An Effective Treatment Method in Periungual and Subungual Warts: Bleomycin Application with Prick Technique

Dear Editor,

Warts of the nail unit are commonly seen in daily dermatology practice. Although, various treatments such as topical salicylic and lactic acids and 5-fluorouracil, cryotherapy, and electrosurgery are frequently used in the treatment of warts, they may not be effective in some cases and can cause permanent nail deformity.[1-3] Because of these difficulties, alternative treatment options are increasing in the literature. Application of bleomycin with the prick technique is an effective and good treatment for nail unit warts.[1-3] Herein, we report two cases of nail unit warts which were resistant to previous therapies and treated successfully with bleomycin using the prick technique.

Case 1

A 19-year-old female patient admitted to our outpatient clinic with periungual and subungual warts, which had been present for two years on her right thumb nail and resistant to many topical treatments and cryotherapy sessions. Following failure of these therapies, intralesional bleomycin with the prick technique was planned for the patient.

The vial containing 15 mg of powdered bleomycin sulfate was diluted with 15 mL of physiological saline. The nail unit was cleaned with povidone iodine. Approximately 0.5 mL of 2% lidocaine was injected bilaterally into the proximal and lateral nail fold junction and along with the lateral nail folds for local anesthesia (distal wing block) and after that a tourniquet was applied to prevent bleeding [Figure 1A]. After the bleomycin solution was dripped onto the wart with an insulin injector [Figure 1B], a large number of holes were drilled with a 27-gauge sterile syringe needle with 1 mm intervals (prick technique), allowing the drug to penetrate into the wart [Figure 1C]. The procedure was performed for two sessions with four-week intervals. Between sessions, the bleomycin solution was stored in the refrigerator at 4°C. In the follow-up of the patient at 12th week, the nail unit wart was completely healed [Figure 1D].

Case 2

A 36-year-old female patient to our outpatient clinic with periungual and subungual warts on her left thumb nail, which had been present for ten years [Figure 2A]. In her history, several methods were applied to the warts. After a punch biopsy which ruled out squamous cell carcinoma, bleomycin treatment with the prick technique was planned for the patient.

With the method described in the first case, 1 IU/mL bleomycin was administered with prick technique for three sessions. In the follow-up at 12th week, the wart was completely healed [Figure 2B].

Bleomycin has antiviral and antitumoral activities by inhibiting DNA and protein synthesis in virus and host cell.[4] Local side effects such as pain, tissue necrosis and onychodystrophy are less likely in prick technique compared to intralesional bleomycin, which is an another method.[4,5]

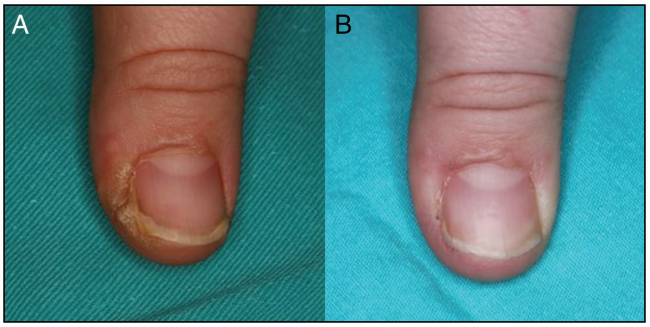

Figure 2: (A) 36-year-old female patient with periungual and subungual warts on her left thumb nail. (B) Follow-up of the patient at 12th week with complete resolution

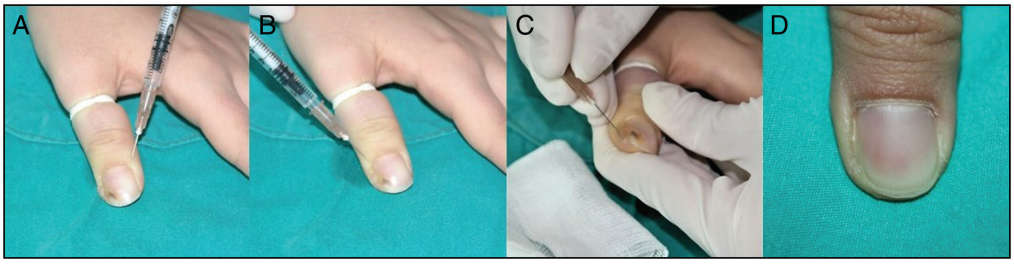

Figure 1: (A) Distal wing block and tourniquet application. (B) Bleomycin application as drops onto the wart. (C) Puncturing into the wart with a syringe. (D) Follow-up of the patient at 12th week with complete resolution

In conclusion, we report two cases of periungual and subungual warts, which were resistant to various treatments and successfully treated with bleomycin application with the prick technique. These cases are presented to emphasize that bleomycin application with the prick technique is an effective treatment option in nail unit warts.

Presentation at a meeting

Cases of the Month in Interventional Dermatology-2.

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Conflicts of interest

There are no conflicts of interest.

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Treatment of Androgenetic Alopecia with Autologous CD200 Positive Cell Suspension

Dear Editor,

Androgenetic alopecia (AGA) is a chronic disorder associated with miniaturization of hair. Research has shown that in AGA, the number hair follicular stem cell number remains the same but the number of proliferating progenitor CD200 and CD34 positive cells is reduced.[1] Gentile *et al.*[2,3] have performed CD200-rich progenitor cell transplant by autologous micrografts using manual mechanical detachment and mechanical device Rigenera™.

Non-cultured melanocyte transplantation (NCMT) is a well-established procedure for treatment of vitiligo. Trypsin enzyme is used to prepare a cell suspension of melanocytes and which is applied to dermabraded vitiliginous skin. Gupta *et al.*[4] have used hair follicles as a source of melanocytes. They used trypsin to separate the melanocytes from hair follicles. They have shown promising results in vitiligo. Incidentally, they found the presence of CD200 positive progenitor cell in their suspension indicating that same suspension may be useful in treatment of AGA. This is a retrospective report of a case where trypsin-isolated single cell suspension was used to treat AGA. A flow cytometric analysis was done.

One patient, aged 22 years, having grade III androgenetic alopecia, was treated with cell suspension of occipital follicular units. 24 mL blood was drawn to prepare platelet-rich plasma (PRP). The patient underwent follicular unit extraction (FUE) of 25 hair follicles (HF) using 0.9 mm punch. HF were washed with saline and collected in DMEM (Dulbecco's modified Eagle's medium) (Melanotrans kit, Cryobank Fertility Research Center, Jalna, Maharashtra, India). HF were incubated in 0.25% trypsin-EDTA (Melanotrans kit) at 37°C for 60 min. Before adding HF to trypsin, they were split longitudinally, slicing through the outer root sheath, to expose the bulge area to the trypsin. During incubation the HF were shaken every 5 min achieving an efficient separation of cells. After 1 h, only keratinous shafts remained in the suspension [Figure 1] and trypsin was inactivated using trypsin inhibitor. The suspension was filtered through a 40 µ filter ensure a single cell suspension. The suspension was then pelleted and redissolved in 5 mL of PRP. 0.05 mL was injected per cm^2 and hair growth was measured using trichoscopy before the procedure and after 8 weeks. Cell suspension from one case was sent for flow cytometry to ascertain the number of CD34 positive cells and CD200 positive cells. HF cells were washed with phosphate buffered saline (PBS) and suspended in 100 µL staining buffer (0.5% bovine serum albumin in PBS). 1 µL of antibody (FITC anti-human CD34 and PE anti-human CD200, BioLegend, San Diego, California) was added per

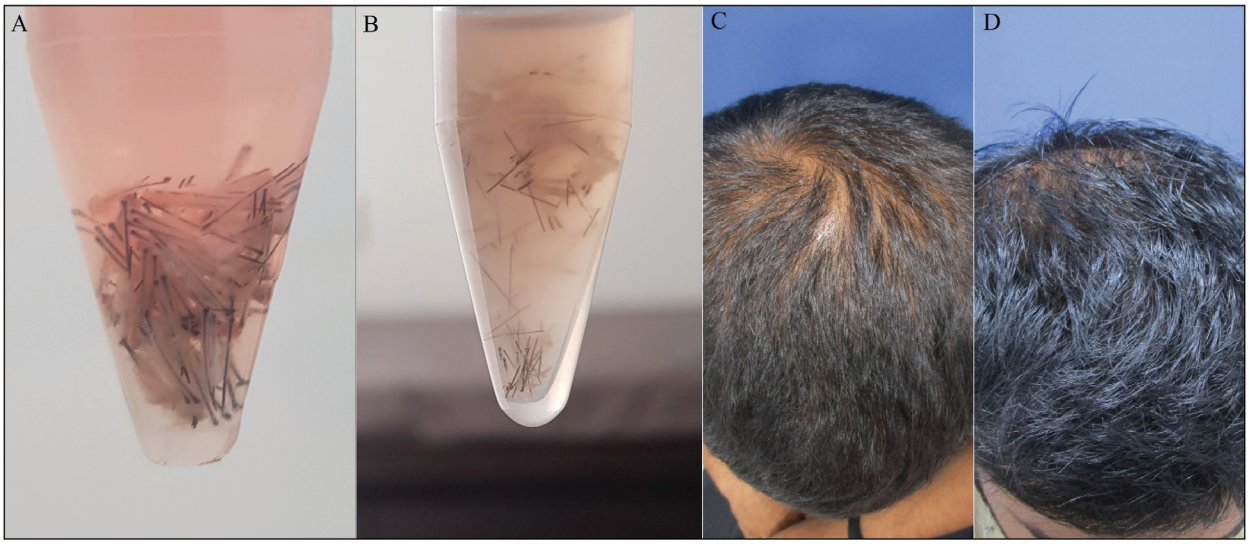

Figure 1: (A) FUE hair follicles before the start of trypsin cell separation. (B) FUE hair follicles after complete disintegration by trypsin. (C) Photograph of the vertex of patient with androgenetic alopecia before treatment. (D) Photograph of the vertex of the patient with androgenetic alopecia 8 weeks after treatment

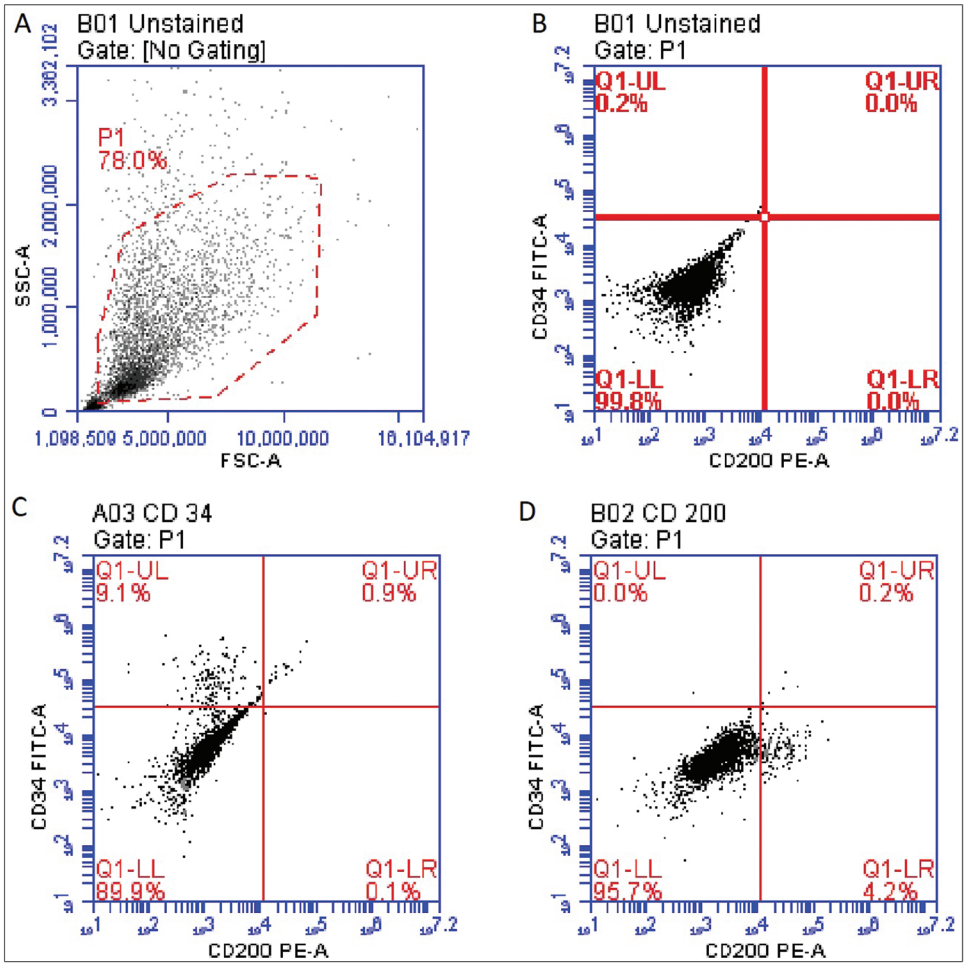

Figure 2: Flowcytometry was done using BD Accuri C6 plus flow cytometer (Becton, Dickinson and Company, Franklin Lakes, New Jersey). (A) The gating strategy developed with the help of unstained cells to identify overall hair follicle cell population and to remove cell debris from analysis. (B) Unstained cells were used to set quadrant gates for quantification of CD34 +ve and CD200 +ve cells. Compensation for fluorescence spill over was carried out using single stained cells. (C) Among the hair follicle cells 9.1% cells were CD34 positive 2D. 4.2% cells were CD200 positive

1×10^5 cells and the cells were incubated in dark for 15 min at room temperature. CD34 +ve and CD200 +ve cells were quantified using flow cytometer. Result showed an increase in hair density of 21 hairs/cm^2. On flowcytometry, 9.1% cells were CD34 positive and 4.2% cells were CD200 positive in the suspension [Figure 2].

In this case, we have quantified CD34 positive and CD200 positive hair follicle progenitor cells. The mechanical separation of cells using Rigenera™ and mechanical detachment of cells as shown by Gentile *et al*., utilize skin biopsy samples from the scalp as a source of progenitor cells and have shown 2.6±0.3% CD200 positive cells in their suspension. A possible higher yield in our case (4.2%) was probably because of use of FUE hair follicles instead of using skin biopsy samples. The innovative method of splitting open the outer root sheath to expose cells in the bulge area also possibly allowed a higher yield. We have repurposed the traditional method of NCMT for AGA. Further studies are required to confirm the viability of the use of this method in the treatment of AGA.

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Conflicts of interest

There are no conflicts of interest.

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A Case of Verruca Plana Juvenile Responding to Blue Light Phototherapy

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Abstract

Verruca plana, especially on the face, is a disease that can cause cosmetic and social concerns. Therapeutic approaches include immunomodulators and keratolytic agents. Blue light phototherapy shows promising results as an alternative treatment. Blue light phototherapy was applied to the patient with verruca plana on her face. We applied solely blue light phototherapy with a wavelength of 420 nm and a distance of approximately 15 cm. This kind of treatment could represent an effective, safe, and well-tolerated approach for the treatment of verruca plana.

Keywords: Blue, light, phototherapy

Introduction

Verruca plana, especially on the face, is a disease that can cause cosmetic and social concerns, leading patients to seek therapy. Commonly used treatment options include cryotherapy, topical retinoids, imiquimod, salicylic acid, topical immunotherapies, photodynamic therapy, etc. However, these therapeutic options may have various side effects, such as hyperpigmentation, edema, scarring, itching, and pain.[1,2] Therefore, there is a need for new effective treatment options with better cosmetic results.

Case Report

A 23-year-old woman presented to our clinic with a 1-year history of multiple verruca, increasing in number over time. Dermatologic examination revealed skin-colored papules with flat tops on the backs of the hands, arms, shoulders, neck, and face [Figure 1]. She was unresponsive to other treatments. Thus, we applied solely blue light phototherapy with a wavelength of 420 nm and a distance of approximately 15 cm to the area [Figure 2]. A total of 10 sessions were applied twice weekly, each session lasting 20 min. No side effects were observed. After 10 sessions, all lesions were completely regressed [Figure 3]. Written informed consent was obtained from the patient before the application.

Discussion and Conclusion

Various treatments are used for verruca plana in clinical practice. However, no treatment has been proven to be 100% effective.[3,4] Therefore, new treatment modalities are being sought that will provide effective and cosmetically better results.

The mechanisms of action of blue light include a decrease in keratinocyte and fibroblast proliferation, as well as the ability to cause regression of human papilloma virus by regulating T-cell functions and cytokine release through chromophores that can be found in its own structure.[5]

In our case, the regression of the existing lesions in a short period of time (5 weeks), obtaining a good cosmetic result, having an easy application method without the need for any photosensitizer, being inexpensive, and having a low risk of side effects support the consideration of blue light as a possible treatment option for verruca plana.

