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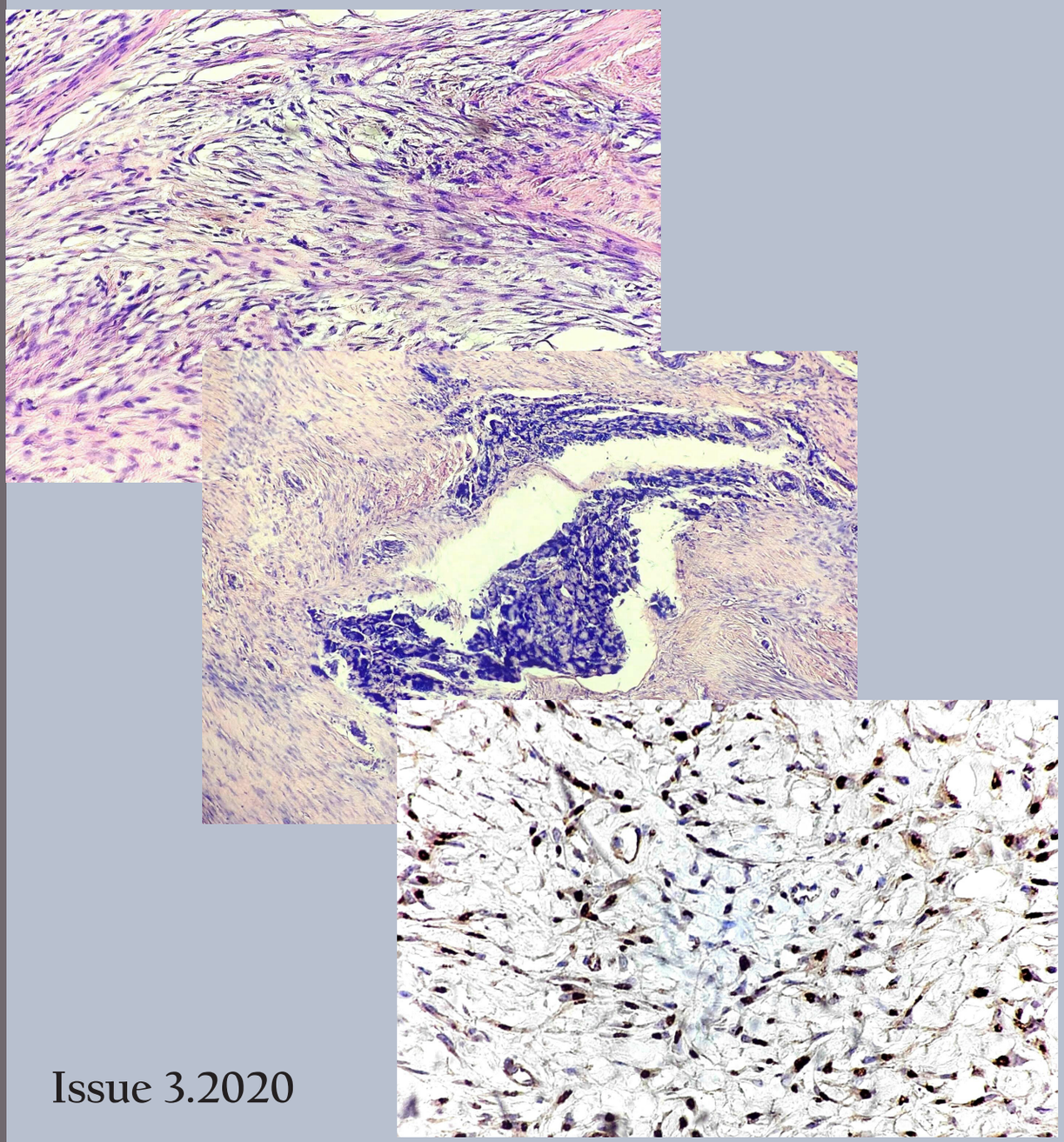

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Original Article

Factors related to response to propranolol treatment for infantile hemangiomas: a cross-sectional study on a Moroccan population

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ABSTRACT

Background: There is no consensus on the optimal duration of propranolol treatment in complicated infantile hemangiomas (IHs), and factors related to its response have not yet been addressed, especially as it relates to the northern Moroccan region. **Aim:** We analyze the factors of good response of IH to propranolol treatment in a Moroccan population; thus, this study is the first to consider an undeveloped country. **Methods:** A descriptive and analytic study was conducted for 9 years in our department. The following parameters were analyzed: epidemiologic, therapeutic, and progressive, as well as the factors responsible for therapeutic response. **Results:** Propranolol treatment for 153 cases of IH was completed. With an average age of 7.9 months, a tuberous form was found in 50.3% of the cases, with 58.1% located on the face. Side effects were minor, and response was good to excellent in 95.6% of the cases. In a univariate analysis, children over 12 months and those with mixed hemangioma, as compared with those with a tuberous form, were less likely to exhibit an excellent response (OR = 0.18 with a 95% CI = 0.03–0.68; and OR = 0.80 with a 95% CI = 0.68–0.94). Excellent response was more prevalent in children treated for more than 6 months (47.8% vs. 11.8%; $p < 0.001$). **Conclusion:** Our study proves the safety and efficacity of propranolol as a treatment of IH. Excellent response was very much correlated with age, clinical form of IH, and the duration of treatment.

Key words: Infantile hemangioma; Propranolol; Factors; Morocco

INTRODUCTION

Infantile hemangiomas (IHs) are vascular tumors that undergo a proliferative phase followed by stabilization and involution [1]. Approximately 10% of IHs require intervention during the proliferative phase since they can grow dramatically or can often locate on the face, which may lead to functional impairment, cosmetical disfiguration, and complications such as ulceration, bleeding, and infection [1,2]. Oral propranolol (OP) is an effective treatment for complicated IHs [1]. Its dosage, effectiveness, and adverse effects, as well as monitoring of treatment, have been widely described [3-7]. There is no consensus on the optimal duration of propranolol treatment for infantile hemangiomas, and factors related to response to propranolol treatment in infantile hemangiomas have not yet been addressed, especially in the northern Moroccan region [3]. The purpose of this study was to evaluate the efficacy and safety of an oral propranolol solution administered for high-risk IHs in a Moroccan population of the minimum age of 6 months up to the maximum age of 24 months in order to analyze factors related to the response of propranolol treatment.

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MATERIAL AND METHODS

Study Population and Data Collection

A prospective observational study was conducted from September 2008 to September 2017 by the dermatological and pediatric services at Hospital Center University Hassan II, located in Fez, Morocco. Data was collected from 153 patients treated for hemangioma with propranolol. The criteria of inclusion were an age of less than 2, hemangiomas with functional risks, namely, orificial locations, hemangiomas complicated by bleeding or ulceration, and those larger than 25 cm2 in nonrisk locations. The criteria of exclusion were medical contraindication to beta-blockers and hemangiomas smaller than 25 cm2 in nonrisk locations. All patients had been receiving short-stay hospitalization where their clinical data and pretreatment assessments were collected. Propranolol treatment was initiated under 24-hour monitoring at 1 mg/kg/day for 1 week. Then, patients underwent evaluation and the dose was increased to 3 mg/kg/day split into 2 doses. The treatment continued until the growth of hemangiomas stopped. Follow-ups were scheduled for once a month for the first year and once every 3 months for the second year. The following parameters were analyzed: epidemiologic, therapeutic, and progressive, as well as the factors of good and bad therapeutic response. Treatment was stopped at a time determined primarily by the lesion regression rate in the reduction of the size and coloration of hemangiomas. Therapeutic evaluation was based on a score [8] ranging from 0 to 3. A score of 3 was an excellent response, if regression was above 75% without any scars; a score of 2 was a good response, if regression ranged between 50% and 75% with or without scars; a score of 1 was a poor response, if regression ranged between 25% and 50% with or without scars; and a score of 0 was no response, if regression was below 25% or no regression was observed. Informed parental consent was systematically collected.

Statistical Analysis

A descriptive analysis of variables was conducted. Categorical variables were summarized by frequencies, and continuous variables were summarized by means and standard deviations.

A univariate and multivariate analysis classified children according to their treatment response: excellent vs. good. The variable of interest was dichotomic and the event was to have an excellent response. The 4 children who had a poor response to treatment or none were excluded from the analysis. Fisher's test and simple logistic regression were used to investigate the association between treatment response and each of the following factors: gender, age, the size and number of hemangiomas, the clinical form of the hemangioma, the presence of ulceration, the progression of hemangiomas, the age at the onset of propranolol treatment, and the duration of the treatment. These factors were selected according to medical knowledge and previous findings [9,10]. Age was included in the analysis as a categorical variable (≤12 months and >12 months).

In multiple logistic regression, association with excellent responses was tested after adjusting for potential confounders and factors cited above (Table 1). Adjusted odds ratios with 95% confidence intervals were provided for each variable. All statistical analyses were performed in the software R, version 3.5.1 (R Core Team (2018). R Foundation for Statistical Computing, Vienna, Austria).

Ethics Statement

Ethical approval was obtained from the ethics committees at Hospital Center University Hassan II in Fez, Morocco. All subjects were informed of the conditions related to the study and gave their written informed consent.

RESULTS

Clinical and Therapeutic Characteristics

Among the 153 children studied and treated for infantile hemangioma with propranolol, 118 (77.1%) were female, and the average age was 8 months (±7.9). Prematurity was reported in 7 (4.5%) children, and congenital malformations in 5 (3.2%) children (Table 2). The tuberous form was the most common (50%), followed by the mixed form (42.4%). More than half (58.1%) of hemangiomas were located on the face, with 3.9% segmental facial hemangiomas; other locations were mostly those of the trunk (5.8%), inguinal areas (5.8%), and head (3.9%). The size of hemangiomas was less than 25 cm2 in 65.3% of cases, and only 9.1% of the patients had more than 3 hemangiomas. As for the other criteria, 22.8% of hemangiomas were in progression, 16.3% displayed ulcerations, while superinfection was reported in 4 (2.6%) cases. More details are described in Table 3.

Table 1: Factors related to an excellent response to propranolol treatment (excellent vs. good). Moroccan study on the factors of response to propranolol treatment for infantile hemangioma. Hospital Center University Hassan II in Fez, Morocco. 2008-2017. N = 149

Factors		Excellent response n(%)	*Univariate analysis**		*Multivariate analysis***	
			Crude OR# (CI95%)##	*p* value	Adjusted OR# (CI 95%)##	*p* value
Gender						
	Male	15(45.4)	1		1	
	Female	44(37.9)	0.73 (0.31-1.73)	0.54	0.78 (0.31-1.92)	0.59
Age						
	≤ 12 months	56 (44.4)	1		1	
	> 12 months	3(13.0)	0.18 (0.03-0.68)	**0.004**+	0.14 (0.02-0.58)	**0.01**+
Size						
	≤ 25 mm	40(41.7)	1		1	
	> 25 mm	19(35.8)	0.78 (0.36-1.64)	0.59	0.85(0.35-2.05)	0.72
Clinical form						
	Tuberous	38 (50.0)	1		1	
	Subcutaneous	3 (30.0)	0.81 (0.59-1.12)	0.21	0.28 (0.05-1.23)	0.10
	Mixed	18 (28.6)	0.80 (0.68-0.94)	**0.01**+	0.43 (0.17-1.05)	0.06
Presence of ulceration						
	No	46 (37.1)	1		1	
	Yes	13 (52.0)	1.82 (0.70-4.80)	0.18	1.14 (0.39-3.26)	0.80
Hemangioma in progression						
	No	45 (39.1)	1		1	
	Yes	14 (41.2)	1.08 (0.45-2.53)	0.84	1.52 (0.57-4.10)	0.39
Number of Hemangioma						
	< 3	55 (40.1)	1		1	
	≥ 3	4 (33.3)	0.74 (0.15-2.95)	0.76	0.43 (0.09-1.73)	0.24
Age at the start of treatment						
	≤ 4 months	27 (40.9)	1		1	
	> 4 months	32 (38.5)	0.90 (0.44-1.85)	0.86	1.46(0.66-3.27)	0.34
Treatment duration						
	≤ 6 months	4 (11.8)	1		1	
	> 6 months	55 (47.8)	6.80 (2.19-28.24)	**<0.001**+	4.77 (1.59-17.77)	**0.009**+

*Fisher test or simple logistic regression. **Multiple logistic regression. #Odds ratio. ##Confidence interval. +a *p* value of less than 0.05 is considered significant

Table 2: Clinical characteristics of the patients. Moroccan study on the factors of response to propranolol treatment for infantile hemangioma. Hospital Center University Hassan II in Fez, Morocco. 2008-2017. N =153

Characteristics	Categories	n(%)
Gender		
	Males	35(22.8)
	females	118(77.1)
Age (months)		8.02(7.9)*
Prematurity		7(4.5)
Congenital malformations		5(3.2)

*Mean (standard deviation)

Propranolol treatment was administered below the age of 4 months for 69 (45.1%) children. A periorificial location was a therapeutic indication in 33.3% of cases. More than two thirds (77.7%) were treated for more than 6 months. The median duration of treatment was 15 months. Response to treatment was good (Fig. 1) to excellent in 95.6% of patients (Fig. 2), poor in one patient (0.6%) (Fig. 3), and no response to treatment was attained in only 3 patients (Fig. 4) (Table 4). No relapses were observed after the end of the treatment.

Factors Related to Excellent Responses to Propranolol Treatment

In a univariate analysis, excellent responses to propranolol treatment were correlated considerably with age, the clinical form of hemangioma, and the duration of treatment. Children of over 12 months and those with mixed forms of hemangioma, unlike those with tuberous forms, had a lesser chance of attaining an excellent response to propranolol treatment (OR = 0.18 with a 95% CI = 0.03–0.68; and OR = 0.80 with 95% CI = 0.68–0.94). Excellent responses were more prevalent among children treated for more than 6 months (47.8% as opposed to 11.8%; $p < 0.001$) (Table 1).

After adjusting for confounding factors, association with excellent responses, age, and the duration of treatment declined but remained significant ($p = 0.01$ and $p = 0.009$, respectively). On the contrary, association with mixed forms disappeared ($p = 0.06$). The size and number of hemangiomas, the progression

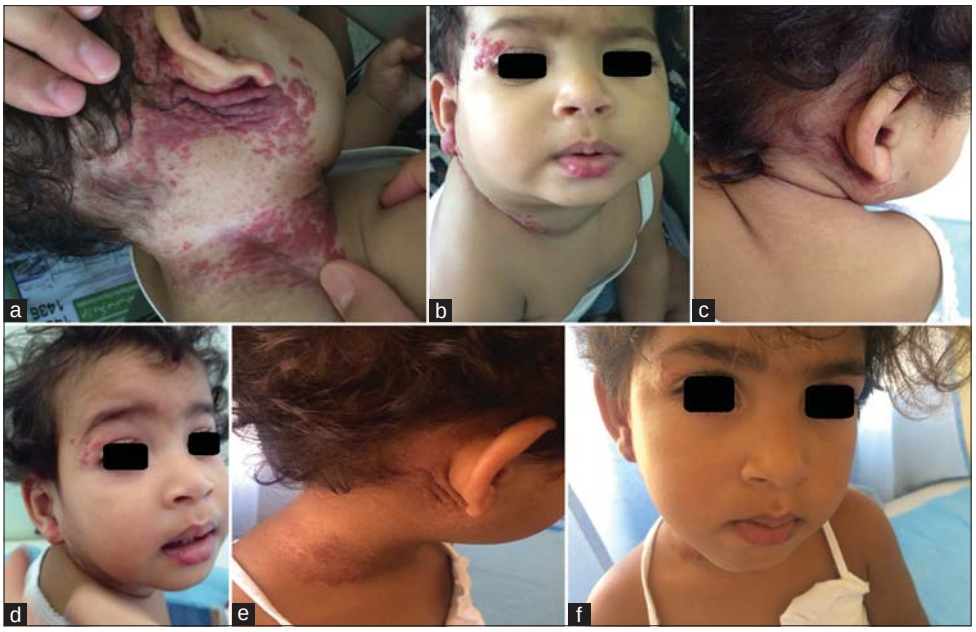

Figure 1: (a, b) Segmental facial hemangioma in a 6-month-old girl prior to propranolol treatment. (c, d) Good response at the age of 12 months after 6 months of propranolol treatment. (e, f) Good response at the age of 18 months after 12 months of propranolol treatment.

Table 3: Characteristics of hemangiomas. Moroccan study on the factors of response to propranolol treatment for infantile hemangioma. Hospital Center University Hassan II in Fez, Morocco. 2008-2017. N =153

Characteristics	Categories	n (%)
Clinical form		
	Tuberous	77(50.3)
	Subcutaneous	11(7.1)
	Mixed	65(42.4)
Site		
	Face	89(58.1)
	Head	6(3.9)
	Neck	3(1.9)
	Nose	1(0.6)
	Lips	2(1.3)
	Trunk	9(5.8)
	Arm	5(3.2)
	Inguinal	9(5.8)
	Buttock	6(3.9)
	Penis	3(1.9)
	Breast	5(3.2)
	Eyelid	2(1.3)
	Multiple sites	13(8.5)
Hemangioma size		
	≤ 25 mm	100(65.3)
	> 25 mm	53(34.6)
Number of hemangioma		
	< 3	139(90.8)
	≥ 3	14(9.1)
Beard hemangioma		1(0.6)
Hemangioma in progression		35(22.8)
Presence of necrosis		2(1.3)
Presence of superinfection		4(2.6)
Presence of ulceration		25(16.3)
Presence of extension		2(1.3)
Presence of bleeding		4(2.6)

Table 4: Therapeutic characteristics of the patients. Moroccan study on the factors of response to propranolol treatment for infantile hemangioma. Hospital Center University Hassan II in Fez, Morocco. 2008-2017. N =153

Characteristics	Categories	n (%)
Age at the start of treatment		
	≤4 months	69(45.1)
	>4 months	84(54.9)
Therapeutic indications		
	Peri-orificiel site	51(33.3)
	Anogenital	3(1.9)
	Breast	7(4.5)
	Miliary hemangioma	3(1.9)
	Scalp	1(0.6)
	Other indications	88(57.5)
Response to treatment		
	Excellent	59(38.5)
	Good	90(58.8)
	Poor	1(0.6)
	Absent	3(1.9)
Treatment duration		
	≤ 6 months	34(22.2)
	> 6 months	119(77.7)
Side effects	Major	0
	Minor (somnolence, and cold hands and feet)	12

of hemangiomas, the presence of ulceration, and the age at the onset of treatment were not significantly associated with treatment response (Table 1).

DISCUSSION

Approximately 10% of IHs require treatment during the proliferative phase due to local complications, a

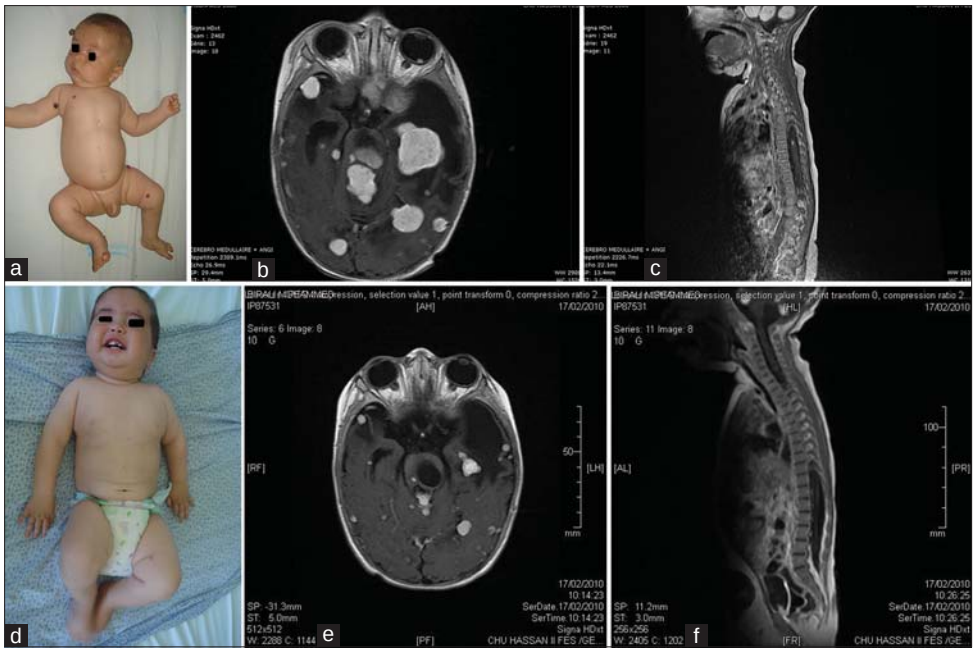

Figure 2: (a) Multifocal infantile hemangioma or hemangiomatosis in a (b) cerebral and (c) spinal location prior to propranolol treatment in a 2-month-old boy. (d) Excellent therapeutic response after 10 months clinically and (e, f) radiologically after 2 months of propranolol treatment.

