Contents

**Original Articles**

- Dietary glycemic load in adolescent girls with and without acne
  *Safoura Shakoei, Nazanin Nasri, Ghazal Shariatpanahi, Rezvan Hashemi* ................................................. 43

- Is there any association between a vitamin D receptor gene polymorphism (*FokI*) and pemphigus vulgaris?
  *Zeinab Aryanian, Mohammad Keramatipour, Nafiseh Esmaili, Azadeh Goodarzi, Arghavan Azizpour, Ifa Etesami, Maede Rayati Damavandi, Ghazaleh Zarrinrad, Somayeh Ahmadloo, Narges Ghandi* .......... 49

- Efficacy of topical application of coumarin on incisional wound healing in BALB/c mice
  *Mohammad Afshar, Mohammadmehdi Hassanzadeh-Taheri, Mahmoud Zardast, Maryam Honarmand* ........... 56

- Assessment of serum vitamin D level in patients with cutaneous warts: a case-control study
  *Azadeh Goodarzi, Mahsa Farshidzadeh, Masoumeh Roohaninasab, Najmolsadat Atefi, Elham Behrangi* ..... 64

- The efficacy of IPL and ammonium lactate 14% versus ammonium lactate alone in the treatment of keratosis pilaris
  *Amir Hossein Siadat, Siamak Rahmani, Fariba Iraji, Ali Asilian, Seyed Mohsen Hosseini, Mohammad Ali Nitforoushzadeh* ..................................................................................................................... 72

**Case Reports**

- Sclerotherapy: a bloodless approach in treatment of pyogenic granuloma in children
  *Manjunath M Shenoy, Amina Asfiya, Malcolm Pinto* ....................................................................................... 76

- Porokeratotic eccrine ostial and dermal duct nevus with extensive linear distribution: a case report
  *Arghavan Azizpour, Zeinab Aryanian, Azita Nikoo, Maryam Nasimi, Azadeh Goodarzi, Ifa Etesami* ........... 80

**Letter to Editor**

- Post dengue hair loss: low prevalence in Thai cases
  *Beuy Joob, Viroj Wiwanitkit* .......................................................................................................................... 83
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1. **Aims and Scope:** The “Iranian Journal of Dermatology” is the official scientific quarterly publication of the Iranian Society of Dermatology.

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6. Letter of Undertaking signed by all the authors.

7. Disclosure regarding source of funding and conflict of interest if any besides approval of the study from respective Ethics Committee/Institution Review Board.

8. Covering Letter

9. Main Manuscript document

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Dietary glycemic load in adolescent girls with and without acne

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INTRODUCTION

Acne is a common inflammatory skin disease, which mostly presents during adolescence. It affects a significant proportion of adolescents, with a prevalence of 79-95% in Western countries. The prevalence of acne has increased among adults in developed countries. It may present, persist, or recur in the third and fourth decades of life.

It predominantly affects the skin and is consequently associated with psychological distress, low self-esteem, poor body image, social withdrawal, and depression. The pathogenesis of acne is complex and attributed to excessive sebum production, follicular hyperkeratinization, intra-follicular colonization by bacteria and yeast, and inflammation.

There is some evidence regarding the involvement of environmental factors, including diet, in the acne pathogenesis. Various hormonal mediators, such as insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and sex hormone-binding globulin (SHBG), are involved in acne development by promoting the growth of

BACKGROUND: Acne is a chronic inflammatory skin disease and a cosmetic problem with considerable emotional and psychological side effects and symptoms, such as pain and pruritus. Some controversies exist concerning the involvement of dietary factors, including glycemic load (GL), in the pathogenesis of acne. Accordingly, we aimed to determine the role of GL and anthropometric measures in acne development among girls aged 12-18 years in Tehran, Iran.

METHODS: In this cross-sectional study, 99 girls (45 girls without acne and 54 girls with acne) aged 12-18 years completed a three-day food record. Anthropometric measures, such as height, weight, waist circumference, and body mass index (BMI), were also assessed. Additionally, an expert dermatologist scored the severity of acne, and then, anthropometric measures and GL were examined in the groups.

RESULTS: The results showed no significant difference in dietary GL, height, weight, BMI, and waist circumference between the groups. Furthermore, the severity of acne was not significantly associated with GL.

CONCLUSION: The present results did not confirm the association between acne and dietary carbohydrates, including GL. However, further research can contribute to determination of the effect of diet on acne and its severity.

KEYWORDS: acne vulgaris, diet, glycemic load, anthropometry

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keratinocytes and sebaceous glands, along with sebum production. There is also controversial evidence regarding the association of dietary patterns with acne pathogenesis. Some studies have indicated the impact of dietary glycemic load (GL) on acne, while some have reported different results. Therefore, the role of GL is particularly intriguing and requires major consideration.

Glycemic load refers to the type and amount of consumed carbohydrates and is interpreted as an index of diet-induced insulin demand. A low glycemic index is associated with the reduction of insulin resistance. Therefore, GL can be implicated in acne development due to diet-induced hyperinsulinemia, in addition to the subsequent cascade of hormonal responses and acne exacerbation. Insulin resistance and increased IGF-1 levels change the concentrations of circulating hormones, binding proteins, and receptors, which are associated with the stimulation of unregulated tissue growth androgens, sebum production, and acne-promoting pathways.

The role of dietary carbohydrates in acne pathogenesis has not been established yet. Evidence suggests no significant difference in the level of glucose, insulin, and dietary GL between acne patients and healthy control individuals. Considering the high prevalence of acne during adolescence and its negative effects on self-image and self-esteem, it is necessary to determine the effective factors in acne, including dietary GL. In this study, we aimed to investigate the possible association of dietary GL with acne in 12- to 18-year-old girls, using a validated questionnaire for GL.

**PARTICIPANTS AND METHODS**

**Participants and study design**

Fifty-four females with acne, aged 12-18 years, were recruited in this study. Forty-five age-matched controls were also selected from the Dermatology Outpatient Department of Ziaean Hospital, which is one of the educational centers affiliated with Tehran University of Medical Sciences, Tehran, Iran. Two groups of patients were excluded from the study: acne patients with a history of acne treatment and patients with a history of systemic disorders, such as diabetes. Patients with acne were divided into three groups by an expert dermatologist: mild (comedonal acne), moderate (papular and pustular lesions), and severe (nodular lesions).

**Anthropometric and clinical data collection**

Demographic and anthropometric measures, including age, waist circumference, weight, body mass index (BMI), and height, were recorded for all participants. Height (cm) was measured using a wall tape in a standing position without shoes. Weight (kg) was measured using a digital scale with minimal clothing. Furthermore, waist circumference was measured at the mid-point between the lower rib margin and the iliac crest in a standing position with normal breathing. Moreover, the participants’ dietary intake was determined using a three-day food record questionnaire, in which they were asked to document their food, snack, and beverage intake completely over three days (two weekdays and one weekend). An expert nutritionist guided them on how to record their food intake. The questionnaires were completed after one week.

All subjects were informed about the study procedures and objectives and provided an informed consent to participate in the study. Additionally, the participants were ensured about the confidentiality of their information. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences and performed in accordance with the Declaration of Helsinki (IR TUMS VCR REC13951849).

The glycemic index of foods was determined, using the International Tables of Glycemic Index and GL Tables. The total carbohydrate and fiber contents of dietary intake were calculated, using the United States Department of Agriculture (USDA) Tables. The dietary information, collected from the food diaries, were converted to grams, and the content of carbohydrate, fiber, and other food components was calculated, using the Modified Nutritionist 4 software. We used the three-day food records to calculate daily GL as follows:

\[
\text{Daily GL} = \text{GI for food item} \times \text{carbohydrate content (g)} / 100
\]

**Statistical methods**

The sample size was calculated with an alpha level of 0.05 and beta level of at least 80% according to previous studies. The calculated sample size
for each group was measured to be 40. Considering the possibility of sample attrition, 55 participants were included in each group. During the study, one patient from the acne group and 10 individuals from the control group withdrew from the study; accordingly, their incomplete information was not included in the final report. Data were analyzed and compared between the two groups, using student’s t-test and one-way analysis of variance (ANOVA) in the SPSS software version 24. P value less than 0.05 was considered statistically significant.

RESULTS

A total of 99 participants were investigated in two groups: acne group (n = 54) and control group (n = 45). Height, weight, waist circumference, and BMI were calculated in the acne and control groups. Our results showed no significant difference between the groups regarding any of the mentioned measurements (Table 1).

Assessment of the adolescents’ dietary intake revealed that generally GL ranged from 55.33 to 199.33, with a mean of 99.91 ± 25.24. The mean GL was not significantly different between the two study groups (98.45 ± 24.88 vs. 101.67 ± 25.85; P = 0.903; Table 2). The association between dietary GL and acne severity was examined in the acne group. ANOVA test did not demonstrate any significant differences in the average GL between the three acne groups (P = 0.406).

Table 1. General and demographic characteristics of the two groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Acne (n=54)</th>
<th>Control (n=45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.07 ± 1.97</td>
<td>14.84 ± 1.44</td>
<td>0.43</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.54 ± 5.74</td>
<td>159.62 ± 7.26</td>
<td>0.51</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.85 ± 13.79</td>
<td>57.96 ± 14.70</td>
<td>0.94</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.37 ± 4.56</td>
<td>22.77 ± 5.02</td>
<td>0.77</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>73.70 ± 7.04</td>
<td>74.18 ± 10.04</td>
<td>0.59</td>
</tr>
</tbody>
</table>

BMI, body mass index; cm, centimeter; kg, kilogram; n, number; SD, standard deviation

Table 2. Glycemic load calculated in the control and acne groups.

<table>
<thead>
<tr>
<th>Glycemic load</th>
<th>Control (n=45)</th>
<th>Acne (n=54)</th>
<th>Mild acne Mean ± SD</th>
<th>Moderate acne Mean ± SD</th>
<th>Severe acne Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic load</td>
<td>101.67 ± 25.85</td>
<td>98.45 ± 24.88</td>
<td>88.98 ± 16.7</td>
<td>101.77 ± 28.48</td>
<td>96.30 ± 18.7</td>
<td>0.903</td>
</tr>
</tbody>
</table>

n, number; SD, standard deviation

DISCUSSION

Our results indicated that dietary GL in Iranian adolescent girls with acne was not higher than that of control subjects. Additionally, no significant association was found between dietary GL and acne severity. Furthermore, BMI and waist circumference of the two groups were not significantly different.

Acne is a common skin disorder affected by different factors, such as genetics, lifestyle and habits, seasonal changes, obesity, and diet. Many studies support the association between dietary carbohydrates and acne development. In this regard, a study compared the inhabitants of Kitua Island (Tanzania) and Paraguay hunters, consuming large amounts of vegetable and meat (with lower levels of carbohydrates), with individuals consuming a Western diet; it was found that acne was rarely found in these populations. Therefore, a low-glycemic diet can protect against acne owing to the absence of hyperinsulinemia and activation of the subsequent endocrine cascade.