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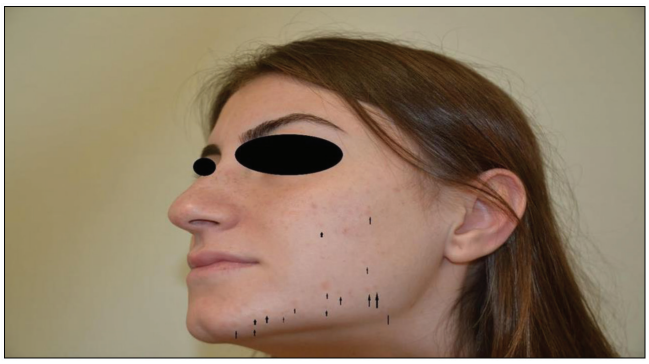

Figure 1: Grouped skin-colored flat papules on the left jawline (black arrow)

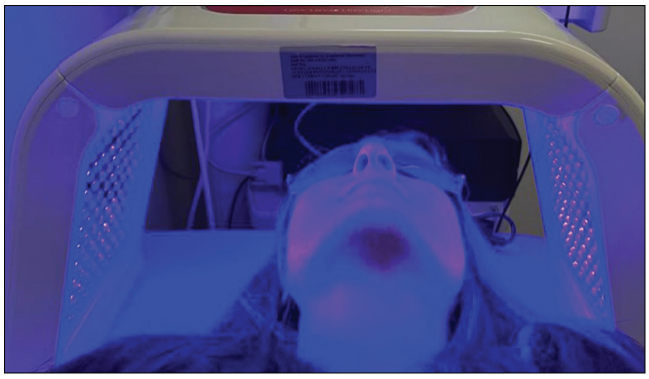

Figure 2: Blue light phototherapy

Declaration of patient consent

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

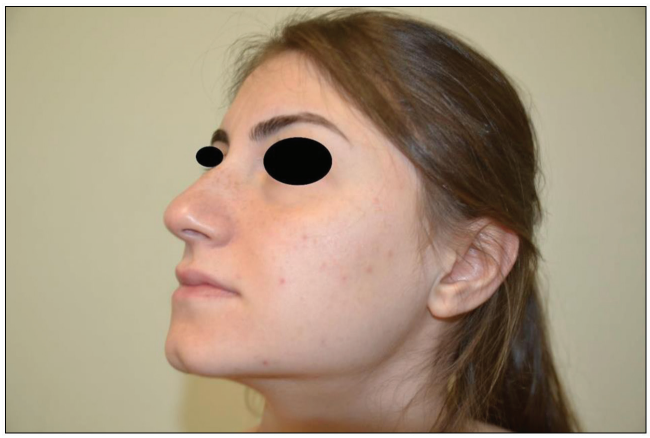

Figure 3: Several papulopustular acne vulgaris lesions in the malar region, with regression of previously existing papules on the left jawline

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Low-dose Apremilast Versus Low-dose Cyclosporine: Antipruritic Efficacy and Reversal of Epidermal Pathology in a Mouse Model of Atopic Dermatitis

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Abstract

Background: Itch control is important in improving the atopic dermatitis patients' quality of life, reducing the damage to the skin barrier, and, thereby, adding to the downregulation of skin inflammation. We aimed to investigate the efficacy of calcineurin inhibition by cyclosporine versus phosphodiesterase-4 inhibition by apremilast in controlling pruritus and reversing skin pathology in an experimental model of atopic dermatitis (AD) induced by oxazolone in mice. **Materials and Methods:** Forty BALB/c female mice were randomly assigned to four groups. AD-like lesions were induced in groups 2, 3, and 4 by repetitive application of oxazolone to the mouse skin. Group 2 mice were left untreated receiving vehicle placebo, whereas those in groups 3 and 4 received cyclosporine (2 mg/kg PO daily) and apremilast (2.5 mg/kg PO twice daily), respectively. Studied mice were subjected to weekly assessment of skin inflammation and scratching behavior for 6 weeks. The oxazolone-treated right ear thickness and skin hydration were measured at the end of the study. Serum immunoglobulin E (IgE) and interleukin (IL)-31 were measured, and biopsies of lesional back skin were obtained for histopathologic evaluation. **Results:** Both cyclosporine and apremilast significantly reduced scratching behavior in treated mice, accompanied by a significant decrease in the elevated levels of IL-31 and IgE by both drugs. IL-31 and IgE suppressions were significantly greater with apremilast. A significant reduction of mean itching started earlier at week 3 with apremilast versus week 4 with cyclosporine. **Conclusion:** We propose that the earlier control of itch observed with apremilast is clinically significant as this will lead to less epidermal damage and that will interrupt the itch-scratch cycle and progression of dermatitis.

Keywords: Atopic dermatitis, cyclosporine, itch, phosphodiesterase-4 inhibitor

Introduction

Atopic dermatitis (AD) is a chronic pruritic inflammatory dermatosis associated with an impaired skin barrier function.[1] Itching is a hallmark of AD to the extent that the disease has been described as an "itch that rashes." Chronic pruritus not only affects the patients' psychological well-being and quality of life but also injures epithelial keratinocytes promoting the release of inflammatory alarmins that activate Th2 cells to release inflammatory and pruritogenic cytokines that augment skin inflammation and pruritus.[2] Controlling AD-related itch is, therefore, considered to be a cornerstone in the management of AD.[3]

AD pruritus is believed to be mediated by the action of nonhistaminergic pathways and, thereby, does not respond to conventional antihistamines. Pruritogens including keratinocyte-derived products, mast cell factors,

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environmental allergens, pathogen-derived molecules, and inflammatory cytokines act on pruritogenic receptors.[2] Immune cells involved in the pathogenesis of AD such as T-helper cell 2 (Th2) lymphocytes, eosinophils, neutrophils, and mast cells activate the pruriceptive pathways through the release of cytokines and neurogenic peptides. The AD-associated interleukin (IL)-31 "itch cytokine" stimulates itch by activation of the receptors on pruriceptive neurons. IL-4 further sensitizes pruriceptive-sensory neurons to direct pruritogens as IL-31.[2] IL-31 also binds to its receptor IL-31RA on keratinocytes maintaining the chronicity of inflammation and atopic itch.[2]

Cyclosporine A (CsA) is a calcineurin inhibitor that acts primarily on T cells to inhibit signal transduction mediated by T-cell receptor activation.[4] It is a commonly used drug for systemic treatment of moderate-to-severe AD unresponsive to topical therapy and oral antihistamines.[5] Phosphodiesterase-4 (PDE4) is involved in the regulation of proinflammatory cytokines through the degradation of cyclic adenosine monophosphate. PDE4 activity was reported to be increased in the inflammatory cells of patients with AD leading to increased production of proinflammatory cytokines and chemokines. Inhibition of PDE4 will, therefore, lead to the reduction of the production of proinflammatory mediators in AD.[6] Apremilast is a PDE4 inhibitor (PDE4I) that is better tolerated, with a more favorable safety profile than cyclosporine.[7] The most commonly reported side effects of apremilast are mild as diarrhea, nausea, upper respiratory infection, and headache with no known end-organ damage.[8] It has been approved by The United States Food and Drug Administration (FDA) for the treatment of plaque psoriasis and psoriatic arthritis. Apremilast has demonstrated a potential as a treatment option for AD.[6,9] In the current study, we compared the potential antipruritic effects of cyclosporine and apremilast in an experimental chronic AD mouse model induced by oxazolone.

Materials and Methods

Animal care measures and experimental procedures were all conducted in accordance with the National Institute of Health Animal Care Guidelines.[10] The research protocol was approved by the institutional ethics committee (IRB 00012098).

Based on the reported mean ear thickness of 0.27 mm in cyclosporine-treated AD mice,[11] 0.39 mm in apremilast-treated AD mice,[12] 0.43 mm in vehicle-administered AD mice,[12] and 0.21 mm in normal mice,[12] the sample size was calculated using the G-power software (Heine University Dusseldorf, Dusseldorf, Germany) using a one-way analysis of variance (ANOVA) analysis, adjusting a power at 80%, level of confidence at 95, and effect size 0.6. The minimum sample size needed to investigate the efficacy of cyclosporine versus apremilast in controlling pruritus and reversing epidermal pathology in oxazolone-induced AD mouse model is 36 female BALB/c female mice (nine per group).

Forty BALB/c 5-week-old female mice were purchased from the animal house of the medical physiology department and housed in clean polypropylene cages at a room temperature of 22–25°C and a 12 h dark/12 h light cycle with free access to food and tap water throughout the experiments. Mice were allowed a period of 1 week for adaptation after which they were randomly assigned to four groups.

Group 1 (normal control mice)

Ten mice in which distilled water was painted to the right ear and shaved rostral back as a single application and continued from day 8 every other day for 6 weeks instead of 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone).

Group 2 (untreated atopic dermatitis mice)

Ten mice were exposed to a single application of 20 µL of 5% oxazolone (Sigma-Aldrich, St. Louis, Missouri) dissolved in a mixture of acetone and olive oil (4:1),[13] which was painted to the right ear and shaved rostral back, to develop acute dermatitis. Starting at day 8, mice were rechallenged by 0.1% oxazolone solution (20 µL applied to the right ear and 40 µL applied to the shaved rostral back) every other day for 6 weeks to develop chronic dermatitis and received vehicle (placebo) daily by gavage feeding for 6 weeks.

Group 3 (cyclosporine-treated atopic dermatitis mice)

Ten mice were similarly challenged as group 2. Starting at day 8, the mice received cyclosporine in a dose of 2 mg/kg/day by gavage in 200 µL of water for 6 weeks (Neoral, Novartis, Switzerland). Three low-dose cyclosporine regimens (2, 5, and 10 mg/kg/day) were initially tested in a pilot study (five oxazolone-induced AD mice per group) and the lowest effective dose with no renal toxicity was chosen, which was 2 mg/kg/day. The three doses were initially tested against a vehicle-treated mouse as regards oxazolone-treated ear thickness. All three doses were associated with decreased ear thickness compared with the control group. Serum creatinine levels were measured. We observed that two mice of the 5-mg/kg/day treated mice developed diarrhea and two of the 10-mg/kg/day treated group demonstrated gingival hyperplasia. None of the mice showed increase serum creatinine levels. All three groups showed decreased ear thickness relative to the control group. We, therefore, selected a low-dose cyclosporine of 2 mg/kg/day.[14] It is known that the risk of chronic cyclosporine nephropathy is minimal with doses less than 5 mg/kg/day,[15] and serum creatinine in

mice receiving 2 mg/kg of CsA was reported to be similar to that of non-CsA-treated mice.[16] In the present study, the oral route for cyclosporine administration was chosen because of its clinical relevance in patient treatment.

Group 4 (apremilast-treated atopic dermatitis mice)

Ten mice were similarly challenged as group 2. Starting at day 8, the mice received apremilast 2.5 mg/kg (Otezla, Amgen, California) dissolved in vehicle and administered in a volume of 5 mL/kg twice daily by gavage feeding for 6 weeks. Similarly, for apremilast, we tested 2.5, 5, and 25 mg/kg twice daily doses in a pilot study (five oxazolone-induced AD mice each). All three doses were associated with decreased oxazolone-treated ear thickness compared with the vehicle-treated group. The 2.5- and 5-mg/kg twice daily treated mice demonstrated no side effects. Three of the 25-mg/kg/day treated mice suffered from vomiting. The 2.5 mg/kg twice daily dose was chosen for the study by virtue of the absence of observed side effects and decreased ear thickness. Furthermore, in a preclinical toxicology study conducted on mice (CC-10004-TOX-004) receiving 10, 100, and 1000 mg/kg/day of apremilast daily, the no-observed adverse effect level (NOAEL) was demonstrated to be 10 mg/kg/gay. Hence, the 2.5 mg/kg twice daily dose employed in our study represents 50% of NOAEL.[17]

All behavioral tests and study measurements were performed by an experimenter blinded to experimental conditions. The following parameters were evaluated:

a) Scratching behavior: Mice were placed individually in acrylic cages. A camcorder (HDR-SR11; Sony, Tokyo, Japan) was positioned above the observation chambers to record the behavior of the mice. Mice were allowed an acclimation period of 1 h, after which a challenge with oxazolone was done, and the mice were quickly returned to the observation chamber. Mice could not see each other during an experiment. The behavior of mice was recorded on video for 40 min with no experimenters present in the observation room, and the number of scratching bouts was assessed by monitoring and counting the replays of each video. A scratching bout was defined as the raising to lowering of a leg, scratching behind the ears was counted, whereas scratching episodes on the face were not counted. One scratching bout was defined as a single or uninterrupted scratching actions of the hindpaws to the neck area that ended with the animals putting the hindpaws back on the floor or licking the hindpaws. Scratching behavior was observed weekly for 6 weeks and expressed as the number of scratching bouts/40 min.
b) Skin hydration was evaluated by EnviroDerm Services Tewameter (Dermal Measurement System EDS12, UK) at the end of the 6th week as an indicator of the epidermal barrier function.[18]
c) Skin inflammation severity scoring was assessed weekly by the Matsuoka scoring system.[19] The severity of the macroscopic clinical signs of dermatitis was measured by the extent of (1) erythema/hemorrhage, (2) scarring/dryness, (3) edema, and (4) excoriation/erosion. The score for each criterion was graded as follows: 0 (none), 1 (mild), 2 (moderate), and 3 (severe).
d) The right ear thickness was measured at the end of the 6th week by using a micrometer (Mitutoyo Corp, Kawasaki, Japan). The micrometer was applied to the right ear edge immediately adjacent to the cartilage bulge, and thickness was recorded. Each measurement was taken twice, and the mean of the two readings was calculated. Measurements were made by a single independent blinded observer to ensure similar pressure and placement of the micrometer.
e) Serologic evaluation: Blood samples from the abdominal aorta of mice were obtained after sacrificing the mice at the end of the 6th week. Serum was collected immediately from the blood by centrifugation and stored at −80°C till laboratory measurements. Serum immunoglobulin E (IgE) and IL-31 concentrations were measured using mouse solid phase standard sandwich IgE enzyme-linked immunosorbent assay (ELISA) (Chongqing Biopsies Co, Ltd, Chongquing, China) and IL-31 ELISA kits (Innova Biotech Co Limited, Chai Wan, Hong Kong) following the manufacturer's instructions. Samples were analyzed in duplicate and expressed in ng/L.[18,20] Blood samples were also obtained after sacrificing the animals for serum creatinine assessment to confirm the absence of renal toxicity.
f) Histopathological examination.

Following scarification, the liver and kidneys were fixed in 10% neutral-buffered formalin for the histopathological examination to confirm the absence of toxicity, and skin specimens were collected from the rostral back skin and ear skin of mice in all groups, then fixed in 10% neutral-buffered formalin solution. After a minimum of 24 h, specimens were subjected to dehydration in ascending grades of ethanol, then cleared in xylene and embedded in paraffin wax. Tissue sections (3–5-μ thick) were cut and stained with hematoxylin and eosin and Masson's trichrome stain according to Bancroft and Stevens,[21] and histopathologically evaluated at (×400).

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (IBM Corp., Armonk, New York). Categorical data were represented as numbers and percentages. The Chi-square test was applied to investigate the association between the categorical variables. Alternatively, the Monte Carlo correction test was applied when more than 20% of the cells had expected counts of less than 5. For continuous data, they were tested for normality by the Shapiro–Wilk test. Quantitative data were expressed as range (minimum and maximum), mean, standard deviation, and median.

The ANOVA test was used for comparing the four studied groups and followed by the post hoc test (Tukey) for pairwise comparison. Pearson coefficient was used to correlate between normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level.

Results

Thickness of the oxazolone-treated ear

The mean thickness of the oxazolone-treated ear in normal control mice was 0.41 ± 0.05 mm compared with 1.0 ± 0.09 mm in untreated AD mice ($P < 0.001$). The thickness of the oxazolone-treated ear was lowest in the apremilast-treated AD mice (0.61 ± 0.07), followed by cyclosporine-treated AD mice (0.64 ± 0.05), and highest in untreated AD mice (1.0 ± 0.09). Both cyclosporine- and PDE4I-treated mice groups had lower ear thickness (0.64 ± 0.05 mm, 0.61 ± 0.07 mm) than the untreated AD mice ($P < 0.001$). The difference between the cyclosporine- and PDE4I-treated mice groups was statistically insignificant ($P = 0.784$) [Table 1 and Figure 1].

Skin inflammation scoring at the 6th week

The mean Matsuoka score was 0 ± 0 and 8.0 ± 0.67 in normal controls and AD-untreated mice, respectively ($P < 0.001$). The mean Matsuoka score was reduced in both cyclosporine-treated mice and apremilast-treated AD mice (2.60 ± 0.52 and 2.20 ± 0.42, respectively) compared with untreated AD mice (8.0 ± 0.67). This difference was statistically significant ($P < 0.001$). The mean Matsuoka score was lower in the apremilast group than in the cyclosporine treatment group, but the difference was statistically insignificant ($P = 0.247$) [Table 1 and Figure 2].