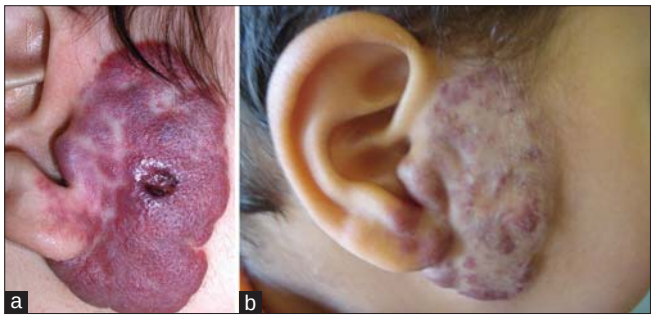

Figure 3: (a) A periauricular and ulcerated hemangioma in a 5-month-old girl prior to propranolol treatment. (b) Poor response at the age of 12 months after 7 months of propranolol treatment.

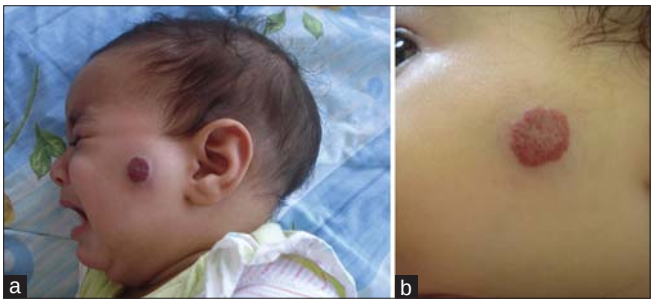

Figure 4: (a) A mixed hemangioma of the cheek in a 6-month-old girl before propranolol treatment. (b) No response after 12 months of propranolol treatment.

life-threatening location, cosmetic issues, or functional risks [3-7]. In 2008, Léauté-Labrèze et al. reported an incidental finding that propranolol could control dramatically the growth of IH. Subsequent studies were conducted and demonstrated that the drug was well tolerated and produced excellent results [6,10] The exact mechanism of oral propranolol treatment for IH has not been elucidated [1,3,4]. There are several proposed hypotheses, including vasoconstriction, decreased renin production, inhibition of angiogenesis, and stimulation of apoptosis. The author concluded that increased apoptosis during the second year of life can offset cellular proliferation and may be involved in initiating the regression of hemangiomas [5,11,12].

This study demonstrated that the efficacy of oral propranolol at 3 mg/kg/day for >6 months in IH Moroccan patients gave good or excellent responses even if the age at the onset of treatment was, in some cases, high. Furthermore, the treatment was deemed relatively safe because almost no side effects were observed in younger patients [6,9]. Indeed, the authors concluded that the use of oral propranolol is well tolerated, effective, and safe in the treatment of IH during the postproliferative phase [4,6,7]. According to the literature, the duration of treatment with systemic propranolol ranges from 1 to 16 months [3,11,12]. In our study, the duration of oral propranolol IH treatment introduced after the proliferative phase ranged from 6 to 24 months, with 15 months as a median duration of treatment. Although propranolol is well tolerated and is rarely associated with adverse reactions [1,3], our study, nonetheless, produced minor side effects, such as somnolence and cold hands and feet. As for criteria such as gender, location, and

clinical form, numerous publications give similar reports [1,3,11,13]. Propranolol withdrawal produced no relapses. The explanation given is the duration of treatment, which was longer and appropriate for the evolution of IH [14,15]. Factors related to excellent responses to propranolol treatment were age, clinical forms of hemangioma, and the duration of treatment. These factors were demonstrated, in some studies, to be factors of relapse [1,3,4,13]. In fact, it is known that therapeutic responses in tuberous forms are more favorable than in mixed and subcutaneous forms. Introducing treatment during the first year of life is a hugely important factor as it coincides with the proliferative phase [3,4,13]. The fact that this phase varies in length between tuberous forms and subcutaneous forms [16-18] could explain the good response of treatment despite a late introduction of treatment, but with a longer duration, that is, for up to 6 months. In our study, late introduction of treatment is explained, on the one hand, by the delay in specialized consultation and, on the other hand, by the little knowledge of therapeutic opportunities of some clinicians. Additionally, the size, number, and progression of hemangiomas, the presence of ulceration, and the age at the onset of treatment were not significantly associated with treatment response. Indeed, in nearly all publications, it is not the size that influences response, but composition and quantity [16-18]. As for ulceration and hemangioma progression, during the first 3–5 months, superficial IH proliferates rapidly. In most cases (80%), the growth is complete by 5 months of age [1,4,9]. In contrast with superficial lesions, deep IH often lags in growth by approximately 1 month. These lesions proliferate for one additional month on average. Despite these well-defined parameters, IHs are very heterogeneous during the early proliferative phase and the growth characteristics of individual IHs can be difficult to predict. By 9 months of age, lesions begin to regress by 10% annually until in complete or partial subsidence [16-18]. Tumor tissue is replaced by fat and fiber components, and the tumoral skin often loosens or scars. In our study, propranolol was 95.6% effective for IH treatment during the proliferative and postproliferative phases. The first results appeared within hours of initiating treatment, and changes in lesion color and softening were observed [1,17,18]. Such involution was not associated with the location of hemangiomas or the age at the onset of treatment. The majority of authors agree that an acceptable response is expected if treatment is introduced within the first 12 months of life. As recently reported by Chang LC et al., most hemangiomas cease growing after 9 months of age. However, there was a small subgroup of hemangiomas with continued growth after this period [12]. Our study was limited to a Morocco population and, as such, represents research in a previously poorly represented country.

CONCLUSION

Although the exact mechanism causing the beneficial effects of propranolol in the proliferative and postproliferative phases are unclear, a better understanding of the mechanism of propranolol-induced regression of IH may allow for more effective treatment. Moreover, an administration of oral propranolol 3 mg/kg/day is safe and effective for IH during the proliferative and postproliferative phases and should be considered a first-line treatment in Moroccan populations. Factors related to excellent responses to propranolol treatment were associated significantly with the age of ≤12 months, tuberous forms, and the duration of hemangioma treatment. Additionally, the optimal duration of treatment should end no sooner than with the regression of IH, that is, more than 6 months since its introduction. Finally, it has to be noted that Morocco is a country unrepresented in these sorts of studies and that genetic factors may also play a role in these treatment mechanisms.

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Statement of Human and Animal Rights

All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 2008 revision of the Declaration of Helsinki of 1975..

Statement of Informed Consent

Informed consent for participation in this study was obtained from all patients.

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Original Article

Normal progressive developmental stages of the pilosebaceous unit in the albino rat's skin - A light microscopic study

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ABSTRACT

Background: Basic histological knowledge of the different components of the pilosebaceous unit is important in the pathologic interpretation of hair disorders. **Aim and Objectives:** Due to paucity of detailed microscopic features and photomicrographs of the pilosebaceous unit (PSU) at one place, this study aimed to review the normal developmental stages and provide microscopic features, along with the photomicrographs of different components of the pilosebaceous unit that could prove useful in certain hair conditions and therapeutic procedures. **Materials and Methods:** Tissue samples were obtained from the site of experimentally induced incisional and excisional skin wounds in such a way that parts of normal skin around these healing skin wounds were included. These samples were processed for routine paraffin serial sectioning. These sections were stained with routine and special stains. Histological features were recorded under different magnification of the Trinocular microscope. **Study Limitation:** The study presented is a part of another research study related to skin wound healing in adult albino rats. **Results:** The general structure of the pilosebaceous unit was shown by routine Haematoxylin and Eosin staining. While the presence and distribution of collagen fibres were demonstrated by Masson's Trichrome and PicroSirius Red with Fast Green staining, type III collagen (reticular) fibres were observed in PicroSirius Red with Silver Nitrate staining and that of elastin fibres were demarcated by Aldehyde Fuchsin with Fast Green staining method. **Conclusion:** In certain conditions and procedures, such as hair disorders, hair transplantation, and follicular drug delivery, where detailed histology of the pilosebaceous unit is required, the present study wherein many special stains were used could prove very useful for quick and easy identification of different types of cells and fibres associated with the pilosebaceous unit.

Key words: Arrector pili muscle; Hair; Hair follicle; Pilosebaceous unit; Sebaceous gland

INTRODUCTION

The pilosebaceous unit (PSU) is a complex structure within the skin that is responsible for hair growth and lubrication of the epithelium [1]. The hair follicle (HF) generates hair and is a reservoir of multipotent stem cells, having the self-renewing capacity during hair cycles, also plays an important role in thermoregulation, physical and immunological protection [2]. Hair follicles also play a vital role in the repair of skin wounds by providing new epithelial cells [3-5]. A complex interaction and communication are known to occur between the epidermis and dermis during embryogenesis [6]. Pilosebaceous units are also known to contain some drugs targeting sites as well. Therefore, this study aims at focusing on normal cyclic changes of follicle formation and microscopic features of different components of the PSU through routine and special stains, to help better understand and plan certain conditions and procedures such as hair transplantation, detection of hair disorders and follicular drug delivery.

MATERIALS AND METHODS

For the study of progressive stages of development in the pilosebaceous unit, skin tissue samples were secured from the sites of experimentally induced incisional and excisional skin wounds in such a way that parts of

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normal skin surrounding these healing wounds were included. These samples were a part of a research study that included a total of forty eight adult albino rats of either sex, each weighing 230-320g, and were performed in the Department of Anatomy, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Surgical Procedures for the Collection of Skin Samples

Horizontal full-thickness incision was made at 2.95 ± 0.17cm in length on the shaved right mid-thigh region. This skin incision was re-approximated with 3-0 Vicryl (2metric–NW2401) absorbable sterilized surgical needled suture USP (synthetic; braided coated polyglactin 910 violet; from Ethicon, manufactured in India by Johnson and Johnson Ltd, Aurangabad). This re-approximated full-thickness incisional skin wound was used for the study of incisional (primary intention) skin wound healing [7]. Dorsal surface of the thoracic region for full-thickness excisional skin wound of 8.5 ± 0.48 mm diameter (an area equivalent to 46.74 ± 0.32 mm^2) on pinched skin fold for the study of excisional (secondary intention) skin wound healing [8].

The skin samples were processed for paraffin sectioning. 5µm thick sections were stained with Haematoxylin and Eosin (H & E), Masson's Trichrome (MT), PicroSirius Red with Fast Green (PSRFG), PicroSirius Red with Silver Nitrate (PSRSN), Periodic Acid Schiff with Haematoxylin (PASH), Verhoeff Van Gieson (VVG) and Aldehyde Fuchsin with Fast Green (AFFG). Histological features under x10, x20, x40, and x100 objective lenses of Trinocular microscope (Olympus, BX40; Japan) were recorded by a digital camera (Sony 18.2 MP, Japan).

Study Limitation

The present study is a part of another research study related to skin wound healing in adult albino rats.

Ethics Statement

The principal study was conducted in the Department of Anatomy, approved by the Institutional Animal Ethical Committee (No. 8937/2014), Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. The animal groups, surgical procedures, and tissue samples for the present study remained the same as those of the principal study.

RESULTS

The general structure of the pilosebaceous unit was shown by routine Haematoxylin and Eosin staining. While the presence and distribution of collagen fibres were demonstrated by Masson's Trichrome and PicroSirius Red with Fast Green staining, type III collagen (reticular) fibres were observed in PicroSirius Red with Silver Nitrate staining and that of elastin fibres were demarcated by Aldehyde Fuchsin with Fast Green staining method. All features of the pilosebaceous unit and its developing stages were depicted by the following figures (Fig. 1 – 11).

Hair placode, Hair germ or follicular bud, Hair peg and Bulbous peg (Fig. 1); Dermal papilla, different components of a hair shaft, bulb and bulge (Fig. 2); Pilosebaceous unit or the follicular unit and Arrector pili muscle (Fig. 3); Segments of the hair follicle (Fig. 4); Perifollicular dermal sheath consists of type III collagen (reticular), elastin fibres (Fig. 5); Components of the hair follicle and hair shaft (Fig. 6); Collagen fibres in the connective tissue sheath (Fig. 7); Elastin fibres in the connective tissue sheath (Fig. 8); Arrector pili muscle (Fig. 9); Progression of growth of the pilosebaceous unit (Fig. 10); Features of the Sebaceous gland (Fig. 11).

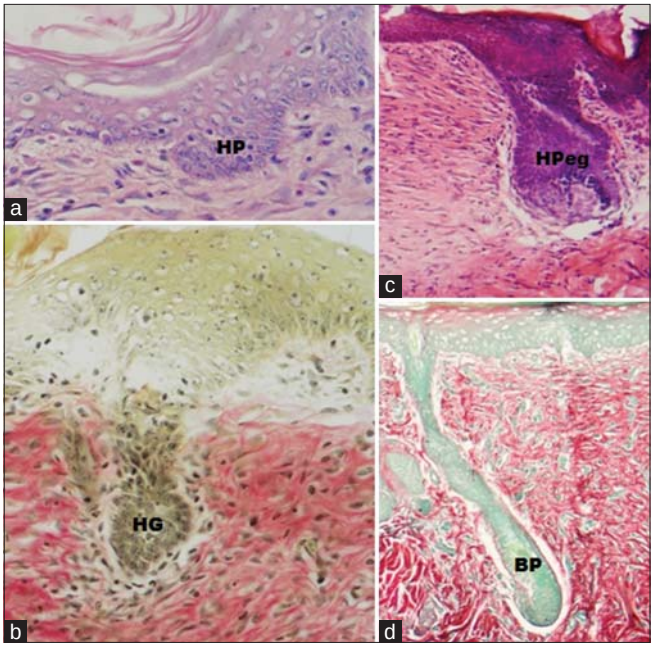

Figure 1: Different developmental stages of hair follicles. a- HP: Hair Placode, Stain: H & E, Magnification x40; b- HG: Hair Germ, Stain: VVG, Magnification x40; c- HPeg: Hair Peg, Stain: H & E, Magnification x20; d- BP: Bulbous Peg, Stain: PSRFG, Magnification x20.

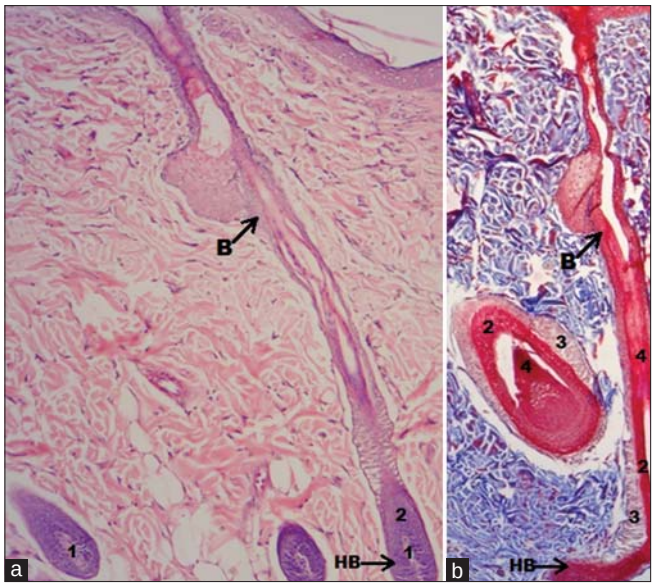

Figure 2: Hair follicle. B→: Bulge, HB: Hair Bulb, 1: Dermal papilla, 2: Inner root Sheath, 3: Outer root sheath, 4: Hair shaft. a- Stain: H & E, Magnification: x20; b- Stain: MT, Magnification x20.

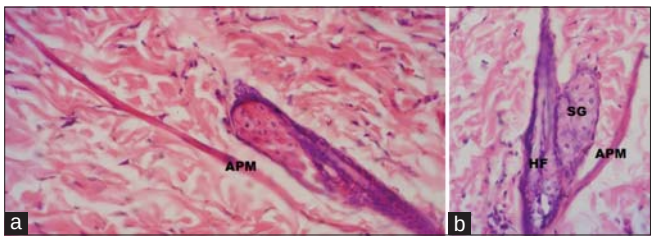

Figure 3: PiloSebaceous Unit (PSU). APM: Arrector Pili Muscle, SG: Sebaceous Gland, HF: Hair Follicle. (a and b)- Stain: H & E, Magnification: x40.

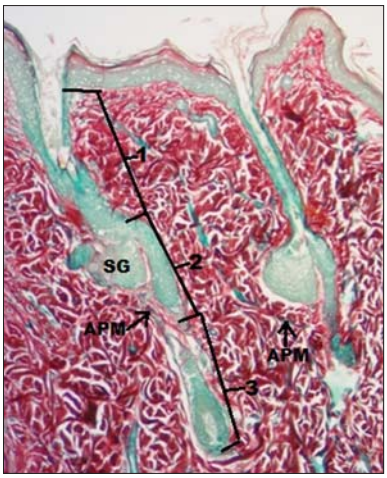

Figure 4: Parts of Hair follicle.1: Infundibulum, 2: Isthmus, 3: Inferior segments, SG: Sebaceous Gland, APM: Arrector Pili Muscle. Stain: PSRFG, Magnification: x20.

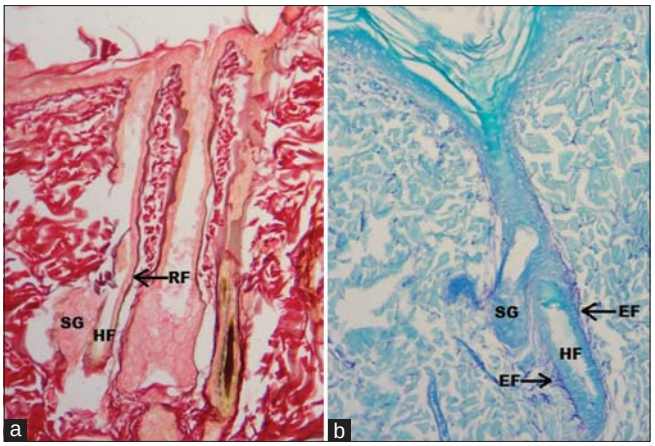

Figure 5: Fibres around the hair follicle. RF: Reticular fibres (Black Colour), SG- Sebaceous Gland, HF- Hair Follicle, EF: Elastin Fibres (Violet Colour). a- Stain: PSRSN, Magnification: x20; b-Stain: AFFG, Magnification x20.

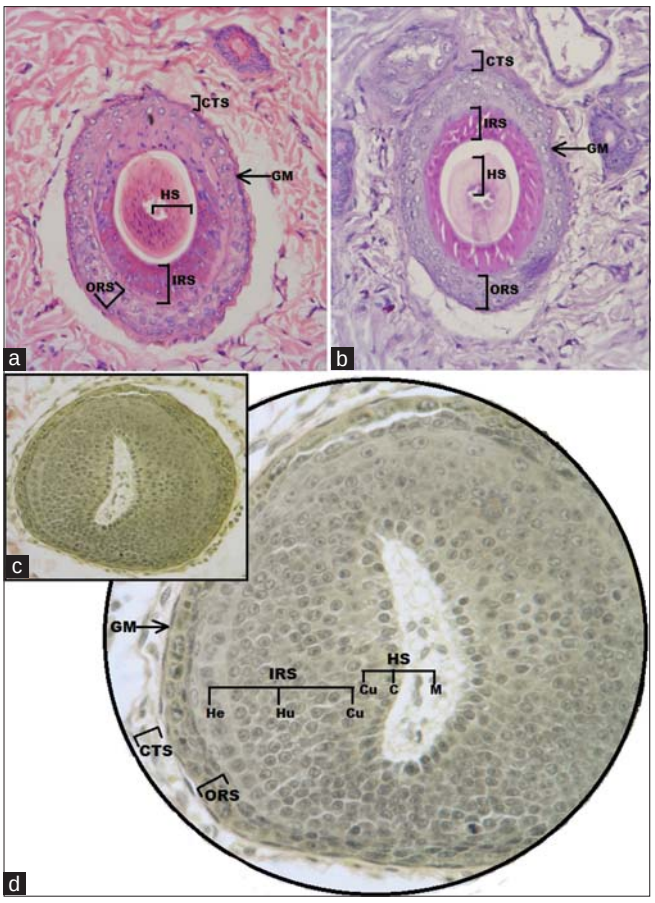

Figure 6: Cross-sections of the hair follicle. CTS: Connective Tissue Sheath, ORS: Outer Root Sheath, IRS: Inner Root Sheath, HS: Hair Shaft, GM: Glassy Membrane. d: IRS- He: Henle's layer, Hu: Huxley's layer, Cu: Cuticle; HS-M: Medulla, C: Cortex, Cu: Cuticle. a- Stain: H & E, Magnification: x40; b- Stain: PASH, Magnification x40; c- Stain: VVG, Magnification: x40; d- Stain: VVG, Magnification: x100.