Ismail et al. found an association between dietary GL and acne in Malaysian young adults. Their study showed that GL was significantly higher in the acne group than in the healthy controls, aged 18-30 years. They reported no significant difference in BMI between the case and control groups. Another community-based study reported that individuals maintaining a Mediterranean diet (a low-carbohydrate diet) were less likely to develop acne. Moreover, a cross-sectional study showed that dietary GL was higher among individuals with acne.
Shakoei et al.

moderate to severe acne (mean age = 21.8 ± 3.5 years), compared to those with mild acnes. The mechanism underlying the association between dietary GL and acne has been well described. A high-GL diet causes hyperinsulinemia with subsequent activation of a signaling cascade, leading to an increase in insulin and IGF-1 activities while reducing IGFBP-3. Reduction of IGFBP-3 significantly increases the bioavailability of IGF-1 as a key stimulator in acne pathogenesis. IGF-1 stimulates lipogenesis and proliferation of keratinocytes and sebocytes. Both insulin and IGF-1 increase the synthesis of gonadal and adrenal androgens. Moreover, IGF-1 reduces the synthesis of SHBG in the liver and occupies androgenic receptors. Consequently, it directly increases the bioavailability of androgens. Androgens promote the production of fat as a factor playing a role in acne development. In addition, IGFBP is a strong pro-apoptotic factor in keratinocytes. Although several studies have been conducted in this area, the association between dietary carbohydrates and acne has not been approved in any investigations, including the current study. In this regard, Kaymak et al. in a prospective cohort study, reported no significant difference in terms of serum glucose, insulin, and leptin content, overall glycemic index, and dietary GL between university students with and without acne. It should be noted that they only focused on dietary carbohydrates, similar to our study. However, they suggested a relationship between high glycemic index and duration of acne, which was not addressed in the current study.

Furthermore, the results of a non-randomized clinical trial demonstrated that acne improved in adolescent boys on both low- and high-GL diets, while the difference between the groups was not significant. They found no significant correlation between changes in acne severity and insulin concentration or sensitivity. They also reported that diet significantly affected IGFBP-1, but not IGFBP-3 or IGF-1. In this respect, Smith et al. conducted four interventional studies to investigate the effect of dietary GL on acne severity. In two clinical trials, low dietary GL significantly reduced acne severity and plasma levels of free androgens, while it increased insulin and IGFBP sensitivity.

Consistent with the findings reported by Smith et al., recent studies including a clinical trial, have provided histopathological evidence regarding the benefits of a low-GL regimen in reducing acne development. They found that low GL was associated with reduced acne severity, sebum production, expression of sterol regulatory element-binding proteins, lipid synthesis, and inflammatory cytokines like interleukin-8. Nevertheless, Pavithra et al. did not find any improvement in acne development following adherence to a low-GL diet. Overall, the impact of weight loss secondary to a low-GL diet has not been examined in any of the mentioned studies, while BMI is an important contributor to acne development.

In a number of studies, the effect of low-GL diet could not be detected when the data were statistically adjusted for BMI changes. In the current study, there was no significant difference in BMI between adolescents with and without acne, which could explain the lack of association between GL and acne development. Additionally, some previous studies demonstrated no significant correlation between BMI and presence of acne or its severity.

The Iranian dietary pattern is high in refined grains, including white rice and bread, potato, tea, whole grains, hydrogenated fats, legumes, and broth, and differs from Mediterranean and Western dietary patterns. It has been revealed that the Iranian diet is not associated with general or central obesity, and the mean GL in the present study is similar to that of previous reports. Although many foods rich in carbohydrates are included in the typical Iranian diet, it appears that some other healthy food groups in this diet, such as legumes and whole grains, could alleviate the effect of high-carbohydrate components. Other factors, such as genetics, tobacco use, and different climatic conditions may be also influential in acne development. Furthermore, differences in the age range of participants may explain the discrepancy between our findings and previous reports.

This study had some limitations. First, we used a three-day food record owing to time and cost constraints, while other food recording methods, such as repeated three-day questionnaire and food frequency questionnaire, would be more effective in calculating GL, especially in patients with chronic diseases. Second, the sample size was calculated to be sufficient to analyze the differences based
on independent t-test. Consequently, the sample size was not large enough for advanced statistical analyses.

CONCLUSION

Chronic skin diseases, including acne, are often accompanied by significant emotional and psychological burdens negatively affecting the individual’s quality of life. Thus, control of modifiable factors to reduce acne is necessary. Change of dietary habits is a controversial treatment option for patients with acne. The results of the present study did not confirm the effects of dietary GL on acne in young girls. Moreover, no significant difference in BMI and waist circumference was observed between the acne and healthy groups.

Acknowledgements

The authors sincerely appreciate Ziaein Hospital for recruiting the participants.

Conflict of Interest: None declared.

REFERENCES


40. Basiotis PP, Welsh SO, Cronin FJ, et al. Number of days of food intake records required to estimate individual and group nutrient intakes with defined confidence. The J Nutr. 1987;117(9):1638-41.
Is there any association between a vitamin D receptor gene polymorphism (FokI) and pemphigus vulgaris?

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Background: Pemphigus vulgaris (PV) is an autoimmune bullous disease of the skin and mucous membranes caused by activation and proliferation of T cells, production of Th2 cytokine profile and pathogenic antibodies. Vitamin D is a probable immunodeviator to Th2, which its actions are mediated through the vitamin D receptor (VDR). FokI is the only single nucleotide polymorphism (SNP) leading to VDR protein with a different structure and function. For the first time, we focused on FokI VDR SNP to evaluate its potential role in the genetic susceptibility to PV, particularly in the Iranian population that has a high prevalence of pemphigus.

Methods: In this case-control study, DNA samples of 122 PV patients and 233 healthy controls were extracted, and FokI genotyping was performed using the PCR-RFLP method.

Results: The mean allele frequencies of F and f alleles in the PV and control groups were 75% and 25%, and 78% and 22%, respectively, showing no significant difference. The genotype frequencies for FF, Ff, and ff genotypes in the case group were 57.4%, 35.2%, and 7.4%, respectively. In the control group, the frequencies were 60%, 36%, and 4%, respectively. Statistical analysis showed no significant difference between the two groups.

Conclusion: The present study concluded the frequencies of F and f alleles as approximately 77% and 23% in the gene pool of the Iranian population. Additionally, it showed no association between the FokI alleles and PV in this population.

Keywords: FokI, pemphigus, polymorphism, vitamin D receptor

INTRODUCTION

Pemphigus is an autoimmune blistering disease affecting the skin and mucous membranes. Pemphigus vulgaris (PV) is the most common form of pemphigus. It is caused by autoantibodies directed against desmosomes, the principal adhesion structures of keratinocytes that are
essential to the stability of stratified epithelia. Binding of autoantibodies leads to destruction of desmosomes, loss of adhesion between epidermal cells (acantholysis), and formation of epidermal blisters 1.

Numerous studies have investigated the immunologic background of these antibody-mediated bullous diseases, with various methods and controversial results, but most of them emphasize on T-helper 2 (Th2) cytokines predominance or at least a mixed Th1/Th2 cytokine balance. It has been proposed that Th2-derived cytokines can modulate the acantholytic process, and this Th2-like response contributes to pemphigus lesions 2,3. Many studies have shown a lower level of vitamin D among PV patients compared to healthy controls 4-8, and the relationship between vitamin D level and disease severity 4,8,9. It has been suggested as an etiological factor that may trigger autoimmunity 10. In addition, it has been revealed that vitamin D metabolites may play a protective role in keratinocytes from detachment and apoptosis 11.

Vitamin D is a potential immunomodulator with various effects on the immune system. It is a probable immunodeviator to Th2, and its actions are mediated through the vitamin D receptor (VDR). The VDR gene is localized on 12q12-14, consists of 11 exons, and spans 75 kb. The active form of vitamin D, 1, 25-dihydroxyvitamin D3 (1,25(OH)2D), binds with high affinity to specific VDRs located in the nucleus of target cells acting as a transcription factor. They regulate the transcription and expression of genes in different vitamin D-responsive tissues, including the epidermis and various immune cells expressing VDR 12. It has been demonstrated that VDR/1,25(OH)2D interferes with the signaling of transcription factors such as NFAT, NF-kB and AP-1 in a dose-dependent way 13. These transcription factors act in the regulation of immunomodulatory genes, such as for numerous cytokines, effector enzymes, adhesion molecules and growth factors 14. The diversity in 1,25(OH)2D-regulated genes and the presence of VDR in multiple cell types reflect the pleiotropic actions of the molecule.

For example, 1,25(OH)2D suppresses cell cycle progression and activation of T lymphocytes and reduces their secretion of cytokines such as IL-2, IL-12, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (Th1 cytokines), which are the hallmark of vitamin D effect on the immune system. These cytokine changes are important in the pathogenesis of several autoimmune diseases like PV 15. In Th2 cells, 1,25(OH)2D also increases the production of IL-4 16. Moreover, 1,25(OH)2D potentially influences maturation, differentiation, migration, and activation of antigen-presenting cells such as dendritic cells and shaping their cytokine secretion patterns 17. Therefore, a possible immune deviation from Th1 to Th2 responses can be postulated. Many researchers have documented that vitamin D can regulate the ongoing immune reaction away from a Th1 toward a Th2 response 16,18.

In humans, several polymorphic variants of the VDR gene have been shown, which are scattered throughout the complete VDR gene region. Among them, the FokI single nucleotide polymorphism (SNPs) as a T/C transition polymorphism (ATG to ACG) at the first of two potential translation initiation codons in exon 2 is the only one resulting in a VDR protein with a different structure and function 19. Moreover, FokI is the only polymorphism not linked to any of other VDR polymorphisms 20. In the presence of the C allele (designated F), translation initiates at the second ATG site and lacks the three NH2-terminal amino acids of the full-length VDR protein. On the contrary, in the presence of the T allele (designated f), translation initiates at the first ATG site and synthesizes the full-length (427 amino acids) VDR protein 19. This polymorphism leads to formation of long f-VDR protein (presence of either two ATG start codons) or short F-VDR (only one start codon owing to a T-to-C substitution) that have different immunological consequences. The short F-VDR results in a higher NFAT- and NF-kB-driven transcription capacity and a more active immune system than the long f-VDR. Therefore, it can be hypothesized that the VDR FokI polymorphism can play a role in the pathogenesis of immune-mediated diseases 21. Numerous studies have evaluated the association between inheritance of FokI polymorphism and genetic susceptibility to various illnesses. They include different autoimmune diseases such as type 1 diabetes mellitus, rheumatoid arthritis, multiple sclerosis, autoimmune thyroid diseases, autoimmune hepatitis, psoriasis, vitiligo, alopecia areata, recurrent aphthous stomatitis, and skin cancer in the field of dermatology 21-23. Nevertheless, an obvious and conclusive association has not yet been identified.
The role of vitamin D in immunomodulation and pathogenesis of autoimmune skin diseases is an open new topic, which is highly discussed. Thus, considering the autoimmune basis of PV and its specific immunologic pattern, and immunomodulatory effects of the vitamin D-VDR complex, along with lack of any association study of vitamin D receptor polymorphisms and this autoimmune skin disease, as well as regarding the fact that Iran is a region with a higher PV prevalence 1, we aimed to evaluate the role of one of VDR polymorphisms, which is the FokI polymorphism in the translation initiation codon of the VDR gene in Iranian patients with PV compared to healthy controls. Determination of the frequency of this polymorphism and its possible relation to PV can improve the knowledge about the genetic background of the diseases.