Mice scratching behavior at the 6th week

The mean scratching score was 9.7 ± 1.25 and 75.8 ± 4.49 in normal controls and AD-untreated mice, respectively ($P < 0.001$). The mean scratching score was reduced in

Table 1: Clinical and laboratory parameters in the studied groups at the 6th week

Clinical/lab parameter	Normal negative control ($n = 10$)	Untreated atopic dermatitis mice ($n = 10$)	Cyclosporine-treated mice ($n = 10$)	PDE4 inhibitor-treated mice ($n = 10$)	P[a]
Thickness of oxazolone-treated ear (mm)					
Mean ± SD (range)	0.41 ± 0.05 (0.34–0.50)	1.0 ± 0.09 (0.88–1.12)	0.64 ± 0.05 (0.58–0.74)	0.61 ± 0.07 (0.53–0.74)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.784$			
Matsuoka score					
Mean ± SD (range)	0 ± 0 (0–0)	8.0 ± 0.67 (7–9)	2.60 ± 0.52 (2–3)	2.20 ± 0.42 (2–3)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.247$			
Scratching score					
Mean ± SD (range)	9.7 ± 1.25 (8–12)	75.8 ± 4.49 (65–81)	21.4 ± 2.41 (18–26)	19.6 ± 2.17 (17–23)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.497$			
Hydration					
Mean ± SD (range)	3.80 ± 0.79 (3–5)	1.0 ± 0 (1–1)	2.0 ± 0.67 (1–3)	2.30 ± 0.67 (1–3)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 = 0.005$*, $P_2 < 0.001$*, $P_3 = 0.699$			
Ig E (ng/mL)					
Mean ± SD (range)	34.2 ± 9.3 (23–49.5)	231 ± 69.2 (168–350)	119 ± 19.4 (94.5–160)	67.95 ± 20.97 (45– 110)	<0.001*
P_0		<0.001*	<0.001*	0.206	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.025$*			
IL-31 (ng/L)					
Mean ± SD (range)	6.90 ± 1.17(5–9)	24.40 ± 0.66(23–25)	15.65 ± 1.03(14–17)	11.85 ± 1.06(10–13)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 < 0.001$*			

SD = standard deviation.

P: P value for comparing between the four studied groups.

P_0: P value for comparing between Negative control and each of the other groups.

P_1: P value for comparing between untreated atopic dermatitis mice and Cyclosporine-treated mice.

P_2: P value for comparing between untreated atopic dermatitis mice and PDE4 inhibitor-treated mice.

P_3: P value for comparing between Cyclosporine-treated mice and PDE4 inhibitor-treated mice.

[a]One way ANOVA test, pairwise comparison between each two groups was done using post hoc test (Tukey).

*Statistically significant at $P \leq 0.05$

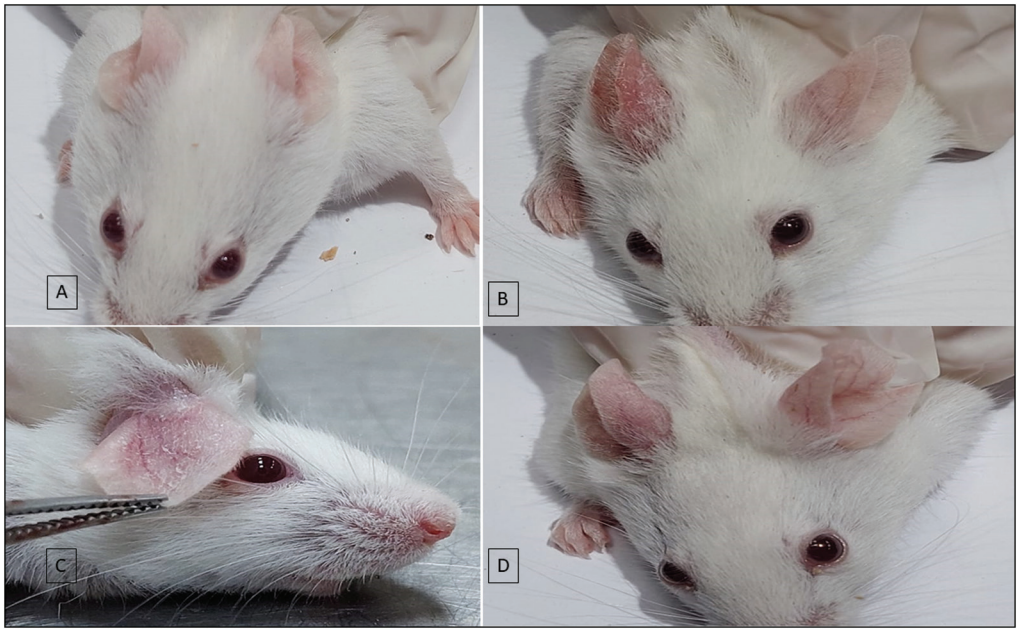

Figure 1: Right ear at the 6th week in (A) normal mice, (B) untreated AD mice, (C) cyclosporine-treated mice, and (D) apremilast-treated mice

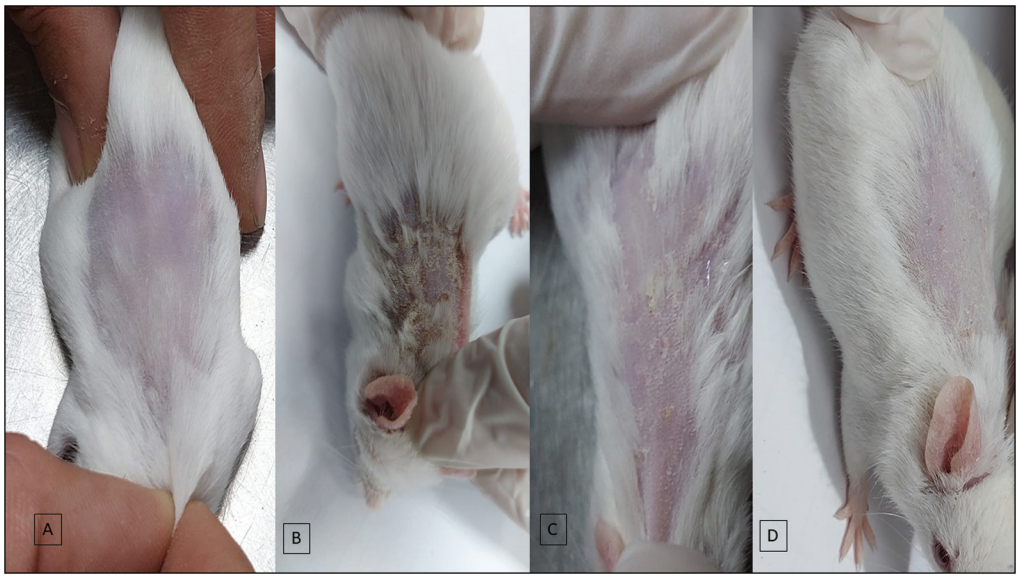

Figure 2: Rostral back skin at the sixth week in (A) normal mice, (B) untreated AD mice, (C) cyclosporine-treated mice, and (D) apremilast-treated mice

cyclosporine-treated AD mice and PDE4I-treated AD mice (21.4 ± 2.41 and 19.6 ± 2.17, respectively) compared with untreated AD mice (75.8 ± 4.49). This difference was statistically significant ($P < 0.001$). The mean scratching score was lower in the PDE4I-treated group compared with the cyclosporine-treated group, but the difference was statistically insignificant ($P = 0.497$) [Table 1].

Scratching scores during different time periods in the three groups

At the end of week 1, the mean scratching scores were 8.0 ± 1.05, 39.70 ± 1.57, 38.40 ± 3.17, and 38.60 ± 2.22 in the normal control, untreated AD, cyclosporine-treated, and apremilasttreated AD mice, respectively. At the of the 4th week, the mean scores were 8.20 ± 0.92, 57.70 ± 3.74, 30.0 ± 3.37, and 27.60 ± 3.06 in normal controls, untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the 5th week, the mean Matsuoka scores were 8.50 ± 0.53, 66.40 ± 3.86, 25.30 ± 3.06, and 22.90 ± 2.69 in normal controls, untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the study, the mean scores were 9.7 ± 1.25, 75.8 ± 4.49, 21.4 ± 2.41, and 19.6 ± 2.17 in normal controls, untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively.

Compared with week 1, the apremilast-treated group showed a significant reduction of the scratching score starting at week 3, which significantly decreased further at weeks 4, 5, and 6. However, the cyclosporine-treated group demonstrated a significant reduction of the scratching behavior starting at week 4 and decreased significantly further at weeks 5 and 6 [Figure 3].

Matsuoka scores during different time periods in the three groups

At the end of week 1, the mean Matsuoka scores were 6.10 ± 0.74, 6.30 ± 0.48, and 6.40 ± 0.52 in the untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the 2nd week, the mean Matsuoka scores were 6.80 ± 0.63, 5.10 ± 0.74, and 5.30 ± 0.48 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. The mean scores at the end of the 3rd week were 7.10 ± 0.74, 4.50 ± 0.71, and 4.50 ± 0.53 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the of the 4th week, the mean scores were 7.50 ± 0.71, 3.50 ± 0.71, and 3.70 ± 0.48 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the 5th week, the mean Matsuoka scores were 7.70 ± 0.48, 2.80 ± 0.42, and 2.80 ± 0.42 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the study, the mean scores were 8.0 ± 0.67, 2.60 ± 0.52, and 2.20 ± 0.42 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. Both the cyclosporine-treated and apremilast-treated mice groups showed a significant reduction of the Matsuoka scores starting at week 2 until the end of the study at the 6th week.

Skin hydration at the 6th week as an indicator of skin barrier function

The mean hydration at the 6th week in normal control mice was 3.80 ± 0.79 compared with 1.0 ± 0 in AD-untreated mice ($P < 0.001$). The mean hydration levels were 2.0 ± 0.67 and 2.30 ± 0.67 in the cyclosporine and apremilast-treated groups, respectively, which were significantly higher than AD-untreated mice ($P = 0.005$ and $P < 0.001$, respectively). The difference between cyclosporine- and PDE4I-treated AD mice groups regarding skin hydration at the 6th week was statistically insignificant ($P = 0.699$) [Table 1].

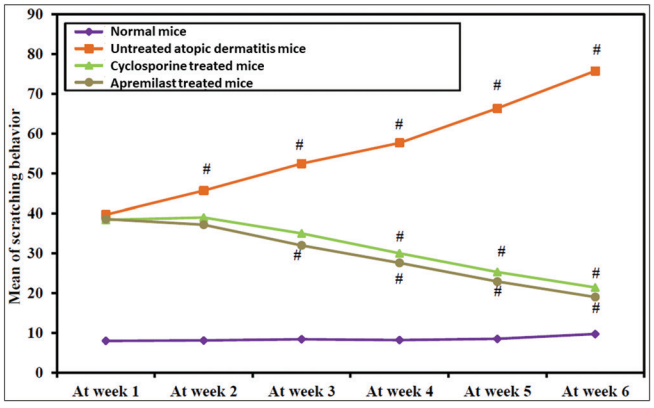

Figure 3: Weekly scratching scores in the studied groups

Serum IL-31 and IgE levels

The mean serum IL-31 was 6.90 ± 1.17 ng/L and 24.40 ± 0.66 ng/L in normal control mice and AD-untreated mice, respectively. This difference was statistically significant ($P < 0.001$). Either group receiving cyclosporine and apremilast had significantly lower mean serum IL-31 (15.65 ± 1.03 ng/L and 11.85 ± 1.06 ng/L, respectively) than untreated AD mice (24.40 ± 0.66 ng/L). This difference was statistically significant ($P < 0.001$). The mean serum IL-31 was significantly lower in AD mice receiving apremilast than in AD mice receiving cyclosporine ($P < 0.001$) [Table 1].

Both the apremilast-treated group and cyclosporine-treated group had significantly lower serum IgE level (67.95 ± 20.97 and 119 ± 19.4, respectively) than AD-untreated mice (231 ± 69.2). The mean serum IgE was significantly lower in AD mice receiving apremilast than in AD mice receiving cyclosporine ($P < 0.001$) [Table 1].

Histopathologic evaluation

Rostral back lesional skin

The mean thickness of the epidermis was significantly lower in normal controls (141.8 ± 47.41 µm) than in the lesional skin in the AD model group (507.3 ± 197.0 µm) ($P = 0.003$). The mean epidermal thickness in the cyclosporine-treated group (303.4 ± 93.15 µm) and apremilast-treated AD mice (134.3 ± 19.87 µm) were significantly lower than the epidermal thickness in untreated AD mice ($P = 0.008$ and $P = 0.002$, respectively). The difference between cyclosporine- and apremilast-treated mice was statistically insignificant ($P = 0.197$) [Table 2 and Figure 4].

The mean number of dermal cell infiltrate was 27.25 ± 4.57 cells/×400 in the normal controls versus 78.0 ± 10.68 cells/×400 in the untreated AD mice group ($P < 0.001$). The mean number of cell infiltrate in the cyclosporine-treated mice (36.75 ± 3.10 cells/×400) and the apremilast-treated mice (49.25 ± 7.76 cells/×400) were significantly lower than the untreated AD mice ($P < 0.001$ and $P = 0.001$, respectively). The difference between cyclosporine- and apremilast-treated mice was statistically insignificant ($P = 0.116$) [Table 2 and Figure 4].

Oxazolone-treated ear skin

The mean epidermal thickness of the right ear skin in the normal control mice was 49.27 ± 0.06 µm and 204.0 ± 4.86 µm in the untreated AD mice ($P < 0.001$). The mean epidermal thickness of the ear skin in each of the cyclosporine-treated (158.7 ± 36.33 µm)

Table 2: Histopathological scores of the lesional back and right ear skin

Histopathologic parameter	Normal negative control (n = 10)	Untreated atopic dermatitis mice (n = 10)	Cyclosporine-treated mice (n = 10)	PDE4 inhibitor-treated mice (n = 10)	P[a]
Lesional back skin					
Thickness of skin epidermis (μm)	141.8 ± 47.41	507.3 ± 197.0	303.4 ± 93.15	134.3 ± 19.87	0.001*
P_0		0.003*			
			P_1 = 0.008, P_2 = 0.002*, P_3 = 0.197		
Number of cell infiltrates (cells/×400)	27.25 ± 4.57	78.0 ± 10.68	36.75 ± 3.10	49.25 ± 7.76	<0.001*
P_0		<0.001*			
			P_1 < 0.001*, P_2 = 0.001*, P_3 = 0.116		
Oxazolone-treated ear skin					
Thickness of skin epidermis (μm)	49.27 ± 0.06	204.0 ± 4.86	158.7 ± 36.33	121.0 ± 18.01	<0.001*
P_0		<0.001*			
			P_1 = 0.037*, P_2 < 0.001*, P_3 = 0.091		
Thickness of skin dermis (μm)	231.8 ± 28.82	874.8 ± 131.4	426.7 ± 40.01	355.9 ± 138.7	<0.001*
P_0		<0.001*			
			P_1 < 0.001*, P_2 < 0.001*, P_3 = 0.745		
Number of cell infiltrates (cells/×400)	20.25 ± 0.96	89.50 ± 5.0	72.75 ± 4.50	46.75 ± 2.06	<0.001*
P_0		<0.001*			
			P_1 < 0.001*, P_2 < 0.001*, P_3 < 0.001*		

SD = standard deviation.

Data were expressed by using mean ± SD.

P: P value for comparing between the four studied groups.

P_0: P value for comparing between negative controls.

P_1: P value for comparing between untreated atopic dermatitis mice and cyclosporine-treated mice.

P_2: P value for comparing between untreated atopic dermatitis mice and PDE4 inhibitor-treated mice.

P_3: P value for comparing between cyclosporine-treated mice and PDE4 inhibitor-treated mice.

[a]One way ANOVA test, pairwise comparison between each 2 groups was done using the post hoc test (Tukey).

*Statistically significant at $P \leq 0.05$

and apremilast-treated mice (121.0 ± 18.01 μm) were significantly lower than in the untreated AD group (P = 0.037 and P < 0.001, respectively). The difference between cyclosporine- and apremilast-treated mice was statistically insignificant (P = 0.091) [Table 2 and Figure 5].

The mean dermal thickness of the ear skin was significantly lower in normal control mice (231.8 ± 28.82 μm) versus untreated AD mice (874.8 ± 131.4 μm) (P < 0.001). The mean dermal thickness of the ear skin in the cyclosporine-treated group (426.7 ± 40.01 μm) and the apremilast-treated group (355.9 ± 138.7 μm) was significantly lower than in the untreated AD mice (P < 0.001). The difference in ear dermal thickness between cyclosporine- and apremilast-treated mice was statistically insignificant (P = 0.745) [Table 2 and Figure 5].

The mean number of cell infiltrate in the normal controls (20.25 ± 0.96 cells/×400) was significantly lower than the untreated AD group cells (89.50 ± 5.0 cells/×400). The mean number of cell infiltrate in the cyclosporine-treated mice (72.75 ± 4.50 cells/×400) and the apremilast-treated mice (46.75 ± 2.06 cells/×400) was significantly lower than in the untreated AD mice (P < 0.001). The mean number of cell infiltrate in the two treated groups was statistically significant (P < 0.001) [Table 2 and Figure 5].