DISCUSSION

Hair follicle (HF) morphogenesis and cycling occur as a result of interactions between epithelial and mesenchymal cells [9]. Hair follicle (HF) morphogenesis and cycling occur as a result of interactions between epithelial and mesenchymal cells [9]. In the embryonic

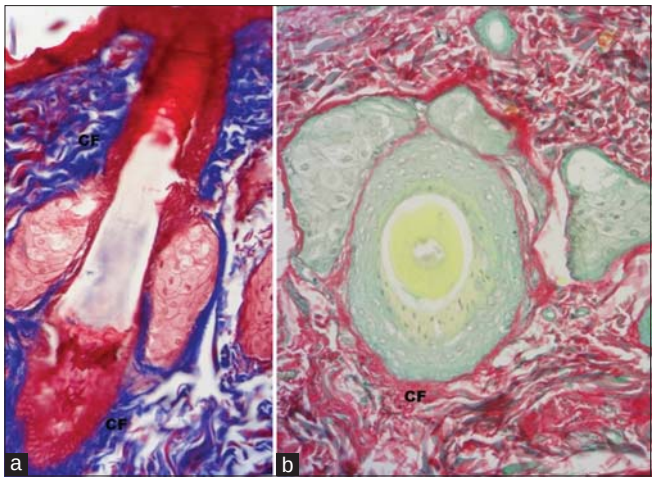

Figure 7: a- Longitudinal section of the hair follicle; CF: Collagen Fibres (Blue Colour); Stain: MT, Magnification: x40. b: Cross-section of hair follicle; CF: Collagen Fibres (Red Colour); Stain: PSRFG, Magnification: x40.

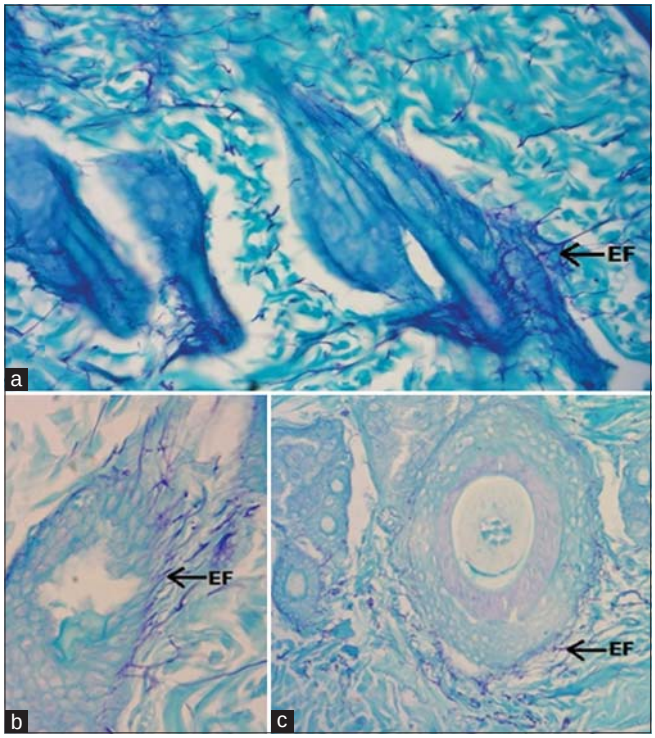

Figure 8: EF: Elastin Fibres (Violet Colour). (a and b): Longitudinal section of hair follicle, a-Magnification: x40; b- Magnification: x100. c- Cross-section of the hair follicle, Magnification: x40. Stain: AFFG

murine model study [10], the hair follicle development is divided into three stages; they are hair placode formation or induction, hair follicle organogenesis and cytodifferentiation. In this present study, all three stages of hair follicle morphogenesis were observed in adult rat skin in different weeks of the skin wound healing process. In the induction stage, local epidermal thickening forms the hair placode (Fig. 1a), and signals from placode cells lead to the development of the dermal condensate [6].

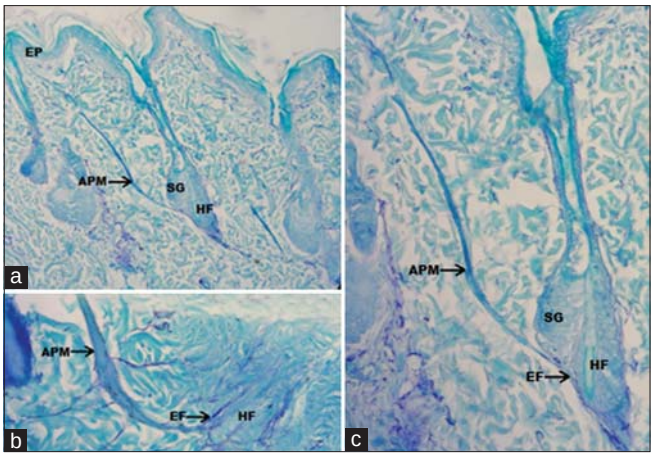

Figure 9: EP: Epidermis, APM: Arrector Pili Muscle, SG: Sebaceous Gland, HF: Hair Follicle, EF: Elastin Fibres (Violet Colour). a- Magnification: x10; b- Magnification: x40; c- Magnification: x20. Stain: AFFG.

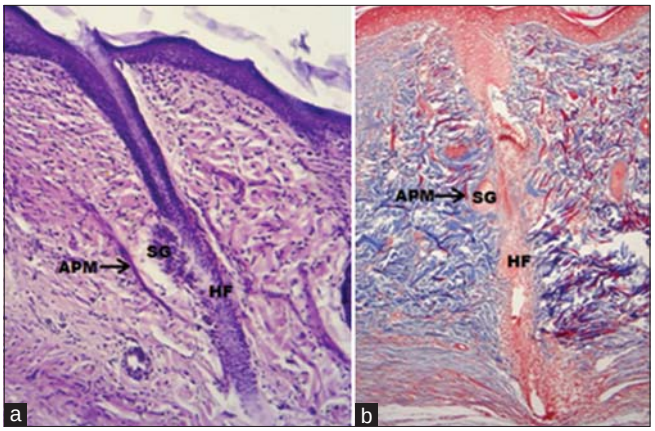

Figure 10: Progression of growth of Pilosebaceous unit. HF: Hair Follicle, SG: Sebaceous Gland, APM: Arrector Pili Muscle. a- Stain: PASH, Magnification: x20; b- Stain: MT, Magnification: x20

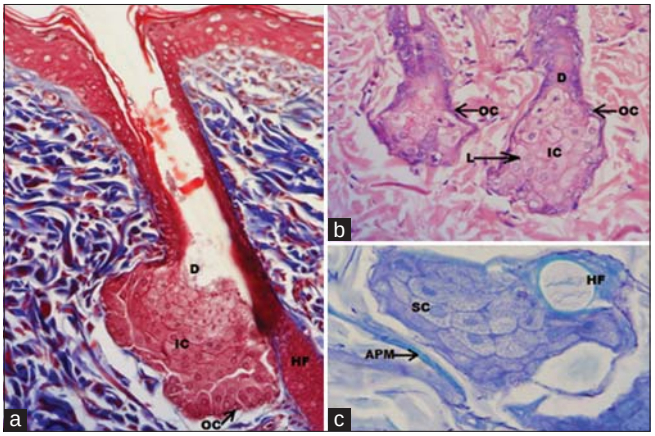

Figure 11: Sebaceous Gland-OC: Outer Cells, IC: Inner Cells, L: Lobule, D: Duct, SC: Sebocyte. HF: Hair Follicle, APM: Arrector Pili Muscle. a- Stain: MT, Magnification: x40; b-Stain: H & E, Magnification: x40; c- Stain: AFFG, Magnification: x40.

In organogenesis, condensation of mesenchymal cells is known as hair germ or follicular bud (Fig. 1b). Dermal

proliferation and invagination of the hair germ lead to the formation of hair peg and bulbous peg (Figs. 1c and 1d). During cytodifferentiation, the bulbous peg surrounds the condensed dermis to form the dermal papilla (DP) [11,12] (Fig. 2). El-Sayyed et al [13] described the prenatal development of all stages of hair follicle morphogenesis in rat's semi-thin skin section by using the routine staining method. However, in our study, we found all these events in adult albino rat's full-thickness skin during the wound healing process in different weeks by using both routine and special staining methods.

In embryogenesis and postnatal life, this dermal papilla regulates the growth and development of the hair follicle [14]. Signals from the DP induce its surrounding epithelial cells to differentiate into the inner root sheath (IRS) and different components of a hair shaft (HS) [2] (Fig. 2).

The pilosebaceous unit or follicular unit consists of hair and its follicle, arrector pili muscle, and sebaceous gland [3,15]. During embryogenesis, hair follicle cells and sebaceous gland originate from the ectoderm but the dermal papillae, the inner and outer root sheaths develop from the mesoderm [9]. Dermal papillae contain numerous small blood vessels, myelinated and non-myelinated nerve twigs. These blood vessels provide nutrition to the growing hair follicle [16]. All these features had been observed in the rodent's hair follicle study [17]. In the present study, we observed only components of the pilosebaceous unit (Fig. 3), but the blood vessels and nerve fibres in the dermal papillae could not be visualized.

The hair follicle has three segments: Infundibulum or dermal pilary canal that is part of the invaginated epidermis extending up to the opening of the sebaceous duct; Isthmus, from the opening of the sebaceous duct to the site of attachment of the arrector pili muscle, and Inferior segment, which extends from the insertion of the arrector pili muscle to the hair bulb [18,19]. Infundibulum and isthmus are permanent regions of the follicle but the lower inferior segment is a variable region. The present study has also found all these segments of the hair follicle (Fig. 4), and this similar finding has recently been reported in a comparative study describing the rodent's hair follicle [17]. Below the infundibulum, the perifollicular dermal sheath consists of type III collagen (reticular) and elastin fibres [19]. While in the rodent's skin study [17] type I and type III collagen and elastin fibres were demonstrated in the dermis, but in the present study collagen (reticular) (Fig. 5a) and elastin fibres (Fig. 5b) have been shown very much in the perifollicular dermal sheath by using special staining methods.

During the skin wound healing process in the adult albino rat, we found all components of the hair follicle. They are the outer root sheath (ORS), inner root sheath (IRS), hair shaft, extracellular matrix, dermal papilla, and connective tissue sheath (CTS) (Fig. 6). A specialized basal lamina called the glassy membrane or dermal sheath is commonly present between connective tissue sheath and follicular epithelium [20] (Fig. 6). Another study [13] reported all these components of hair follicles in the 19-day prenatal development stage of the rat. In this present study, we observed that the CTS is connected to the dermis and consists of collagen fibres running in various directions along with some elastin fibres, which possibly helps in the movement of hair follicles [19,21]. The presence of collagen and elastin fibres in the connective tissue sheaths of the rat's hair follicles had been reported by Maynard and Downes [22]. Available photomicrographs of the mice skin study [23] focused on only a general arrangement of collagen fibres by Masson's trichrome and elastin fibres by Verhoeff method fibres in the skin. The present study mainly focused on the distribution of fibres around the hair follicles themselves. The collagen fibres were visualized by Masson's Trichrome and PicroSirius Red with Fast green stainings (Fig. 7) and elastin fibres were observed with Aldehyde Fuchsin with Fast green staining method (Fig. 8).

Dermal papillae, hair melanocytes and matrix are localized at the proximal end of the hair follicle to form the bulb (Fig. 2). The bulge is an extension of ORS and contains localized epithelial and melanocytic hair follicle stem cells [9,15] (Fig. 2). Experimental group in the nude mice skin study [24], the newly formed hair follicle contains the IRS, ORS, hair bulb and matrix.

The basic ultrastructural features of rodents and human skin are remarkably similar at physiological and anatomical levels [17]. Interestingly, all hair follicle's epithelial components and layers of the hair shaft in the adult albino rat's skin were also noticed in the present study (Fig. 6). The epithelial components contain inner (internal) and outer (external) root sheaths. Outer root sheath (ORS) is made up of rounded and nucleated double-layered cells, but in the upper part of the follicle, it contains multilayered cells and the arrector pili muscle attached to this sheath [16]. The inner root sheath (IRS) supports the deeper part of the hair follicle and extends up to the level of isthmus. This

sheath contains three layers from within outwards are Cuticle, Huxley's layer, and Henle's layer [5,16]. The cuticle layer consists of keratinized squamous cells which interlock with hair cuticle to stabilize the hair and direct the upward growth of the IRS and hair shaft [5]. Cells in the Huxley's layer containing one to three layers of flattened nucleated cells, but Henle's layer consists of a single layer of cubical cells with flattened nuclei [20]. Hair shaft consists of medulla, cortex and cuticle from within outwards. The medulla is present only in thick hairs and consists of irregular shaped cornified cells containing vacuoles and air cavities present within and between the cells [19]. In the cortex, the cuboidal cells differentiate into keratin-producing cells. The cuticle is formed by cornified squamous cells, acts as a protective barrier and provides strength and protection to the hair shaft [16,25]. All microscopic features of the epithelial components and hair shaft of the present study were found to be in agreement with another rat's skin histological study [22].

The arrector pili muscle (APM) (Fig. 3) is a bundle of smooth muscle cells, attached from the bulge region of the hair follicle to the basement membrane of the epidermis marked by the presence of elastin fibres at both ends [19] (Fig. 9). Numerous nerve fibres are present at the site of muscular attachment of the muscle to the follicle [26]. Contraction of the arrector pili muscle helps in the elevation of hair and squeezing the sebum into the hair follicle, which was found to be in agreement with the findings reported earlier in the animal study [17].

Sebaceous gland develops from the ectodermal cells in the wall of the hair follicle [27] (Fig.10). The gland consists of lobules with sebocytes and ducts. One or more lobules are connected to the base of the follicular infundibulum by a single duct [28]. Each lobule contains outer or basal and inner cells. The small outer, cuboidal cells are a single layer of proliferative cells that rests on the basement membrane and large rounded inner cells filled with lipids [19]. The gland secretes sebum, which can be characterized by antimicrobial activity [25], thermoregulatory role and maintains the softness of the skin and hair. The features of the rat's sebaceous gland had been reported by Maynard and Downes [22], which are very similar to those observed in the present study (Fig.11).

In the present study, we observed the events of hair follicle morphogenesis and microscopic features of the pilosebaceous unit in the adult albino rat during different weeks of skin wound healing. Due to the paucity of photomicrographs of events of hair follicle morphogenesis and the pilosebaceous unit in one place, we used the routine and special staining methods to explain most of the microscopic features supported with photomicrographs. The special staining methods that were used proved to be very useful for quick identification, determining the location, orientation and density of different types of cells, and fibres associated with the pilosebaceous unit.

CONCLUSION

The review and observations of the histological features of all stages of the follicle formation in the adult albino rat's skin are expected to be useful in the interpretation of certain hair disorders, application of follicular drug delivery, and the hair transplant procedure.

ACKNOWLEDGEMENTS

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Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

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Our Dermatology Online

Original Article

The scenario of Lepra reaction at the Tertiary Level Hospital in a Hilly State and our experience with its management

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ABSTRACT

Background: Leprosy is a curable disease but, due to the presence of bacilli in the tissues, altered immune response hypersensitivity reactions may develop, which can increase the morbidity rates of the disease. We planned this study to observe the types of leprosy hypersensitivity reactions, their onset, presentation and response to treatment. This retrospective study was conducted over a 2-year period - from January 2015 to December 2016 - to evaluate the scenario of Lepra reaction in Hansen patients in Indira Gandhi Medical College in Shimla, Himachal Pradesh, India. **Results:** A total of 66 patients were registered as new cases of Hansen's disease. Lepromatous leprosy (LL) was the most common spectrum. A total of 41 patients (62.1%) developed a reaction either at presentation or during the course of the disease. Infection as a trigger of the reaction was elucidated in 6 patients, while trauma and vaccination triggered a reaction in 2 patients. Type 1 reaction (T1R) was observed in 18.18% of patients, while type 2 reaction (T2R) appeared in 43.93% of patients. The most common age group for T1R was 30-45 years, while for T2R it was the ages of 15-30. Grade 1 deformity (G1D) was present in 30 patients, grade 2 deformity (G2D) was present in 26 patients, while grade 2 deformity of eyes was diagnosed in 6 patients. A significant number of patients (36.5%) who developed reactions were relieved by the standard World Health Organisation's (WHO) regimen of prednisolone. Adjuvant drugs in the form of clofazimine, thalidomide and methotrexate were given to non-responders. **Limitation:** Due to the short follow up period, we did not observe late reactions. **Conclusion:** In our study, multibacillary leprosy was more common, and the younger age group was involved, thus leading to more deformities, stigma and impaired quality of life. Hansen's disease is a slowly progressive, curable disease, but an interruption by hypersensitivity reactions can alter the course of the disease, which may lead to deformities, hence the need for it to be managed vigorously.

Key words: Leprosy; Lepra reaction; Triggers; Deformities

INTRODUCTION

The causative agent of leprosy, *Mycobacterium leprae*, was identified by Armauer Hansen in 1873 [1]. Leprosy has a predilection for skin and peripheral nerve. Despite being mildly infectious and curable, the course of the disease is often complicated by potential intermittent hypersensitivity reactions called Lepra reactions that may aggravate the nerve damage and lead to deformities and disabilities, something that has been deeply associated with the social stigma connected with the disease [2]. Reactions often cause the symptoms that compel patients to seek medical attention for the first time. Though we have successfully eliminated leprosy from India, with a current prevalence rate of 0.66 in a population of 10000, and an annual new case detection rate of 9.71 in a population of 100,000 [3], Lepra reactions remains the major problem in the management of Hansen patients. A reaction may occur anytime during the course of the illness or after the release from treatment. Therefore it is not only important that the prevalence of leprosy decreases but the control and management of reactions is equally important.

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MATERIAL AND METHODS

A retrospective data analysis of all leprosy cases registered at the Department of Dermatology, Venereology and Leprosy of our Institute of the Tertiary Care Hospital, Indira Gandhi Medical College in Shimla, Himachal Pradesh, India that additionally takes care of looking after leprosy patients. The study period lasted from January 2015 to December 2016. The diagnosis of leprosy was based on the Ridley-Jopling classification, which is based on a detailed morphological, bacteriological (Acid Fast Bacilli in lesions/nasal smears), immunological (Lepromin test) and histopathological examination [4]. Also, cases were classified according to the WHO criteria as multibacillary (MB) leprosy if there are more than 6 skin lesions or positive bacillary index (BI) and paucibacillary (PB) if there are less than 5 skin lesions and negative BI [3]. All these diagnosed cases were started on multidrug treatment (MDT) as per the WHO regime. A detailed medical history and physical examination, including general physical examination, vitals, cutaneous, nerve examination, eye, joints, mucosa and other systemic examination were recorded at the first visit and a monthly follow-up until the completion of MDT and a three-monthly one for 2 years after that were also ordered. This information was collected and entered into an excel spreadsheet. All these patients were subjected to a routine haematological and biochemical examination at baseline and every three months till the completion of the therapy. Type 1 reactions were defined as an acute exacerbation, characterised by cutaneous lesions with redness and swelling or acute nerve tenderness with or without motor or sensory loss or just oedema of hands and feet. Type 2 reactions were defined as multiple, tender, erythematous nodules/ plaques with/without neuritis/constitutional symptoms/involvement of other organs such as eyes, testes, joints, or bones [5]. All the diagnosed cases of Hansen's disease were evaluated for the type of reaction, onset, clinical presentation, course of disease, deformities, frequency of occurrence of reactions, triggering factors and management of reactions. All patients with mild reactions were treated with rest and diclofenac in a twice-daily dose. Those with severe reactions were initially treated with 40 mg of prednisolone, followed by 30 mg, 20 mg, 15 mg, 10 mg, and 5 mg. Each dose was given for a period of 2 weeks. Patients who developed reactions after 3 months of the control of the reaction were labelled as recurrent T2R and those who developed a reaction within 3 months were labelled as chronic T2R. Recurrent ENL was managed with the WHO regimen of tapering doses of prednisolone along with clofazimine, a 100 mg dose TDS for 1 month, followed by a BD dose and OD dose for 1 month each [6]. For chronic reactions, thalidomide and clofazimine were started along with prednisolone.

Data analysis

Data were entered into an excel spreadsheet. Statistical analysis was done using Epi info 7.2.2 (the Centre for Disease Control and Prevention). All discrete variables were expressed as percentages or proportions. Continuous variables were presented in Mean ± SD.

RESULTS

Over a period of 2 years, 66 patients were diagnosed with Hanse's disease (Table 1). Males outnumbered females in a ratio of 3:1 (Table 1). The majority of patients were in the spectrum of Lepromatous Leprosy (LL) (48.5%) followed by Borderline Lepromatous (BL) (27.2 %) (Table 1). A reaction was seen in 41 (62.1 %) patients. The time of presentation of these patients is shown in Table 2. The maximum level of patients presented to us with a reaction (65.8%) at the time of the diagnosis of Hansen's. T2R was more commonly observed in 43.93% (Table 3). T1R were the most common in the BL spectrum and T2R in LL disease (Table 3) The patients' history was analysed to find any triggering factors. In most of the cases (80.48%), no triggering factors were present. Tubercular

Table 1: Sex distribution of cases diagnosed as leprosy and spectrum of disease

Group	Male	Female	Total
TT	1		1
BT	9	4	13
BL	11	7	18
LL	25	7	32
Pure neuritic	1		1
Indeterminate	1		1
Total	48	18	66

TT: Tuberculoid leprosy; BT: Borderline Tuberculoid leprosy; BL: Borderline lepromatous; LL: Lepromatous leprosy.