PARTICIPANTS AND METHODS

Participants and study design

The study was conducted within a year on PV patients referred to Razi Hospital, which is a tertiary referral dermatology clinic at Autoimmune Bullous Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran. All patients with PV were included until sample size was reached. Diagnosis of PV was made based on the clinical, histological and direct immunofluorescence study of cutaneous biopsies. Demographic and clinical data of the patients were recorded. Unrelated healthy individuals were also randomly selected as the control group. Patients with any systemic autoimmune diseases such as diabetes mellitus and thyroid disorders, and multiple sclerosis were excluded from this study. In controls, people, who had a history of autoimmune blistering diseases or a family history of such disorders in first-degree relatives or being affected by any other systemic disease, were excluded. This study was approved by the Ethical Committee of Tehran University of Medical Sciences. Written informed consent was obtained from the patients and controls before sampling.

DNA extraction and genotyping of the FokI polymorphism

Genomic DNA was extracted from 5 ml peripheral blood collected in EDTA vacutainers by the modified salting out method. DNA was stored at -20°C until use. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were performed for genotyping of single nucleotide substitution at the FokI (rs2228570) position of the VDR gene. VDR gene polymorphic FokI site (exon 2) was amplified by PCR. The primers 5’-AGCCACGTATGCTAGCCCATATAC-3’ (VDR-F) and 5’-CTCTTGGGTGAGGAAGCTGTGAT-3’ (VDR-R) were used (Pishgam Biotech Co., Tehran, Iran) to amplify exon 2. The PCR conditions were 30 cycles at 95°C for 1 min for denaturation, 1 min at 64°C for annealing and 1 min at 72°C for the extension. The PCR products were analyzed on 2% agarose gels containing ethidium bromide (0.5mg/ml) and visualized under a UV transilluminator. A 100 bp ladder (Fermentase, Germany) was used as a marker. Exon 2 PCR products were then digested with the FokI restriction endonuclease enzyme at 37°C for 1 h. Digested products were run on 2% agarose gels (voltage 120v) and then stained with silver nitrate. The genotypes were classified as FF homozygotes (absence of the FokI site resulted in one fragment of 557 bp); Ff heterozygotes, fragments of 557 bp, 494 bp, and 63 bp; and ff homozygotes (presence of the FokI sites resulted in two fragments of 494 bp and 63 bp). Two additional cutting sites existed for the FokI enzyme other than the FokI site resulting in two 128 and 63 bp bands in all digested samples with any genotypes (Figure 1).

Figure 1. Restriction fragment length polymorphism (RFLP) results of FokI polymorphism on agarose gel electrophoresis. Lane 1, 100 bp DNA ladder. Lane 2, undigested PCR product. Lanes 3,4,6,9,10,11 FF homozygote genotypes. Lanes 5,7,8 Ff heterozygote genotypes, and lanes 12,13 ff homozygote genotypes
Statistical methods

Statistical analysis was performed using the SPSS software Version 17.0 (SPSS Inc). Chi-Square test and ‘Yates’ correction were used to compare the frequencies of genotypes from patients and controls. If five or fewer persons were present per group, Fisher’s exact two-tailed test was used. P-value less than 0.05 was considered statistically significant.

Ethical considerations

The written informed consent was obtained from all participants.

RESULTS

In this study, a total of 122 Iranian patients with PV were recruited. They were 74 females and 48 males, age range 16–73 years, mean age 44.5 years, and mean age of disease onset 40.8 years. The patients were from different geographical regions of Iran and were referred to Razi Hospital. The distribution of three clinical phenotypes of the disease was as follows: mucous membrane form without skin involvement, 32 patients; both skin and mucous membrane involvement, 76 patients; and skin involvement without mucosal involvement, 11 patients.

FokI genotyping was carried out in the group of patients with PV and 233 healthy control subjects. Homozygous cleavage by FokI produced two fragments, 63 and 494 bp, whereas the heterozygotes displayed all three 557, 63 and 494 bp bands; therefore, three genotypes FF, Ff and ff were specified. Table 1 shows the frequencies of FF, Ff and ff genotypes in PV patients and control healthy subjects. The distribution of FokI genotypes in the PV group demonstrated no statistically significant differences compared to the healthy control group (P = 0.35).Allele frequencies of F and f were 75% and 25% in the patient group, and 78% and 22% in the control group, respectively. The comparison of patients and controls revealed no significant differences in F and f alleles frequencies (P = 0.31).

In Tables 1 and 2, you can see the allele frequencies based on the patients’ age of disease onset and site of involvement, respectively. We concluded that the frequencies of F and f alleles were approximately 77% and 23% in the gene pool of the Iranian population (Table 3). The comparison of FF, Ff and ff genotypes among three phenotypic groups of the patients and between the two age groups (≤ 40 and > 40 years) at the disease presentation demonstrated no significant difference (P = 0.49 and 0.13 respectively).

DISCUSSION

According to epidemiological studies conducted in different ethnic populations and family studies, the genetic background can influence the occurrence of pemphigus 24. A relatively strong

Table 1. Allele frequencies of FokI polymorphisms according to the age of disease onset in patients with PV.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>≤40 years</th>
<th>&gt;40 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>41 (63.1%)</td>
<td>28 (50%)</td>
<td>69 (57%)</td>
</tr>
<tr>
<td>Ff</td>
<td>18 (27.7%)</td>
<td>25 (44.6%)</td>
<td>43 (35.5%)</td>
</tr>
<tr>
<td>ff</td>
<td>6 (9.2%)</td>
<td>3 (5.4%)</td>
<td>9 (7.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 2. Allele frequencies of FokI polymorphisms according to the site of involvement in patients with PV.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mucosal</th>
<th>Pemphigus type</th>
<th>Cutaneous</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>21 (65.6%)</td>
<td>5 (45.4%)</td>
<td>42 (55.3%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Ff</td>
<td>10 (31.3%)</td>
<td>4 (36.4%)</td>
<td>28 (36.8%)</td>
<td></td>
</tr>
<tr>
<td>ff</td>
<td>1 (3.1%)</td>
<td>2 (18.2%)</td>
<td>6 (7.9%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32 (100%)</td>
<td>11 (100%)</td>
<td>76 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Genotype and allele frequencies of FokI polymorphisms in patients with PV and controls.

<table>
<thead>
<tr>
<th>FokI genotype</th>
<th>Patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>70 (57.4%)</td>
<td>141 (60%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Ff</td>
<td>43 (35.2%)</td>
<td>83 (36%)</td>
<td></td>
</tr>
<tr>
<td>ff</td>
<td>9 (7.4%)</td>
<td>9 (4%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>122 (100%)</td>
<td>233 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allelic frequency of FokI</th>
<th>Patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>183 (75%)</td>
<td>365 (78%)</td>
<td>0.35</td>
</tr>
<tr>
<td>f</td>
<td>61 (25%)</td>
<td>101 (22%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>244 (100%)</td>
<td>466 (100%)</td>
<td></td>
</tr>
</tbody>
</table>
genetic predisposition to PV exists in certain ethnic groups such as Mediterranean, Persian and Ashkenazi Jewish descents. PV is an antibody-mediated autoimmune blistering disease that is probably caused by Th2 cells, and the vitamin D-VDR complex is identified as a strong immune-regulator, which can deviate immune response to the Th2 cytokine profile. In recent decades, cascade of studies has been launched to assess the role of VDR polymorphisms in the genetic background of different diseases.

Although there were numerous association studies evaluating the VDR gene polymorphism and various diseases, including various autoimmune diseases of different body systems, to the best of our knowledge, this is the first study investigating the association between the VDR gene polymorphism and the genetic susceptibility to PV. A number of polymorphisms have been identified in the VDR locus: an exon 2 initiation codon polymorphism detected with FokI restriction enzyme, BsmI, Tru9I, and Apal restriction fragment length polymorphisms located between exons 8 and 9, the TaqI located in exon 9, and a PolyA polymorphism downstream of the 3′-untranslated region. Nevertheless, as previously mentioned, FokI is the only one leading to the VDR protein with a different structure and function as well as the only VDR polymorphism not linked to other VDR polymorphisms.

Overall, it has been documented that the VDR FokI polymorphism can affect immune system behavior, with more active immune cells in the presence of the short F-VDR protein, thereby playing a possible role in immune-mediated diseases. Therefore, we focused only on the FokI site. We found no relationships between PV and the FokI RFLP VDR genotype in Iranian population. Our results suggest that this SNP, despite its associations with many other autoimmune disorders and dermatological conditions is not involved in the pathogenesis of pemphigus vulgaris and even the frequencies of the genotypes are similar in different phenotypes of the disease. It also does not affect the disease age of onset.

A number of previous reports are available on the VDR FokI polymorphism in Iranian population, and two studies investigated autoimmune diseases. Naderi et al. indicated that the frequencies of FF, Ff and ff genotypes were 54%, 38.7%, and 7.3%, respectively, in Iranian control subjects, and the frequencies of F and f alleles were 73% and 27% in their study. They suggest a probable association of the FokI polymorphism in the VDR gene, and the susceptibility to Crohn’s disease in the Iranian population. Mohammadnejad et al. found the frequency of 55%, 40% and 5% for FF, Ff and ff genotypes, respectively in the control group, and the frequencies of F and f alleles were 75% and 25% in their study. No significant association was observed between FokI VDR SNP and the susceptibility to type 1 diabetes mellitus in their survey in the Iranian population. The genotypes and allele frequencies found in our control group for FokI were similar to those seen in the control group of both above-mentioned studies, supporting validation of our results. Since there are many articles regarding association of dermatologic disorders with VDR gene polymorphism, which could be related to the disease susceptibility or clinical courses.

CONCLUSION

Our findings didn’t identify the role of the VDR gene in the pathogenesis of PV, but we investigated only one site among various sites of the VDR gene and only the vulgaris variant of the pemphigus group of diseases. More extensive surveys on other polymorphisms of the VDR gene, such as BsmI, Tru9I, Apal, TaqI and PolyA, and on other pemphigus variants such as pemphigus foliaceous are necessary. Furthermore, to conclude that there is no relationship between VDR gene polymorphism and PV, the VDR FokI should be further studied in other populations and larger groups, since a marked ethnic difference has been revealed in the VDR genotype. However, this preliminary study may provide insights into the VDR gene and pathogenesis of PV.

Acknowledgements

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Conflict of Interest: None declared.

REFERENCES

Association between VDR gene FokI & pemphigus vulgaris


Efficacy of topical application of coumarin on incisional wound healing in BALB/c mice

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INTRODUCTION

Skin is a vital organ for humans because of preventing loss of water, bleeding, and invasion of microorganisms and regulation of body temperature 1. Wound healing is a restorative process that involves hemostatic, inflammatory, proliferation, and remodeling phases. At the inflammatory phase, inflammatory response is organized by granulocyte and lymphocyte cells. The subsequent proliferation phase is formed by angiogenesis, epithelialization, formation and accumulation of fibroblasts and collagen synthesis of the extra cellular matrix (ECM). At the end of wound healing, remodeling phase is formed that involves fundamental changes in the collagen structure 2.