Discussion

AD is a common dermatologic disease with a worldwide prevalence of about 34% in children and 10% in adults.[22] The disease is characterized by an impaired barrier function, eczematous dermatitis, and chronic itching.[23] Reduction of pruritus contributes to barrier repair and suppression of cutaneous inflammation and is, therefore, considered a cornerstone in the AD management.[24,25]

Interestingly, itching in AD does not respond to systemic antihistamines. It has been postulated that the atopic itch is conveyed through nonhistaminergic sensory nerves. These nonhistaminergic sensory nerves are believed to be stimulated primarily by inflammatory mediators central to AD pathogenesis. Released alarmins TSLP, IL-33, and IL-25 stimulate itch and activate both innate and adaptive immune responses that accentuate the predominant Th2 inflammatory

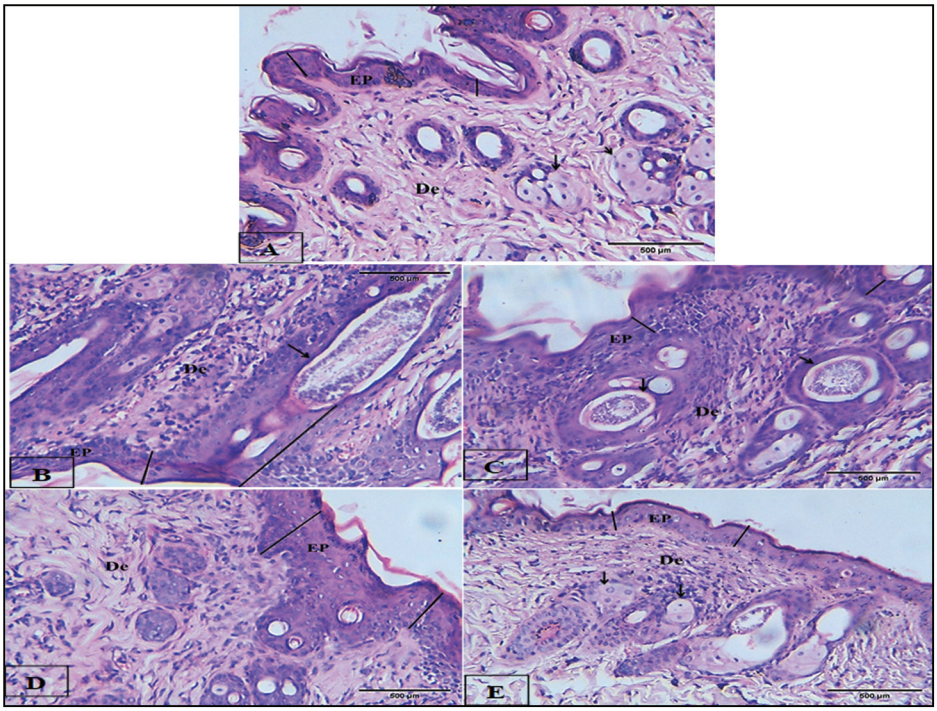

Figure 4: Rostral back skin sections in (A) normal mice showing external thin layer of epithelium (EP) over the dermis (De) cell layer and the sebaceous glands are fully developed (black arrows). (B and C) untreated AD mice showing loss of normal structure with marked hyperplasia of the epidermis and epidermal thickening (EP), and dermal infiltrate (De) consists of neutrophils, eosinophils, and lymphocytes and spongiosis (intraepidermal edema) are seen and most glandular tissue is seen as a cystic structure (black arrows). (D) Cyclosporine-treated mice showing almost normal structure with hyperplasia of the epidermis and epidermal thickening (EP) still present and dermal infiltrate (De) consists of neutrophils, eosinophils, and lymphocytes and spongiosis (intraepidermal edema) are seen. (E) Apremilast-treated mice showing almost normal structure with thin layer of EP over the De cell layer with mild dermal infiltrate (De) consists of neutrophils, eosinophils, and lymphocytes and the sebaceous glands are fully developed (black arrows)

immune response in AD and stimulate the generation of pruritus.[24] IL-31, also known as the itch cytokine, is believed to play an important role in the pathogenesis of atopic itch. It is produced by Th2 cells and acts on IL-31 receptors on sensory nerves generating itch sensation. The binding of IL-31 to its receptors on sensory nerves also stimulates the branching of the sensory nerves and also decreases the stimulatory threshold to IL-31 and other pruritogens. This increased sensitivity of sensory nerves is believed to be responsible for the chronic itch and perpetuation of the itch-scratch cycle.[24]

We employed an AD mouse model in BALB/c 5-week-old female mice based on outside–inside theory and compared the efficacy of cyclosporine and apremilast in the inhibition of pruritus and cutaneous inflammation. Our untreated AD mice demonstrated increased scratching behavior and decreased skin hydration compared with normal control mice. Mice in the employed AD mouse model demonstrated skin inflammation evidenced clinically by significantly higher Matsuoka score, and histopathologically by increased epidermal and dermal thickness, significant dermal inflammatory infiltrate of the oxazolone-treated ear skin and increased epidermal thickness and evident dermal cellular inflammatory infiltrate of the lesional back skin. These observations are in agreement support that repetitive extracutaneous application of the haptenoxazolone induces sensitization. This repetitive exposure provokes a Th2 immune response with several AD-like features such as scratching behavior and eczematous dermatitis. It also induces increased epidermal and dermal thickness and an inflammatory dermal infiltrate with several ultrastructural changes of decreased expression of skin differentiation proteins, decreased stratum corneum ceramide content leading to decreased stratum corneum hydration, and increased transepidermal water loss.[26]

The untreated AD mice also demonstrated significantly higher mean serum IgE and IL-31 levels than the normal control mice. The Th2 inflammatory response induced by repetitive oxazolone application AD stimulates B cells to produce IgE that binds with IgE receptors on several immune cells such as mast cells, basophils, and eosinophils inducing further production of cytokines, chemokines, histamine, and leukotrienes, maintaining and exacerbating the inflammatory response and clinical manifestations of AD. In fact, elevated IgE is regarded as a key immunologic feature of AD.[27-29] IL-31 is also known to be predominantly produced by Th2 cells.[25] A meta-analysis by Lu *et al*.[30] reported that serum IL-31

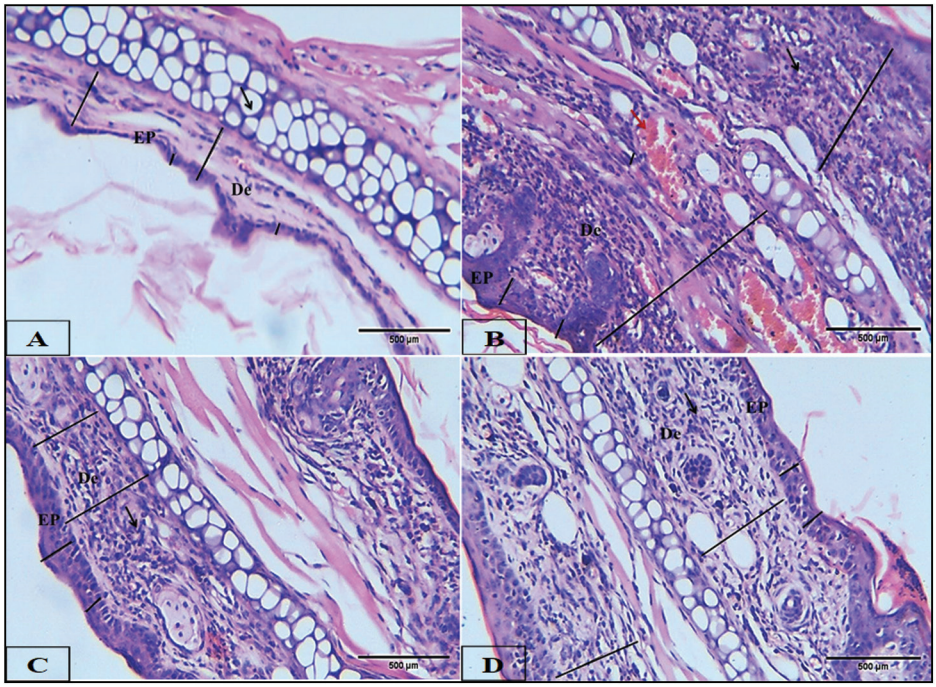

Figure 5: Ear skin sections in (A) normal mice showing normal thin epidermal layer of epithelium (EP) over the dermal layer (De) cell layer and cartilage (black arrow). (B) Untreated AD mice showing loss of normal structure with marked hyperplasia of the epidermis, epidermal (EP) and subepidermal thickening, and dermal dense cellular infiltrates dermis (De) of primarily mononuclear and some polymorphonuclear cells and spongiosis (black arrow) and sever hemorrhage (red arrow). (C) Cyclosporine-treated mice showing almost normal structure with hyperplasia of the epidermis and epidermal thickening (EP) still present and dermal infiltrate (De) consists of mononuclear and some polymorphonuclear cells and spongiosis (black arrow). (D) Apremilast-treated mice showing almost normal structure with thin epidermal layer of EP over the De cell layer with mild dermal infiltrate (De) consists of mononuclear and some polymorphonuclear cells and spongiosis (black arrow) and hyperplasia of the dermal cell layer

is significantly higher in AD patients than in normal controls. Available data suggests that IL-31 plays a possible role in AD pathogenesis and generation of itch.[25,31]

The cyclosporine-treated AD mice in this study demonstrated a significant reduction of dermatitis severity and increased skin hydration compared with untreated mice as evidenced by a significantly lower mean thickness of oxazolone-treated ear skin, mean Matsuoka score, and a higher mean epidermal hydration score. In agreement with our observations, Ko *et al*.[32] reported that intraperitoneal injection of CsA (5 mg/kg) significantly reduced dermatitis severity and transepidermal water loss in the AD mice model. These effects are the result of CsA-mediated T-lymphocyte activation and transcription of IL-2 and other cytokines involved in AD.

Our results show that cyclosporine-treated AD mice had significantly lower mean serum IgE and IL-31 levels than untreated AD mice. Lucae *et al*.[33] suggested that serum IgE levels in AD patients parallel the degree of skin inflammation, which explains the reduction of serum IgE following the reduction of skin inflammation with cyclosporine treatment. Cyclosporine is a calcineurin inhibitor that inhibits the activation of nuclear factor of activated T cells, decreasing T-lymphocyte activation and cytokine transcription of interferon-gamma (IFN-γ)/TH1– and IL-4/IL-13/IL-5/TH2–producing T cells and associated products including IL-31.[4,34]

The cyclosporine-treated AD mice in our study also demonstrated significantly lower epidermal and dermal thickness and lower dermal inflammatory infiltrate of the oxazolone-treated ear skin. The rostral back skin of cyclosporine-treated mice also showed a significantly lower epidermal thickness and significantly less dermal inflammatory infiltrate compared with AD mice. Ko *et al*.[32] reported that intraperitoneal injection of CsA (5 mg/kg) significantly reduced the epidermal thickness of treated mice. Similarly, Khattri *et al*.[4] reported that regenerative hyperplasia of the epidermis of AD skin was reversed with CsA as evidenced by reductions in epidermal proliferation and differentiation markers. This might be secondary to the CsA-mediated reduction of factors regulating epidermal hyperplasia (IL-19, IL-22, fibroblast growth factor, and vascular endothelial growth factor) and TH2/IL-13–, IL-19–, and IL-22/IL-17–modulated genes (S100A7-9 and PI3/elafin).

We reported a significantly lower scratching score in cyclosporine-treated mice than in untreated AD mice. Ko *et al*.[32] similarly reported that intraperitoneal injection of

CsA (5 mg/kg) significantly reduced scratching behavior and a number of scratching bouts. The inhibition of itch-related cytokines, such as IL-31, improved skin barrier function, reduction of acanthosis, and dermal inflammatory cell infiltrate to explain the antipruritic effects of cyclosporine treatment.

We reported a significantly lower thickness of oxazolone-treated ear skin and mean disease severity scores (Matsuoka scores) and improved barrier function (skin hydration) in apremilast-treated mice compared with AD mice. Schafer *et al*.[12] showed that apremilast of 2.5 mg/kg twice daily significantly reduced ear swelling in two models of dermatitis. Bissonnette *et al*.[35] showed that topical PDE4I reversed improved skin barrier function in terms of decreased transepidermal water loss.[35] Apremilast inhibits T-helper 1 and T-helper 17 cells through inhibition of IL-12 and IL-23 release from monocytes, respectively. Furthermore, it decreases prostaglandin E2-suppressing Th2 cell response. Inflammatory cytokines such as IFN-γ and tumor necrosis factor-alpha released from Th1 cells, IL-4 and IL-13 released from Th2 cells, and IL-17 and IL-22 released from Th17 cells are, thereby, decreased.[8] This inhibition of T-cell immune responses explains the observed reduction of clinical signs of inflammation.

We demonstrated significantly lower epidermal and dermal thickness and less dense dermal inflammatory infiltrate of the oxazolone-treated ear skin of apremilast-treated mice compared with the untreated AD group. The rostral back skin of apremilast-treated mice also showed a significantly lower epidermal thickness and dermal inflammatory infiltrate than AD mice. It was shown that mice ears topically treated with apremilast microemulsion exhibited less inflammatory cell infiltrate and a normal stratum corneum comparable with normal skin were observed.[36] The reduction of epidermal hyperplasia supports a role of apremilast in normalizing epidermal homeostasis and integrity regulation of epidermal keratinocytes.

We demonstrated that apremilast-treated AD mice had significantly lower serum mean IgE and IL-31 levels than untreated AD mice. Expression of PDE4 isoforms in the AD skin was found to be three-fold greater than in healthy skin,[12] and elevated PDE activity has been demonstrated in leukocytes from patients with AD.[6] The reduction of serum IgE probably reflects the reduction of skin inflammation. Mohan *et al*.[37] reported that apremilast treatment normalized IL-31 production. Apremilast inhibits T-helper 2 and 17 immune responses. Therefore, IL-4– and IL-17–dependent IL-31 production from keratinocytes is subsequently decreased.[8]

We reported a significantly lower mean scratching score in apremilast-treated mice than in untreated AD mice. Recent clinical trials highlighted the potential for apremilast in the treatment of AD and AD-related itch.[6,8] This can be explained by the inhibition of IL-4– and IL-17–dependent IL-31 production from keratinocytes contributing to the relief of pruritus,[8] in addition to decreased skin inflammation, improved barrier function, and the reduction of inflammatory cells that directly release itch-related mediators, such as NGF, cytokines, and proteases.

To the best of our knowledge, this is the first study to compare the efficacy of itch control between the commonly used low-dose cyclosporine and apremilast. Apremilast treatment was associated with significantly lower mean serum IgE and IL-31 levels than cyclosporine treatment. There was also a significantly less dermal inflammatory infiltrate in the ear skin of apremilast-treated mice compared with cyclosporine-treated mice. We observed that the dermatitis severity scores (mean Matsuoka scores and thickness of oxazolone-treated ear skin) were lower with apremilast; however, the difference was not statistically significant. Skin barrier function as assessed by hydration despite being higher with apremilast than cyclosporine treatment, the difference was not statistically significant. The histopathologic assessment showed no significant difference regarding epidermal, dermal thickness, or dermal infiltrate of the back skin. We suggest that both apremilast and cyclosporine showed comparable efficacy in reducing the severity of skin inflammation and decreasing epidermal and dermal hyperplasias. However, the apremilast-treated group showed a more rapid significant reduction of the scratching score starting earlier at week 3 after treatment. The cyclosporine-treated group demonstrated a significant reduction of the scratching behavior starting later at week 4. This might be secondary to a greater reduction of mean serum IL-31 levels and a greater reduction of the dermal inflammatory infiltrate that interacts with sensory nerve fibers in the atopic skin as reported in our study. This early control of pruritus was similarly reported by a post hoc analysis of phase 3 clinical trials of a topical PDE4I (crisaborole), which demonstrated an early improvement of pruritus.

The study is limited by the use of low-dose cyclosporine with minimal renal risk and a known apremilast dose representing 50% of the no-observed side defect dose. Higher doses are expected to exhibit more clinical efficacy. We believe that the earlier control of itch observed with apremilast is clinically significant as this will lead to less epidermal damage and that will interrupt the itch-scratch cycle and progression of dermatitis.[32,38,39] We suggest that apremilast is promising for the control of pruritus, reducing inflammation, and improving the skin barrier function. Studies employing different doses of apremilast owing to its favorable safety profile may help optimize dosing to reduce pruritus in AD patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Article

CD30 and PD-1 in Mycosis Fungoides

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Abstract

Introduction: Mycosis fungoides (MF) is the most common cutaneous lymphoma, accounting for 50% of all cutaneous lymphomas. Programmed death-1 (PD-1; CD279) is a marker of follicular helper T cells and is expressed by the neoplastic T cells of some types of malignant lymphoma, including MF. About 30% of primary cutaneous T-cell lymphomas are CD30 positive and have a broad spectrum from lymphomatoid papulosis to primary cutaneous anaplastic large cell lymphoma. CD30 expression in MF is important for diagnostic purposes, prognostic value, and a therapeutic perspective. In this study, PD-1 and CD30 expression in early MF lesions has been examined, and its relationship between prognosis and survival has been questioned. The byproducts could be unorthodox treatment options. **Methods:** We prospectively applied immunohistochemically CD30 and PD-1 antibodies to the biopsies at our institution. We statistically evaluated the relationship between the expression rates of CD30 and PD-1 in atypical lymphocytes, with recurrence and survival. **Results:** This research with 119 patients was able to show a statistically significant relationship between CD30 and recurrence with PD-1 and poor survival. **Conclusions:** When evaluated together with treatment options, CD30 and PD-1 are markers that may guide the clinical follow-up of aggressive MF cases.