Table 2: Onset of reactions after MDT

	Onset of T1R (No. of patients)	Onset of T2R (No. of patients)
At the time of presentation	10	17
0-3 months	0	1
4-6 months	1	2
7-9	0	1
10-12	0	4
After getting RFT	1	4
Total	12	29

lymphadenitis in one, respiratory tract infections (RTI's) in two, urinary tract infections (UTI's) in two patients, filariasis in one, post BCG vaccination in one and a roadside accident in one were the only cases with triggering factors. Among the age distribution, the most common age group for T1R was 30-45 years which included 5 patients (41.6%) while the most common age group for T2R was 15-30 years which included 12 patients (41.37%) (Table 4). Regarding the deformity of patients, grade 2 deformity (G1D) of hands and /or feet was quite common (26 patients). Eyes involvement was seen in 6 patients (9.09%). Grade 0 deformity was observed in 10 (15.15%) patients while 84.8 % of patients had some deformity or the other (Table 5).

The management of patients who developed reactions during the course of the disease was also analysed. There were three patients (7.31%) with a mild and 92.6% with a severe reaction. It was observed that 15 patients (36.6%) who developed a severe reaction were relieved with a single course of tapering steroids as per the WHO schedule. Poor compliance and interruption of steroid therapy with a subsequent Lepra reaction was observed in 13 (31.7%) patients. Recurrent T2R was seen in 8(19.5%) patients. Out of these cases of the recurrent reaction, 5 (12.1%) cases reported improvement with no further reaction episodes with prednisolone and clofazimine tapering doses, while in 3 (7.3%) cases thalidomide had to be added. There were 3 (7.3%) patients with chronic T2R.

Table 3: Prevalance of reaction

Type of leprosy	T1R	%	T2R	%
TT	1	1.51	-	
BT	4	6.06	3	4.54
BL	7	10.60	11	16.67
LL	-		15	22.73
Total	12	18.18	29	43.93
Grand total	41 out of 66 patients (62.12%)			

TT: Tuberculoid leprosy; BT: Borderline Tuberculoid leprosy; BL: Borderline lepromatous; LL: Lepromatous leprosy.

Table 4: Age distribution of reaction

Age (years)	Type 1		Total	Type 2		Total
	Male	Female		Male	Female	
0-14	0	0	0	0	0	0
15-30	3	0	3	6	6	12
31-45	4	1	5	8	0	8
45-60	1	0	1	5	0	5
>60	3	0	3	3	1	4
Total	11	1	12	22	7	29

Table 5: Type of deformity

Type of deformity	No. of patients	Prevalence
Grade 1	30	45.45
Grade 2	26	39.39
Grade 2 eyes	6	9.09

DISCUSSION

Leprosy is a disease known for its low infectivity and chronic course. Although it appears to be a benign infectious disease, it is often complicated by immunologically mediated hypersensitivity reactions called Lepra reactions which may lead to greater inflammation and damage. Three types of Lepra reactions can be recognized, T1R, T2R and Lucio phenomenon. Risk factors for T1R are a borderline group of patients, patients with previous episodes of reactions, female, of old age, patients with multiple or disseminated patches or large facial patches, starting with MDT which can lead to a breakdown of Bacilli and release of bacterial antigens, immunotherapy and the hepatitis B or C infection [2]. Clinically T1R can be observed as erythema, oedema and tenderness of a pre-existing lesion associated with nerve tenderness and oedema of extremities. Risk factors for T2R are LL with skin infiltration, anti-leprosy drugs except clofazimine, bacteriological index (BI)>4, age < 40 years, intercurrent infections, trauma, surgical intervention, stress, immunization, pregnancy, parturition, and drugs like potassium iodide. T2R reaction is characterized by the sudden appearance of crops of evanescent pink coloured tender papules, nodules or plaques variable in size. These skin lesions are known as Erythema Nodosum Leprosum (ENL). ENL lesions are associated with systemic complaints in the form of a fever, myalgia, arthralgia, orchitis, ocular complaints etc. Lucio phenomenon is a rare form of Lepra reaction observed in Lucio leprosy [2]. It can be characterized by slightly indurated red-bluish plaques on the skin with an erythematous halo which may later develop into a necrotic eschar which detaches easily to reveal an irregularly shaped ulcer but patients remain afebrile. NLEP guidelines on reactions have clearly differentiated between mild and severe T1R, T2R, as well as basic differences between the two types of reaction [7].

Implementation of MDT has brought a decline in the incidence of reactions. Vijaya Kumaran et al. in their study observed a fourfold reduction in the incidence of late Lepra reactions in patients who received MDT *versus* patients only undergoing dapsone monotherapy [8].

Age and sex distribution of our study compared to other studies is displayed in Table 6 [9-13]. As shown in Table 6, the most common age group involved in most of the studies was 20-40 year. This could be because this age group, being the most physically active group, is more prone to get in contact with leprosy patients and to develop reactions. Ortiz et al observed that only 2.98% of patients were in the paediatric age group.[14] In our study, the majority of patients were in the spectrum of Lepromatous Leprosy (LL) which is in contrast to other studies, except the one in our state. Other studies from our and neighbouring country observed BT to be the most common [9-11]. The reason could be that cases of BT Hansen's are more easily recognized and managed at the periphery while difficult cases were referred to our tertiary health centre. Another reason is that our state is a hypo-endemic area for Hansen's disease so LL patients dominated. Puri et al also observed multibacillary leprosy to be more common (75.6% versus 24.4%). [15]

In our study, the overall prevalence of Lepra reaction was 62.12%. Out of total leprosy patients, 41% of patients (65.8% of total patients with reactions) had a reaction at the time of diagnosis and 7.5 % developed it after stopping MDT. Most of the other studies, as shown in Table 6, have reported a much lower incidence of leprosy reactions [9-12]. Sharma et al reported 51.6% patients of T1R and 76. 5 % of T2R were in a reaction at the time of diagnosis.[9] In our case, most of T1R first had a reaction at the time of diagnosis (83.3 %) while only 58.6 % in T2R. Similarly, in a study by Sallodkar and Kalla [13], 33% of the patients presented with a reaction during their first visit while Brakel et al [16] observed that 59% of their patients had T1R at presentation. Similarly, Manandhar et al, in their study of reactions, observed that 34% of the patients had T2R at the time of presentation, 32% developed it within 6 months and 19% - after one year of treatment [17]. The higher rate of reaction cases in our centre may be due to difficult terrain, physical exertion and more. Also, most of our patients belonged to the financially challenged socio-economic group, migrant labourers, who only presented when they were symptomatic. Also, we had a greater number of patients displaying type 2 reaction, compared to other studies. This could be because of a greater number of multibacillary patients in our study.

In our study, T1R was seen in the maximum BL>BT>TT (10.60%,6.06%,1.51% respectively). Sharma et al had the maximum prevalence of T1R in BB>BL>BT>LL (23.3%, 18.18%, 6.25%, 3.25% respectively) [9]. Brakel et al also observed the maximum T1R in BT Hansen> BL (34.15%,30.6% respectively) [16]. In our study, the relatively higher incidence of T1R in BL could be because we had a greater number of patients in the BL spectrum and no patient in BB.

In our study, the highest prevalence of T2R was found in LL >BL>BT (22.73%,16.67%,4.54%). The findings were similar to Sharma et al [9] where the frequencies of T2R in LL and BL patients were 22.8% and 4.5% respectively, with LL patients having a significantly higher value than BL patients. Brakel et al also observed a similar pattern [16]. The high bacillary load in LL predisposes the patient to type 2 reaction.

In our study, 80.48 % of patients had no specific triggering factor. Similarly, in a study by Prasannan et al, no triggering factor was identified in 80-84 % of patients in T1R and T2R [18].

A timely management of reactions is very important in preventing the progression of sensorimotor deficit and the subsequent development of deformity. We had a high incidence of deformities in our patients, which can be because of a high percentage of patients presented with reactions and also non-compliance with the treatment of reactions since recurrent and chronic reactions increase the risk of deformities.

Lepra reactions are managed predominantly with the help of corticosteroids, thalidomide, clofazimine, and steroid-sparing agents like methotrexate, azathioprine, cyclosporine, mycophenolate mofetil etc [6]. Prednisolone and thalidomide prove effective

Table 6: Comparison of various parameters in different studies

Study	Sex M:F	Age group maximum involved (years)	Commonest spectrum	Reaction (%)	Type 1 (%)	Type 2 (%)
Ours	2.66:1	T1R (30-45) T2R (15-30)	LL (48.48)	62.12	29.26	70.7
Sharma [9]	2.79	-	BT (63.7)	12.8	63.3	36.7
Chhabra [10]	2.3	21-40	BT (56.3)	37.5	81.06	19.7
Singh [11]	2.08	-	BT (32.9)	14.6	57.6	43.4
Jindal [12]	3	20-40 (47.8%)	LL (33.12)	-	-	-
Salodhkar [13]	2.42		Polar leprosy (50)	11	19.2	80.1

LL: Lepromatous leprosy; BT: Borderline Tuberculoid leprosy.

in the management of acute reactions. A long-term use of prednisolone, which may be required in the control of reactions, may lead to steroid-induced side effects and a recurrence of lesions after the withdrawal of steroids. Clofazimine has to be added in recurrent and chronic reactions because of its deposition in the reticuloendothelial system and macrophages, which interferes with the capacity of macrophage to process and present antigens, thereby preventing their mobilization and activation, and Interleukin-2 release.

Limitation

Due to the short follow up period, we could not find the true incidence of late reactions. Secondly, because the patients were coming from remote areas, the standard dose schedule of prednisolone at the referral centre given by Girdhar et al. [18] was not followed. Thirdly, the poor financial condition of the patients restricted us in using thalidomide or other steroid-sparing agents in the majority of patients.

CONCLUSION

In the end, we can conclude that leprosy, though it is a benign and chronic disease, it is very commonly interrupted by reactions which can be more damaging than the disease itself. The recognition of warning signs of a reaction is very important. Deformities can be prevented by early diagnosis and prompt treatment of these reactions.

Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

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Our Dermatology Online

Brief Report

Elaboration and subsequent application of a protocol for measuring reduction in a patient with Hashimoto thyroiditis

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ABSTRACT

Background: Hypothyroidism is the result of insufficient production or absence of production of thyroid hormones. Weight gain is one of the side effect of this autoimmune disease. In this paper a protocol for the reduction of measures for a Hashimoto thyroiditis patient was performed and the obtained results were analyzed. **Material and Methods:** The patient is female of 26 years of age, with an initial weight of 63.1 kg and a height of 160 cm. Body mass index (BMI) of 24.6. She presents hyperlipodystrophy in the abdominal area and thighs and cellulitis in the thigh area. **Results:** There was a weight loss associated with measurement of the reduction of abdomen and thighs. **Conclusions:** Treatments consisting in measuring reduction help in reducing body measurements, but following a good diet and practicing exercise are also required.

Key words: Hashimoto thyroiditis; Measurement of reduction; Aesthetic biomedicine; Nutrition

INTRODUCTION

Chronic autoimmune thyroiditis is the most frequent thyroiditis and is the most common cause of hypothyroidism in countries where diet provides a sufficient supply of iodine [1]. It is also called chronic lymphocytic thyroiditis or Hashimoto's thyroiditis.

Up to 95% of cases occur in women [1]. It affects people of all ages, especially those in their 30s and 50s. The incidence of this thyroiditis has increased exponentially over the last 50 years, which may be related to the increase in iodine content in the diet [2].

The natural course of the disease is the gradual loss of thyroid function [3]. Among patients with this condition who exhibit moderate increases in TSH and in the presence of anti-thyroid antibodies, hypothyroidism affects up to 5% of the general population.

The main clinical manifestations are the signs and symptoms of hypothyroidism [4].

Due to the insidious evolution of the disease, the doctor will find patients with less specific complaints, such as: weight gain (moderate), constipation or fatigue [5].

MATERIALS AND METHODS

The initial consultation and subsequent treatments were developed in Clínica Áurea – Clínica de Biomedicina Estética, Portugal.

In the initial consultation, all the procedures were explained, as well as side effects, expected results and contraindications.

The patient signed informed consent.

The patient is a female of 26 years of age. Initial weight of 63.1 kg and a height of 160 cm. Body mass index (BMI) of 24.6.

She presents hyperlipodystrophy in the abdominal area and thighs and cellulitis in the thigh area.

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In the initial consultation, the measurements described in Table 1 were taken, which will serve as a reference for each treatment session and to facilitate the evaluation of results. Initial photos were also taken (Figs. 1a and 1b).

The patient reported that she did not want to perform mesotherapy treatments, because she had already done it in the past and it had been quite painful.

Although the area being evaluated was the abdomen, a thigh protocol was also performed (Table 2 and 3).

The patient was provided with information regarding the foods that she could consume and those that would have to be avoided, based on the analysis of the potential triggers of autoimmune diseases upon the outcomes of previous research (Table 4).

Ethics Statement

The procedures followed were in accordance with the ethical standards of the responsible Committee on Human Experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2000 and 2008.

RESULTS

After 12 treatment sessions, the results are as follows (Figs. 2 - 4).

Final photos were also taken (Figs. 5a and 5b).

DISCUSSION

The patient presented at the initial consultation a weight of 63.1 kg and height of 160 cm, with a BMI of 24.6.

Table 1: Measurements at the first consultation

Date	Weight (Kg)	%Fat mass	%Water	Visceral fat	Muscle mass (Kg)	Basal Mean Index (KJ)	
	63,1	30,9	48,7	3	41,4	1349	
01/08/2018	**Abdomen (cm)**	**Waist (cm)**	**5 cm below belly button (cm)**	**Right thigh (cm)**	**Left thigh (cm)**	**Right arm (cm)**	**Left arm (cm)**
	88	77	101	60	61	32	32

Table 2: Measurement of reduction in the abdomen

	Treatment	Protocol
Session 1	Cavitation + Pressure therapy	20 min of Cavitation + 30 min of Pressure therapy
Session 2	Radiofrequency	20 min
Session 3	Pressure therapy + Cryotherapy	Cooling gel on the abdomen + 30 min of Pressure therapy
Session 4	Electrostimulation + Massage	25 min of Electrostimulation in the abdominal area. Immediately afterwards a modeling massage
Session 5	Cavitation + Pressure therapy	20 min of Cavitation + 30 min of Pressure therapy
Session 6	Radiofrequency	20 min
Session 7	Cavitation + Pressure therapy	20 min of Cavitation + 30 min of Pressure therapy
Session 8	Radiofrequency	20 min
Session 9	Electrostimulation + Massage	25 min of Electrostimulation in the abdominal area. Immediately afterwards a modeling massage
Session 10	Cavitation + Pressure therapy	20 min of Cavitation + 30 min of Pressure therapy
Session 11	Pressure therapy + Cryotherapy	Cooling gel on the abdomen + 30 min of Pressure therapy
Session 12	Radiofrequency	20 min

Table 3 : Measure reduction for thighs

	Treatment	Protocol
Session 1	Pressure therapy	30 min.
Session 2	Pressure therapy+Cryotherapy	Gel with cold effect on the abdomen + 30 min of Pressure therapy
Session 3	Radiofrequency	15 min in each zone (right front leg, right back leg, left front leg, left back leg)
Session 4	Pressure therapy+Cryotherapy	Cooling gel on the abdomen + 30 min of Pressure therapy
Session 5	Pressure therapy	30 min.
Session 6	Radiofrequency	20 min
Session 7	Electrostimulation+Massage	25 min of Electrostimulation. Immediately afterwards a modeling massage
Session 8	Pressure therapy	30 min.
Session 9	Pressure therapy+Cryotherapy	Cooling gel on the abdomen + 30 min of Pressure therapy
Session 10	Pressure therapy+Cryotherapy	Cooling gel on the abdomen + 30 min of Pressure therapy
Session 11	Radiofrequency	15 min in each zone (right front leg, right back leg, left front leg, left back leg)
Session 12	Electrostimulation+Massage	25 min of Electrostimulation. Immediately afterwards a modeling massage

Table 4: Foods recommended and advised against based on the analysis of the potential triggers of autoimmune diseases

Consume		Avoid
Almonds	Lactose-free Yoghurts	Caffeine
Asparagus	Lettuce	Flax seeds
Bean	Lobster	Green tea
Beef	Orange	Lactose
Brewer's yeast	Oysters	Pasta, rice, bread (based on wheat)
Canned tuna	Pasta and brown rice	Raw vegetables: cabbage, corn, broccoli, Brussels sprouts, cauliflower and spinach
Carrot	Peanuts	Soy and Derivatives
Chicken meat	Plowing	Sugar consumption
Cockle	Pork liver	Sweet potato
Cooked shrimp with no sauces	Prunes	
Corn	Pumpkin	
Cucumber	Quinoa	
Dark bread	Saltwater fish	
Dried seaweed	Strawberries	
Egg yolk	Sunflower seeds	
Garlic	Walnuts	

Figure 1: (a) Initial photo: frontal. (b) Initial photo: lateral.

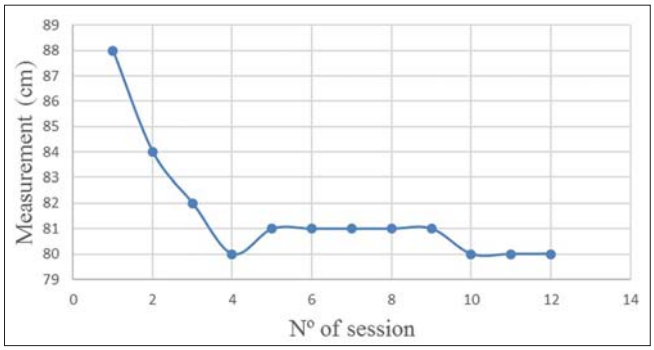

Figure 2: Measurement of the abdomen throughout the sessions.

According to the BMI chart, the patient (26) has normal weight for her height. After analyzing the percentage table of fat mass for females, she is in the "healthy" range.

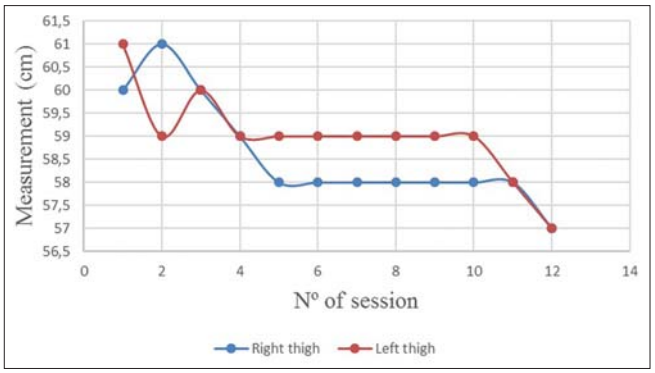

Figure 3: Measurement of the thighs throughout the sessions.

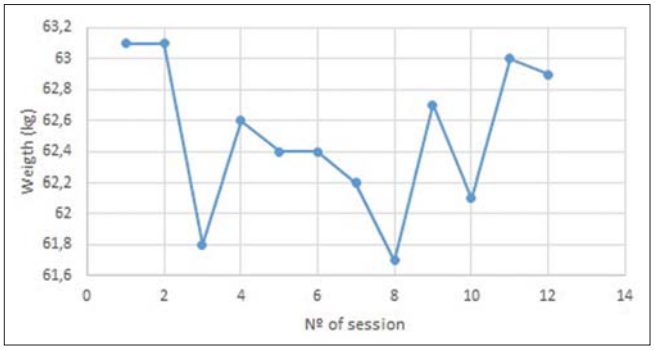

Figure 4: Weight measures along the sessions.

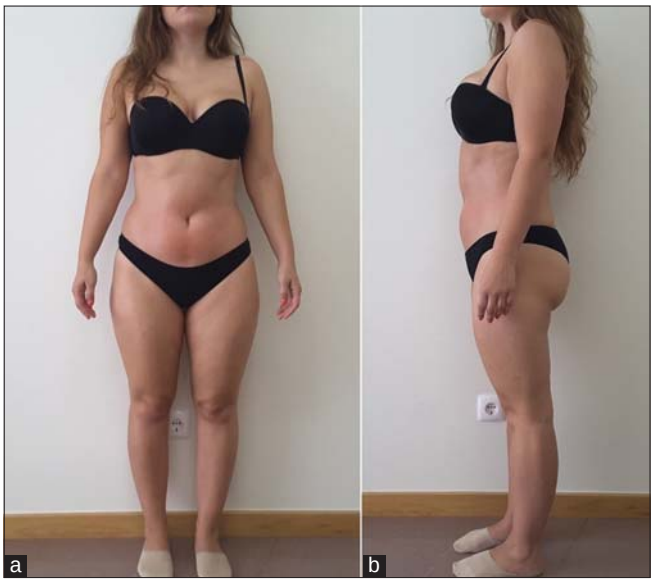

Figure 5: (a) Final photo: frontal. (b) Final photo: lateral.