Various factors affect wound healing, including oxygenation (tissue perfusion), infection, age, stress, nutrition, sex hormones, ECM, proteases, and cytokines 1,2. Increasing the rate of wound repairing leads to positive financial and health
results, which is the main aim of medical science in recent decades. One of the most important ways to achieve this goal is to reduce inflammation and wound infections.

The use of herbal medicines has grown enormously because of their low cost and side effects and also easier preparation compared to chemical drugs. Furthermore, many plants have anti-inflammatory and anti-infective properties. Hence, the plant-related medicine industries are expanding in the world.

Coumarin C9H6O2 belongs to a group of polyphenolic compounds which are benzopyrone derivatives, and in nature, there are two glycosylated and free forms. Benzopyrone is divided into alpha and gamabenzopyrone sub-types, and coumarin belongs to the alpha-banzopyrene subtype. Coumarin has anti-cancer, anticonvulsant, anticoagulant, and immune regulator properties. It also has anti-inflammatory, anti-oxidant, anti-viral, ant-bacterial, and anti-fungal effects. Among the investigations conducted on these properties, there are some important studies which took the natural and synthetic coumarin derivatives with their anti-inflammatory/antioxidant activities into account. These studies indicated that coumarin can reduce tissue edema and inflammation. It also induces high degree of antioxidant activity. Not only coumarins have some effects on the formation and removal of reactive oxygen species (ROS), but also the processes involving free radical-mediated injuries have been affected. Furthermore, prostaglandin biosynthesis, which takes part in fatty acid hydroperoxy intermediates, is inhibited with coumarin and its 7-hydroxy-derivative. Also, the results from the study of anti-inflammatory effect of MC13 (a new coumarin compound extracted from the leaf of Condiment Murraya extract) showed that this compound inhibits the production of lipopolysaccharide (LPS) that is produced by various inflammatory mediators such as nitrite oxide, IL6, and TNFα.

These studies consider the anti-inflammatory/anti-oxidant properties of coumarin, a good candidate to accelerate the wound healing process; however, there have been no precise studies on the therapeutic effects of this substance on the wound repairing process so far. Therefore, in this study, we set to investigate the usefulness of coumarin in accelerating wound healing in mice.

MATERIALS AND METHODS

Preparation of ointment

Coumarin (code: C85557-5G) was purchased from Sigma-Aldrich Company. 1% or 2% cream containing this substance with the basis of cold cream (eucerin) was prepared and used. Nitrofurazone cream and other creams were purchased from the Behnam Company in Tehran.

Experimental animals

In this study, sixty mature (2.5 months of age) male BALB/C mice weighing 25 ± 5 gr were used. The animals were provided from the Experimental Medicine Research Center of Birjand, University of Medical Sciences (BUMS). Each mouse was kept individually in a clean cage. Environmental conditions were controlled by daily replacement of cages, 12:12 h light-dark cycle, 22 ± 1°C temperature, and an average air humidity of 40 to 45%. Mice had free access to water and food. The number of mice and method of this study were determined based on previous studies.

Surgical procedure

The mice were initially anesthetized with intraperitoneal (I.P.) injection of Xylasine 2% and ketamine 70 mg/kg, and the hair of dorsal thoracic skin was completely shaved. Then, the shaved area was disinfected with 10% iodine (Iran Drug Production Company) under sterile conditions (observing all the rules of surgery). A full-thickness incisional wound (2 cm length) was created on dorsal thoracic skin of the mice, using a ruler and a sterile blade.

Afterwards, the mice were divided into five groups: the first and second groups were treated with cream containing coumarin 1 and 2% and the third and fourth groups as the positive and negative controls which received nitrofurazone 0.02% and cold cream (eucerin), respectively. The fifth group was not treated (sham). The first to fourth groups were dressed twice daily at 8:00 am and 8:00pm for 14 days.

Histological study

On days 4, 7, 10, and 14 after the treatment, three
animals from each group were sacrificed with an overdose of anesthetics, and incisional wounds areas were removed for histopathological studies. The samples were embedded in paraffin after fixation in 5% formaldehyde solution, dehydrated in ethanol series with excessive concentration, and cleared in xylene. Then, 5 μm serial sections of specimens were prepared by rotary microtome (Leits, Italy) and stained with hematoxylin and eosin (H & E) and specific staining of Masson’s trichrome. Finally, the slides assessed under light microscope (Olympus, BX54) in order to evaluate the epithelialization, granulation, and collagenization.

Statistical analysis

The quantitative data were reported as mean ± standard error of the mean. One-way ANOVA test was used to compare the groups, and Tukey test was used in case of significant results. Data were analyzed with SPSS statistical software (ver. 19). Results were considered statistically significant at P < 0.05. Image J software was used for quantitative data and numerical analysis of the number of fibroblast cells, epithelialization, and granulation in different stages of repair. Evaluation of inflammation and collagenization areas were estimated by semi-quantitatively score of - to +++, based on the following score system; - low, -/+ low to mild, +/- very mild, + mild, ++ moderate, and +++ severe.

Ethical considerations

The ethical standards of working with laboratory animals were designed based on the protocol of work with laboratory animals approved by Birjand University of Medical Sciences with the approved code (455171) (Ethics Code: ir.bums.REC.1396.81).

RESULTS

Study samples on the 4th day

The mean of the inflammatory cells decreased significantly in the treated groups with coumarin 1% and 2% and also nitrofurazone compared with the negative control and sham groups. This parameter also showed a slight decrease in the experimental groups compared to the positive control group (P < 0.05; Table 1, Figure 1).

Study samples on the 7th day

Inflammatory cells in the experimental groups showed significant decrease compared with the negative and sham control groups (P < 0.05; Table 1, Figure 1). Also, the total number of inflammatory cells in these groups decreased significantly compared to the nitrofurazone group (P < 0.05; Table 1, Figure 1).

Study samples on the 10th day

The findings on the 7th day indicated that the mean of fibroblast cells also increased in the experimental groups compared to the negative and sham control groups (P < 0.05; Table 2, Figure 2). On the other hand, only the mean number of fibroblasts in the coumarin 2% treatment group was significantly higher than the nitrofurazone group (P < 0.05; Table 2, Figure 2).

Study samples on the 14th day

The density of collagen fibers in the experimental groups was higher than the all control groups (Table 1, Figure 2). On this day the mean of granulation tissue formation was significantly increased in the experimental groups in comparison with negative and sham control groups and also nitrofurazone group (P < 0.05; Table 2, Figure 2).

Table 1. Efficacy of topical application of coumarin on inflammation phase and collagen density in the wound repair model.

<table>
<thead>
<tr>
<th>Groups/Days</th>
<th>Studied parameters</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumarin1%</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Coumarin2%</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Eucerin</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sham</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Inflammatory cells (Inf. C) and collagen density (Col. D) were estimated by the following score system; - low, +/- low to mild, +/- very mild, + mild, ++ moderate, +++ severe.
Efficacy of coumarin on wound healing

inflammatory and fibroblast cells, collagen density, granulation, and epithelization. The total number of inflammatory cells in the experimental groups decreased significantly in comparison with the control groups ($P < 0.05$; Table 1, Figure 1). The mean number of fibroblast cells in the experimental groups demonstrated a significant increase compared to the control groups ($P < 0.05$; Table 2, Figure 2).

Table 2. Efficacy of topical application of coumarin on fibroblast proliferation, epithelialization, and granulation tissue ($\mu m^2$) in the incision wound repair model.

<table>
<thead>
<tr>
<th>Studied parameters</th>
<th>Groups/Days</th>
<th>7</th>
<th>10</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroblast proliferation (number)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coumarin 1%</td>
<td></td>
<td>15.20 ± 2.52*#</td>
<td>38.40 ± 5.91*#</td>
<td>28.90 ± 5.85*#</td>
</tr>
<tr>
<td>Coumarin 2%</td>
<td></td>
<td>19.50 ± 1.77*#</td>
<td>44.50 ± 3.17*#</td>
<td>29.40 ± 5.98*#</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td></td>
<td>12.40 ± 3.23</td>
<td>27.60 ± 4.90*</td>
<td>18.70 ± 6.16*</td>
</tr>
<tr>
<td>Eucerin</td>
<td></td>
<td>11.60 ± 2.71</td>
<td>13.10 ± 4.86</td>
<td>12.10 ± 4.86</td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td>9.90 ± 1.79</td>
<td>10.90 ± 1.79</td>
<td>8.90 ± 1.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelialization (µm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coumarin 1%</td>
<td></td>
<td>-</td>
<td>17.16 ± 2.11*#</td>
<td>25.25 ± 3.11*#</td>
</tr>
<tr>
<td>Coumarin 2%</td>
<td></td>
<td>-</td>
<td>23.65 ± 1.79*</td>
<td>26.57 ± 3.48*#</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td></td>
<td>-</td>
<td>15.96 ± 1.92*</td>
<td>18.62 ± 1.88*</td>
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<tr>
<td>Eucerin</td>
<td></td>
<td>-</td>
<td>11.17 ± 2.08</td>
<td>12.66 ± 1.78</td>
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<tr>
<td>Sham</td>
<td></td>
<td>-</td>
<td>8.90 ± 0.97</td>
<td>9.10 ± 1.17</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Granulation tissue (µm²)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coumarin 1%</td>
<td></td>
<td>12545.71 ± 4519.03*#</td>
<td>23770.23 ± 2397.99*#</td>
<td>22198.69 ± 3820.91*#</td>
</tr>
<tr>
<td>Coumarin 2%</td>
<td></td>
<td>13394.35 ± 3514.45*#</td>
<td>24476.02 ± 7007.80*#</td>
<td>23196.29 ± 1959.65*#</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td></td>
<td>9227.23 ± 985.91</td>
<td>16004.09 ± 2075.76*</td>
<td>13507.67 ± 3520.29</td>
</tr>
<tr>
<td>Eucerin</td>
<td></td>
<td>6248.78 ± 1281.43</td>
<td>12547.70 ± 1510.85</td>
<td>11194.85 ± 2616.32</td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td>2886.63 ± 957.33</td>
<td>8631.52 ± 1312.44</td>
<td>7094.19 ± 2120.01</td>
</tr>
</tbody>
</table>

Values are expressed as the Mean ± SEM. P-value: * $< 0.05$ compared with the negative and sham control groups. P-value: # $< 0.05$ compared with the positive control group.
Based on Masson’s trichrome staining, the density of collagen fibers in the two experimental groups was significantly higher than the control groups (Table 1, Figure 2). In addition, the mean area of granulation significantly increased in both experimental groups in comparison with the control groups ($P < 0.05$; Table 2, Figure 2). Besides, the thickness of epithelialization significantly increased in the experimental groups compared to the control groups ($P < 0.05$, Table 2).

**Study samples on the 14th day**

It was indicated that the mean number of fibroblast cells significantly increased in the experimental groups compared to the control groups ($P < 0.05$; Table 2, Figure 2). The density of collagen fibers in the two coumarin experimental groups was significantly higher than the negative and sham control groups. However, there was no difference between these experimental groups and the positive control group (Table 1, Figure 2). Furthermore, the mean area of granulation significantly increased in the treatment groups with 1% and 2% coumarin compared to the control groups ($P < 0.05$; Table 2, Figure 2). There was a significant increase in the mean of epithelialization in the two coumarin experimental groups in comparison to the control groups ($P < 0.05$; Table 2).

