a negative control. Labeling reactions were performed in the dark for 45 min at 4°C. The cells were then resuspended in PBS containing 2% FCS at 3×10^6/ml. All samples were analyzed immediately by a FACSCalibur flow cytometer (Becton-Dickinson USA). The experiments were repeated at least twice using the same conditions and settings. Cells positive for CD 49f and CD 29 accepted as IESCs and they were used in this study.

Wound healing model

The animals were anesthetized using chloroform inhalation. After cleaning the hair, skin was disinfected by ethyl alcohol.

Full-thickness skin specimens, including subcutaneous tissue were excised using the four different 5 mm punch (total area: 28 mm^2) biopsy device (Stiefel) from the dorsal side of rats.

Transportation of the stem cells

After isolation, stem cells were transported to the wound. Cytoline-2 microcarrier is macroporous and composed of the polyethylene and silica, was used for this procedure. Cytoline-2 microcarriers yield higher stem cell concentrations which can be sustained for a long time in the transplanted area. The stem cells at the 2–4 passages were used for loading onto the cytoline-2. Microcarriers were sterilized with 98% ethanol and spread over the 2 cm diameter culture flask. 2 mL cell suspension was plated into 2 cm diameter culture flask in two stem cell treatment group. They were incubated at 37°C with 5% CO_2 and shaken every 30 min over a period of 6 h to attach the cells onto the surface of the microcarrier instead of the base of the culture flask. After this step, 100.000 cells in one cytoline were transported into the wound area for each application. Approximately, 6–8 microcarries were seated in for each area (average 10^6 cells for each application). The exposed skin was covered by Tegaderm (3M Tegaderm™ Non-Adherent Contact Layer, USA). The wound area was washed and new stem cells transported to the wound area every 3 days until complete wound healing. In control group, 10 wounded rats were received 0.5 ml PBS (P5498, Sigma USA) injections without any stem cells each application.

The evaluation of wound healing

Wound size

Wound contraction was measured in 7th, 14th, 21th, and 28th postoperative days as a percentage reduction in area. Decreasing in the wound area was measured regularly by tracing the wound margin using on a tracing paper. The tracing is then placed onto graph paper, and the number of squares was counted. The total wound area was noticed each control and determined the differences from the initial measures of the wound area. The percentage of wound closure was calculated as (area of the original wound-area actual wound)/area of the original wound X100.

Time of complete healing

Time of the complete healing was recorded as the day on which wound healed completely. Healing was considered complete when the hairy skin covered the entire wound area and mean duration were noticed for complete healing was calculated for all groups.

Photographic documentation

The wounds were photographed at days 0, 7, 14, 28 with the rats in the prone position by using a digital camera (Canon EOS 100 D).

Statistical methods

Data were statistically analyzed to evaluate the difference between the groups. Data are presented as mean values ± SD. The results were considered statistically using the Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results

Phenotypic characterization of stem cells

Flow cytometer analysis of the BMSCs from passage 2 showed that these stem cells expressed CD29 but were negative for CD 45. Flow cytometer showed the presence of marker CD29 expression more than 80% and absence of CD45 expression (<1% of cells). It was considered that the major population of detected cells were BMSCs (Not shown). On the other side, two-color flow cytometric analysis of CD29 and CD49b expression in ESCs was noticed. At passage 3, most of the IESCs showed high levels of CD 29 and CD 49b expressions (93% and 88% respectively) suggesting that the most of the cultured cells were IESCs [Figure 1].

Wound size

Digital photographs of the macroscopic gross differences of the wound area are shown in Figure 2. It was found that wound closure increased significantly in two stem cell groups compared to control toward to 14 days. On day 14, faster recovery was observed in stem cell treated groups compared with the control group. Healing of the wound area on day 21 is faster stem cell groups than the control group ($P = 0.18$ for BMSC and $P = 0.23$ for ESC). On day 28, healing of the diabetic wound in ESC and BMSC groups completely finished. Mean values of wound area (mm^2) in the diabetic wound of rats were shown in Table 1. There were no significant differences between the BMSCs ($P = 0.115$) and IESCs ($P = 0.085$) according to the closure of the wound area at day 21.

The percentage wound closure was shown in Figure 3. Wounds closure rate of healing found 80% in IESCs and 78% in BMSCs groups and 60% in the control group on day 21. Furthermore, there were significant differences between diabetic rats treated with PBS (Group I), and stem cell (BMSCs and IESCs)-treated groups ($P < 0.05$).