Keywords: CD30 ligand, mycosis fungoides (MF), programmed cell death-1 receptor (PD-1)

Introduction

Mycosis fungoides (MF) is the most common cutaneous lymphoma, accounting for 50% of all cutaneous lymphomas.[1] It has been also shown that MF patients have an increased risk of developing other malignancies, such as lymphomatoid papulosis (LYP), primary cutaneous anaplastic large cell lymphoma (PCALCL), Hodgkin's lymphomas, or nonlymphoid neoplasia.[2]

The neoplastic lymphocytes of MF usually show a T-helper phenotype. Immunohistochemical studies are robust and helpful in demonstrating this. CD3, CD4, and CD8 could be the antibodies to start with. The CD4/CD8 ratio should be evaluated at the level of the epidermis and dermis. The normal ratio of CD4/CD8 is usually between 2:1 and 4:1, and a ratio of more than 10:1 is considered abnormal. The CD4/CD8 ratio should always be evaluated with CD3 because the background of Langerhans cells and macrophages can cause CD4 overexpression.[3] Rarely in early MF, an aberrant CD4+/CD8+ or CD4−/CD8− phenotype can be seen.[4,5] Double-negative cases can be positive with programmed death-1 (PD-1).[6] The earlier algorithms proposed a loss of more than 90% for CD7 and more than 50% for CD2, CD3, or CD5, to be considered abnormal. The detection of a high CD4/CD8 ratio and a low (generally <25%) CD8/CD3 ratio is critical in an appropriate clinicopathological setting for MF.[7] We have investigated the effectiveness of CD3, CD4, and CD8 ratios in the diagnosis of early stages of MF, by applying to naïve and early lesion biopsies. This way strengthening the database of the study was also aimed.

PD-1 (CD279), a membrane molecule, one of the B7 family receptors, is expressed from germinal center-related T cells in normal or reactive lymphoid tissues.[8] Its role as an inhibitory factor makes us consider it creates an immune-free environment and encourages

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the progression of the neoplastic cells.[9] Although the reported results of PD-1 in MF are conflicting, studies have accelerated with the widespread use of anti-PD-1 agents (nivolumab, pembrolizumab, etc.) in other malignancies.[8,10-12]

CD30 is a type 1 transmembrane glycoprotein molecule and is a member of the tissue necrosis factor superfamily. About 30% of primary cutaneous T-cell lymphomas are CD30+ and have a broad spectrum from LYP to PCALCL.[6] CD30 expression in MF is important for three main reasons: diagnostic purposes, prognostic value, and a therapeutic perspective. The percentage of CD30+ cells rarely reaches the 75% cutoff value necessary for the diagnosis of anaplastic large cell lymphoma.[7] Negativity for CD30 has been related to a poor prognosis in transformed MF. Brentuximab vedotin (BV), an anti-CD30 monoclonal antibody, has been a treatment alternative in recent years.[13-15] In an international, open-label, randomized, phase 3, multicenter trial by the ALCANZA study group and others is one of the major trials for anti-CD30 treatment in PCTCL. In the MF group, more than 50% of the patients have an objective response.[16]

In this study, PD-1 and CD30 expression in early MF lesions has been examined, and its relationship between prognosis and survival has been questioned. Prognostic markers for MF are still not well established, and these markers could be important for prognostication. The byproducts could be unorthodox treatment options.

Materials and Methods

Patient selection and clinical parameters

Retrospectively, the pathology archive was searched between 2008 and 2014, and prospectively, the dermatology clinic added the patients to the study in 2015. The first biopsies of the patients were included as naïve biopsies in the study as the diseases of these patients may progress over the years and could be affected by their treatments. Patients that did not have follow-up biopsies were excluded from the study. The clinical characteristics of the patients were obtained from the hospital management system. Informed consent was obtained from patients routinely before their biopsies. Local ethics committee approval of the institution has been obtained in 2015 for the research.

Morphological parameters

Histopathological evaluation was made from archive hematoxylin and eosin slides by two pathologists. On morphological base, biopsies that were characterized by basilar or disproportionate epidermotropism, epidermal cerebriform cells, Pautrier's microabscesses, epidermal lymphocytes larger than dermal lymphocytes, and dermal lichenoid or band-like lymphocytic infiltration were selected. Archive slides were also reevaluated for CD3, CD4, and CD8 expressions.

Immunohistochemical study

CD30 (mouse monoclonal, clone: Ber-H2, 1:40, DAKO, Glostrup, Denmark), PD-1 (Rabbit monoclonal, clone: SP269, 1:100, Spring Bioscience, Pleasanton, California, USA), CD3 (Rabbit monoclonal, clone: 2GV6, ready to use, Ventana, Arizona, USA), CD4 (Rabbit monoclonal, clone: SP35, ready to use, Ventana), and CD8 (Rabbit monoclonal, clone: SP57, ready to use, Ventana) staining were performed on deparaffinized, rehydrated tissue sections obtained from formalin-fixed and paraffin-embedded tissue blocks, using an automated slide stainer (Ventana-XT, Arizona, USA). Antigen retrieval was performed in a citrate buffer. Tonsil tissue was used as a positive control for the markers.

The expression of the atypical cell population was noted for all markers by percentage. If the expression was less than 10%, it was considered negative.

Statistical analysis

Statistical analysis was performed using SPSS 15.0 software (SPSS Inc., Chicago, Illinois). Categorical variables were presented as frequencies and percentages and were compared using Fisher's exact test. Results were considered significant at the $P < 0.05$ level.

Results

Clinical data

Among 1228 biopsies of 185 patients with the criteria of having paraffin blocks belonging to the first biopsies of the patients in the archive, having multiple biopsies of the patients, and having MF diagnosis in at least a biopsy, 119 patients were chosen. All the biopsies were from patch lesions. The total number of biopsies of these 119 patients between 2008 and 2016 was 824 [Figure 1]. The average number of biopsies was 7 (range: 2–18). A total of 75 (63%) were male and 44 (37%) were female. The mean age of the patients was 53 (range: 21–81). The average follow-up period of the patients was 4 years (range: 0–9). The patient with the shortest follow-up died 3 months after diagnosis.

Histopathologically, eight (7%) cases were diagnosed as folliculotropic MF, two (2%) had large cell transformation on their initial, and three (2.5%) had large cell transformation on follow-up. Three (2.5%) had pigmented purpuric dermatosis-like MF. A total of 42 (35%) patients were nonrecurrent, whereas 77 (65%) had a recurrence. Four (3%) died from MF and three (3%) from non-MF or unknown causes.

It was found that 38 (31.9%) patients received narrowband ultraviolet B, 10 (8%) phototherapy, and 1 (1%)

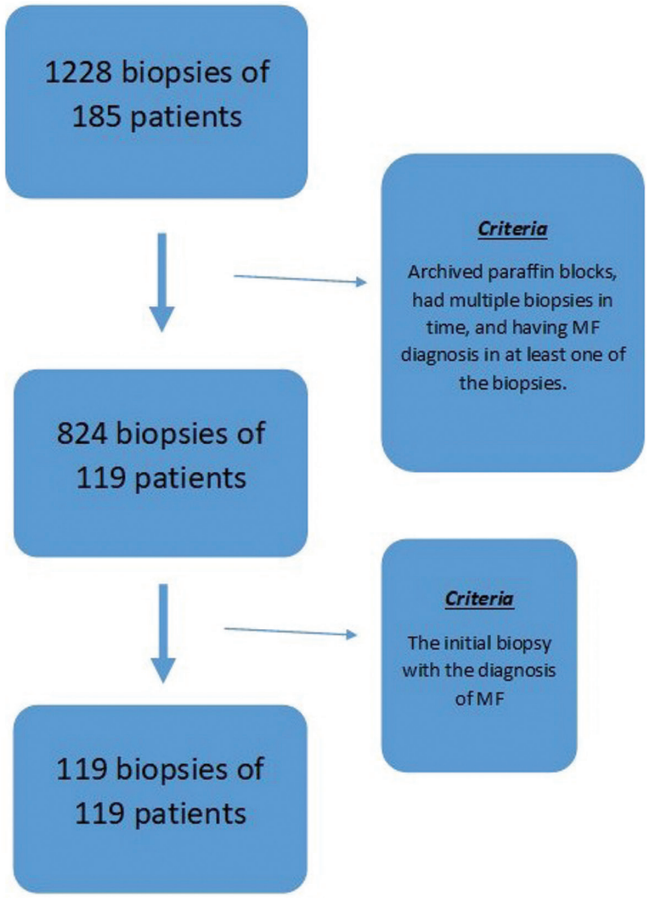

Figure 1: Flow chart of samples selected for the research

radiotherapy. Treatment of 70 (59%) patients was not available from the hospital management system [Table 1].

Immunohistochemical findings

In all cases, CD3, CD4, and CD8 expressions were evaluated in the epidermis and dermis separately. Diffuse CD3 expression was observed in intraepidermal and dermal infiltration in all biopsies. Both epidermal and dermal expression ratios of CD4/CD8 were increased (mean 4/1) in 99 (83%) biopsies [Figure 2], whereas CD4 and CD8 expression rates were similar in 20 (17%) cases. There was recurrence in 66 (55%) in the group with high CD4/CD8 expression rates, and 11 (9%) in the similar CD4/CD8 group. There were no statistically significant differences between the two groups in terms of recurrence (P = 0.32) [Table 2]. In the CD4/CD8 similar group, two (2%) were CD30+, three (3%) were PD-1+, and only one (1%) was both CD30+ and PD-1+.

CD30 expression was observed in 27 (22.6%), and 92 (77.4%) were negative [Figure 3]. Within the CD30+ group, recurrence was observed in 22 (19%), whereas nonrecurrent was 5 (4%). The expression rates of the recurrent group were 10%–50% [Figure 4]. However, the expression rates of the nonrecurrent group were 10%–20%. Within the CD30− patients, recurrence was observed in 55 (46%), whereas nonrecurrent was 37 (31%). There was a statistically significant relationship between CD30 expression and recurrence (P = 0.041) [Table 3]. Among the CD30+ patients, it was striking that the patient with 40% expression had large cell transformation in follow-up biopsies, but the patient with 50% did not.

PD-1 expression was observed in 22 (19%), and 97 (81%) were negative [Figures 4 and 5]. Among PD-1+ patients, recurrence was observed in 16 (14%), whereas nonrecurrent was 6 (5%). Within the PD-1− patients, recurrence was observed in 61 (51%), whereas nonrecurrent was 36 (30%). The expression rates of the recurrent and nonrecurrent groups were between 10% and 90%. There was no statistically significant relationship between PD-1 expression and recurrence (P = 0.46) [Table 3].

Table 1: Demographic data, histologic significant information, and immunohistochemistry results

Demographic data		Results (%)
Number of biopsies from each patient	Min.	2
	Max.	18
	Ave.	7
Sex	Male	75 (63%)
	Female	44 (37%)
Age	Min.	21
	Max.	81
	Ave.	53
Biopsy site	Abdomen	49 (41%)
	Back	21 (18%)
	Leg	15 (13%)
	Thigh	11 (9%)
	Arm	7 (6%)
	Gluteal	5 (4%)
	Unknown and other	11 (9%)
Follow-up period	Min.	3 months
	Max.	9 years
	Ave.	4 years
Therapy	Narrowband ultraviolet B	38 (32%)
	Phototherapy	10 (8%)
	Radiotherapy	1 (>1%)
	Unknown	70 (59%)
Histology and immunohistochemistry		Results (%)
Diagnosis	Classic MF	103 (86%)
	Folliculotropic MF	8 (7%)
	Large cell transformation	5 (4,5%)
	Pigmented purpuric dermatoses-like MF	3 (2,5%)
CD4/CD8 ratio	Min.	1/1
	Max.	25/1
	Ave.	4/1
CD30 expression	Positive	27 (23%)
	Negative	92 (77%)
PD-1 expression	Positive	22 (18%)
	Negative	97 (82%)

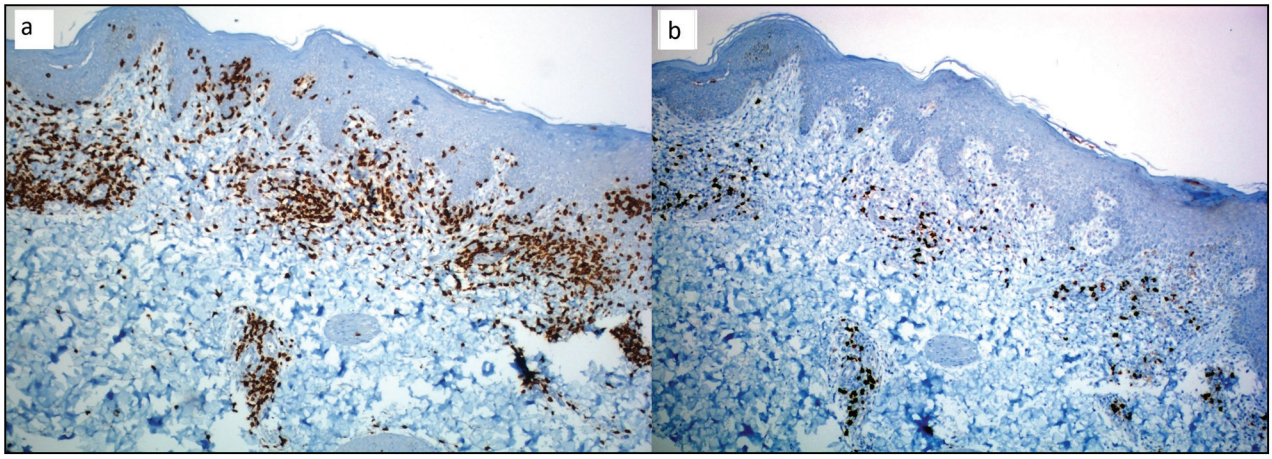

Figure 2: CD4 and CD8 immunohistochemistry staining (magnification, ×100). The high percentage of CD4 expression in atypical lymphocytes of mycosis fungoides (a) compared with CD8 (b) in the same areas of interest

Table 2: Relationship of CD4/CD8 ratio and recurrence

	CD4/CD8 ratio high	CD4/CD8 ratio similar	*P* value
Recurrent	66 (55%)	11 (9%)	0.32
Nonrecurrent	33 (28%)	9 (8%)	
Total	99	20	

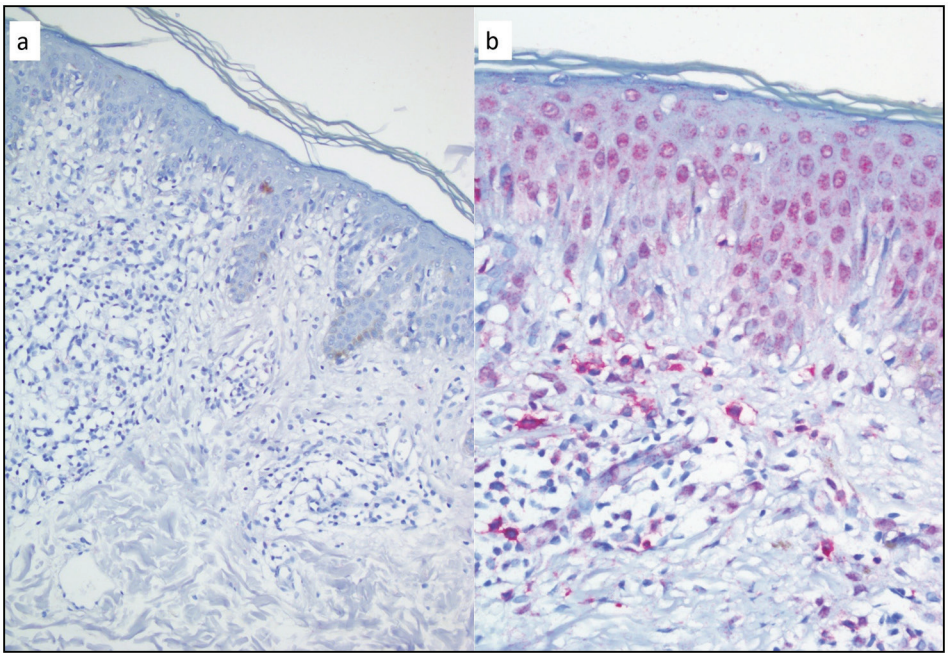

Figure 3: CD30 immunohistochemistry staining (magnification, ×200). (a) Negative. (b) Positive

Only five (4%) of the patients were both CD30+ and PD-1+. A total of 75 (63%) were CD30−/PD-1−. Among CD30+/PD-1+ patients, recurrence was observed in four (3%), whereas nonrecurrent was one (1%). Within the CD30−/PD-1− patients, recurrence was observed in 43 (36%), whereas nonrecurrent was 32 (27%). Among CD30− or PD-1− patients, recurrence was observed in 73 (61%), whereas nonrecurrent was 41 (35%). Within the CD30−/PD-1− patients, recurrence was observed in 43 (36%), whereas nonrecurrent was 32 (27%). There was no statistically significant relationship between dual CD30/PD-1 positivity and recurrence in both (P = 0.66 and P = 0.40) [Table 3].

All MF-caused ex-patient biopsies were PD-1+. The expression rates were 30%–90%. Their ages were between 58 and 81 years old. The follow-up period of these patients

was 1–7 years [Table 4]. One ex-patient developed Sezary syndrome (SS) on follow-up. A statistically significant difference was found between PD-1 expression and survival ($P = 0.001$) [Table 5].