**DISCUSSION**

Wound is defined as a structural and functional breakdown of skin normal continuity and integrity, resulting in damage from physical, chemical, and biological factors. Wound healing is a natural restorative response to injury. This process is performed based on collaboration of cellular and molecular events that involve the infiltration of cells into the wound, cell proliferation, and also synthesis and accumulation of new ECM. These events occur during the three phases of skin repair including hemostatic-inflammation, proliferation, and remodeling.

The results showed that coumarin had positive effects during various phases of wound healing, especially inflammatory and proliferative phases. One of the most important events that occurs at the beginning of wound healing is the phenomenon of inflammation. The inflammatory process involves cell and hormonal parts. Neutrophils, macrophages, and lymphocytes are the most important inflammatory cells that appear at the wound area at this stage. Our study revealed
Efficacy of coumarin on wound healing

that inflammation and infiltration of related cells from the beginning of the study on the fourth day were less in the coumarin-treated groups compared to all other control groups, and this trend was also observed in the following days.

Coumarins with a fused benzene and α-pyrone rings are found in plants and constitute a major group of phenolic derivatives. Over 1300 secondary metabolites of this compound have been identified, chiefly in green plants, fungi, and bacteria. Studies have shown that coumarin, as a phenolic compound, has anti-inflammatory, anti-oxidant, and antibacterial properties. Among these effects of coumarin, its anti-inflammatory properties have been confirmed in numerous studies as following: Iranshahi et al. conducted a study in which antioxidant, anti-inflammatory, and lipoxygenase inhibitory activities of the prenylated coumarin, umbelliprenin were evaluated. It was revealed that umbelliprenin exhibited antioxidant and lipoxygenase inhibitory properties. In a study conducted by Kafaj et al. on therapeutic effects of leaf extract of Plantago lanceolata on infected burn-wound in mice, the beneficial effects of this extract on wound healing process could be due to the presence of phenolic compounds, such as coumarin, which has anti-inflammatory properties and consequently accelerate the process of wound repairing.

Another experimental study also demonstrated that the substance (S)-(+)-decursin, a biological compound of coumarin (extracted from the glacial plant), reduced IgE levels of inflammatory cytokines in comparison with the control group, concluding that it may be valuable as a therapeutic drug for the treatment of atopic dermatitis. Additionally, Luchini et al. noted that coumarin and 4-hydroxycoumarin prevent the glutathione depletion that occurs as a consequence of the colonic inflammation and intestinal oxidative stress and may be effective in the treatment of ulcerative colitis in rats. Similarly, the present study confirmed the anti-inflammatory effects of coumarin during wound healing and acceleration of the wound healing process due to this positive feature.

In the proliferative phase which consists of the activation and migration of fibroblasts and production of glycosaminoglycan, proteoglycan, and collagen fibers, the granulation tissue is formed. This phase of repair is very important and sensitive. Moreover, in this stage, epithelialization is accelerated due to the formation of granulation tissue. So, any compound that can affect the rate and extent of the granulation tissue formation can also change the rate of wound healing. Our findings showed that coumarin could increase the area of granulation tissue formation and the epithelialization rate on days 7, 10, and 14 in the experimental groups compared to the control groups. Hence, these properties can accelerate wound healing.

Kiran et al. evaluated Sesamum indicum L. seed and sebum on wound healing activity in rats and demonstrated that this plant, due to coumarin content as one of the most effective ingredients, had better repairing effects on increasing granulation tissue and wound closure and also significantly reduced the epithelialization period compared to treated groups with Aloe vera as a standard control.

Ilango et al. carried out a study on wound healing. They found that the fruit pulp of Limonia acidissima L. (Rutaceae) had anti-oxidant activities in rats due to the content of flavonoids, tannins, glycosides, coumarin, and saponins. They demonstrated a significant increase in the number of fibroblasts, collagenization, granulation tissue weight, wound contraction, wound breaking strength, and decreasing of epithelialization period in the experimental group compared with the control group.

The Plantago lanceolata leaf ointment has been reported to be effective for enhanced repair in burn-wound by increasing the angiogenesis and granulation tissue formation as well as reducing the epithelialization period due to the content of specific compounds such as coumarin, glycoside, and phenol.

Kim et al. explored the anti-inflammatory and ulcerative healing effects of Stellera chamaejasme L. This herb is used as a medicinal plant in China. Most of its compounds include flavonoids, lichen, and coumarin. This herbal extract was proved to affect the acceleration of wound healing process via increasing the expression of TGF-β1.

One of the most important components of the granulation tissue is fibroblasts which are involved in the production of collagen and ECM of granulation tissue. We also focused on these cells and manifested that the number of fibroblast cells...
on days 7, 10, and 14 increased considerably in the two coumarin experimental groups compared to the control groups. Concentrations 2% of this compound demonstrated better effects in comparison to two different percentages of coumarin.

There is also limited evidence to confirm the positive effects of coumarin-based herbal extracts on fibroblast cells and collagen fibers’ synthesis. Lee et al. conducted a study to promote the synthesis of collagen fibers using the root of Angelica dahurica, showing that the three types of coumarin derivatives isolated from this plant stimulated collagen biosynthesis in human fibroblasts. Shiravi et al. investigated the effect of henna leaves’ extract (Lawsonia inermis) on skin wound healing and revealed that the extract of this plant with chemical compounds such as multiple phenolic glucosides, coumarin, beta-cytosterol, and alkaloids, as the medicinal plant parts, could produce more collagen fibers, higher density of fibroblasts, and blood vessels and could also reduce healing periods.

Some studies also point to the mechanism of possible effects of coumarin on the number of fibroblasts. Martino et al. evaluated the effect of Hibiscus syriacus extract (rich in flavonoids and coumarins) on skin wound healing and demonstrated that this extract could increase the closure rate of wound area via increase of neoeipidermis and stimulation of the expression of wound-accelerated markers (TGFβ). Moreover, this extract increased the expression of genes involved in hydration, homeostasis, and production of collagen and also increased keratinocytes and fibroblasts in the cell group treated with this extract.

Research on coumarin confirms that this substance causes vasodilatation and increases blood supply, which affects the applied capacity of fibroblasts and increases collagenization.

CONCLUSION

The results of this study confirmed that the coumarin compound plant has a highly positive effect on different stages of wound healing processes, including inflammatory and proliferative phases. The evidence indicates that coumarin would be a good candidate for wound healing. Hence, more supplementary studies are suggested to investigate the mechanism of coumarin effect during the process of skin wound healing.

Acknowledgment

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Conflict of interest: None declared.

REFERENCES

Efficacy of coumarin on wound healing


Assessment of serum vitamin D level in patients with cutaneous warts: a case-control study

Background: The use of vitamin D and its analogues in the treatment of warts has been to be effective and painless without any considerable side effects, unlike some other routine safe and effective therapeutic modalities such as cryotherapy that in some cases is really difficult to perform due to related pain, especially in the children. Owing to a probable relationship between the deficiency of serum vitamin D and warts, this research aimed to find the association between level of serum vitamin D and warts.

Methods: This case-control study was conducted on 56 subjects in Rasoul Akram Hospital, Tehran, Iran, for 18 months. The subjects were randomly divided into case and control groups, including 28 age and sex-matched subjects in each of them. The required data were extracted through a questionnaire. Finally, the data were analyzed in the SPSS software V.22 using tests such as Mann-Whitney and Pearson correlation.

Results: In this study, 56 people were examined, and 28 subjects were included in both case and control groups. The mean serum level of vitamin D in both case and control groups was 23.564 and 31.593, respectively. The association between serum levels of vitamin D in the group with and without wart was not significant in both men and female groups. Serum vitamin D levels were compared in two groups of under 18 years old and between 18 and 50 years old, which were not statistically significant.

Conclusion: According to our results, there is no significant relationship between serum vitamin D levels and existence of warts. This relationship is not statistically significant at the level of age and sex variables, and there is no difference in age and gender between the case and control groups.

Keywords: wart, human papillomavirus, vitamin D, gender, age

INTRODUCTION

Warts are common skin lesions, which are solid, skin-colored or gray-brown bumps with a rough surface and a few millimeters of thickness. They can be single or multiple. In addition, if they join, they can make plaque and regular shapes on a rough surface. Some kinds of them are all over the body and have a larger size and amount than those of normal warts. They are usually painless. If they become dense or exist around the joints and nails, they can be painful. Sometimes, their surface is covered with full of fissures; therefore, it can cause pain.

Formation of warts is the most common effect of human papillomavirus (HPV). HPV is a small duplex virus having more than 100 types. It causes warts after infection of squamous epithelial
cells and results in DNA replication inside them.

The tumor is made by polymorphic viruses and can be created in various parts of the body, such as the skin of the hands and feet, the mucosa and the skin of the genital, larynx and oral mucosa. However, virus replication occurs in areas where keratinocytes are quite distinct, such as the spinosum, granular and basal layer of squamous epithelium. First, it targets these cells and replicates there in a hidden form. With this replication, it can cause hyperplasia and hyperkeratosis all over the body.

No specific information is available on the incidence of non-genital warts, but studies have revealed that it is more prevalent in children and adolescents. In 2013, one study in the United States reported a prevalence of wart among person from birth to 17; the highest one was 6.8% in children aged 9 to 11.

Additionally, two population-based studies reported the prevalence of wart to be 84.1% in the United States and 12.9% in Russia. Other studies reported the prevalence of 12% in children aged 6-4 years in the United Kingdom, and 24% in adolescents aged 18-16 in Australia.

No proven treatment exists for wart diseases. Most HPV infections can be treated with special tools having direct destructive effects on lesions, including cryotherapy. All of these requested methods are used to treat warts, but they are painful, expensive and time-consuming, and none of them is the gold standard. One of the newest methods is the use of vitamin D. Several studies have investigated the association between vitamin D deficiency and bacterial and viral infectious diseases. The effect of vitamin D on the treatment of infectious diseases was also studied. In 2011, a successful treatment of warts by topical active vitamin D in a patient with a transplanted kidney was reported. In another case report, a successful treatment of anogenital warts was reported in a newborn with topical active vitamin D.

In one study on 21 patients with warts, intrallesional injection of vitamin D eventually caused 81% of patients to show complete disappearance of warts. For this reason, intrallesional injection of vitamin D was proposed as an alternative treatment modality. Intrallesional injection of an immunotherapy agent into warts can stimulate the immune system against HPV cells. Vitamin D regulates the proliferation and differentiation of epidermal cells. It has a receptor on melanocytes, keratinocytes, fibroblasts and immune cells. Therefore, it enhances cytokine production. In addition, El-Taweel et al. showed that Vitamin D could be successfully used in topical and injectable forms.

According to various studies, the use of vitamin D and its analogues in the treatment of warts has been effective, but treatments like cryotherapy are difficult to perform. In addition, considering the higher prevalence of skin warts in children and the pain concerns of treatments in this age group, finding a safe and non-invasive therapy is necessary. Therefore, due to a probable relationship between the deficiency of serum vitamin D and warts, we aimed to design a case control study to investigate the association between the level of serum vitamin D and warts; so if it is found any relationship between vitamin D level deficiencies and wart, hypothetically correction for a normal range and/or use of vitamin D analogues could be a potential protective strategy or adjuvant therapy for warts. To conduct this study precisely, and owing to the importance of demographic variables, we examine this relationship in different sexes and age groups.