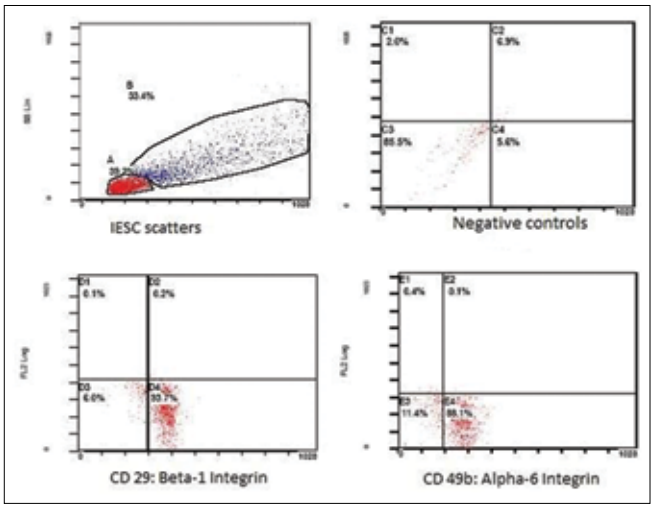

Figure 1: Flow cytometric analysis of cultured Interfollicular epidermal stem cells. Most of the stem cells highly expressed the surface markers, CD 29 and CD 49 (93% and 88% resp.)

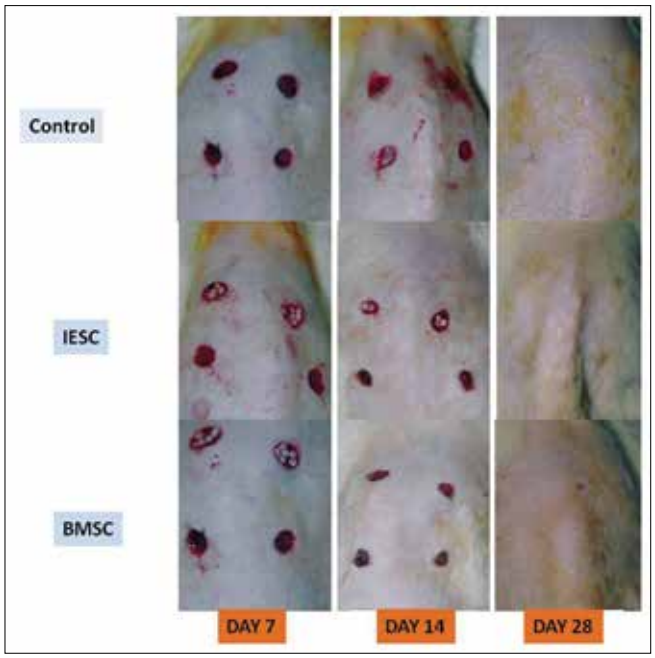

Figure 2: Representative images of diabetic wound healing treated with placebo, bone marrow-derived mesenchymal stem cell and interfollicular epidermal stem cell at different times at day 7, 14 and 28. The wound size was significantly reduced in bone marrow-derived mesenchymal stem cell and interfollicular epidermal stem cell group at day 14 as compared with the phosphate buffered saline control group. On day 28, all diabetic wounds in each group completely healed

Table 1: Mean Values of wound area (mm²)

Groups	Wound area in post wounding days			
	7th day	14th day	21th day	28th day
Diabetic control	27.1±3.5	16.4±4.7	11.5±1.9	2.5±0.9
Bone marrow SC	22.0±3.3	11.8±2.2	5.3±0.9	0.3±0.2
Interfollicular ESC	23.4±3.2	12.1±1.9	5.7±1.1	0.4±0.1

Complete healing time

Differences between the stem cells treated and non-treated group were noticed on the 7th day, 14th day, and 21th day. Complete wound-healing time was 19.4 ± 2.85 days in BMSCs, 20.3 ± 3.45 days in IESCs and 24.7 ± 4.17 days in the control groups. There were significant differences between diabetic rats treated with PBS and treated with stem cells groups ($P < 0.05$). Complete healing was completed on the 28th day in all groups [Figure 3].

Discussion

Foot ulcers in diabetes are a serious complication of diseases due to impaired wound healing process.[1,2] The wound healing process depends on multiple factors, including cell migration, extracellular matrix deposition, chemokine, and growth factor secretion.[5,6] There is an abnormal inflammatory response, impaired vascularization, defective collagen metabolism and dysfunction of the growth factors in diabetes.[4]

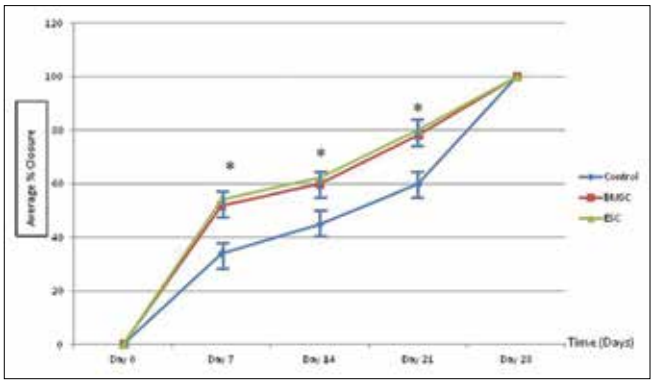

Figure 3: Percentage wound closure rates. Healing of the diabetic ulcer is expressed percentage closure (mean ± standard error). Wound sizes were measured at day 0, 7, 14, 21, 28. Bone marrow-derived mesenchymal stem cell and interfollicular epidermal stem cell-treated wounds showed greater percentage closure at day 7, 14, 21 compared with phosphate buffered saline controls. On day 28, all wounds in each group were completely healed. *Indicates a significant difference ($P < 0.05$)