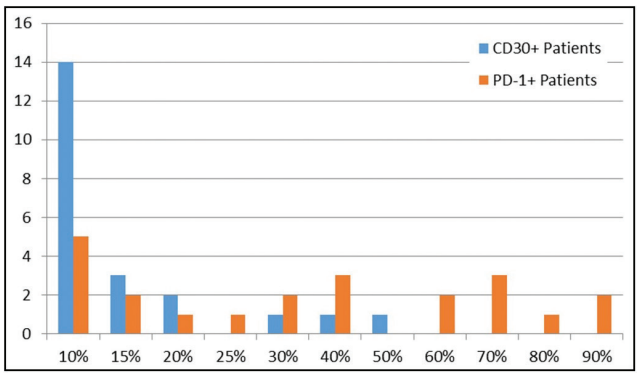

Figure 4: CD30 and PD-1 positivity percentages of patients

CD30 expression was observed in 27 (22.6%) cases and was not observed in 92 (77.4%). PD-1 expression was observed in 22 (18%) of the case, and was not observed in 97 (82%). These are the percentages of the expression within positive biopsies

Discussion

MF is a cutaneous T-cell lymphoma with a generally silent and protracted course. In many cases, a definitive diagnosis can be made only after a careful clinicopathological correlation. The similarity with inflammatory dermatoses has been confusing for dermatologists and pathologists. It has been important to identify clinical and pathological parameters that will facilitate differential diagnosis between early stages of MF and dermatoses and predict the prognosis of early-stage MF. For this purpose, there is a need for safer and more effective biomarkers.[17]

Immunohistochemistry-guided immunophenotyping is not only important for diagnosis but also important to identify the baseline profile. Most of the cases display T-helper CD3+, CD4+, CD8−, and TCR β+ phenotype characteristics of mature memory cells of the Th2 subtype. A high CD4/CD8 ratio in the lymphocytic infiltrates of clinically suspicious lesions is considered highly suggestive for the histopathologic diagnosis of MF and very helpful in clinically suspicious cases. However, CD4/CD8 dual positivity and CD4/CD8 dual

Table 3: Relationship between CD30 and PD-1 with recurrence

	CD30 positive	CD30 negative	*P* value	PD-1 positive	PD-1 negative	*P* value	CD30/ PD-1 dual positive	CD30 or PD-1 negative	*P* value
Recurrent	22 (19%)	55 (46%)	0.041	16 (14%)	61 (51%)	0.46	4 (3%)	73 (61%)	0.66
Nonrecurrent	5 (4%)	37 (31%)		6 (5%)	36 (30%)		1 (1%)	41 (35%)	
Total	27	92		22	97		5	114	

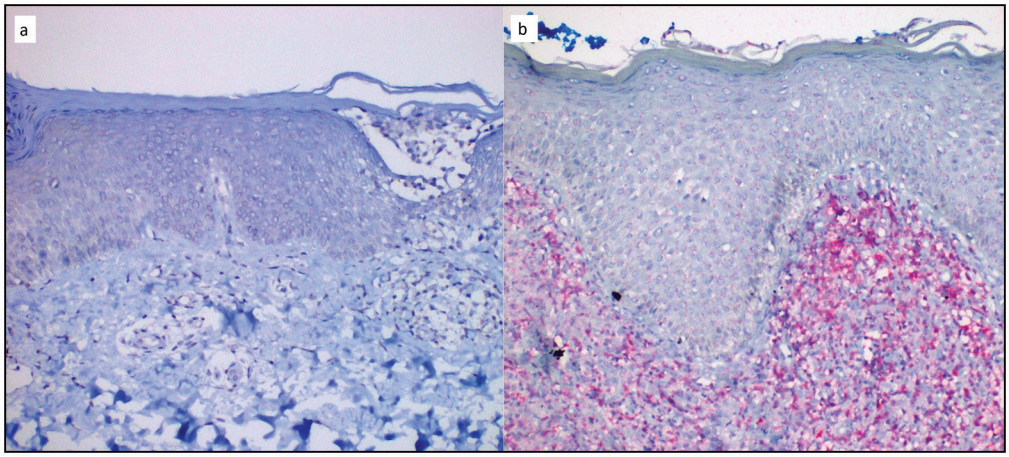

Figure 5: PD-1 immunohistochemistry staining (magnification, ×200). (a) Negative. (b) Positive

Table 4: Summary of clinicopathologic features of ex-patients with mycosis fungoides caused death

	Sex	Age	Epidermal CD4/ CD8 ratio	CD30 expression	PD-1 expression percentage	Number of biopsies through follow-up	Follow-up period (year)
Patient 1	Male	72	8	Negative	60	17	3
Patient 2	Female	66	5	Negative	30	5	7
Patient 3	Male	58	1	Negative	90	2	1
Patient 4	Male	81	5	Negative	30	4	1

Table 5: PD1 expression and survival summary

Survival	PD-1 +	PD-1 −	Total
Ex	4	0	4
Alive	18	94	112
Total	22	94	116

The Fisher exact test $P = 0.001$

negativity are rarely seen variants.[4] Expression of PD-1 has been reported in a double-negative case, suggesting a possible follicular T-helper origin.[5] The expressions of CD3, CD4, and CD8 in this study is compatible with the literature. The CD4/CD8 ratio was increased in 113 cases. Six were CD4/CD8 dual positive, and there was no dual negativity.

CD30 is one of the important markers that is sometimes used in the immunohistochemical evaluation of MF. Although large cell transformation is primarily a morphologic concept, CD30 expression has been linked to it. But it is important to remember that CD30 expression does not define large cell transformation. Only 30%–40% of transformed MF cases show more than 40% CD30 expression. CD30+ cells are rarely found in the epidermis in the patch stage.[18]

Scarisbrick *et al.*[19] found no significant difference in survival between CD30+ and CD30− cases in their series of 1275 advanced-stage MF and SS patients. Again, in Scarisbrick *et al.*'s[19] series of 100 early-stage MF lesions, there was no significant difference between survival rates and CD30 expression. This finding is inconsistent with the results of this study of 119 patients. CD30 expression was observed in 27 (23%) cases in our study, and there is a statistically significant relationship between CD30 expression and recurrence ($P = 0.041$). Additionally, some studies have shown that CD30 expression is a better prognostic marker for both transformed and nontransformed MF.[20,21] In the study by Benner *et al.*[22] consisting of 130 transformed MF patients, CD30 negativity was reported to reduce survival. Talpur *et al.*,[23] in their study of 187 MF-transformed cases, found that CD30 expression of over 10% indicates a good prognosis, even though it shows transformation. In our study, statistically significant results could not be obtained due to the small number of transformed lesions. It is thought that more effective results about the relationship between CD30 expression and recurrence can be obtained in studies involving numerous transformed MF patients.

In our study, a CD30+ case had LYP diagnoses in continuing biopsies. This situation led us to question the place of MF in the classification of CD30 (+) lymphoproliferative diseases.

The other important issue regarding CD30 is BV, which received Food and Drug Administration approval in 2017 as an anti-CD30 antibody-drug conjugate for use in primary cutaneous large cell lymphoma and MF showing CD30 expression. In the phase II study of 32 patients by Kim *et al.*,[24] patients with CD30 expression of more than 5% in the tissue showed a significantly higher response to the drug than those with CD30 expression of less than 5%. Therefore, the demonstration of CD30 in an aggressive MF patient can be important to provide treatment opportunities.

In a study of 26 cases by Kantekure *et al.*,[25] PD-1 was expressed in all lesions in the early patch and plaque stage, whereas expression decreased in MF cases that transited to the tumor phase. In their case reports, Ogunrinade *et al.*[26] emphasized that a case with a high PD-1 expression showed a poor prognosis. Here, CD30 negativity and transformation were also observed and were associated with poor prognosis. Cetinozman *et al.*,[10] in their study that included 60 MF and 27 SS patients, PD-1 expression was observed in 89% of SS patients, whereas PD-1 expression was observed in only 13% of MF patients. In this study, over 50% of the PD-1 expression rate was accepted as positive, and no information was given about the prognosis of the patients.[10] The diversity of PD-1 at varying rates in our study and the literature shows that a cutoff value of this marker has not yet been established and that the mean values should be determined by studying more patients.

In our study, PD-1 expression was observed in 22% of the cases, and it was noted that survival was significantly reduced in these cases. Although a significant relationship between PD-1 expression and recurrence could not be demonstrated, it suggests that patients with PD-1 expression should be followed closely in terms of poor prognosis.

In conclusion, we applied CD30 and PD-1 antibodies to the first biopsies of follow-up MF patients. We statistically evaluated the relationship between the expression rates of atypical lymphocytes with CD30 and PD-1, and recurrence and survival. We have shown that CD30 expression is a statistically significant dependent marker for recurrence, and a statistically significant relationship has been shown between PD-1 expression and poor survival. When evaluated together with treatment options, CD30 and PD-1 are markers that may guide the clinical follow-up of aggressive MF cases.

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Conflicts of interest

There are no conflicts of interest.

Ethical approval

Local ethics committee approval of our institution has been obtained on 2015 for the research with the number of 16.

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Original Article

The Role of Salusins and Interleukin 12 Family in the Rosacea Pathogenesis

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Abstract

Aim: Salusins and recently discovered interleukin (IL)-12 family members (IL-35 and IL-39) have been investigated in various disorders associated with chronic inflammation. The aim of this study was to evaluate the roles of salusin-alpha (α), salusin-beta (β), IL-35, and IL-39 in the pathogenesis of rosacea. **Methods:** This study is a single-center, prospective case–control study performed in a tertiary healthcare institution. Salusin-α, salusin-β, IL-35, and IL-39 were analyzed by enzyme-linked immunosorbent assay method from venous blood of 50 rosacea patients who did not receive any treatment and 50 age-matched healthy controls, and the test results were compared between the two groups as statistically. **Results:** Patients in the rosacea group (female:male ratio = 1.9:1; median age: 56 years) had significantly higher mean salusin-α, IL-35, and IL-39 levels compared with the control group (female:male ratio = 2.1:1; median age: 41 years). There was no statistically significant difference between the two groups in terms of salusin-β levels. **Conclusion:** The increased vascularity and Th1-mediated inflammation might be possible explanations for the elevated salusin-α and IL-39 levels in rosacea patients. On the other hand, the higher mean IL-35 level detected in the same group was an unexpected finding due to the immunosuppressive effect of the cytokine. Recently, targeted therapies have become popular in many inflammatory diseases. In this context, salusins, IL-35, and IL-39 seem to be possible molecules that could be modified for therapeutic reasons in the future in the treatment of rosacea.

Keywords: Interleukin 12, interleukin 35, interleukin 39, rosacea, salusin-α, salusin-β

Introduction

Rosacea is a chronic inflammatory cutaneous disorder that usually occurs in adults between 20 and 50 years old.[1-3] The etiopathogenesis of rosacea is poorly understood. Genetic predisposition, environmental triggers, immune dysregulation, inflammatory reactions to cutaneous microorganisms, neurovascular dysregulation, and vascular dysfunction are the possible underlying factors. Various triggers are known to aggravate rosacea symptoms, such as ultraviolet exposure, diet, smoking, alcohol consumption, obesity, and stress.[4-7]

In addition, rosacea has been associated with several disorders such as inflammatory bowel disease, malignancies, metabolic, autoimmune, allergic, urogenital, and cardiovascular disease (CVD).[8,9] However, there is no clear explanation for these associations. The chronic inflammatory nature of rosacea and the vascular dysfunction in its pathogenesis might play a central role in the development of comorbid disorders.[8]

Salusin-alpha (α) and salusin-beta (β) are mediators that were first identified in the human embryo and are expressed in a variety of tissues, including vascular tissues.[10] In studies conducted on psoriasis vulgaris, rheumatoid arthritis (RA), and CVD, it has been reported that salusin levels differed in patient groups compared with the controls.[11-13] Thus, there may be changes in salusin levels in rosacea, as well.

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Interleukin (IL)-35 and IL-39 are recently discovered ILs belonging to the IL-12 family.[14,15] IL-35 generates an immunosuppressive effect by increasing T-regulatory (Treg) cell proliferation and inhibiting T-helper (Th) 17 cell differentiation.[15] IL-39 is another proinflammatory cytokine whose expression is increased in some chronic inflammatory skin disorders such as psoriasis and atopic dermatitis.[16]

To the best of our knowledge, the salusin-α, salusin-β, IL-35, and IL-39 levels have not been studied in patients with rosacea. We aimed to define the relationship between the levels of salusin-α, salusin-β, IL-35, IL-39, and rosacea.

Materials and Methods

Fifty patients with rosacea who were followed up at 2–3 months intervals in our tertiary dermatology outpatient clinic were enrolled in the study as the patient group, whereas 50 subjects from a similar age group were included in the control group. None of the subjects in the patient group had received topical or systemic treatment for rosacea. The exclusion criteria were tobacco consumption (including passive smoking), history of any chronic inflammatory disorder, known malignancy or active acute/chronic infection, and use of corticosteroids or other immunosuppressive therapy. Written and verbal consent of the patients, who voluntarily agreed to participate, was taken before the study.

Serum samples were obtained from the patients and the control group from the venous blood. Salusin-α, salusin-β, IL-35, and IL-39 were studied by enzyme-linked immunosorbent assay method. The test results were statistically compared between the two groups. SPSS program for Windows, Version 14.0. (SPSS Inc., Chicago, IL, USA) was used for the statistical evaluation, and $P < 0.05$ was accepted as statistically significant.

The study has been approved by the Ethics Committee of Çanakkale Onsekiz Mart University Faculty of Medicine (approval date/number: 23.09.2020/12-29). The financial source of the study was provided by Çanakkale Onsekiz Mart University Scientific Research Projects Unit with project number 3542.

Results

The demographic profile and clinical characteristics of the subjects in the rosacea and control groups are summarized in Tables 1 and 2, respectively.

Among 50 patients in the rosacea group, 33 were female, and 17 were male (female:male ratio = 1.9:1). The median age of the rosacea group was 56 years (age range: 32–79). In the control group, 34 were female, and 16 were male (female:male ratio = 2.1:1), with a median age of 41 years (age range: 28–70). Hypertension (HT) (n = 5; 10%), diabetes mellitus (DM) (n = 5; 10%), and hyperlipidemia (HL) (n = 1; 2%) diagnoses were present in the patient group.

The median disease duration in rosacea patients was 8.5 years (range: 1–35 years). Erythematotelangiectatic rosacea (n = 38;76%) was the predominant subtype, whereas ocular rosacea was the least commonly observed phenotype (n = 3; 6%). The Malar region was exclusively involved.

The mean salusin-α, IL-35, and IL-39 levels were significantly higher in the rosacea group compared with the control group. However, there was no statistically significant difference between the two groups regarding salusin-β levels [Figure 1 and Table 3].

Discussion

Salusins are recently discovered bioactive peptides associated with oxidative stress. They are biosynthesized from prosalusin under the influence of tumor necrosis factor (TNF)-α, which is generated by triggered inflammatory cells.[17,18] Conflicting results were presented in the literature regarding the salusin levels in the context of disorders, in which oxidative stress and TNF-α play a significant role. These are particularly CVD and other inflammatory disorders such as RA, multiple sclerosis, and psoriasis, which are demonstrated to coexist with rosacea.[10,11,13,19-21]

The antiatherogenic effect of salusin-α and proatherogenic effect of salusin-β have been established in the light of the study reporting elevated salusin-β and reduced salusin-α levels in atherosclerotic diseases.[21] Correspondingly, Erden *et al.*[11] observed lower salusin-α and higher salusin-β levels in psoriasis patients compared with the control group.

Table 1: Demographic characteristics of the rosacea and control groups

Characteristics	Rosacea (n = 50)	Control (n = 50)	P value
Age (years) median (range)	56 (32–79)	41 (28–70)	**<0.001**
Gender, n (%)			
Female	33 (66)	34 (68)	0.832
Male	17 (34)	16 (32)	
Concomitant disease, n (%)			
DM	5 (10)	0 (0)	**<0.001**
HT	5 (10)	0 (0)	
HL	1(2)	0 (0)	
Family history, n (%)			
Yes	50 (100)	50 (100)	
No	0 (0)	0 (0)	
Smoker, n (%)			
Yes	50 (100)	50 (100)	
No	0 (0)	0 (0)	
Alcohol intake, n (%)			
Yes	50 (100)	50 (100)	
No	0 (0)	0 (0)	

DM: diabetes mellitus, HL: hyperlipidemia, HT: hypertension, SD: standard deviation.

Statistically significant values are highlighted in bold

Rosacea is a systemic disorder that might coincide with other inflammatory disorders. Vascular dysregulation, immune function impairment, increased oxidative stress, and TNF-α are implicated in its pathogenesis.[1-4] A meta-analysis of 13 studies on 50,442 subjects evaluating the relationship between rosacea and metabolic syndrome revealed an association of rosacea with HT and HL. However, no clear relation was identified with DM or CVD.[22] In our rosacea patients, DM (n = 5), HT (n = 5), and HL (n = 1) diagnoses were present, whereas they were not detected in the control group. Spoendlin *et al*.[23] reported decreased rosacea risk in patients with advanced DM. Since vasodilation is a major component of rosacea, they attributed this finding to insufficient vasodilatation frequently encountered in these patients.[23] In another study, the ultrasonographical examination of the vascular structures in the facial region of rosacea patients reported no occlusion, unlike in atherosclerosis, but an increased dermal and hypodermal vascularity compared with the control group.[24] This might be a possible explanation for the elevated salusin-α levels in our rosacea patients.