The results confirm that the patient has lost volume and weight, a fact that is noticed by the loss of centimeters in both the abdomen and the thighs (Figs. 3 - 5).

The BMI was not altered.

The patient confirms that she notices improvements in the cellulitis in the thigh area and that the skin is much smoother.

CONCLUSION

The lack of improvement in the percentage of fat mass (initial: 30.9, final 33.6) is due to the fact that the patient confirmed that she did not exercise during the treatment and did not follow the recommendation of foods to eat and avoid.

These treatments help in reducing the body measurements, but it is also necessary to follow a good diet and practice exercise. The patient was advised to attend nutrition consultations and exercise to continue to reduce weight.

The majority of subjects with thyroid disease experience problems in maintaining normal body weight and have a higher body mass index and waist circumference than healthy individuals [6].

The role of diet in autoimmune diseases seems to be important but the true extent of its influence and the therapeutic potential are still largely unknown in this context.

Physical exercise is of equal importance to enhance the results that the treatments provide.

However, these two major reasons are not responsible alone for weight loss. If there is a hormonal imbalance, which many people with Hashimoto's thyroiditis experience, then it will be extremely difficult to lose a significant amount of weight by eating and exercising alone [7]. Eating a lot of refined foods and skipping meals affects two of the major hormones in the body, cortisol and insulin [8]. If these habits continue for many years, this will put stress on the adrenal glands and can eventually lead to insulin resistance [9]. Until this is corrected, even with a perfect diet and exercise pursued on a daily basis, losing weight will be difficult.

Many people with Hashimoto's thyroiditis and other types of hypothyroidism also have an imbalance in the ratio between the hormones estrogen and progesterone, which can also lead to weight gain and thus make it difficult to lose weight [10]. The patient was advised to consult with a competent endocrine doctor, as they will be able to detect a hormonal imbalance.

Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible Committee on Human Experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

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Our Dermatology Online

Case Report

Ezrin may confine neutrophil orientation in a chemotactic gradient towards the vessels in a case of Sweet syndrome

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ABSTRACT

Sweet syndrome is a neutrophil dermatosis. Here we present a case of a 63 male who presented with tender, reddish purple macules and papules on his trunk. Skin biopsies were taken for hematoxylin and eosin stain (H&E), immunohistochemical staining (IHC) and direct immunofluorescence examination (DIF). H&E stains demonstrated the histologic features previously described in SS, along with other findings. Strong expression of CD31, CD34, Von Willebrand Factor, podoplanin and vimentin (vessels markers) as well as Ham 56, CD68 and myeloperoxidase were seen. The DIF showed expression of ezrin in the vessels and in the areas overwhelmed by neutrophils. Our data suggest that ezrin may confine neutrophil orientation in a chemotactic gradient towards the vessels and may play an import role in Sweet syndrome. We also showed that vascular involvement, skin appendageal inflammation and cluster damaged dermal extracellular matrix maybe also part of the Sweet syndrome.

Key words: Sweet syndrome; Neutrophilic dermatosis; Ezrin; CD31; Von Willebrand factor; Podoplanin; HAM 56; Neutrophil transmigration

INTRODUCTION

Acute febrile neutrophilic dermatosis (Sweet syndrome) (SS), was first described in 1964 by Robert Douglas Sweet and later studied by multiple authors. This disease usually occurs in middle-aged women and can occur after non-specific infection of the respiratory or gastrointestinal tract. SS is considered by some authors to have three clinical settings: a), classical (or idiopathic), b), malignancy-associated and 3), drug-induced, especially including bortezomib medication. SS has a histiocytoid variant. Some people associate SS with immature neutrophils, and some others with rare extracutaneous manifestations including cardiovascular involvement, coronary artery occlusion, involvement of the eyes, joints, and oral mucosa as well as systemic manifestations involving the lungs, liver, kidneys, and central nervous system. Clinically, SS syndrome usually presents clinically with raised erythematous plaques with pseudo-blistering and occasionally pustules may occur on the face, neck, chest, and extremities, accompanied by fever and general malaise [1-4]. Ezrin (also known as cytovillin or villin-2), is a cytoplasmic peripheral membrane protein and is a member of the ezrin, radixin and moesin (ERM) protein family. Ezrin is a membrane of the cytoskeleton linker protein that plays a key role in cell surface structure adhesion, migration, and organization. Ezrin has been shown to play a role in neutrophil migration [5,6]. Therefore we decided to test for the presence of ezrin in a patient skin biopsy.

CASE REPORT

A 63 male presented with a diffuse eruption on the trunk that began as red –purple maculo-papules

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that later became confluent, forming tender plaques (Fig. 1). The skin lesions were accompanied by mild fever. The clinical diagnosis was Sweet Syndrome (SS), and dapsone was used for treatment. Skin biopsies were taken for hematoxylin and eosin stain (H&E), direct immunofluorescence examination (DIF) and immunohistochemical (IHC) staining, and these tests were performed as previously described [7,8]. In brief, for the DIF, we used, in addition to the routine antibodies to IgA, A, M, E, D, C1Q, C3C, albumin and fibrinogen, an antibody to ezrin (3C12), Cat # MA5-13862 from Invitrogen, at 1:75 dilution. We used for its secondary antibody a Texas red goat anti-mouse IgG secondary antibody both from (Carlsbad, California, USA). For the IHC we used antibodies to Von Willebrand Factor, clone F8/86, to podoplanin clone D2-40, to CD68, clone EBM11, CD31, clone JC70A, CD34 Class II clone QBEnd 10, and rabbit anti-human myeloperoxidase all from Dako, Carpinteria, CA, USA). We also used macrophage (HAM-56) mouse monoclonal antibody 279M-1 HAM-56 from Cell marque (from Sigma-Aldrich, (Rocklin, CA, USA). The tests were performed in a Leica Bond Max machine.

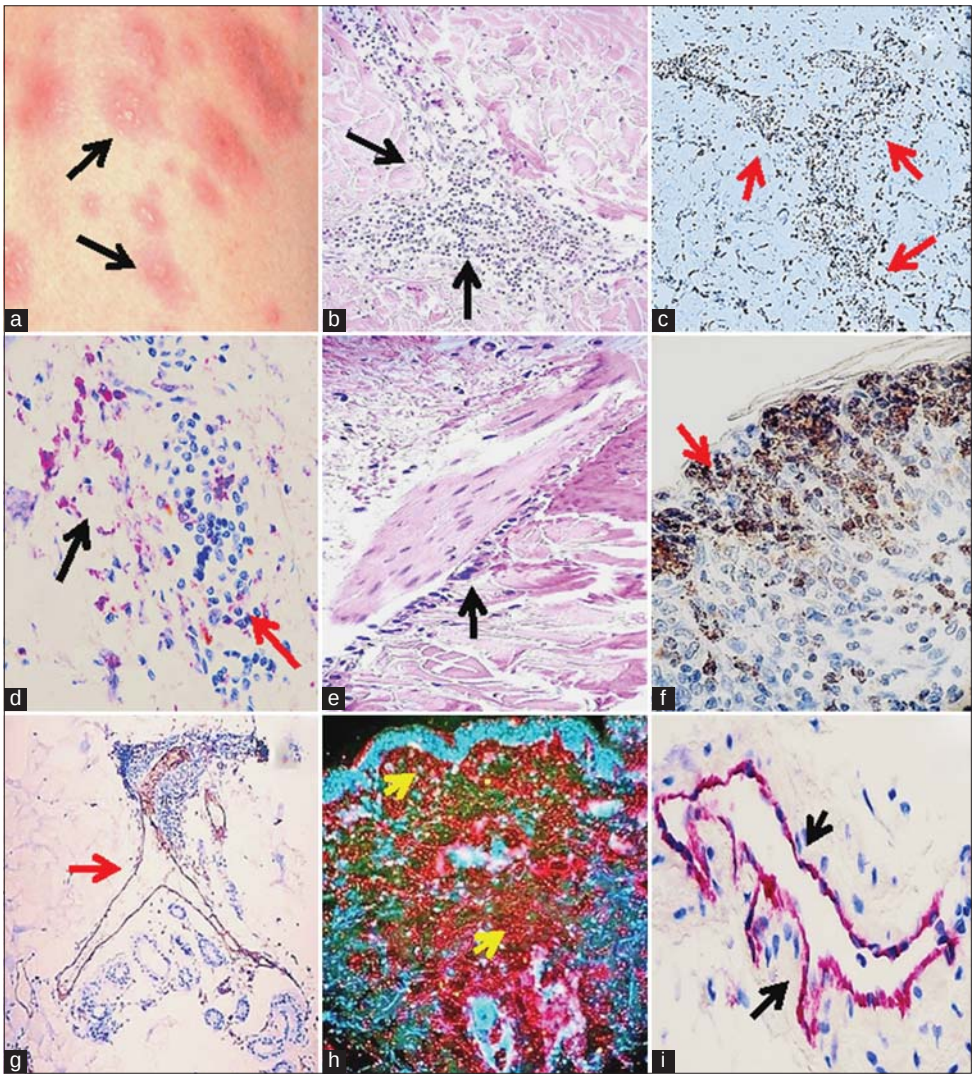

Figure 1: (a) Shows multiple erythematous plaques and vesicles in the legs of the patients (black arrows). (b) H&E stain shown in the papillary dermis, a strong infiltrate of numerous neutrophils with an admixed with few cells including lymphocytes, histiocytes and occasional eosinophils in the dermis, (black arrows, 200X). (c) IHC stains showing positive stain with HAM 56 in the vessels where the neutrophils and other inflammatory cells were seen, (dark stain, red arrows). (d) Double IHC stain showing positive stain with in fuchsia with myeloperoxidase (+++) (black arrow) cells also stain with CD68 near the vessels in brown (red arrow) (400X). (e) H&E stain shows neutrophils infiltrate in a liner pattern along the piloerector muscle (black arrow) (400X). (f) IHC stain positive in the corneal cluster with myeloperoxidase (++++) (dark stain, red arrow) (400X). (g) IHC stains with CD31 in an elongated and dilated vessel (dark stain, red arrow) surrounded by neutrophils. (h) DIF shows overexpression of ezrin along the dermal vessels and those neoformed in the dermis (red stain, yellow arrows), (100X). The nuclei of the cells were stain in blue with 4',6-diamidino-2-phenylindole (DAPI). (i) IHC showing a dilated and deformed upper dermal lymphatic positive for D2-40 (++++),(fuchsia stain), (black arrows).

The H&E stain demonstrated focal subcorneal collections of neutrophils with focal papillary dermal edema and the presence of early subepidermal vesiculation due to dermal edema (Fig. 1). Within the papillary dermis, a dense infiltrate of neutrophils was observed, admixed with lymphocytes, histiocytes and occasional eosinophils. Abundant cellular debris was also observed. Newly formed vessels were detected in the dermis. Of interest, a dense inflammatory infiltrate was also seen around the pilosebaceous glands and the arrector pili muscles, mainly consisting of neutrophils, as well as scattered lymphocytes, histiocytes and eosinophils (Fig. 2). Multiple skin appendages were damaged and they appeared to be decreased in number and size. The dermal extracellular matrix was also damaged in multiple spots, especially near the mesenchymal-endothelial cells junctions (Fig. 2). Focal leukocytoclastic debris was also seen, but frank vasculitis was not identified. The DIF was negative for IgG, IgA, IgM, IgE, complement/C1q, complement/C3, albumin, and fibrinogen. Ezrin was very positive (++++) in the area where the majority of neutrophils were seen in the dermis, as well as where the newly formed vessels were observed with the routine H&E staining (Fig. 1).

The IHC stains for vascular markers dermis (Von Willebrand factor, CD31, CD34, vimentin, and D2-40) were strongly expressed throughout the entire. Most of the dermal vessels demonstrated altered shapes. The neutrophils were found in the same areas of the cells positive for HAM 56 and CD68 (Fig. 1). Neutrophils were also present around most of skin appendages. Neutrophilic dust and cellular debris were found in the dermis (Figs. 1 and 2). HAM-56 was positive in cells around the vessels, around the sweat glands and was extremely positive around the mesenchymal-endothelial cells junction's dermal vessels (colocalizing with extracellular dermal damage). Myeloperoxidase was also positive in the epidermis (++++), and inside the vessels (++++) (Fig. 1). DIF of the skin shows overexpression of ezrin (++++) (in red) in the dermal vessel and in the areas where the neutrophils were seen (Fig. 1).

STATEMENT OF ETHICS

Although Institutional Review Board (IRB) approval for a case report is not needed, the US Health Insurance. Portability and Accountability Act of 1996 (HIPAA). Privacy Rule restricts how protected health information(individually identifiable health information) is disclosed and nothing about this report violates those rules.

DISCUSSION

In this report we describe a case of Sweet syndrome with histopathologic features previously not described such as alteration of vessels with neovascularization, a peri-appendageal inflammatory infiltrate of neutrophils and damage of several skin appendages. We also describe the expression of Ezrin in the areas populated by neutrophils including the vessels in the dermis. In this case we observed histologic features of Sweet Syndrome displaying perfect colocalization of epidermal blisters with a myeloperoxidase marker. The presence of myeloperoxidase in the blisters suggests that this enzyme may contribute to the blister formation (when present). We also detected an important finding occurring with the neovascularization in the dermis

Additionally we observed the damage of multiple skin appendages. These two findings have not been described before in Sweet syndrome. Additionally the expression of vascular markers (e.g. Von Willembrand factor, CD31, CD34, vimentin, and D2-40) and the colocalization of HAM-56 within close proximity to the vessels may indicate some possible antigen presentation involving endothelial and other vessels components.

Of interest, this is the first description of the presence of Ezrin being overexpressed in most of the dermal vessels within the areas where neutrophils were seen. Ezrin links the cortical cytoskeleton to the plasma membrane and plays a role in regulating changes in cell shape. Recently, a study reported that NSC668394 (a pharmacological inhibitor) inhibits a key step for ezrin activity, i.e. phosphorylation at threonine 567. The authors also pointed out that in neutrophils, another key regulatory step is the $Ca2^{+}$-mediating cleavage of ezrin by calpain. The authors furthermore showed that neutrophils with NSC668394-inhibited ezrin phosphorylation remained both phagocytic and chemotactically competent. However, phagocytosis was slightly impaired and chemotaxis could not be maintained over longer periods with aberrant morphology. The authors presented evidence that although phosphorylation of ezrin plays a minor role in limiting the rapid changes in cell shape in neutrophils, inhibition of ezrin phosphorylation by NSC668394

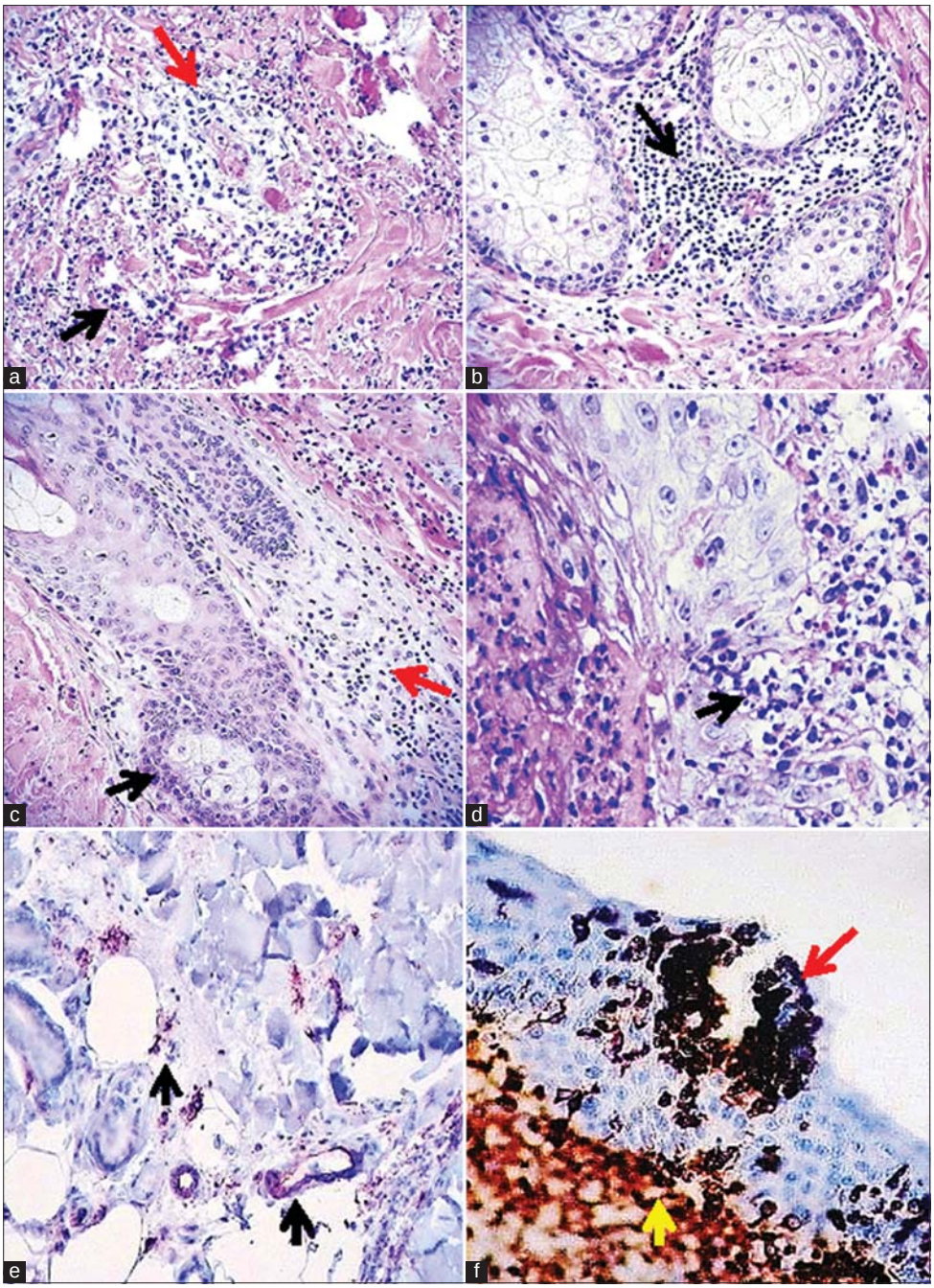

Figure 2: (a) Through d, H&E stains. a. shows a large dilated dermal vessel (red arrow) altered in shape and surrounded by neutrophil dust debris (black arrow) (400X). (b) The sebaceous glands were surrounded by mostly neutrophilic but also some lymphohistiocytic infiltrate (black arrow), (400X). (c) The pilosebaceous glands are damage showing some sebaceous gland atrophy (black arrow) and inflammatory cells are also appreciated (red arrow) (400X). (d) The black arrow points toward neutrophil debris in the sebaceous gland (1000X). (e) IHC stain positive at the mesenchymal endothelial cells junctions with HAM 56 (purplish stain, black arrows) (400X). (f) IHC stain with vimentin (staining the neutrophils at the intra-epidermal blister (black arrow), and in the dermis under the blister (400X), (yellow arrow).

prevented multiple and prolonged shape changes during extended chemotaxis [6].

It is known that during inflammation, the selectin induced sluggish rolling of neutrophils on venules cooperates with chemokine signaling to mediate neutrophil recruitment into tissues. It has been also suggested that the pathophysiological roles of ezrin/radixin/moesin proteins can alter the leukocyte rolling as shown in in mice deficient in moesin, a member of the ezrin-radixin-moesin family [9,10].

In this report we also showed that HAM-56 and CD68 positive cells were present at the mesenchymal-

endothelial cells junctions near altered dermal extracellular matrix clusters. Under inflammatory conditions or during interactions with other cell subsets, neutrophils inherently express or can de novo produce the receptors needed for antigen presentation. For several authors these observations support that neutrophils have the capacity to function as antigen presenting cells [11]. Some authors also have demonstrated that tumor necrosis factor-α and IL-17A activates and induces pericyte-mediated basement membrane remodeling in human neutrophilic dermatoses [12]. Indeed, in this case we observe that the neutrophils and their products had strong affinity to the vessels. It is conceivable that they released inflammatory arsenals causing the shape alterations to vessels as well as those that gave rise to the skin damaged appendages.

We conclude that in this case of Sweet syndrome, the vessels markers are strongly expressed, altered in their shape and that ezrin is co-localized. This protein may play a significant role in the neutrophil migration. Further studies are needed.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Our Dermatology Online

Case Report

A rare diagnosis of idiopathic eruptive macular pigmentation with dermoscopy. A case report

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ABSTRACT

Idiopathic eruptive macular pigmentation (IEMP) is a rare disease of the paediatric age group characterised by hyperpigmented brownish to black macules; they are asymptomatic and involve mainly the trunk, back and extremities with no preceding inflammatory condition or any previous exposure to drugs. We report a case of a 7-year-old healthy boy with brown to black asymptomatic macules on the back and chest. The lesions persisted for only a few months. The changes were discrete with individual lesions ranging from 1-2 cm. Histology showed prominence of the pigment layer with increase in the dermal collagen and sparse lymphocytic inflammation of the dermis.