Tissue engineering technologies have been widely used for the past 3 decades. It can provide many treatment options for wound healings instead of the traditional skin grafting.[8,10] Stem cells are regarded the master cells, capable of self-renewal properties, and promise enormous potential for tissue repair and regeneration.[9,24] The development of novel stem cell-based therapies shows promising results for the treatment of diabetic wound healing. The therapeutic potential of stem cell is due to promote to secrete proregenerative cytokines and other mediators.[8,9]

Many sources of stem cells, such as BMSCs, adipose-derived stem cells, and ESCs, were used extensively for wound healing.[8,10,11] BMSCs are fibroblast-like self-renewing stem cells in the bone marrow. These cells are nearly 10% of the hematopoietic stem cells in a number. BMSCs have been used widely successful in a streptozotocin-induced diabetic rat wound healing model.[20,25-27] The effects of the BMSC on diabetic wound healing were due to improving dermal matrix deposition, granulation tissue formation, and promote angiogenesis.[16,18,28,29] Addition to these effects, BMSCs may trans-differentiate into epidermal keratinocytes and differentiated skin.[27]

On the other side, ESCs are crucial for wound coverage and restoring epidermal maintenance.[26,30-32] ESCs are primitive, unique, multipotent stem cells. Different ESCs coming from the hair follicle, isthmus, infundibulum, and interfollicular epidermis contribute to wound healing.[7,11,24,33,34] Keratinocytes are derived from two different stem cell populations in the skin: IESC, located in the basal layer and hair follicular bulge stem cells (HFSC), located in the outer root sheath.[7,33,34] Unipotent IESC and their progenitors are essential for maintaining for interfollicular epidermis, therefore, basal layer is not only mitotically active layer and but also the interfollicular epidermis have its own stem cell population in the skin. IESCs possess high level of ß1 integrin and α6 integrin. Two

sources of the stem cells provide keratinocytes in the restore the wounded area.[34] In response to injury, cells from hair follicles and basal layer have been shown to migrate from stem cell area to the wound site.[32,34] It has been proposed that IESC-derived stem cells are the major long-term contributors to wound healing compare to HFSC.[21,30] Wound healing in normal skin is dependent on the replicative properties of IESC. IESC and their progenitors begin to proliferate and restore the barrier function of the epithelium.[34] On the contrary, Langton *et al*. suggested that keratinocytes from IESC contribute healing process without hair follicle stem cells.[35] There are some explanations about the effect of the IESC on wound healing. The differentiation of stem cells in a wound process is organized by the epigenetic mechanism. Epigenetic regulations of ESCs will be demonstrated enormous potential for diabetic wound healing.[36,37]

Previously, cultured autologous keratinocytes were used as single-cell sources for wound healing as first treatment modalities.[38,39] Repeated regular applications of the autologous keratinocytes and keratinocytes differentiated from embryonic and adipogenic stem cells were found effective treatment for wound healing in diabetes.[40,41]

The present study was the first experimental animal study using IESCs therapy for diabetic wound healing process compared to BMSCs population. IESCs and BMSCs groups were found equally effective in the treatment of streptozotocin-induced diabetic wound healing. The percentage wound closure increased significantly in two stem cell groups compared to control group at day 7, day 14, and day 21. On the other hand, the healing time of the wound area on day 21 is faster in two stem cells groups than the control group.

There are some concerns that we have to take into account before stem cell therapy for diabetic ulcers. It has also remained unclear which stem cell population, and delivery methods provide most effective methods. First, a selection of the most appropriate stem cell populations is a very important point for the treatment of the diabetic wounds. It can be speculated that IESCs are the most suitable stem cell source for wounds. Second, effective delivery methods are permitted to use to protect the stem cell source and provide functional enhancement. We used Cytoline-2 microcarriers for transplantation of the stem cell delivery. Cytoline-2 microcarriers yield higher stem cell concentrations which can be sustained for a long time in transplanted area. It may be useful to compare microcarriers to other delivery methods in the next experimental studies.

Conclusion

Our study demonstrated that the administration of IESCs was found equally effective as BMSC in the treatment of the diabetic wound healing. In the future, the availability of skin stem cells from the skin samples could provide new opportunities without the risk of immune rejection. The regenerative capacity of ESC from the skin will create new opportunities to develop stem cell-based therapies for wounds. It will be helpful to provide safer and more effective therapies for diabetic ulcers.

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Conflicts of interest

There are no conflicts of interest.

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