**MATERIALS AND METHODS**

**Participants and study design**

This case-control study was conducted on 56 subjects in Rasoul Akram Hospital, Tehran, Iran, for 18 months. This study had case and control groups, including 28 subjects in each of them. Furthermore, the participants were age-matched in two groups (under 18 years old as children and 18-50 years old as adults). According to gender, they were also matched in both case and control groups. The case group was selected from patients with wart referred to the Dermatology Clinic of the hospital, based on clinical examinations and diagnostic tests of warts. Moreover, the control group was randomly selected from staffs of the hospital. Inclusion criteria were the age, gender, diet, physical activity and sun exposure in the normal range of the patients.

**Data collection methods**

After finalizing the subjects, based on these variables, the required data were extracted through a questionnaire. It contains some questions about...
age, sex, number of warts, involvement area, amount of daily activity and outside exercise, diet, usage of food supplements, sun exposure and vitamin D intake. In addition, the patient was requested to test vitamin D levels. The hospital’s laboratory measured this amount using the ELISA technique. In the process of blood sampling, 5 cc blood was taken from the patients. This process is performed in accordance with the principles of safety and health. After bleeding, the patient is placed in a sitting position for 5 to 10 minutes, and then left with a good general condition. Finally, laboratory reports were recorded on a checklist.

**Ethical considerations**

In all steps taken in this study, the principles of the Declaration of Helsinki (ethical principles for medical research involving human subjects) and Iran University of Medical Sciences Ethics Committee were followed (IR.IUMS.FMD.REC 1396.8921215072). The study was approved by Iran University’s Ethics Committee. The process of blood sampling from the patients was performed with the written informed consent.

**Statistical methods**

The data were entered into the statistical analysis software, SPSS, version 22, and were then statistically analyzed. The results for the quantitative variables were reported in Mean ± SD format, and the ordinal qualitative variables were reported in percentages. The Mann-Whitney U test was used.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Frequency</th>
<th>Percentage</th>
<th>P-Value</th>
</tr>
</thead>
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<td>Case=15</td>
<td>53.6</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control=12</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Case=13</td>
<td>46.6</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control=16</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td>Case</td>
<td>Less than 18=12</td>
<td>42.9</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18-50=16</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Less than 18=12</td>
<td>42.9</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18-50=16</td>
<td>57.1</td>
<td></td>
</tr>
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<td>0.001</td>
</tr>
<tr>
<td></td>
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<td>71.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
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<td>21.4</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>High</td>
<td>3</td>
<td>10.7</td>
</tr>
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<td>Diet with dairy products</td>
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<td>0.001</td>
</tr>
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<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>2</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Low</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium</td>
<td>28</td>
<td>89.3</td>
</tr>
<tr>
<td>Sunscreen use</td>
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<td>78.6</td>
<td>0.221</td>
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<tr>
<td></td>
<td>No</td>
<td>6</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
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<td>60.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>11</td>
<td>39.3</td>
</tr>
<tr>
<td>Sun exposure</td>
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<td>0.001</td>
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<tr>
<td></td>
<td>Medium</td>
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<td>60.7</td>
<td></td>
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<td>4</td>
<td>14.3</td>
<td></td>
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<td>Low</td>
<td>4</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium</td>
<td>24</td>
<td>85.7</td>
</tr>
<tr>
<td>Wart</td>
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<td>20</td>
<td>71.4</td>
<td>0.696</td>
</tr>
<tr>
<td></td>
<td>More than 5</td>
<td>8</td>
<td>28.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Demographic data of participants (Case: patients with wart, Control: matched individuals without wart).
RESULTS

In this study, 56 people were examined, and 28 subjects were included in both case and control groups. The age of the participants was in the range of 5 to 46. The mean age in the case and control groups was 24.67 ± 13.61 and 25.36 ± 13.59, respectively. The patients were age-matched in the two groups, under 18 with 12 participants and 18-50 years old with 16. All demographic information for both case and control groups are summarized in Table 1. Furthermore, the mean serum level of vitamin D in both case and control groups was 23.564 and 31.593, respectively (Table 2). The serum level of vitamin D in the case group does not follow the normal curve in our study (Figure 1).

The amount of vitamin D levels was divided into three levels, which the highest percentage was approximately the insufficient level with 53.6% and 46.4% in both case and control groups, respectively (Table 3).

Furthermore, the number of warts in the case group was determined in two levels. First, one was less than five with 71.4% in 20 individuals, and the second one was above five with 28.6% in eight individuals. Additionally, the association between serum vitamin D level and warts was not statistically significant (P = 0.696).

Daily activity in both case and control groups was the highest in the middle level with 71.4% and 89.3%, respectively. According to the uses of sunscreen, 21.4% in the case group and 39.3% in the control group use it. Other information collected is sun exposure, divided into three levels: low, moderate, and high. In both case and control groups, the average level was 60.7% and 85.7%, respectively.

The dairy diet is also ranked again at three levels: low, middle and high. In the case group, it was 75% at the middle level, and in the control group, the highest was 89.3% again in the middle group.

Furthermore, the rate of involvement in different parts of the body was examined. Hands had
the highest percentage with 39.3% involvement (Figure 2). The serum level of vitamin D was determined by gender in patients with warts (case group). The average in both male and female groups was 22.307 and 25.015, respectively (Table 4). Then, the serum level of vitamin D in the groups with and without warts were compared to each other, and the association was not significant in both male and female groups with \( P = 0.435 \) and with \( P = 0.726 \), respectively (Table 5). In both case and control groups, the association between serum vitamin D level and age was measured, which was not statistically significant in both groups \( (P = 0.658, P = 0.107, \text{respectively}) \) (Table 6).

**Table 3.** Vitamin D levels status in the case and control groups.

<table>
<thead>
<tr>
<th>VitD_Range</th>
<th>Case and Control</th>
<th>Vit D level</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Valid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>&lt;10</td>
<td>6</td>
<td>21.4</td>
<td>21.4</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>10&lt;D&lt;30</td>
<td>15</td>
<td>53.6</td>
<td>53.6</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>Sufficient</td>
<td>D&gt;30</td>
<td>7</td>
<td>25.0</td>
<td>25.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>28</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Valid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>&lt;10</td>
<td>5</td>
<td>17.9</td>
<td>17.9</td>
<td>17.9</td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>10&lt;D&lt;30</td>
<td>13</td>
<td>46.4</td>
<td>46.4</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td>Sufficient</td>
<td>D&gt;30</td>
<td>10</td>
<td>35.7</td>
<td>35.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>28</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.** Serum level of vitamin D in case and control groups in terms of gender.

<table>
<thead>
<tr>
<th>Group/Gender</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>Variance</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22.307</td>
<td>16.499</td>
<td>272.246</td>
<td>18.60</td>
<td>2.7</td>
<td>67</td>
</tr>
<tr>
<td>Female</td>
<td>25.015</td>
<td>13.892</td>
<td>193.006</td>
<td>22.90</td>
<td>5.4</td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27.433</td>
<td>19.53</td>
<td>381.446</td>
<td>21.65</td>
<td>8.9</td>
<td>77</td>
</tr>
<tr>
<td>Female</td>
<td>34.712</td>
<td>28.40</td>
<td>806.789</td>
<td>22.65</td>
<td>7.3</td>
<td>90</td>
</tr>
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</table>

**Table 5.** Mean serum levels of vitamin D with respect to gender in cases with and without warts.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Group (Case and Control)</th>
<th>N</th>
<th>Mean rank</th>
<th>Sum of ranks</th>
<th>( P )-value</th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td>Vit. D</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>15</td>
<td>12.93</td>
<td>194.00</td>
<td>0.435</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12</td>
<td>15.33</td>
<td>184.00</td>
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<td></td>
<td>Total</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Vit. D</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>13</td>
<td>14.38</td>
<td>187.00</td>
<td>0.726</td>
</tr>
<tr>
<td></td>
<td>Control</td>
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<td>15.50</td>
<td>248.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Number, N; vitamin, Vit
In the case group, serum vitamin D levels were compared in two groups of under 18 years old and between 18 and 50 years old, which were not statistically significant ($P = 0.164$). In addition, this comparison was made in the control group, which did not show a significant correlation ($P = 0.246$).

**DISCUSSION**

Aktas et al. conducted a study to evaluate the effect of vitamin D injection on the treatment of warts. The results of their study showed that injections of vitamin D into the lesion could be effective in the treatment of warts 9. Moscarelli et al. reported a case study in order to determine the successful treatment of warts via local vitamin D activation in a patient with a kidney transplant. Eventually, they observed that active vitamin D had a significant effect on the treatment 15. Furthermore, El-Taweel’s recently published paper states that vitamin D has been successfully used in locally injectable forms 14.

According to the results of our study, the mean serum level of vitamin D in both case and control groups was 23, and about 50% of the subjects in both groups had insufficient levels. This deficiency of vitamin D and the development of warts show the importance of this issue.

In this regard, the results of a study by Liu et al. showed that vitamin D regulates some endogenous antimicrobial peptides in immune cells. This action leads to the potential role of vitamin D in regulating immune responses to various infectious diseases 16.

Additionally, Monto et al. in their study mentioned the hypothesis. They showed that the normal level of vitamin D in the host body contributed to the regulatory functions of the immune system against developing viral respiratory infections. These functions work through suppression of excessive responses to cytokines and increase the purification of various microbial species from the body 17. These studies demonstrate the importance of vitamin D in preventing warts and are aligned with our study.

In addition, the number of warts in the case group was determined at two levels, which were lower than 5% with 71.4% and higher than 5% with 28. In 2013, one study in the United States reported a prevalence of warts among person with age from birth to 17. The highest one was 6.8% in children aged 9 to 11 6. Moreover, two population-based studies showed that the prevalence of wart was 84.1% and 12.9% in the United States and Russia, respectively. Other studies reported the prevalence of 12% in children aged 6-4 years in the United Kingdom and 24% in adolescents aged 18-16 in Australia 5. These results are slightly different from our study results due to differences in samples and populations.

According to the results of our study, approximately 70% of the subjects had moderate levels of sun exposure. Paskit et al., from 1974 to 1977, showed that exposure to sunlight has a significant effect on the level of vitamin D. They also found that in the winter, levels of this vitamin were more dependent on receiving the light than nutrition 13. Cannele et al. concluded in their study that vitamin D deficiency could cause flu and respiratory infections in the winter due to lack of ultraviolet light 18. Being consistent with our results, the results of these studies demonstrate the importance of exposure to the sun to provide adequate levels of vitamin D.

In our study, the amount of using sunscreen in both case and control groups was 21.4% and 39.3%, respectively. Russell et al. showed a correlation between vitamin D levels and skin moisture. According to this correlation, low level of vitamin D can decrease the average amount of skin moisture. Topical use of cholecalciferol improves skin moisture and improves the clinical grading of dry skin 19.

In addition, the rate of involvement in different

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**Table 6. Vitamin D level status in case and control groups according to age groups (children and adults).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Less than 18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between 18 to 50</td>
<td></td>
</tr>
<tr>
<td>Case (P=0.658)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit. D range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Insufficient</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Sufficient</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Control (P=0.107)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit. D range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Insufficient</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Sufficient</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>16</td>
</tr>
</tbody>
</table>
parts of the body was examined. Hands had the highest percentage. This indicates that some organs are more exposed to sunlight. Therefore, they have higher risk of developing warts, and it is better to use sunscreen for these places.