Key words: Macules; Eruptive; Hyperpigmentation; Asymptomatic

INTRODUCTION

Idiopathic eruptive macular pigmentation (IEMP) is a rare condition with only a few cases published so far. It is characterised by the presence of pigmented asymptomatic macules which mainly involve the trunk, face and extremities and are usually found in the paediatric age group. These macules gradually resolve spontaneously over time and may take months to years for resolution. IN 1978 Degos et al. provided the first description of this condition. It was further described in English by Sanz De Galdeano in 1996 [1]. This laid the criteria for the diagnosis of the condition, with the following points:

1. Eruption of brownish black, discrete, non-confluent, asymptomatic macules involving the neck, trunk and extremities in children and adolescents.
2. Absence of any preceding lesions.
3. No previous drug exposure.
4. Epidermal hyperpigmentation of the basal layer with dermal melanophages without any basal cell damage
5. Normal mast cell count.

In this report, we have a 7-year-old boy fulfilling the criteria for this condition.

CASE REPORT

We report a 7-year-old male patient who reported to the outpatient department of dermatology due to multiple hyperpigmented lesions on the back and chest (Fig. 1). There was gradual increase in size and number of these hyperpigmented macules. There was no history of any drug intake in this patient. On examination multiple asymmetrical hyperpigmented brown to black macules on the back and chest were observed. The size ranged from pin-point to 1-2 cm in diameter. These macules were asymptomatic with no preceding skin lesions. There was no history of itching and there was absence of scaling as well. Some lesions had a velvety surface. The patient also had multiple patches of hair loss on the scalp ranging 1-4 cm in diameter. The patient was in good health and no comorbidity was seen.

The patient was further investigated using dermatoscopy which revealed brown to black pigmented granules and dots scattered across an accentuated pigment network (Fig. 2).

Trichoscopy of the scalp demonstrated the presence of black dots, cadaver hairs, an occasional exclamation

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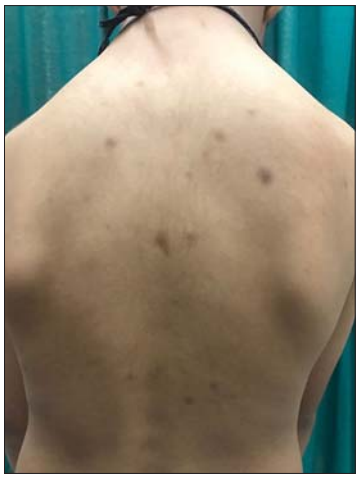

Figure 1: Multiple asymmetrical hyperpigmented brown to black macules on the back of a 7-yeard-old male

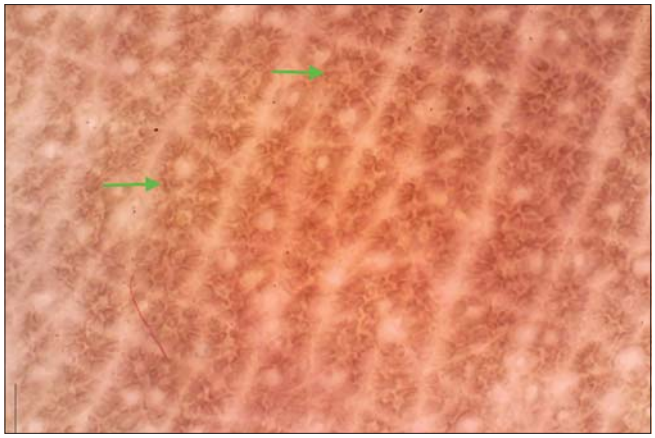

Figure 2: Brown to black pigmented granules and dots were seen scattered across an accentuated pigment network (green arrows) on dermoscopy (DL4, polarised).

point hair and a clinical diagnose of alopecia areata was made.

The patient was further evaluated with a skin biopsy which was taken from the back. Histological description showed epidermis with a prominent pigment layer due to basal cell hyperpigmentation. Moderate acanthosis could also be seen (Fig. 3). The underlying dermis showed insignificant inflammation with sparse lymphocytic infiltrate. There was also an increase in dermal collagen (Fig. 4).

Normal mast cell count was seen in this patient, further fulfilling the said criteria for this condition.

The patient was first treated with topical steroids on the back and chest with no response and was later sent for observation without any topical or systemic treatment and assurance. Spontaneous resolution

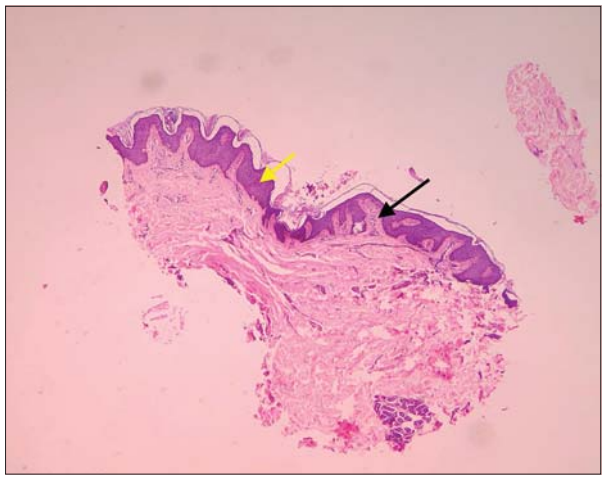

Figure 3: Moderate irregular acanthosis (yellow arrow), and sparse superficial lymphohistiocytic infiltrate in the upper dermis (black arrow) (H & E x10).

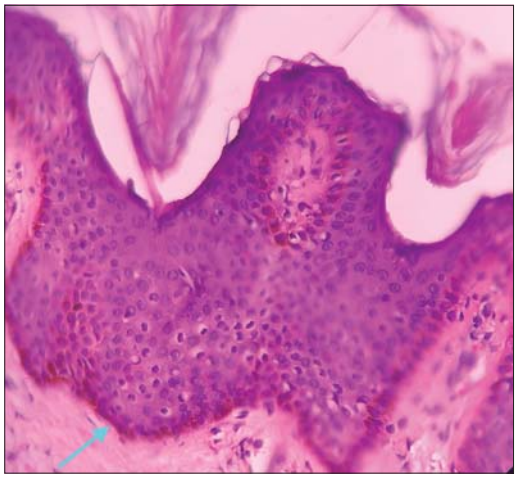

Figure 4: Basal layer hyperpigmentation (blue arrow), dermis showing sparse lymphohistiocytic infiltrate and increase in collagen (H & E ×40).

began 3 months after stoppage of any topical medication.

Alopecia areata was treated with topical application of steroids and tacrolimus with a positive response.

DISCUSSION

Idiopathic eruptive macular pigmentation (IEMP), a rare disease of children and adolescents is a benign self-limiting condition where remission may take from months to years. Differential diagnosis of the condition includes lichen planus pigmentosus, erythema dyschromicum perstans and urticaria pigmentosa. On biopsy, IEMP could be differentiated from the other conditions. Lichen planus pigmentosus and erythema dyschromicum perstans show basal cell

vacuolization with individually necrotic keratinocytes at the dermoepidermal junction with melanophages scattered in papillary dermis. Urticaria pigmentosum gives a positive Darier's sign which is negative in IEMP. The condition has been underreported probably due to reluctance to perform biopsy in children and because of smaller awareness of the condition among the treating physicians. The pathogenesis of IEMP is unknown; however, hormonal factors may play a role as the condition is mainly present in the peripubertal age group. There has been a case report of this condition in a patient as young as 1 year old while a case of a 50-year-old has also been reported [2,3]. Most of the reported cases involve young patients. As already mentioned, the criteria for the diagnosis of this condition have been laid down by Sanz De Galdeano [1]. Our case fulfilled the above-mentioned criteria. It must also be mentioned that only a handful of cases of similar nature have been reported [4-6] among the Indian population. The indent of this case report is to create awareness about this condition among treating physicians of all specialties to avoid misdiagnosis and unnecessary management of this self-limiting condition.

Consent

Examination of the patient was conducted according to the Declaration of Helsinki.

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

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Source of Support: Nil, **Conflict of Interest:** None declared.

Our Dermatology Online

Case Report

Desmoid-type fibromatosis arising in the inguinal region in a young woman: a case report from a histopathological perspective

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ABSTRACT

Fibromatoses generally comprise a broad spectrum of myofibroblastic proliferation of similar histomorphology. Desmoid-type fibromatoses (desmoid tumors) are locally aggressive lesions which principally involve deep soft tissue structures. The author describes a 35-year-old female, who was found to have a nodular subcutaneous resistance arising in the upper portion of the right inguinal region. The tumor measured 40 x 25 x 15 mm and adhered to the aponeurosis of the abdominal internal oblique muscle. Histology revealed a dense mass of proliferating spindle-shaped myofibroblasts of uniform appearance. At the periphery, the lesion was poorly circumscribed and infiltrated an adjacent striated muscle. A diagnosis of desmoid-type fibromatosis was made. Desmoid tumor is a quite rare oncologic entity. Although it never metastasizes, it can lead to significant morbidity due to locally aggressive behaviour with a striking tendency to recur. Every patient once treated for deep fibromatosis requires long-term follow-up.

Key words: Desmoid-type fibromatosis; Desmoid tumor; Beta-catenin

INTRODUCTION

Fibromatoses generally comprise a broad group of myofibroblastic proliferation of similar histomorphology whose biologic behaviour is intermediate between that of benign fibrous lesions and fibrosarcomas [1]. According to the WHO (World Health Organisation) classification of tumors [2], they are categorized as neoplasms of uncertain behavior (codes 8813/1, 8821/1 and 8822/1). Various pathologic entities that represent this group occur mostly in adults with a predilection for certain body sites. Based on anatomical distribution, fibromatoses are divided into two major groups with several subdivisions (Table 1) [1]. Superficial (fascial) fibromatoses are slowly growing and of small size. They usually arise from the fascia or aponeurosis. In contrast, deep (musculoaponeurotic) fibromatoses are rapidly growing tumors that often attain a large size, but do not metastasize [1]. As the name indicates, they arise from musculoaponeurotic stromal elements and mainly involve deep soft tissue structures. The synonyms for deep fibromatoses are desmoid tumors (desmoids) or aggressive fibromatosis. The former denomination is derived from a macroscopic appearance of lesions, as the term "desmoid" originates from the Greek word "desmos", meaning tendon-like. The term aggressive fibromatosis emphasizes a biological behavior that tends to be more aggressive, accompanying by a high recurrence rate [1-3]. Desmoid tumors are quite infrequent in routine clinical practice. In this journal, two articles addressing a superficial [4] and an intraabdominal fibromatosis [5] have been published. In the present paper, a case of subcutaneous desmoid-type fibromatosis in a young woman is described.

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CASE REPORT

A 35-year-old female manifested with a subcutaneous resistance arising in the upper portion of the right inguinal region. She claimed that the lesion had been present for a few months, during which it had increased in size. On physical and CT examination, it appeared as a nodular subcutaneous tumor mass measuring 3-4 cm in diameter. It stuck to aponeurosis of the abdominal internal oblique muscle. An overlying skin was intact. The presumptive clinical diagnosis was a benign tumor of the soft tissue. A surgical extirpation of the lesion was done following a plastic reconstruction of the inguinal canal. Grossly, the solid tumor measured 40 x 25 x 15 mm, it was white-grayish in color and elastic in consistency. Histology revealed a dense mass of proliferating spindle-shaped cells that was tightly fixed with the aponeurosis (Fig. 1). The proliferation consisted of elongated (myo)fibroblasts of uniform appearance without hyperchromasia or atypia (Figs. 2 and 3). The nuclei were small, sharply defined, some with inconspicuous nucleoli. Mitotic rate was very low (1 mf per 10 HPF) and proliferation activity (Ki-67 index) did not exceed 5%. The tumor cells were usually separated from one another by sparse collagen fibers, but many sections with an extensive hyalinization or keloid-like structures were also visible. Although the cellularity varied from area to area, overall, the lesion was rich in cells. An interesting feature were remnants of degenerated striated muscle cells forming multinucleated giant cell aggregates, that were entrapped within the tumor tissue (Fig. 4). At the periphery, the lesion was poorly circumscribed and infiltrated an adjacent striated muscule. By immunohistochemistry, the tumor cells showed a focal cytoplasmic positivity for alpha-smooth muscle actin (Fig. 5) and almost diffuse nuclear positivity for beta-catenin with moderate to strong staining intensity (Fig. 6). Based on the histopathological findings and immunophenotype, a diagnosis of desmoid-type fibromatosis was made. Because the patient came from another district and was managed in her permanent residency region, no information about her further clinical workup was available at the time of writing this article.

DISCUSSION

Desmoid tumors (DTs) account for 0.03% of all neoplasms [3,6] and less than 3% of all soft tissue tumors [3]. The estimated annual incidence in the general population is 2 – 4 cases per million

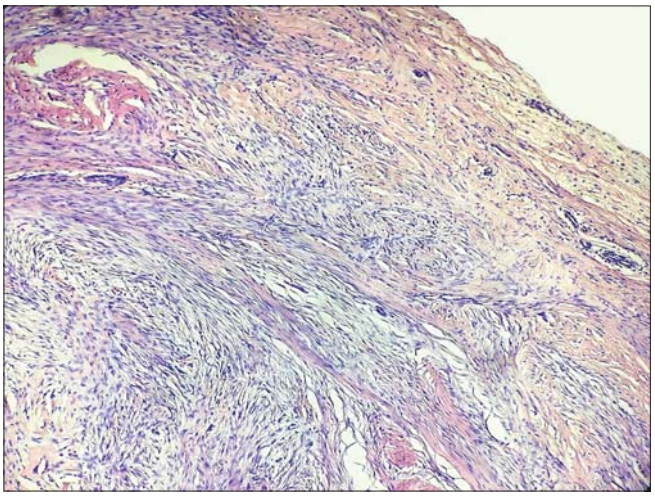

Figure 2: Interlacing bundles of elongated tumor cells separated by variable amount of collagen. (H&E, 100x).

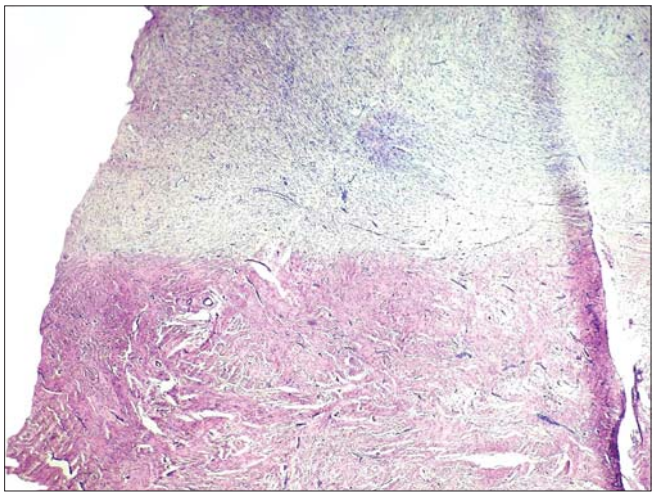

Figure 1: Tumor tissue (upper part) was tightly fixed with the aponeurosis (lower part) (H&E, 40x).

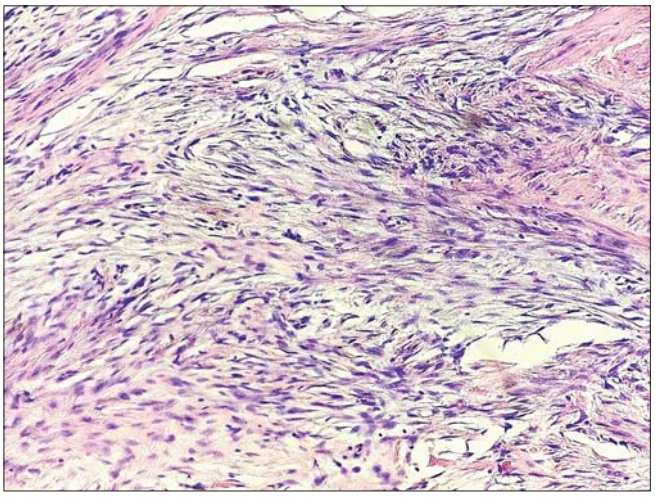

Figure 3: High power view of tumor cells with uniform features and no mitosis. (H&E, 100x).

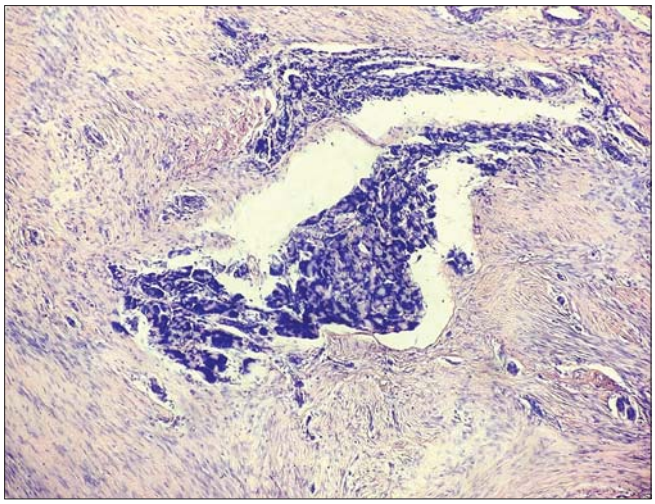

Figure 4: Degenerated muscle giant cells entrapped within the tumor tissue (H&E, 100x).

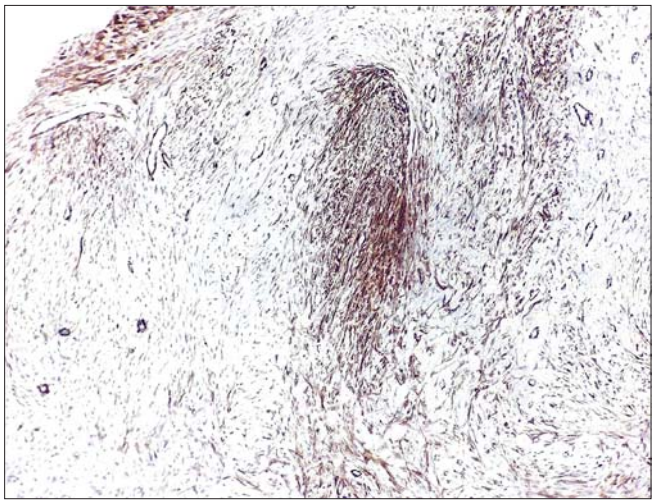

Figure 5: Cytoplasmic positivity of tumor cells for alpha-smooth muscle actin. (clone 1A4, 40x).

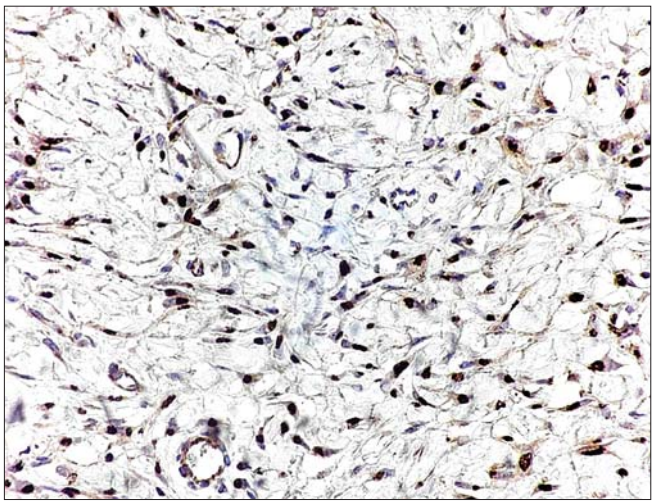

Figure 6: Nuclear positivity of tumor cells for beta-catenin. (clone 15B8, 200x).

Table 1: The anatomical location-based classification of fibromatosis. (from ref. 1)

A. Superficial (fascial) fibromatoses
Palmar fibromatosis (Dupuytren's disease)
Plantar fibromatosis (Ledderhose's disease)
Penile fibromatosis (Peyronie's disease)
Knuckle pads
B. Deep (musculoaponeurotic) fibromatoses
Extra-abdominal fibromatosis (extraabdominal desmoid)
Abdominal fibromatosis (abdominal desmoid)
Intraabdominal fibromatosis (intraabdominal desmoid)
-pelvic fibromatosis
-mesenteric fibromatosis
-mesenteric fibromatosis in Gardner syndrome

inhabitants [3,6]. Most DTs arise sporadically, whereas a minor proportion (ca. 8%) is associated with familiar adenomatosis polyposis/Gardner syndrome [3,7]. Mutations in either the APC (adenomatous polyposis coli) or beta-catenin genes are likely to be a major driving force in the formation of DTs and progression of disease [6]. DTs may be found at virtually any body site. In a study conducted by Zreik et al. [7], among 165 individuals with deep fibromatosis, extraabdominal tumors were the most frequent (68%) followed by abdominal wall (16%). The anatomic distribution and gender predominance of the lesions are age-related. In children, DTs occur with equal frequency in men and women, and most are extra-abdominal. Individuals between puberty and the age of 40 tend to be female, and the abdominal wall is the favoured site of involvement. Later in adulthood, these tumors are equally distributed between extra-abdominal and abdominal locations and develop equally in both genders [2]. The clinical course of DTs is quite variable [6]. Although unable to metastasize, these highly infiltrative and locally destructive lesions have a significant tendency to recur, with recurrence rates ranging from 20 to 39% [6].