Table 2: Disease characteristics of the rosacea group

Parameters	Values
Age of onset (years) median (range)	44 (27–61)
Disease duration (years) median (range)	8.5 (1–35)
Rosacea subtype, *n* (%)	
Erythematotelangiectatic	38 (76)
Papulopustular	4 (8)
Ocular	3 (6)
Phymatous	5 (10)
Anatomical localization of lesions, *n* (%)	
Forehead	24 (48)
Nose	46 (92)
Malar region	50 (100)
Chin	21 (42)
Eyes	3 (6)
Others	0 (0)
Treatment use for rosacea in the last 3 months, n (%)	
No	50 (100)
Yes	0 (0)

SD: standard deviation

Similarly, Özgen *et al*.[13] observed high salusin-α levels in RA and Behçet disease (BD), whereas in another study, they conducted on systemic lupus erythematosus (SLE) and systemic sclerosis (SS) patients salusin-α levels were reported to be low.[25] Researchers have suggested that salusin-α might play a role in the inflammatory pathway of Th1-mediated diseases because RA and BD are Th1-dependent, whereas SLE and SS are Th2-dependent.[13] Th1-mediated inflammation in the rosacea pathogenesis might be another reason for the elevated salusin-α levels in the rosacea group of our study.

Besides their impact on the hemodynamic system and atherosclerosis pathogenesis, salusins exhibit mitogenic activities. Salusin-β induces the expression of growth-related genes such as c-myc and c-fos.[17] It also stimulates the proliferation of vascular smooth muscle cells, fibroblasts, and muscle cells.[17] The antiapoptotic effects of the salusins were established in the study of Xiao-Hong *et al*.[26] as well. Considering the high salusin-α levels in our study, it might be suggested that salusins stimulate the growth and proliferation of inflammatory cells and induce inflammation by inhibiting apoptosis in rosacea. Meanwhile, the augmented vascularization in areas of intense erythema may be attributed to the same mechanism.

Another major outcome of our study was the higher levels of IL-35 and IL-39 in the rosacea group compared with the control group. IL-35 is among the recently identified members of the IL-12 family. It is mainly secreted from

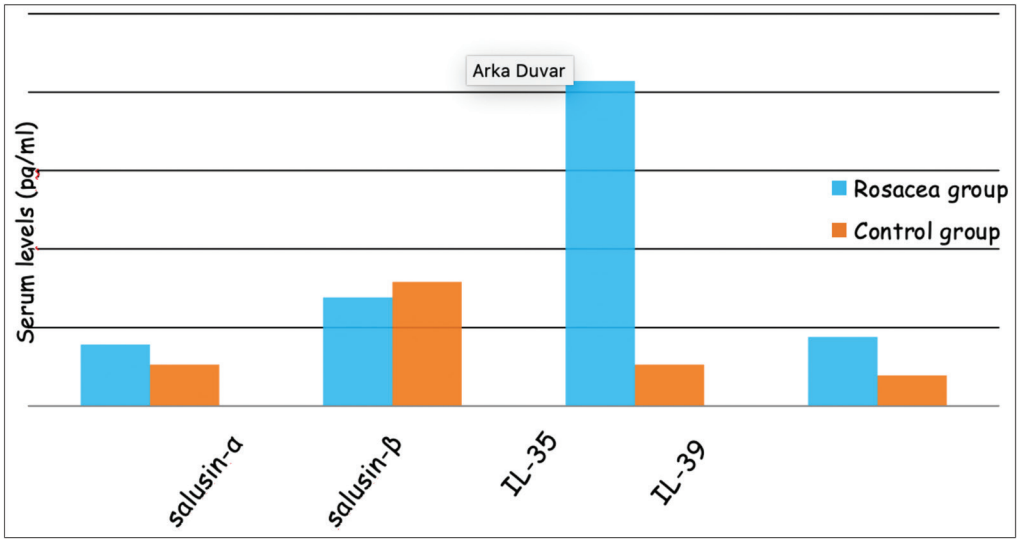

Figure 1: Salusin-alpha, salusin-beta, interleukin-35, and interleukin-39 levels in rosacea and control groups

Table 3: Comparison of rosacea and control groups regarding salusin-alpha, salusin-beta, interleukin-35, and interleukin-39 levels

Variables	Rosacea	Control	Total	*P* value
	n = 50 (50%)	*n* = 50 (50%)	*n* = 100	
Salusin-alpha[a] (pg/mL)	784.532 ± 272.112	529.972 ± 198.694	254.559 ± 47.649	**<0.05**
Salusin-beta[a] (pg/mL)	1386.508 ± 516.870	1585.547 ± 263.436	413.759 ± 98.343	0.414
Interleukin-35[a] (pg/mL)	4142.590 ± 662.478	529.972 ± 198.694	2557.043 ± 100.82	**<0.05**
Interleukin-39[a] (pg/mL)	881.561 ± 358.634	391.597 ± 104.891	489.963 ± 52.843	**<0.05**

[a]Data represented as mean ± SD.

Statistically significant values are highlighted in bold

Treg cells. By increasing Treg proliferation and preventing Th17 differentiation, IL-35 suppresses the release of IL-17 and consequently causes immunosuppression.[27,28] Thus, it has been investigated in disorders such as RA, psoriasis, SLE, SS, and dermatomyositis, in which Th17 plays a role in the inflammatory cascade, and contradictory results have been reported.

The majority of the studies on psoriasis and RA showed low levels of IL-35, as expected.[29-33] On the other hand, in several reports of SLE, SS, and dermatomyositis patients, IL-35 was detected at higher levels than controls.[34] In two further studies, patients with inactive SLE were shown to have higher IL-35 values than the ones with active SLE,[35,36] whereas Qiu *et al*.[37] noted a decline in IL-35 levels in their SLE patients following systemic corticosteroid therapy. A similar inconsistency prevails regarding IL-35 levels in the setting of SS. The higher IL-35 levels were detected in SS patients with pulmonary fibrosis compared with individuals without pulmonary fibrosis.[38] However, another study revealed elevated IL-35 levels in early phase SS compared with the late phase.[39]

Similar to SLE patients, Zdanowska *et al*.[31] observed a reduction in IL-35 levels in psoriasis patients managed with adalimumab therapy compared with pretreatment values. The conflicting data on the IL-35 levels in the disorders mentioned above with similar pathogenetic mechanisms might be due to the fluctuations in the disease activity and immunosuppressive treatments employed. The significantly higher mean IL-35 level in our rosacea patients was also an unexpected finding, which necessitates further investigation.

IL-39 is another newly discovered member of the IL-12 family. It has a heterodimeric structure consisting of IL-23p19 and Ebi3 subunits. Unlike IL-35, IL-39 has a proinflammatory effect. IL-39 is mainly secreted from B cells stimulated with lipopolysaccharide, whereas other immune cells such as dendritic cells and macrophages have been reported to express IL-39 mRNA.[40,41]

A limited number of studies are present on IL-39 in the literature, each highlighting a different action of the molecule. Luo *et al*.[41] reported an increase in IL-39 levels in patients with acute coronary syndrome compared with the control group. The Ebi3 subunit was detected at lower levels in SS patients than the controls in another study. The authors concluded that this led to increased collagen deposition and fibrosis.[42] IL-39 levels were significantly higher in the rosacea group in our study, which had been expected due to its proinflammatory effect.

The limitation of our study is the limited number of patients, whereas the prospective case-control design is its main strength.

Conclusion

The increased vascularity and Th1-mediated inflammation might be possible explanations for the elevated salusin-α and IL-39 levels in rosacea patients, whereas the higher mean IL-35 level detected in the same group was an unexpected finding. The targeted therapies have become popular for inflammatory disorders as the underlying pathogenetic mechanisms are increasingly clarified. Salusins, IL-35, and IL-39 seem to be possible molecules that might be modified for therapeutic reasons in the future. Further large-scale studies are warranted to draw more precise conclusions.

Ethical statement

The study was approved by the Clinical Research Ethics Committee of Çanakkale Onsekiz Mart University Rectorate (approval date: 23.09.2020, approval no:12-29)

Declaration of patient consent

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

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Conflict of interest

There are no conflicts of interest.

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Original Article

Examining the Use of Cosmetic Products and the Awareness of Healthy Life among University Students

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Abstract

Aim: Cosmetics may be harmful to human health due to the substances they contain. University students commonly use these products. Health promotion and maintenance are closely associated with gaining healthy lifestyle behaviors, too. People need to be aware of the benefits of changing their lifestyles if they want to maintain their health and ward off illnesses. The health awareness of individuals can play a significant role in giving up their unhealthy behaviors and developing conscious behaviors. The aim of this study was to examine the use of cosmetic products and the awareness of healthy life among university students who commonly use cosmetics. **Materials and Methods:** This was a descriptive and cross-sectional study conducted with university students between May 2021 and February 2022. The population consisted of 2757 students, and the sample of the study consisted of 422 students who attended a foundation university and met the inclusion criteria. The data were collected from the students using an Information Form and the Healthy Life Awareness Scale over Google Forms. The data were analyzed in the Statistical Package for the Social Sciences (SPSS) software program using descriptive statistics and Mann–Whitney *U* test, a nonparametric test. **Results:** The students had a mean age of 20.24 ± 2.09 years; approximately 78.1% of them were females and they were commonly using cosmetics every day (62.3%), the most frequently used oral and dental care products (20.7%), and hair care products (20.2%). Half or fewer of the participants were aware of the symbols specific to cosmetic products, such as consumed within *x* days of opening, shelf life, protection against ultraviolet rays, and compliance with the standards. The awareness of healthy life was above the moderate level (59.0 ± 9.6) and was higher in those who were females and paid attention to the products' protection band, color/odor changes, and expiration date. It was observed that one-third of the participants shared their items with others, and one-quarter of them used items of others. Those who did not share their own cosmetics with others and did not use those of others had a higher awareness about healthy life. **Conclusion:** It was concluded that students who had high awareness of healthy life used cosmetic products more consciously. In order for young people to gain healthy life behaviors and to prevent the unnecessary use of cosmetic products, it is recommended to establish information platforms, particularly on the internet and social media, and to organize training programs that include their families and individuals in their immediate circle and also peer learning.

Keywords: Cosmetics, healthy life, awareness, university students

Introduction

Cosmetics are the products one can apply on the body (e.g., skin, nails, body hair, hair, lips, genitalia, teeth, and mouth) to clean the given area, improve one's smell and appearance, and keep oneself in good condition.[1,2] They include personal care, hair care, oral care, makeup, and nail care products as well as moisturizers, fragrances, depilatories, and sunscreens.[1-3]

Today, there are a great number of cosmetic products of different qualities manufactured by various companies.[3] These products contain more than 10,000 ingredients that may cause many diseases.[3-5] These products may cause both acute and long-term side effects.[6]

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Most of the cosmetics are applied directly to the skin; therefore, dermal exposure is the most critical pathway for the emergence of their potential harmful effects. Also, the use of cosmetics around the mouth or hand-to-mouth contact may lead to oral exposure.[3] Product exposure can cause mild or severe allergic reactions, some skin problems (e.g., acne, contact dermatitis, and urticarial), hormone disorders, eye, skin, and respiratory irritation, neurotoxicity, cancer, congenital abnormalities, developmental and reproductive disorders, and infertility.[1-8] Due to the potential adverse effects of cosmetics on human health, it can be asserted that their use is one of the healthy lifestyle behaviors.

University students commonly use these products.[9-11] A study conducted with students attending the faculty of health sciences reported that they used cosmetic products at a rate of 91%.[12] Another study conducted with female students reported this rate as 97.8%.[13] Young people use these products mainly to feel beautiful and boost their self-confidence.[11]

Health promotion and maintenance are closely associated with not only preventing diseases but also gaining healthy lifestyle behaviors. Healthy lifestyles are defined as managing the behaviors that affect a person's health and choosing behaviors that are appropriate for their own health status while organizing daily activities. People need to be aware of the benefits of changing their lifestyles if they want to maintain their health and ward off illnesses. The health awareness of individuals can play a significant role in giving up their unhealthy behaviors and developing conscious behaviors.[14] The aim of this study was to examine the use of cosmetic products and the awareness of healthy life among university students. The number of similar studies on cosmetic products and awareness of healthy life is limited; therefore, this study is original as it would both contribute to the literature and be guiding for future studies.

Research questions

1. What are the characteristics of students using cosmetic products?
2. What is the awareness level of students about healthy life?
3. Do students' awareness levels of healthy life vary based on gender and how they use cosmetic products?

Materials and Methods

The study was conducted based on descriptive and cross-sectional design.

Place and time of the study

The study was conducted with the students attending a foundation university located in the Central Anatolia Region, Turkey, between May 2021 and February 2022.

Population and sample

The population consisted of 2757 students attending the Faculties of Medicine, Dentistry, Pharmacy, and Health Sciences and Vocational School of Health Services in the field of health as well as the Faculties of Law, Political Sciences, Economics and Social Sciences, Fine Arts and Architecture, and Communication, Vocational School of Justice and Vocational School in other fields within the body of a foundation university. The sample size was determined according to the formula for the sample with a finite population:[15]

$$n = \frac{N \cdot t^2 \cdot p \cdot q}{d^2 \cdot (N-1) + t^2 \cdot p \cdot q},$$

where n is the number of individuals to be included in the sample, p is the frequency of occurrence of the case analyzed = 50% (calculated by assuming that the occurrence rate of the case examined in accordance with the related literature would be 50% as it could not be reached), q is the frequency of nonoccurrence of the case analyzed = 50%, t is the theoretical value found from the t table at certain degrees of freedom and the identified error level = 1.96 (the theoretical t value at $\alpha = 0.05$ for ∞ degrees of freedom), d is standard error of the rate to be determined in the study = 0.05:

$$n \cong \frac{2757 \times 1.96^2 \times 0.5 \times 0.5}{0.05^2 \times (2757-1) + 1.96^2 \times 0.5 \times 0.5}.$$

The minimum sample size to be reached in the study was determined as 338 students. A total of 422 students were reached in the study.

Inclusion criteria for the students were determined as follows; being 18 years of age or older, having no visual impairment making reading difficult, being able to understand the statements on the scale and questionnaire, and being voluntary to participate in the study.

Data collection tools

The data were collected using an information form prepared by the researchers and the "Healthy Life Awareness Scale."

- *Information form:* This form, prepared by the researchers based on the literature,[9,16,17] consists of six questions on the sociodemographic characteristics of the participants and 15 questions on their characteristics related to the use of cosmetic products.
- *Healthy Life Awareness Scale (HLAS):* The scale, which was developed by Ozer and Yilmaz in 2020, consists of 15 items and 4 subscales (change, socialization, responsibility, and nutrition). Each item is anchored with a 5-point Likert-type scale as (1) strongly disagree, (2) disagree, (3) undecided, (4) agree, and (5) strongly agree. The score of the change subscale is obtained by

summing the scores of items 1–5 as in the socialization subscale with items 6–9, in the responsibility subscale with items 10–12, and in the nutrition subscale with items 13–15. The lowest and highest scores of the scale are 15 and 75, respectively. A high score signifies a high level of healthy life awareness. Ozer and Yilmaz[14] conducted its Turkish validity and reliability study in 2020 and determined that the scale is a valid and reliable tool for determining the healthy life awareness levels of individuals. The Cronbach's alpha reliability values for the subscales of the scale are 0.70 for the change subscale, 0.71 for the socialization subscale, 0.74 for the responsibility subscale, and 0.61 for the nutrition subscale.[14] In this study, Cronbach α values were 0.79 for the change subscale, 0.79 for the socialization subscale, 0.77 for the responsibility subscale, 0.78 for the nutrition subscale, and 0.87 for the overall scale.

Data collection

Data collection tools were delivered to the students online through Google Forms. First, they were informed about the research team and the purpose of the study, and that no personal information would be required in the study, their responses would only be used for scientific purposes, they could withdraw from the study at any time, their participation in the study would have no effect on the educational process, and their information would be kept confidential. As a prerequisite of the process, they were asked to check "I agree" in response to the statement "If you have read the above information and are voluntarily participating in this study." In this way, the students acknowledged that they gave informed written consent. Then they filled out data collection tools online.

Data analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 22.0 (IBM Corporation, Armonk, New York). Data were represented in number, percentage, mean, standard deviation, and minimum and maximum values as descriptive statistics. Kolmogorov–Smirnov test was used to test whether or not the data were normally distributed. The statistical significance level was accepted as 0.05. The difference between dependent and independent variables was analyzed using the Mann–Whitney *U* test, a nonparametric test. The data were assessed at a confidence interval of 95% and a significance level of $P < 0.05$. Cronbach α coefficient was used to analyze the reliability. The results of the study were reported according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist.

Ethical considerations

The approval (IRB no. 74791132-604.01.01-1187) from the Non-Invasive Clinical Trials Ethics Committee of Ankara Medipol University as well as institutional permission was obtained. The participants' informed consents were obtained. The authors who conducted the Turkish validity and reliability study of the scale granted their permission for its use in the study. All steps of the study were conducted in accordance with the principles of the Declaration of Helsinki.