The serum levels of vitamin D in the groups with and without wart were compared to each other, which were not statistically significant in both genders. This issue suggests that the examination and follow-up of warts should be noticed in both genders with the same importance. Elaine et al. conducted a study in different age and sex groups. They showed that genital wart reductions were associated with the use of human papillomavirus vaccine. Furthermore, the prevalence of warts decreased over the years, and this drop occurred in females with different ages and in males aged 20 to 24 years old.

Moreover, the comparison of vitamin D in both groups was made based on age, and no significant relationship was found between them in both groups. This result suggests that warts may occur at any age. As humans grow up, more precise criteria are needed to determine the exact causes of warts. Raghukumar et al. conducted a study aiming at assessing the safety and efficacy of intracellular vitamin D in the treatment of warts. It is concluded that intravenous vitamin D is effective in the treatment of warts; however, aging can reduce the effect. Contrary to our results, this study showed that aging was clearly an obstacle to the recovery of warts with vitamin D.

CONCLUSION

Consequently, despite the positive effect of vitamin D analogues on the treatment of warts, according to our results, there is no significant relationship between serum vitamin D levels and warts. This relationship is not statistically significant at the level of age and sex variables, and there is no difference in age and gender between the case and control groups.

Further studies in this field may also consider BMI to match the case and control groups to each other. It should also be noted that choosing control group from a larger population with various diets, in contrary to this research’s second age group of controls, may lead to more realistic results. Both of these proposals were the limitations of this research, which can be resolved in future studies.

Acknowledgements

We would like to show our gratitude to Rasoul Akram Clinical Research Development Center (RCRDC) for its technical and editorial assists.

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REFERENCES

Serum vitamin D level in patients with cutaneous warts


The efficacy of IPL and ammonium lactate 14% versus ammonium lactate alone in the treatment of keratosis pilaris

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INTRODUCTION

Keratosis rubra pilaris is a common condition with an estimated percentage of 40% involvement in the population. This condition is inherited as an autosomal dominant trait and more commonly involves patients with dry skin. Positive family history is observed in 67% of the patients. In this condition, the infundibulum of the hair follicles is filled with hard keratin ball. The lesions of the keratosis pilaris are worsened during the winter and tend to be improved during warm weather of the summer.

Different types of treatment have been suggested for this condition such as use of mild cleansers like baby and glycerinated soaps, use of emollients, and keratolytics, including urea, salicylic acids and lactic acids.

Ammonium lactate is a compound of lactic acid and ammonium hydroxide. Lactic acid is one of the alpha hydroxy acids that can remove stratum corneum from the epidermis. This compound has

Background: Keratosis rubra pilaris is a common condition with an estimated percentage of 40% involvement in the population. This condition is inherited as an autosomal dominant trait and more commonly involves patients with dry skin. In the current study, we evaluated the efficacy of intense pulsed light (IPL) plus ammonium lactate versus ammonium lactate alone in the treatment of keratosis pilaris.

Methods: A total of 50 patches were selected in 10 patients. If there were two similar patches with the same color and same location, each of them was randomized to receive ammonium lactate (Kerapil cream®) or ammonium lactate (Kerapil cream®) +IPL. Treatment was performed for 3 months, and improvement was rated by physicians and patients one month and two months after the intervention.

Results: The grade of improvement in the IPL + ammonium lactate was not significantly higher than ammonium lactate alone group as rated by blinded physicians at week 4 (P > 0.05). However, the score of improvement was also higher, as rated by the patients, in the IPL + ammonium lactate versus ammonium lactate alone (P < 0.05) at week 8.

Conclusion: Our results demonstrated that addition of IPL to ammonium lactate could improve the clinical response of keratosis pilaris lesions.

Keywords: Ammonium lactate, intense pulsed light therapy, keratosis pilaris

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high moisturizing effect and is commonly used in conditions such as dermatitis, pruritus and inflammation, and some inherited diseases like ichthyosis.

Intense pulse light (IPL) is a light source emitting non-coherent light with the wave lengths of 500–1200 nanometers (nm) and pulses with different duration and different energies. Intense pulse light has been used for the treatment of different dermatologic conditions such as acne, melasma, telangiectasia, and sebaceous hyperplasia.

In the current study, we evaluated the efficacy of IPL plus ammonium lactate versus ammonium lactate alone in the treatment of keratosis pilaris.

**MATERIALS AND METHODS**

This was a randomized, non-blinded clinical trial performed at Isfahan University of Medical Sciences and Skin Diseases and Leishmaniasis Research Center clinics from 2016 to 2017 (Research no: IRCT2016061428448N1). The ethical committee clearance and informed consent were achieved. All of the selected patients had a history of keratosis pilaris. All of the selected patients were female, and only patients who were older than 5 years old were recruited. The lesions were randomized to receive ammonium lactate (Kerapil cream®, Noreva Laboratories, France) or IPL + ammonium lactate (Kerapil cream®) using the simple randomization method.

Exclusion criteria included history of sensitivity to ammonium lactate, pregnancy, lactation, aggravation of the lesion after ammonium lactate or IPL treatment, history of skin cancer or connective tissue disorder and non-compliance of the patients for treatment. A total of 50 patches of keratosis pilaris were enrolled in our study. The patches were located on extensor arms, lateral thighs and buttocks. The mean of age was similar in the two groups and was 25.6 as the patches of both cases and controls were selected in the same patients.

Cooling using ice pack was performed between the passes to prevent possible burning. For IPL, Solari Device (Lutronic company), with the contact cooling temperature of −5°C was used.

Standard digital photograph was taken at baseline, 4 weeks and 8 weeks after the last treatment. Two dermatologists who did not perform the laser procedure evaluated the response through images. Global improvement score (according to keratotic papules, roughness, hypopigmentation, and erythema) was evaluated using the grading system for improvement: Grade 1, 1–25% improvement (minimal); grade 2, 26–50% improvement (moderate); grade 3, 51–75% improvement (good); grade 4, > 75% improvement (excellent).

Patient satisfaction was assessed at the end of the study (8th week of the follow-up). They were requested to rate the improvements of their lesions according to grading scores (0: unsatisfied; 1: poor; 2: fair; 3, satisfied; 4, extremely satisfied).

**Statistical methods**

Fisher exact test was used to compare continuous data between two dependent samples. All analyses were conducted using STATA version 13 (Stata Corp., College Station, Texas, USA). A p-value of 0.05 or less was considered statistically significant.

**RESULTS**

A total of 10 healthy female patients with 50 patches of keratosis pilaris were enrolled in our study. The patches were located on extensor arms, lateral thighs and buttocks. The mean of age was similar in the two groups and was 25.6 as the patches of both cases and controls were selected in the same patients.

The grade of improvement in the IPL + ammonium lactate group (group B) was not significantly higher than that in the ammonium lactate alone group (group A) as rated by blinded physicians at week 4 (P = 0.07). However, the score of improvement was also higher as rated by the patients, in the IPL + ammonium lactate versus ammonium lactate alone at week 8 (P = 0.04; Table 1). All patients rated improvement of their lesions according to the patients' satisfaction score (Table 2, Figure 1).
DISCUSSION

Keratosis rubra pilaris may cause a sense of cosmetic disfigurement for patients; therefore, many different modalities of treatments have been suggested for these patients. Rodríguez-Lojo et al. evaluated the efficacy of IPL in four female patients with keratosis pilaris atrophicans using 570-nm filter. Additionally, 75% and 100% reductions in the severity of erythema were observed in all patients with no side effect and no recurrence after 10 months of follow up; reduction of roughness was also obtained in the patients. The results of this study were consistent with the results of our study and confirmed the efficacy of IPL in reduction of erythema and roughness of keratosis pilaris lesions; however, our selected cases were affected by keratosis rubra pilaris and not by keratosis pilaris atrophicans.

One recent study showed successful treatment of keratosis pilaris rubra with pulsed dye laser.

Table 1. Global assessment of keratosis rubra pilaris improvement in IPL+ammonium lactate group (group B) versus ammonium lactate alone (group A) as rated by blinded dermatologists.

<table>
<thead>
<tr>
<th>Grading</th>
<th>4 weeks of follow-up n = 50</th>
<th>8 weeks of follow-up n = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>1 (&lt;25% improvement, minimal)</td>
<td>15 (60.0)</td>
<td>7 (28.0)</td>
</tr>
<tr>
<td>2 (26–50% improvement, moderate)</td>
<td>6 (24.0)</td>
<td>9 (36.0)</td>
</tr>
<tr>
<td>3 (51–75% improvement, good)</td>
<td>4 (16.0)</td>
<td>9 (36.0)</td>
</tr>
<tr>
<td>4 (&gt;75% improvement, excellent)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25 (100)</td>
<td>25 (100)</td>
</tr>
</tbody>
</table>

P-value 8 weeks = 0.040 < 0.050; P-value 4 weeks = 0.07 > 0.05. Number, n.

Table 2. Patients’ satisfaction in the IPL + ammonium lactate group (Group B) versus ammonium lactate alone (group A) at 8-weeks follow-up.

<table>
<thead>
<tr>
<th>Patients’ grading</th>
<th>Group A n = 25</th>
<th>Group B n = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (unsatisfied)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 (poor)</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>2 (fair)</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>3 (satisfied)</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>4 (extremely satisfied)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Number, n.

Figure 1. Clinical improvement in a patient in the IPL and ammonium lactate group at baseline (a), week 4 (b), and week 8 (c).
IPL and ammonium lactate in the treatment of keratosis pilaris

(PDL). In this study, eight patients were treated with PDL for keratosis pilaris rubra, and all of them showed noticeable improvement after one to four treatments. The authors concluded that PDL was effective, easily accessible, and underused therapy in the treatment of keratosis pilaris rubra. The results of this study were also consistent with the results of the current study; however, in our study, we used IPL instead of PDL that may provide less expensive treatment as compared to PDL. Furthermore, to achieve maximum efficacy in reduction of roughness, we used ammonium lactate cream.

Vachiramon et al. used fractional CO2 laser for the treatment of adult patients with keratosis pilaris. In this study, fractional CO2 laser was performed to one side of the arm, and the contralateral arm served as control, and the patients were followed for 3 months. The results demonstrated that 30% of lesions on the laser-treated side had moderate to good improvement, and keratotic papules and hyperpigmentation responded better than the erythematous component. However, four patients with Fitzpatrick skin type V developed transient pigmentary alteration. In the current study, we used IPL instead of fractional CO2 laser having less down time and may be more effective for the erythematous component of keratosis pilaris. As CO2 fractional laser was more effective on keratotic papules, we suggest that in another study, the efficacy of IPL plus CO2 fractional laser is evaluated for patients with keratosis pilaris.

The efficacy of diode laser was also assessed in the treatment of keratosis pilaris. Eighteen patients were randomized to receive 2 passes of laser treatment on the right or left arm for 3 treatments. Significant improvements in skin texture and roughness/bumpiness in keratosis pilaris patients were observed, but baseline erythema was not improved. We recommend that the efficacy of IPL plus diode laser be evaluated for patients with keratosis pilaris.