As it has been demonstrated in the present case, desmoid-type fibromatosis usually consists of the fascicles with slender, spindled cells of uniform appearance set in a collagenous stroma [1,2]. However, the histopathologic pictures may vary between individual lesions. Zreik et al. have recognized seven distinct morphologic patterns: conventional, hypocellular/hyalinized, staghorn vessel, myxoid, keloidal, nodular fasciitis-like, and hypercellular. In their study [7], the mean number of patterns per case was two, with some lesions exhibiting up to five patterns. Of note, abdominal and extraabdominal tumors had a significantly higher percentage of the conventional pattern compared with intraabdominal tumors. Conversely, intraabdominal lesions had a significantly

higher percentage of the keloidal. Awareness of the spectrum of histologic patterns is essential to prevent misdiagnosis, especially in small biopsy samples with limited tissue volume. In challenging cases, immunohistochemistry and molecular analysis may help to clarify the diagnosis [7]. In desmoid tumors, the cells stain variably for muscle specific actin and smooth muscle actin, and are typically negative for desmin, h-caldesmon and S100 protein [2]. Of importance, virtually all deep fibromatoses have somatic beta -catenin or adenomatous polyposis coli (APC) gene mutations leading to intranuclear accumulation of beta-catenin [8]. As a result, the vast majority (80-100%) of them show nuclear positivity for beta-catenin detected by immunohistochemistry [8-10]. In 2005, Bhattacharya et al. [8] investigated 21 deep fibromatosis cases with monoclonal beta-catenin antibody and compared with a plethora of other mesenchymal lesions of similar histomorphology, such as low-grade fibromyxoid sarcoma, leiomyosarcoma, various other fibrosarcoma variants, myofibroma/myofibromatosis, nodular fasciitis, and scars. While all examples of deep fibromatosis displayed nuclear beta-catenin staining, all other lesions lacked it, showing only cytoplasmic accumulation. They concluded [8] that beta-catenin immunohistochemistry could separate deep fibromatosis from entities in the differential diagnosis. However, this somewhat contradicts with the study of Carlson and Fletcher [9], who detected nuclear beta-catenin immunopositivity in 80% of cases of sporadic deep fibromatosis, but also in a lower percentage of superficial fibromatoses, low-grade myofibroblastic sarcomas, solitary fibrous tumors, infantile fibrosarcomas, desmoplastic fibrosarcomas, and gastrointestinal stromal tumors. They stated [9], that nuclear staining for beta-catenin was supportive, but not definitive for the diagnosis of desmoid fibromatosis.

Since beta-catenin signalling pathways plays a key role in the pathogenesis of this disease, there have been attempts to disclose a potential prognostic importance of beta-catenin immunoreactivity in the tumors. However, several studies have demonstrated conflicting results until now [10-12]. In a recent paper of Korean authors [11], the 5-year progression free survival rate was 100% in the group with a high nuclear beta-catenin intensity and 62.5% in the group with a low nuclear beta-catenin intensity, although showing no significant difference. Hamada et al. [12] demonstrated that a higher nuclear expression of beta-catenin was significantly associated with a poor response to meloxicam treatment. Other investigators have not found a significant correlation between nuclear beta-catenin expression and the outcome of COX-2 inhibitor therapy [10]. To answer the question whether immunohistochemical evaluation of this marker could have a prognostic value in clinical practice might require further studies.

CONCLUSION

Desmoid-type fibromatosis is a quite rare oncologic entity. Although this tumor never metastasizes, it can lead to significant morbidity due to locally aggressive behaviour with a striking tendency to recur. Every patient once treated for deep fibromatosis requires long-term follow-up. Nuclear beta-catenin expression in tumor cells is an important immunohistochemical feature for differential diagnosis, but further research is needed to elucidate whether it may also serve as a predictive marker.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Our Dermatology Online

Case Report

Lesional IgE in flares induced by drugs in a patient with autoimmune lupus erythematosus

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ABSTRACT

Flares in systemic lupus erythematosus (SLE) may be triggered by multiple factors. Here we present a case of a flare in an SLE patient triggered by drugs. A 53 year old female with SLE presented to the dermatologist with an annular, scaly, itchy rash with vesicles on her body and with malaise, fever and joint pain. The rash started after she began receiving sulfasalazine and erythromycin for a "non-healing leg ulcer". The H&E demonstrated subcorneal blisters with neutrophils, hyperkeratosis and follicular plugging. The IHC and DIF displayed reactivity with IgE in the upper dermal vessels, and among cell junctions in the epidermis. The DIF also demonstrated staining in the blister with multiple immunoglobulins, and fibrinogen in the basement membrane. The remainder of the basement membrane zone displayed the classic lupus band. We demonstrate that the IgE deposits in skin from lupus patients with a flare occurring suddenly after receiving new medications may indicate a superimposed allergic reaction. Our case shows for the first time that IgE needs to be studied in confirmed lupus patients with a new medication associated flare.

Key words: Flares in lupus, IgE, drug induced lupus flares

Abbreviations: Systemic lupus erythematosus (SLE), hematoxylin and eosin (H&E), immunohistochemistry (IHC), basement membrane zone (BMZ) direct and indirect immunofluorescence (DIF, IIF), myeloperoxidase (MPO).

INTRODUCTION

Lupus flares may occur abruptly and without clear cause, and patients frequently notice a return of the symptoms they previously experienced. Drug-induced lupus flares are different from classic lupus erythematosus [1].

Drug-induced lupus is different from lupus flares in patients with known autoimmune lupus erythematous [2-7]. To our surprise, we searched the MeSH headings (previously similar to key words), and also specifically used search field tags (also termed qualifiers in the Pubmed data base from 1980 until today) using the terms for "cutaneous lupus flares", "markers", and/or "immune histopathologic skin lesion due to medication"; the response yielded few reports. Minimal data was available on features in the skin during flares of systemic lupus erythematosus (SLE). Here we describe a patient with chronic, controlled lupus that experienced flares concomitant with medications used for an unhealed ulcer on her leg.

CASE REPORT

We describe a 53 year old Caucasian female who presented to the dermatologist for the sudden presence of an annular, scaly, itchy eruption with erythematous plaques and some raised ridges over the entire body, especially affecting the trunk, arms, and the face. She had a history of 14 years of systemic lupus erythematosus (SLE) treated with tacrolimus 0.5% topically, prednisone 10 milligrams daily orally, Plaquenil® (hydroxychloroquine) tablets

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of 200 milligrams/day, and iron. In addition, she was taking multiple medications including carbamazepine, trazodone and triamterene-hydrochlorothiazide. Two new medications had been recently added: sulfasalazine and erythromycin for a "non-healing ulcer on her leg". Upon presentation of the eruption, some studies were performed, including a metabolic panel showing a low chloride level 96 mEq/L, (97-110 mEq/L), a lymphopenia 9.1 um^3, (17-480 um^3), a monocytopenia 2.6%, (4-10%) and a granulocytosis 88.3$10^3/mm^3$ units, (1.2-6.8 $10^3/mm^3$ units). The diagnosis of a drug induced lupus flare in a patient with classic SLE was made, and the following medications were added: Verdeso® (desonide) foam, Vanos®, (fluocinonide), Claritin® (loratadine) and Atarax® (hydroxyzine).

Skin Biopsies

Skin biopsies were taken for hematoxylin and eosin staining (H&E), direct immunofluorescence (DIF) and for immunohistochemistry (IHC) as previously described [8-11]. For the DIF we classified our findings as previously described [3-6], i.e., negative (-), weakly positive (+), positive (+++) and strongly positive (++++).

IHC

We performed IHC as previously described [8-11], utilizing multiple monoclonal and polyclonal antibodies including rabbit anti-human IgG (code AR0423), fibrinogen (code F0111), IgD (code IR517; Southern Biotechnology, Birmingham, Alabama, USA), IgE (code A0094; Vector Labs, Bellingham, Washington, USA), and myeloperoxidase, all from Dako (Carpinteria, California, USA) unless noted. We utilized the following Dako mouse anti-human monoclonal antibodies: CD8, clone C8/144B, CD31, clone JC70A, CD68, clone EBM11, and myeloperoxidase (MPO).

Statement of Ethics

Institutional Review Board (IRB) approval for a case report is not needed. However, the US Health Insurance Portability and Accountability Act of 1996 (HIPAA) Privacy Rule restricts how protected health information (individually identifiable health information) on a patient is protected. Compliance with patient privacy, institutional rules, and federal regulations were followed. No photos or illustrations that contain identifiable features are included the case report, and the case(s) described in the report are not so unique or unusual that it might be possible for others to identify the patients involved.

The H&E staining demonstrated mild epidermal hyperkeratosis with minimal follicular plugging and focal subcorneal blisters containing neutrophils and some eosinophils. No evidence of bacteria or fungi was observed (Fig. 1a). Occasional necrotic keratinocytes were also noted in the epidermis. A mild interface infiltrate of lymphocytes and histiocytes was seen in the dermis. Periadnexal infiltrates were also visualized around eccrine glands, as was a perivascular infiltrate. Small blisters were also seen in the basement membrane zone (BMZ).

On DIF examination, the corneal layer (especially in the upper side of the blister), was positive with C3C, fibrinogen IgD, IgA and IgM (+++). Deposits of IgG, IgA, IgM, and IgE were seen at the basement membrane zone (BMZ) (+) in the typical lupus band stain for SLE. IgE was seen around the upper dermal vessels and well as around cell junctions in the epidermis (+++). Fibrinogen and C3C were also positive (in a shaggy pattern at the BMZ) (+++). Cytoid bodies positive for IgG, IgM, IgE and IgA (++) were observed (Fig. 1b, black arrow; 200X and Fig. 1e, red arrows; 100X). The upper dermal vessels were positive with fibrinogen (++++), and IgD. The eccrine glands ducts were positive with fibrinogen (++++), and IgE (++).

With IHC, MPO was strongly expressed on sides of the nascent corneal blisters (+++) (Fig. 1c). Fibrinogen was positive in the corneal blister (+++), and also in both upper and lower neurovascular plexus in the dermis (+++). Using double color IHC, positive staining for IgD was observed in the corneal layer as well as in the vessels, colocalizing with CD31 in the dermal vessels (Fig. 1d). CD8 was positive in the inflammatory infiltrate around dermal vessels at several levels but more strongly around the vessels near the eccrine ducts. Fibrinogen was positive in the dermal papillae, at the adjacent BMZ as well as in the upper dermis. The vessels showed polarization towards the dermal papillae, shown using CD31 in the vessels. CD68 was mostly negative, as well as CD4. Summarizing the main findings, we noted IgA, MPO and fibrinogen deposits in the corneal blisters and IgD, IgE and fibrinogen in the dermal vessels.

DISCUSSION

Lupus flares often present when exacerbating factors occur in patients with known autoimmune

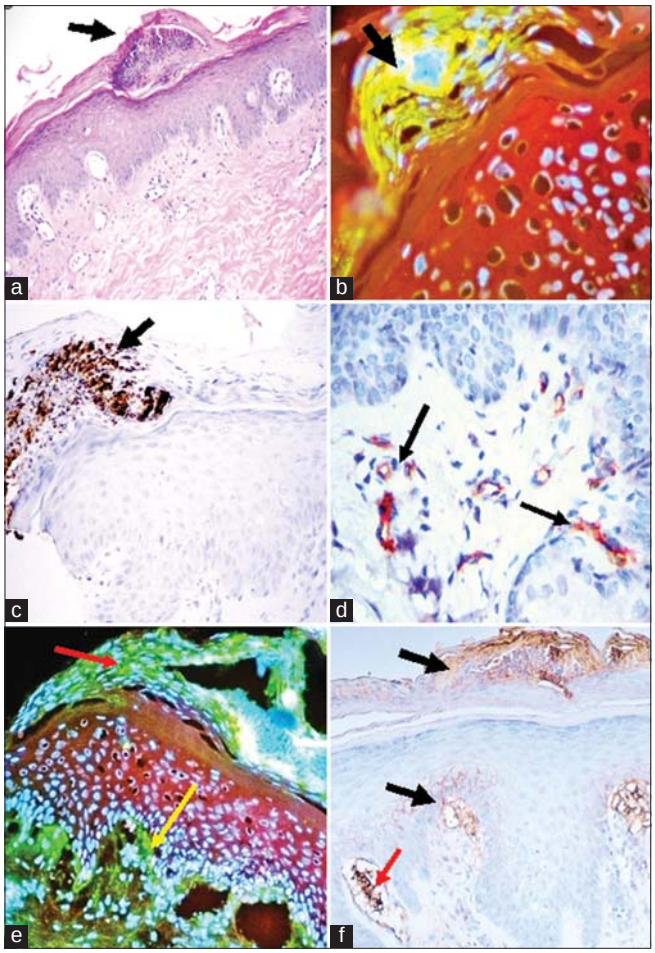

Figure 1: a. The H&E staining demonstrated mild epidermal hyperkeratosis with minimal follicular plugging and focal subcorneal blisters with luminal neutrophils (black arrow; 100X). b. The corneal layer especially on the upper side of the blister was DIF positive with C3C, fibrinogen IgD, IgA and IgM (+++) (green-yellow staining; black arrow; 400X). c. IHC showing positive staining with MPO in a subcorneal blister (brown staining; black arrow). d. IHC positive stain with IgE (red-brownish stain) in the upper dermal vessels (black arrow). e. DIF shows deposits of IgE in the upper dermal vessels as well as in epidermal cell junctions (green staining; red arrows; +++) (400X). In blue are the nuclei of the cells stain with Dapi f. Positive staining with FITC conjugated fibrinogen in the corneal layer (red arrow), as well as along the BMZ (green staining; yellow arrow; 400X)

lupus [1,8-12]. In our case, this patient was taking multiple medications and the new ones (added for her ulcer; sulfasalazine and erythromycin) are well known of being associated with lupus flares. Fortunately, neither serositis, lung and/or joint symptoms that can be often seen in lupus flares were present [1,9].

The most common known triggers for a lupus flare include ultraviolet light, sulfa drugs, which make a person more sensitive to the sun, such as: trimethoprim-sulfamethoxazole; diuretics and sun-sensitizing tetracycline drugs such as minocycline, penicillin or other antibiotic drugs such as amoxicillin and ampicillin. Other possible triggers and/or flare factors include emotional or physical trauma, weakness, viral infections, cold weather, winter low vitamin D, cigarette smoke, alcohol, occupational exposures (silica, mercury, pesticides, and solvents), hormones, pregnancy and vaccinations. Drug-induced lupus erythematosus flares usually resolve within days to months after removal of the trigger drug (s) in a patient with lupus [1,8-12]. Leg ulcers occur in systemic lupus erythematosus (SLE) due to vasculitis, antiphospholipid antibodies, and, rarely, pyoderma gangrenosum or calcinosis cutis [2,4].

In our case, the medications given for the patient's ulcer were likely the triggering factor. Of interest the presence of IgE around the vessels and in the cell junctions of the dermis are indicative of some form of allergic reaction to the new medications given to the patients. Recent studies using drug-specific immunoglobulin E (IgE) antibodies have been reported to be have a potential correlation with the putative triggering factors. The authors propose that this test could be modified and applied to assess drug-specific IgE antibodies in the event of drug-related anomalies such the one presented in our case [13].

Based on our findings, skin lesional drug induced lupus flares show differences with lupus. In lupus is common to see deposits of fibrinogen mostly at the BMZ, part of the "lupus band". Here, and in other cases [9], we also appreciated deposits of immunoglobulins, complement and MPO in the corneal layer above nascent blisters, as well as in the dermal vessels. We speculate that the epigenetic response is created by an inflammatory shift, specifically induced by the flaring agents.

We conclude that in lupus patients with drug induced flares caused by new medications, the presence of IgE in lesional skin should be added as a tool to augment the classic lupus band. As in our case, other biomarkers such other immunoglobulins, complement, fibrinogen and MPO may help to confirm the identity of the flare triggers.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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Our Dermatology Online

Case Report

Severe hypoglycemia and fainting caused by the use of levofloxacin in a nondiabetic patient

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ABSTRACT

Fluoroquinolone-type antimicrobials can cause hypo- or hyperglycemia in certain patients. We reported the case of a young male with no history of any condition and use of medication present with cellulitis. He developed hypoglycemic episodes with levofloxacin. Levofloxacin can cause new onset hypoglycemia and fainting in non-diabetic patients. This study suggests that levofloxacin can cause hypoglycemia even in nondiabetic and previously healthy patients. The seriousness of hypoglycemia and the few reported deaths also underscore the importance of early detection and appropriate management of patients with hypoglycemia.

Key words: Fluoroquinolone; Hypoglycemia; Levofloxacin

INTRODUCTION

Fluoroquinolones have been widely used for the treatment of community- and hospital-acquired infections. These drugs are known to cause glycemic disturbances mainly in patients with diabetes taking either oral hypoglycemic agents or insulin [1,2]. Gatifloxacin was banned in India on March 18th, 2011 because it poses a 17 times higher risk of developing serious hyperglycemia. Although uncommon, hypoglycemia has also been reported with fluoroquinolones. Hypoglycemia typically occurs within the first 3 days of fluoroquinolone therapy and has also been reported after the first dose of either intravenous or oral administration [1]. Most of the patients who developed hypoglycemia following fluoroquinolone use had risk factors, such as old age, diabetes, renal insufficiency, and a concomitant use of hypoglycemic drugs, especially sulfonylureas [2]. Fluoroquinolones have caused at least 67 cases of life-threatening hypoglycemic coma, including 13 deaths and permanent or disabling injuries, according to an internal safety review by the food and drug administration. Most cases (44) were associated with levofloxacin [3] and also new neuropsychiatric side-effects related to fluoroquinolones, including disturbances in attention, memory impairment, and delirium. Considering these findings, the agency will strengthen warning labels on all fluoroquinolones, especially for older people [3]. We reported a case of hypoglycemia associated with levofloxacin administration in a patient without diabetes and history of any medication therapy.

CASE REPORT

A 30-year-old male without any history of disease or use of medication came to our office with pain and swelling in his left leg (Fig. 1). MRI reported a collection of soft tissue in his left leg. He was treated empirically with a levofloxacin tablet of 500 mg once a day due to the

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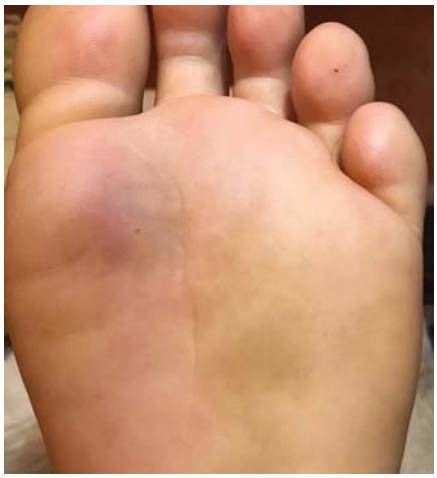

Figure 1: Cellulitis of left foot (plantar).

diagnosis of cellulitis. After the first dose of levofloxacin, he experienced weakness and dizziness, and after the second dose of levofloxacin, he unexpectedly fainted and was quickly taken to the emergency room. The blood sugar level was detected at 45 mg/dl. He received one vial of dextrose 50% in addition to the standard intravenous dose of saline serum 500 cc. Levofloxacin was discontinued after 2 days, after it was suspected to have caused hypoglycemia. After the discontinuation of levofloxacin, the symptoms of hypoglycemia did not reoccur.