Results

The mean age of the students was 20.24 ± 2.09. Overall, 78.1% of them were females, 69.4% were studying in health-related departments, and 75.8% graduated from high schools other than health vocational high schools

Table 1: Distribution of descriptive characteristics of the students (n = 422)

Characteristics	Mean ± SD	Median	Min–Max
Age (year)	20.24 ± 2.09	20	18–30
		n	**%**
Gender	Female	331	78.1
	Male	91	22.6
Faculty/college	Field of health	293	69.4
	Other fields	129	30.6
Graduated high school	Health vocational high school	102	24.2
	Other	320	75.8
Financial status	Income less than expense	71	16.8
	Income equal to expense	287	68.0
	Income more than expense	64	15.2
Place of residence	In dormitory	49	11.6
	With family	335	79.4
	Home alone/with friends	38	9.0
Employment status	Employed	31	7.3
	Unemployed	391	92.7
Total		**422**	**100**

[Table 1]. It was determined that 62.3% of the students used cosmetics every day, and they most frequently used oral and dental care products (20.7%), followed by hair care products (20.2%). They obtained information on cosmetics from user comments (23.7%), immediate circle (22.6%), and social media (19.0%), respectively. They mostly purchased these products from cosmetic chain stores (31.0%) and pharmacies (22.0%). Even though the participants' knowledge of the symbols on the products varied, they were aware mostly of the symbol of recycling (87.7%), followed by flammable-explosive (82.9%). The data on the cosmetic product usage habits of the students participating in the research are given in Table 2. The side effects experienced were reported only local symptoms such as acne on the skin, spotting, redness, dryness, itching, burning sensation, allergy, and dandruff on the scalp. None of the participants reported any systemic side effects.

The participants had a mean score of 59.0 ± 9.6 in the Healthy Life Awareness Scale, which was above the moderate level. Their subscale mean scores were 18.5 ± 4.1 for the change subscale, 16.6 ± 3.1 for the socialization subscale, 13.1 ± 2.2 for the responsibility subscale, and 10.78 ± 3.1 for the nutrition subscale. Cronbach α reliability coefficient of the scale was 0.87 for the overall score and between 0.77 and 0.79 for the subscales [Table 3].

Table 4 compares the healthy life awareness levels of the students based on gender and how they used cosmetic products. The mean scores of the change and responsibility subscales and the total mean score of the scale were significantly higher in female students than in their male counterparts ($p < 0.05$). The students who paid attention to the protection band obtained a higher total score and higher mean scores from all the subscales than the students who did not ($p < 0.05$). The difference between the students who used the products even if their color/odor changed and the scale total score was statistically significant ($p < 0.05$). Those, who did not use the expired products, had higher scores in the change subscale of Healthy Life Awareness Scale and higher total score compared to those who used ($p < 0.05$). Total scores of those who did not share their personal items with others and used the items of others, as well as their mean scores of the socialization, responsibility, and nutrition subscales, were statistically significantly higher ($p < 0.05$) [Table 4].

Discussion

A limited number of studies in the literature indicated that university students widely used cosmetic products in Turkey, Saudi Arabia, and India.[9-11] Although a study conducted with students of health sciences in Tanzania reported the rate of using cosmetics as 91%,[12] another study conducted with female students in Ethiopia found this rate as 97.8%.[13] The results of the study revealed that university students used commonly cosmetic products (62.3% every day). Even though the majority of the participants in this study were females, males today are also interested in personal care, and thus, they commonly use cosmetic products.[18] Young people use these products mainly to feel beautiful and boost their self-confidence.[11] It is an expected result that the students would use cosmetics often to make themselves feel beautiful/good and boost their self-confidence. This would especially hold true during university, as people at that age value what others think about them.

This study reported that the students most frequently used oral and dental care products, followed by hair care products. These products are essential components of daily personal care for people of all ages and both genders. The related studies have also revealed similar results.[9,16] The high rate and frequency of using cosmetic products are considered to be significant in informing individuals about the effects of oral and dental care products and hair care products on health and ensuring that they make informed choices.

As information technologies have advanced in recent years, university students have started to prefer information technologies as information sources to learn more about cosmetics.[12,19] In this study, it was determined that the majority of the participants obtained information about cosmetics from user comments, their immediate circle, and social media. In the study by Al-Hindi *et al.*,[19] a great majority of the participants reported that they learned about cosmetic procedures mostly from social media, TV, and friends. In their study, Kureh *et al.*[12] reported that students mostly referred to their family members, friends, media, and the internet as sources of information about the negative effects of cosmetics. These results reveal not only the sources of information used by university students but also the sources that should be taken into consideration in planning trainings on cosmetics. In this sense, it is important to employ innovative methods in interventions for this group, which heavily uses mobile technologies and social media.

Demirci and Demirci-Aksoy[16] stated in their study that the majority of consumers were not aware of the symbols on the labels of cosmetic products. Even though the participants' knowledge of the symbols on the products varied, they were aware mostly of general symbols such as recycling (87.7%) and flammable-explosive (82.9%) used on packages along with cosmetics. The fact that half or fewer of the participants were aware of the symbols specific to cosmetic products, such as consumed within *x* days of opening, shelf life, protection against ultraviolet rays, and compliance with the standards, indicated that they needed more information on this subject. Possible side effects are more likely to be seen in products that have

Table 2: Distribution of the students' characteristics of using cosmetic products (n = 422)

Characteristics		*n*	%
Frequency of using cosmetic products	Everyday	263	62.3
	Once every 2 days	113	26.8
	Once a week	37	8.8
	Once a month or less	9	2.1
Most frequently used cosmetic product[a]	Oral and dental care products	405	20.7
	Hair care products	394	20.2
	Hand body care products	385	19.7
	Face care products	327	16.7
	Makeup products	281	14.4
	Products used in the genital area	90	4.6
	Foot care products	71	3.6
Source of information on cosmetic products[a]	User comments	316	23.7
	Immediate circle (family, relatives, and friends)	302	22.6
	Social media	254	19.0
	Dermatologist	211	15.8
	Media	151	11.3
	Cosmetologist	101	7.6
Purchase point of cosmetic products[a]	Chain stores on cosmetics	355	31.0
	Pharmacies	252	22.0
	Supermarket	181	15.8
	E-commerce websites	153	13.4
	Natural-organic product markets	109	9.5
	Catalog sales	75	8.4
Being aware of symbols on these products[b]	Quantity	167	39.6
	Consume within *x* days of opening	244	57.8
	Flammable, explosive product	350	82.9
	Shelf life	192	45.5
	Protection against UVA rays	185	43.8
	Recycling	370	87.7
	Organic certified product	288	68.2
	No animal testing	249	59.0
	Standardized product	129	30.6
	See additional information	226	53.6
	Copyright	67	15.9
Factors that affect the purchase of cosmetics*[a]	Dermatological testing	354	19.6
	Free of chemical substances	304	16.9
	No harm to animals	270	15.0
	Reputable brand	262	14.5
	Herbal-natural content	252	14.0
	Organic-halal certificate	215	11.9
	More grams	45	2.5
Purchase of unnecessary cosmetics	Yes	90	21.3
	No	332	78.7
Use of tester	Yes	141	33.4
	No	281	66.6
Paying attention to the product protection band	Yes	399	94.5
	No	23	5.5
Using a product even if its color/odor changes	Yes	25	5.9
	No	397	94.1
Using an expired product	Yes	35	8.3
	No	387	91.7
Sharing personal products with others	Yes	137	32.5
	No	285	67.5

Table 2: Continued

Characteristics		*n*	%
Using products of others	Yes	109	25.8
	No	313	74.2
Believing that it negatively affects body	Yes	225	53.3
	No	197	46.7
Having side effects	Yes	161	38.2
	No	261	61.8

[a]More than one option are marked

[b]Correct responses to the symbols are presented

Table 3: Students' mean scores for Healthy Life Awareness Scale and its subscales and reliability coefficients

The scale and its subscales	Mean ± SD	Median	Min–Max	Cronbach α
Healthy Life Awareness Scale	59.0 ± 9.6	60	15–75	0.874
Change	18.5 ± 4.1	19	5–25	0.794
Socialization	16.6 ± 3.1	17	4–20	0.793
Responsibility	13.1 ± 2.2	14	3–15	0.765
Nutrition	10.78 ± 3.1	11	3–15	0.782

not been kept under appropriate conditions. Therefore, it is important to know the meanings of the symbols on cosmetic labels and to obey them for the health and safety of users.

A great majority of students in this study claimed that when purchasing these products, they looked for whether or not dermatological tests are conducted, they are free of chemicals, any animal gets harmed during testing, they are of respected brands, and they contain herbal components. The study by Kureh *et al.*[12] with students who attended health sciences reported that the participants used trendy and fashionable cosmetics, giving attractiveness and beauty and boosting their self-confidence, and primarily skincare ones. Shah *et al.*[20] also determined in their study that 60% of female students checked the quality of cosmetics before purchasing them. Although these results reflect the main factors that should be considered when purchasing cosmetics, they also draw attention to the fact that students use fashionable products that they believe make them more attractive and beautiful.

Cosmetic products, even if they contain natural ingredients, can cause not only acute but also long-term side effects.[1,6] In this study, 46.7% of the participants did not believe that cosmetic products had adverse effects on the body. The fact that almost half of the participants believe that cosmetic products have no negative effects on their bodies poses an important threat to health protection and promotion.

It is necessary for individuals to be aware of changing their lifestyles in order to maintain their health and ward off illnesses. This may allow them to give up unhealthy behaviors and develop positive health behaviors.[14] In their study, Gokbulut and Bal[21] found that the HLAS mean score of individuals aged between 18 and 25 was 59.08 ± 9.59. This study similarly revealed that the HLAS mean score of the students was 59.0 ± 9.6, which was above the average. Among the subscales of the HLAS, the change subscale had the highest mean score; whereas, the nutrition subscale had the lowest mean. The study by Mansur and Ertaş[22] indicated that while the highest mean score was observed in the change subscale, the socialization subscale had the lowest mean score, followed by the nutrition subscale with the second lowest mean score. The results are compatible with the literature, and healthy life awareness of university students should be raised. Also, the students' low mean scores in the socialization and nutrition subscales suggested that they had low life awareness in these subscales.

It is important for individuals to notice the changes in their bodies and the effects of negative health behaviors on their health in order to display preventive health behaviors and health-promoting behaviors.[14] The female students obtained significantly higher mean scores in change and responsibility subscales and higher total mean scores than those of their male counterparts. Accordingly, female students were more sensitive to changes in their bodies and aware of the effects of negative health behaviors on their health than their male counterparts. At this point, it is observed that it is necessary to support men more than women to notice the possible effects of health behaviors on their bodies.

People need to be careful about the use of cosmetics in order to improve their production, sale, and use.[2] In a study with female university students, it was observed that 48.6% of the participants shared their items with others.[13] In this study, it was observed that one-third of the participants shared their items with others, and one-quarter of them used items of others. When the

Table 4: Comparison of Healthy Life Awareness Scale total and subscale mean scores of the students in terms of gender and some characteristics of using cosmetic products

Characteristics	Healthy Life Awareness Scale														
	Change			Socialization			Responsibility			Nutrition			Total		
	Mean ± SD	Median	Statistics	Mean ± SD	Median	Statistics	Mean ± SD	Median	Statistics	Mean ± SD	Median	Statistics	Mean ± SD	Median	Statistics
Gender															
Female	18.9±3.9	19.0	*U* = 11609.5	16.9±2.8	17.0	*U* = 13230.5	13.2±2.1	14.0	*U* = 12842.0	10.9±3.1	11.0	*U* = 13094.0	59.9±8.8	61.0	*U* = 12084.0
Male	17.0±4.6	18.0	*P*=0.001	15.9±3.8	17.0	*P* = 0.072	12.6±2.6	13.0	*P* = 0.026	10.3±3.1	11.0	*P* = 0.055	55.7±11.5	58.0	*P* = 0.004
Paying attention to the product protection tape															
Yes	18.7±3.9	19.0	*U* = 3284.5	16.8±2.8	17.0	*U* = 2931.5	13.2±2.1	14.0	*U* = 3151.0	10.9±3.0	11.0	*U* = 2945.0	59.6±8.8	60.0	*U* = 2652.5
No	15.7±5.9	16.0	*P* = 0.021	13.6±5.2	14.0	*P* = 0.003	11.2±3.7	11.0	*P* = 0.009	8.7±3.7	9.0	*P* = 0.004	49.2±15.2	52.0	*P* = 0.001
Using a product even if its color/odor changes															
Yes	16.8±5.2	18.0	*U* = 3930.5	15.2±3.9	16.0	*U* = 3855.5	12.0±3.1	13.0	*U* = 4183.5	10.5±3.1	10.0	*U* = 4712.5	54.6±11.3	56.0	*U* = 3675.0
No	18.6±4.0	19.0	*P* = 0.080	16.8±3.0	17.0	*P* = 0.058	13.1±2.1	14.0	*P* = 0.175	10.8±3.1	11.0	*P* = 0.671	59.3±9.4	60.0	*P* = 0.029
Using an expired product															
Yes	16.6±4.5	18.0	*U* = 5005.5	16.1±3.4	16.0	*U* = 6035.5	12.4±2.7	13.0	*U* = 5843.5	10.0±3.4	10.0	*U* = 5644.5	55.1±10.1	56.0	*U* = 5026.5
No	18.7±4.0	19.0	*P* = 0.010	16.7±3.0	17.0	*P* = 0.280	13.1±2.2	14.0	*P* = 0.164	10.9±3.0	11.0	*P* = 0.100	59.4±9.5	60.0	*P* = 0.011
Sharing personal products with others															
Yes	18.1±4.0	18.0	*U* = 17807.5	16.0±3.0	16.0	*U* = 15277.5	12.7±2.3	13.0	*U* = 16478.0	10.2±3.0	11.0	*U* = 16259.5	57.0±9.1	58.0	*U* = 15571.0
No	18.7±4.2	19.0	*P* = 0.143	17.0±3.0	17.0	*P* = 0.001	13.2±2.2	14.0	*P* = 0.007	11.0±3.1	11.0	*P* = 0.005	60.0±9.7	61.0	*P* = 0.001
Using products of others															
Yes	18.3±4.0	19.0	*U* = 16396.5	15.9±3.2	16.0	*U* = 13450.0	12.5±2.4	13.0	*U* = 13669.5	10.3±3.0	11.0	*U* = 14545.5	57.0±9.6	58.0	*U* = 14031.0
No	18.6±4.2	19.0	*P* = 0.545	16.9±3.0	17.0	*P* = 0.001	13.3±2.1	14.0	*P* = 0.001	11.0±3.1	11.0	*P* = 0.021	59.8±9.5	61.0	*P* = 0.006

* $p < 0.05$, Mann–Whitney *U* test

participants' characteristics of sharing personal care products and using products of others were compared with the healthy life awareness levels, it was observed that the participants who did not share their products with others had higher mean scores in the change, socialization, responsibility, and nutrition subscales, and higher total mean score than those who did. Besides, it was observed that there was a significant difference in the scale total score and in all subscales of the scale, except for change. It is an expected situation that individuals with high healthy life awareness would not share cosmetic products with others. In one study, it was found that infectious agents in make-up products can survive from a few hours to a few days and infections can be transmitted from person to person through makeup products.[23] These reasons point out that it is important to raise awareness that cosmetic products, particularly makeup supplies, are personalized and individuals should not share these supplies with others.

Cosmetics adversely affect health due to the chemicals they contain, which particularly affects women who heavily use cosmetics.[3-5,10,24] The studies have shown that continuous and long-term use of cosmetics cause some health problems such as fungal infections, contact dermatitis, dry eyes, congenital abnormalities, developmental and reproductive disorders, hair loss, dizziness, and lung injury.[3-5,10,24,25] In the study, it was observed that HLAS total score was high in those who paid attention to color and odor changes in cosmetics. The most important thing to protect yourself from the harmful effects of cosmetic products is to avoid using questionable products. Therefore, it is expected for students who follow this basic principle to have a high awareness of healthy life.

It is an important issue in maintaining health that the products offered for human consumption are not used after their expiration dates. The study by El-Gilany and Taref[26] reported that 88.7% of female students did not use expired cosmetics. In this study, 91.7% of the participants stated that they did not use expired cosmetics. Moreover, HLAS scores of those who did not use the expired ones were higher than the scores of those who did. It was thought that the students had high awareness level on this subject.

When the comparison of paying attention to the protection band with the levels of healthy life awareness was examined, it was observed that the participants who did not share their products with others had higher mean scores in the change, socialization, responsibility, and nutrition subscales, and higher total score than those who did, resulting in showing a significant difference. Raising awareness of individuals about healthy life can reflect on different spheres of their lives. It is an expected situation that as the awareness of the individual raises, he/she avoids behaviors that would negatively affect his/her health.[9] It is believed that if individuals have a high awareness of healthy life, they would make conscious choices about cosmetic products, hence protecting and improving the health of individuals and society.

Limitations

This study is limited to only students who attended a foundation university. Also, male students thought that only women use cosmetic products. This situation may have affected their participation in the study. Another limitation of the study is that female dominance is very high in the study group due to the fact that women are more willing to participate in the study.

Conclusion and Recommendations

The results of the study revealed that the students with high awareness of healthy life displayed more positive behaviors toward the use of cosmetics. In order for young people to gain healthy life behaviors and to prevent the unnecessary use of cosmetic products, it is recommended to establish information platforms, particularly on the internet and social media and to organize training programs that include their families and individuals in their immediate circle and also peer learning. It may be beneficial to carry out works to encourage male students to participate in training programs in order to raise their healthy life awareness. It is believed that introducing symbols on cosmetic products to the public and making them understandable would enable people to make healthier choices about these products.

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Conflicts of interest

There are no conflicts of interest.

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