CONCLUSION

Overall, regarding the results of our study, we suggest that combination use of the IPL plus keratolytic cream or some methods of treatment such as lasers and microdermabrasion could be effective for patients with keratosis rubra pilaris. It is recommended that further studies be conducted with a larger number of patients and a longer follow-up period to better evaluate the efficacy of IPL in the treatment of keratosis rubra pilaris.

Conflict of interest: None declared.

REFERENCES

INTRODUCTION

Pyogenic granuloma (PG) is a benign vascular tumor, which can present on the skin or mucous membranes. The name is a misnomer, since it does not have an infective etiology nor does it contain granulation tissue. PG grows rapidly to form a red papule or exophytic nodule which has a tendency to bleed profusely on minor trauma. The surface of the lesion is susceptible to bleeding and ulceration. The exact etiology of PG is unknown. It is believed that an unknown stimulus triggers endothelial proliferation and angiogenesis, leading to rapid growth. Various treatment modalities are available, however, none of them is effective. Sclerotherapy is defined as the targeted elimination of small vessels, varicose veins and vascular anomalies by the injection of a sclerosant. Since PG involves the proliferation and growth of blood vessels, sclerotherapy was tried as a treatment modality. Accordingly, we report a case of PG occurring on the scalp in a child successfully treated with sclerotherapy.

CASE PRESENTATION

A 12-year-old boy presented to the outpatient department with chief complaint of a proliferating lesion over the scalp since 3 months. At the beginning, it started as a small reddish nodule, which progressed rapidly to the present size. It was associated with bleeding on trivial trauma. The bleeding was profuse and stopped only after applying firm pressure for several minutes. There was no history of trauma prior to the onset of the lesion. No history of similar lesions in the past or elsewhere in the body was noted. The patient had not taken treatment elsewhere.

On examination, a single exophytic growth measuring approximately 4 × 4 cm was present over the right temporal scalp. The lesion was pinkish red in color with soft and edematous consistency. Surrounding skin was normal, and the lesion was not attached to the underlying bone (Figure 1).

Based on the history and clinical examination, a provisional diagnosis of pyogenic granuloma was made. The patient was treated with cryotherapy for three sittings; however, there was minimal
improvement.

Regarding the vascular nature of the lesion, sclerotherapy with sodium tetradecyl sulfate was considered a treatment modality. Informed consent was obtained. The area was cleaned with antiseptic. The procedure was carried out without any local anesthesia. The sclerosant used was 3% sodium tetradecyl sulfate diluted with sterile water in a ratio of 1:4 and injected into the lesion with the help of an insulin syringe. It was given until there was visible blanching of the lesion (Figure 2). Thereafter, pressure was applied with gauze for 3-4 minutes. Minimal bleeding was noted during the procedure. The patient tolerated the procedure well. The injection was repeated at 2 weekly intervals for 4 sittings. The lesion significantly decreased in size at the end of 2 months (Figure 3). No complication was noted after the procedure. Electrocautery was performed to remove the shriveled PG lesion (Figure 4). No recurrence of the lesion was seen even at 6-month follow-up.

DISCUSSION

Poncet and Dor for the first time described
pyogenic granuloma (PG) in 1897. It was earlier known as ‘botryomycosis hominis’. Hartzell, in 1904, coined the term ‘pyogenic granuloma’ (PG) or ‘granuloma pyogenicum’; it is also known as Crocker and Hartzell’s disease, lobular capillary hemangioma, and granuloma gravidarum (GG) in pregnant women.

Pyogenic granuloma has a worldwide distribution. Although, they occur in any age group, they are more prevalent in children and adolescents. It accounts for 0.5% of all childhood skin nodules. The mean age of onset is 6.7 years in children; with 42% of cases occurring by 5 years of age.

A higher frequency is seen in the second and third decade of life, with a peak prevalence in women. The commonest locations are the lips, tongue, buccal mucosa, gingiva, and palate. Extraoral sites include the skin of the upper and lower extremities, scalp, face, mucous membrane of the nose, eyelids, and genitalia.

Clinically, it begins as a red solitary lesion, which grows rapidly over weeks to months and then stabilizes in size. It can be pedunculated or sessile and rarely exceeds 1 cm in diameter. PG can present on the skin or mucosal surface and has a characteristic acanthotic base known as the “epithelial collarette.” The lesion is painless, but it can grow rapidly and can bleed on minor trauma.

Although the diagnosis is basically clinical, some differential diagnoses include keratoacanthoma and other epithelial neoplasms, inflamed seborrhoeic keratoses, melanocytic naevi, melanoma, Spitz nevi, viral warts, molluscum contagiosum, angioma, glomus tumour, eccrine poroma, Kaposi sarcoma and metastatic carcinoma. On histopathological examination, lobular proliferation of small vessels is seen erupting through a break in the epidermis to produce a globular pedunculated tumor. Various treatment modalities include curettage and cautery, coagulation of the base with diathermy, excision and sutures, cryotherapy with liquid nitrogen or nitrous oxide and lasers. Although sclerotherapy is not a very popular procedure to treat PG, this procedure has been tried with success regarding the vascular nature of the lesion.

The commonly used sclerosants are polidocanol and sodium tetradecyl sulfate owing to their efficacy and safety profile. Some of the complications of sclerotherapy include hyperpigmentation, transient burning pain, telangiectatic matting, cutaneous necrosis, ulceration, thrombophlebitis, pulmonary embolism or inadvertent arterial injection, which are mostly seen with varicose veins and haemorrhoids. Although sclerotherapy is a relatively safe procedure, Nicolau syndrome is a potentially catastrophic complication.

CONCLUSION

The authors found that sclerotherapy was a novel method to treat pyogenic granuloma and was relatively safe. The technique is painless, bloodless, and economical, requires minimal surgical expertise and can be done as an outpatient procedure. There is no need for local anaesthesia or any dressings after operation. It can be used as an alternative to conventional methods such as surgery, lasers, radiofrequency ablation and electrodessication. The method can be employed in the treatment of PG in children, patients with comorbidities, and as an initial treatment for large PG in order to shrink the size of the tumor. Hence, we conclude that sclerotherapy may be tried as treatment modality.
in patients with pyogenic granuloma more so in the pediatric population.

Conflict of Interest: None declared.

REFERENCES


Sclerotherapy in treatment of pyogenic granuloma
Porokeratotic eccrine ostial and dermal duct nevus with extensive linear distribution: a case report

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INTRODUCTION

Porokeratotic eccrine ostial and dermal duct nevus (PEODDN) is an extremely rare disorder of keratinization involving acrosyringium and hair follicles. It is presented clinically by discrete keratotic papules on palms, soles or distal extremities arranged in a linear pattern along Blaschko lines. The lesions resemble comedones but are found at locations free of pilosebaceous follicles. PEODDN is present at birth in most cases, but they may appear during adulthood 1,2. Histopathologically, it is characterized by cornoid lamella, a column of parakeratotic cells over an epidermal invagination that is almost always located overlying an eccrine duct with a dilated acrosyringium 3. Considering that PEODDN is a very rare skin condition with less than 50 cases reported previously in the literature, we present a case with this disorder with an unusual presentation of extensive PEODDN lesions along the lower extremity 4.

CASE PRESENTATION

A 25-year-old young woman presented with multiple keratotic papules on her left sole. In
continuity with the lesions on her sole, a linear plaque following Blaschko lines was extended up to her buttock. The lesions were present since her birth and were asymptomatic. The patient had consulted to a dermatologist at the age of 9, and the mid-portion of the linear plaque on her calf and lower thigh had been resected. Nevertheless, the remaining lesions were stable until our first visit. In her clinical examination, multiple keratotic papules were present on her left sole, and a linear verrucous plaque following Blaschko’s line was distributed over her left leg and her left buttock resembling linear verrucous epidermal nevus. The linear plaque was interrupted on her lower thigh and popliteal fossa due to the past resection (Figures 1a-c). Our patient was otherwise healthy with no significant past medical history; the physical examination and routine lab data were also normal. Two incisional biopsies were taken from her skin lesions. Linear verrucous epidermal nevus, linear porokeratosis, linear lichen planus, linear psoriasis and linear Darier’s disease were considered as primary clinical differential diagnoses. Histopathology showed hyperkeratosis and acanthosis with cornoid lamella overlying the acrosyringium with dyskeratotic cells in upper epidermis under the porokeratotic column (Figures 2a, b). Regarding both clinical and histopathological findings, PEODDN was determined as the proper diagnosis. The patient was referred to the laser clinic for ablative erbium laser therapy.

**DISCUSSION**

PEODDN is an extremely rare dermatological disorder. Its pathogenesis is unknown, but some studies regarded genetic mosaicism and abnormal keratinization as the possible mechanisms of the disease. Recently, it has been proposed that PEODDN is probably a mosaic form of Keratitis ichthyosis deafness (KID) syndrome. The mutant gene in KID syndrome, GJB2, encodes connexin 26, which is a gap junction protein. The condition is usually present at birth, but cases of PEODDN presenting during adulthood have been reported. It is commonly presented clinically as keratotic papules on palms and soles, but when other sites are involved, keratotic plaques are reminiscent of linear verrucous epidermal nevus, as they were seen in our patient. Furthermore, cutaneous lesions of PEODDN extended to the proximal leg in our patient, which is a rare presentation in this disease.

Although PEODDN is considered a benign congenital hamartoma, an association between PEODDN and a variety of disorders, such as Bowen’s disease, squamous cell carcinoma, sensory polyneuropathy, deafness and hyperthyroidism, have been reported. Histopathologically, PEODDN is identified by cornoid lamella appeared over acrosyringia. The treatment options are ablative methods, particularly laser therapy, and for localized lesions, excision by surgery is
recommended. Laser therapy with ultrapulse CO2 laser and erbium/CO2 laser has been reported as a successful treatment.

CONCLUSION

In the present report, the rarity of PEODDN and interesting presentation of the lesions on the sole of our patient with extension in a linear Blaschko pattern to the leg, thigh and buttock resembling verrucous linear epidermal nevus, promoted us to present this case.

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Conflict of interest None declared.

REFERENCES

Post dengue hair loss: low prevalence in Thai cases

Dear Editor,

Dengue is a very common tropical infection in tropical Asia. The disease can cause acute febrile illness with hemorrhagic complications. The post dengue problem is also observable, and hair loss is an important disorder. In a report from Norway, 45% of the dengue patients had hair loss after infection. In another report from Taiwan, the same percentage, 45%, of patients were noted with post dengue hair loss. Here, the authors attempted to summarize the magnitude of the problem among Thai patients. Data on 1-month post dengue follow-up on 143 dengue patients in our medical center were analyzed, and it was found that the post dengue hair loss was observed in only 7 cases giving the rate equal to 4.9%. For a longer follow-up, in 3 months, 6 months, 12 months and 15 months, there is no other additional case presented with the problem of post dengue hair loss. Interestingly, the observed rate in Thailand where the disease is highly endemic is significantly lower than those reported in patients in non-endemic areas. The exact underlying factors contributing to the difference are unknown, and this is an interesting issue for further study. The heterogeneity of dengue epidemiology is also an important consideration. To ensure these results, newer studies conducted in different regions of the world should be evaluated.

Conflict of interest: None declared.

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REFERENCES