DISSCUSION

Hypoglycemia is a rare but known, potentially adverse effect of fluoroquinolone therapy [4]. Published reports are available for hypoglycemia in connection with ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, and clinafloxacin [1]. Several published case reports have especially implicated levofloxacin as the causative agent of hypoglycemia, with some resulting in fatal outcomes [4]. Due to hypoglycemia having the potential to lead to serious morbidity and mortality, it is important for clinicians to recognize risk factors associated with this adverse event and increase monitoring or choose an alternative therapy when appropriate [5]. Several risk factors may predispose patients to hypoglycemia while being treated with fluoroquinolones, including diabetes, a concomitant use of sulfonylureas or insulin, renal insufficiency, and old age, which is generally defined as patients aged more than 65 [4]. However, our patients have no risk factors. To our knowledge, levofloxacin poses a higher risk of hypoglycemia compared to other fluoroquinolones. The mechanism that causes fluoroquinolones to induce hypoglycemia has not yet been fully elucidated. However, in vitro and animal model studies have provided evidence on proposed pharmacodynamic pathways that modulate insulin secretion. These studies, in addition to the known pharmacokinetic profile of levofloxacin, provide potential explanations for this clinical scenario [4]. Glucose-stimulated insulin secretion from pancreatic beta-cells is controlled by ATP-regulated potassium (KATP) channels, comprised of Kir6.2 and SUR1 subunits [6]. In vitro studies suggest that fluoroquinolones block this channel, resulting in membrane depolarization. This leads to an influx of calcium through voltage-gated calcium channels and a subsequent increase in insulin release, glucose, and hypotonicity-induced cell swelling stimulates an insulin release from pancreatic β-cells but the mechanisms are poorly understood. Recently, Piezo1 was identified as a mechanically-activated nonselective Ca2+ permeable cationic channel in a range of mammalian cells. As cell swelling-induced insulin release could occur through the stimulation of Ca2+ permeable stretch-activated channels, Deivasikamani et al showed that Piezo1 agonist channel induces insulin release from β-cell lines and mouse pancreatic islets could be suggesting a role for Piezo1 in cell swelling-induced insulin release. Hence Piezo1 agonists have the potential to be used as enhancers of insulin release [7]. These similar molecular mechanisms occur when sulfonylureas attach to the sulfonylurea receptor 1 subunit on the KATP channels. This results in the same downstream signaling, resulting in the exocytosis of insulin secretory granules via calcium signaling [8]. In conclusion, fluoroquinolones affect the function of the mitochondria in pancreatic beta cells which may diminish the insulinotropic effect of KATP channel closure and contribute to the hypoglycemic episodes [9].

CONCLUSIONS

This study suggests that levofloxacin can cause hypoglycemia even in nondiabetic and previously healthy patients. The seriousness of hypoglycemia and the few reported deaths also underscore the importance of early detection and appropriate management of patients with hypoglycemia. Taking into consideration the risk of hypoglycemia and other previously identified neuropsychiatric adverse effects [10], clinicians should avoid prescribing fluoroquinolones to patients who have other treatment options for acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, and uncomplicated

urinary tract infections because, for these patients, the risks outweigh the benefits [11].

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Our Dermatology Online

Case Report

Bazex syndrome in a patient with pulmonary adenocarcinoma

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ABSTRACT

The paraneoplastic acrokeratosis or Bazex syndrome is a rare paraneoplastic dermatosis generally associated with malignancy, most often with squamous cell carcinoma of the upper aerodigestive tract, characterized by acral psoriasiform lesions. Amelioration of the eruption usually requires treatment of the underlying malignancy. We report the case of a Moroccan woman followed up for pulmonary adenocarcinoma who received treatment based on palliative chemotherapy; she later developed a cutaneous eruption. The clinical, biological and histological elements were in favor of paraneoplastic dermatosis (Bazex syndrome). Paraneoplastic acrokeratosis has been traditionally described as a paraneoplastic entity mainly associated with malignant tumor. Treatment of the underlying malignancy is the basis for managing paraneoplastic Bazex syndrome.

Key words: Bazex syndrome; Paraneoplastic syndrome; Squamous cell carcinomas

INTRODUCTION

Paraneoplastic acrokeratosis (Bazex syndrome), described by Bazex in 1965, is a rare paraneoplastic condition. It is strongly associated to various internal malignancies, including squamous cell carcinoma of the upper aerodigestive tract, but also in a number of other tumors. In this report, we describe a rare case of a 63-year-old Moroccan female who developed Bazex syndrome a year after being diagnosed with lung adenocarcinoma with cerebral metastasis.

CASE REPORT

A 63-year-old woman, was diagnosed with lung adenocarcinoma in May 2018. She first underwent radiation therapy (10 cycles received). The follow up imaging revealed cerebral metastasis of her lung adenocarcinoma. The patient then received palliative chemotherapy (carboplatin and paclitaxel). After her third cure of chemotherapy, she developed erythematous lesions on the hands and face.

On physical examination, the patient presented a symmetric non-pruritic erythematous and keratotic erythematous plaque with fine borders covered by fine scales. The lesions were located on her face (cheeks and nose), helices of her ears and acral areas (fingers and toes). There was no megacapillary on periungual dermoscopy (Figs. 1 and 2).

The main differential diagnosis consisted of mixed connective tissue disease, systemic lupus erythematosus, dermatomyositis, hand-feet syndrome related to chemotherapy and Bazex syndrome.

Skin biopsy histology and direct immunofluorescent were not significative as well as for biological investigations for differential diagnosis. Hence, the diagnosis of acrokeratosis paraneoplastica (Bazex syndrome) was established. We prescribed topical corticosteroids with a slight regression of symptoms.

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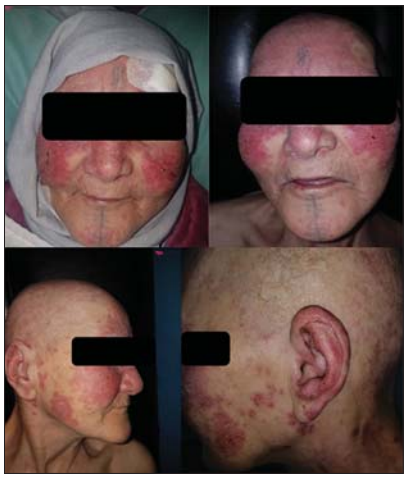

Figure 1: Lesions erythematous and keratotic erythematous plaque with fine borders covered by fine scales, located on face (cheeks, nose and ears).

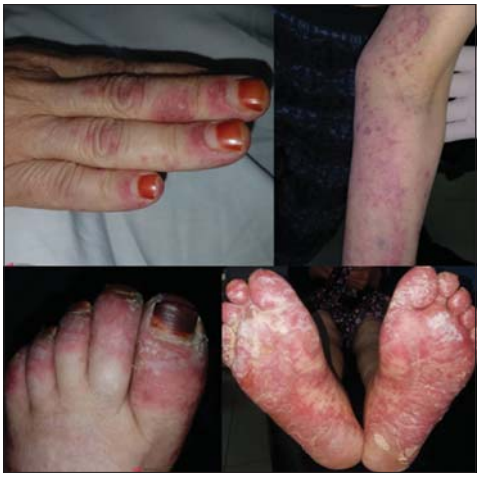

Figure 2: Lesions erythematous and keratotic erythematous plaque with fine borders covered by fine scales, located on the acral areas.

DISCUSSION

Bazex syndrome is a rare paraneoplastic skin condition, which is most prevalent with squamous cell carcinomas of the upper aerodigestive tract [1]. In a systematic review of the literature that identified 77 patients with Bazex syndrome, the underlying neoplasms were squamous cell carcinoma of the head and neck in 39 percent and squamous cell carcinoma of the lung in 11 percent, followed by gastrointestinal adenocarcinoma, genitourinary tumors, lymphomas and lung adenocarcinoma in 3.5 percent [2]. A review of the literature by Antonio Crucitti et al. showed that of 143 cases, only 5 had Bazex syndrome with pulmonary adenocarcinoma [3], a 6th case has been reported in the literature by Jing Zhao in 2016 [4].

In other studies reported by Rodrigues et al., this disease rarely affects female patients, with only 16 occurrences in the largest case series up to date. Cases accompanying lung adenocarcinoma in female patients as reported here by our patient are extremely rare, the first case of a female patient with pulmonary adenocarcinoma was reported in 1989 [5].

Typical clinical manifestations of Bazex syndrome include psoriasiform eruptions that favor the acral such as the fingers (61%), toes (39%) and nose (63%) with tenderness but no pruritus in most cases [4]. This condition normally progresses in 3 stages: cutaneous lesions on the ears, fingers, nails and palmoplantar keratosis. Remarkably, the underlying malignancy tends to display its symptoms during the stage of palmoplantar keratosis. The biological, histological and immunological findings of Bazex syndrome are unspecific, as in this patient. The underlying mechanism of this paraneoplastic syndrome in uncertain. A mixed reaction between specific types of tumours and cutaneous antigens may be responsible. The growth factors released by highly proliferative cancer cells and neoplasm associated with zinc deficiency might be implicated in the occurrence of this cutaneous paraneoplastic disorder [6].

In most patients, cutaneous manifestations of Bazex syndrome precede the diagnosis of malignancy by several months. However, the malignancy is diagnosed concurrently or before Bazex syndrome manifestations in approximately one-third of cases, as in our patient [7].

The lesions of Bazex syndrome are frequently resistant to targeted therapies but treatment of the neoplasm usually leads to resolution to the cutaneous findings. In our patient, we noticed a minor regression of cutaneous symptoms after chemotherapy. However, there are reports of patients in whom the cutaneous manifestations persist following successful therapy.

CONCLUSION

Our case demonstrates the rare occurrence of Bazex syndrome in a female patient diagnosed while being treated for lung adenocarcinoma with brain metastasis. Treatment of the underlying malignancy is the basis for managing paraneoplastic Bazex syndrome.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Our Dermatology Online

Case Report

A case of verrucous carcinoma of the back in a 17-year-old immunocompetent

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ABSTRACT

Verrucous carcinoma (CV) occurring in the oral cavity and in the genital area is induced by HPV. Our study describes a rare case of cutaneous CV manifested by a botiomyoma in a 17-year-old boy. Having no particular history, he consulted in dermatology at the Dupuytren Hospital in Limoges, for a tumor in the form of a nodular protrusion of angiomatous color in the lower back. Tumor biopsy and histological analysis revealed verrucous carcinoma. Our observation shows two originalities. The age of our patient is well below the average described by the literature as well as the unusual location of lesions. Verrucous carcinoma is a low-grade malignancy associated with HPV that can occur at any age and can be localized on all integuments.

Key words: Verrucous carcinoma; Adolescence; Back

INTRODUCTION

Verrucous carcinoma (VC) is a variant of well-differentiated squamous cell carcinoma and is considered to be associated with human papillomavirus (HPV) infection. Most VCs occur in the oral cavity, genital area or plantar surface [1,2]. VCs are rarely detected in the trunk area; the incidence of dermal VC from sites other than the inguinal region, buccal and leg is unknown. Surgical resection is the preferred treatment strategy for VC patients [1]. The present study describes a rare case of cutaneous VC of the back manifesting as a botiomyoma in a 17-year-old boy.

CASE REPORT

A 17-year-old sexually active heterosexual, nonsmoking, phototype IV male with no particular antecedent was sent to a dermatology consultation at Dupuytren Hospital in Limoges in November 2016 with a skin tumor in the lower back (Fig. 1). The patient reported that the lesion had appeared a month earlier with a painless papule and had gradually increased in size. On examination the tumor was in the form of a nodular lesion, budding angiomatous color, bleeding in places and measuring 6 cm in diameter. Anamnesis and clinical examination did not reveal suspicious lesions of papillomatosis or other changes. We discussed botriomycoma. Excisional biopsy of the lesion was performed, the histological examination of which showed a squamous mucosa with a papillomatous, acanthotic, hyperplastic, hyperkeratotic center with invagination of the keratin producing horny cysts. The dermis facing is very inflammatory seat of a dense infiltrate rich in lymphoplasmocytes, histiocytes and granulocytes. The epithelial basement membrane is nibbled by the inflammatory infiltrate and there are some casings of squamous cells isolated within the chorion, without basement membrane (Fig. 2). All these histological elements have concluded to warty squamous cell carcinoma. Complete patient assessment with abdominal ultrasound and MRI for abdominal cysts was negative. Serologies for hepatitis, HPV and HIV were also negative. The same is true for

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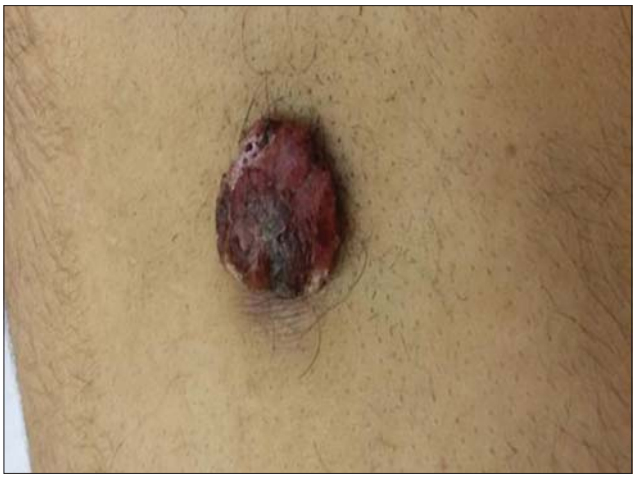

Figure 1: Lesion of verrucous carcinomas simulating botriomyoma.

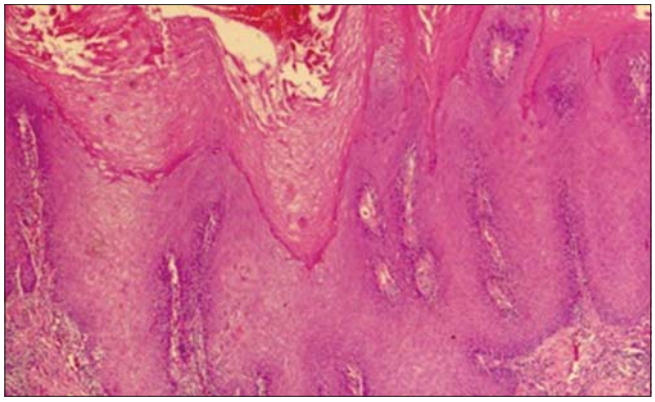

Figure 2: Histological picture of verrucous carcinoma.

immunohistochemistry. The operative follow-up was uneventful and the patient healed well. Progress after 3 months postoperatively was without recurrence.

DISCUSSION

This observation draws its originality from two facts: occurrence in adolescence and localization on the back. Warty squamous cell carcinoma usually occurs in adulthood. Several authors report an age range between 63 and 91 years with a median age of 70 [2-5]. On the other hand, some authors have indicated 43 and 47 years in their case reports [1,6]. Our patient was only 17; such a young age is not reported in the literature. Male predominance has been found in the literature. According to the series of Terada [3] a verrucous lesion was observed in (70%) men and (30%) females. Similarly Kumar [2] mentioned a male predominance (94.7%). Our patient was male. In the majority of cases, the localization of verrucous carcinoma was the oral cavity, the genital area and the foot [1]. Some authors have described atypical localizations such as the neck, the armpits, the thigh and the buttocks in the fribro-epithelial polyp form [6-8]. Also, Terada [3] described 5 cases of VC of the skin in Japan. The sites were the hand, lip, face and foot sole. In our case, the lesion is located in the back. In addition, our patient underwent surgical resection without recurrence in a follow-up of three months. Surgical excision is the treatment of choice for small and well-defined lesions [1,3,7]. The effectiveness of several therapeutic is associated with the inclusion of radiotherapy, laser, immunotherapy, photodynamic therapy have been described in the literature [2,5]. The prognosis for recurrence of this tumor is controversial. According to Penera's review of literature [1] recurrence occurred between 4 months and 3.5 years and also depended on the types of therapeutic measures used.

CONCLUSION

This study presents a rare case of cutaneous VC simulating a botriomycome appearing in the back, an unusual localization for this type of tumor and an exceptional finding in a young man of 17 years. Unlike VC lesions that occur at more common sites of development and in the elderly, HPV infection has not been identified in this case.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Our Dermatology Online

Opinion Article

Sunscreen: "Do-It-Yourself" (DIY) does not mean enough protection

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ABSTRACT

Skin cancer can affect anyone regardless of their gender, race, or age. It is estimated that every 1 in 5 Americans will develop skin cancer in their lifetime. In May 2019, the journal Health Communication published a study on Pinterest pins tagged with the labels "homemade sunscreen" and "natural sunscreen." The second most common ingredient listed was essential oils (48.7%), with the most recommended being raspberry and lavender. Kaur and Saraf (2010) explain that the SPF of volatile herbal essential oils varies between 1 and 7, while that of non-volatile essential oils varies between 2 and 8. Therefore, DIY sunscreen based on essential oils does not offer enough sun protection and dermatologists should be concerned about such influence of social media on skincare trends. In addition, family doctors, dermatologists, and the relevant healthcare providers should be prepared and open to talk to their patients about sun safety and address their concerns regarding sun protection, as this can help reduce the risk of skin cancer in the long run.

Key words: Essential oils; Sunscreening agents; Sun protection factors

Skin cancer can affect anyone regardless of their gender, race, or age. It is estimated that every 1 in 5 Americans will develop skin cancer in their lifetime [1]. Exposure of skin to UVB light can cause DNA damage, leading to the destruction of keratinocytes and malignant transformation. If the damaged cells are affected less severely, cell-cycle progression can be halted and nucleotide excision repair (NER) can begin to undo the DNA damage. Cells that were not irreversibly damaged and can be successfully repaired will survive. However, keratinocytes will undergo apoptosis and produce "sunburn cells" if no appropriate repair process can take place successfully [2].

According to the American Academy of Dermatology (AAD), everyone needs to use sunscreen for protection against the excessive harmful ultraviolet radiation (UVR) of the sun. Sunscreen options include creams, gels, lotions, ointments, sprays, and wax sticks, and are categorized into chemical and physical sunscreens. Chemical sunscreens absorb sunrays and normally contain oxybenzone, avobenzone, octocrylene, homosalate, octinoxate, and/or octisalate. Physical sunscreens deflect sunrays and contain zinc oxide and/or titanium dioxide [1]. According to the journal Scholars Academic Journal of Pharmacy, recipes for homemade and DIY sunscreen include all-natural ingredients, such as aloe vera, tomato, pomegranate, and cucumber, but also oily ingredients such as shea butter, soybean oil, evening primrose oil, almond oil, jojoba oil, and carrot seed oil [3].

In May 2019, the journal Health Communication published a study on Pinterest pins tagged with the labels "homemade sunscreen" and "natural sunscreen." The second most common ingredient listed was essential oils (48.7%), with the most recommended being raspberry and lavender. Researchers analyzed 189 relevant pins and discovered that 68.3% of them were homemade sunscreen recipes offering insufficient UVR protection. Claims about sun protection factor (SPF) made in 33.3% of pins ranged between 2 and 50, most frequently between 30 and 40. Additionally, 41.8% of the pins had been saved anywhere from once to more than 21,000 times, with a mean of 808.4 saves, which proves to be a substantial number. According to Dr. Lara McKenzie, the lead author of the study and principal investigator at the Centre for Injury Research and Policy at Nationwide Children's

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Hospital, many of the recipes claim SPF levels of up to 50, yet the ingredients used have not been scientifically proven to offer broad-spectrum coverage. According to Merten, who conducted the research together with Dr. Lara, the amount of mineral scientifically proven to be effective and present in DIY sunscreens—for instance, zinc oxide—might not be sufficient and may not be mixed properly [3]. Furthermore, Kaur and Saraf (2010) mention that the SPF of volatile herbal essential oils varies between 1 and 7. These include olive oil, coconut oil, castor oil, almond oil, mustard oil, chaulmoogra oil, and sesame oil. On the other hand, the SPF of nonvolatile essential oils varies between 2 and 8. Examples include peppermint oil, tulsi oil, lemongrass oil, lavender oil, orange oil, lemon oil, tea tree oil, eucalyptus oil, and rose oil [4].

The U.S. Food and Drug Administration (FDA) recommends sunscreen with an SPF of at least 15. On February 21, 2019, the FDA proposed sunscreen regulation changes. The two main ingredients of sunscreen—i.e., zinc oxide and titanium dioxide—which are "generally recognized as safe and effective" (GRASE) are proposed to be effective and safe for sunscreen use. A sunscreen would also require an SPF of 15 or higher to be labeled as broad-spectrum. As the SPF increases, the broad-spectrum protection increases as well. Broad-spectrum sunscreen is important to provide protection from both UVA and UVB light. The AAD highly recommends the use of broad-spectrum, SPF-30, water-resistant sunscreen. In short, there are recognized public health benefits of sunscreen use [1]. Even though essential oils are known to possess some level of SPF inherently, it is exceptionally low and, thus, they do not meet the minimum recommended by the FDA or ADD and do not provide adequate UVR protection. The FDA mentions that sunscreens lacking an SPF of at least 15 or not being broad-spectrum are proven to only prevent sunburn, but can, nonetheless, increase the risk of skin cancer and early skin aging [1]. Research has found that essential oils may have medical properties—remedial, antimicrobial, anti-inflammatory—but they have also been found, among other things, to be toxic or lead to photosensitivity [5].

The rise of DIY sunscreen could be due to the misconceptions or misinformation surrounding sunscreen. According to the Journal of Clinical Pharmacy and Therapeutics, the state of Hawaii will be banning two major ingredients of sunscreen—oxybenzone and octinoxate—from January 1, 2021. This is due to the direct and indirect association of sunscreen with coral toxicity, which was supported by mechanism studies and concentration estimates [6]. The AAD verified that the FDA did not mention that many sunscreen ingredients are unsafe. The proposed rule by the FDA is for manufactures to provide more data regarding the safety of certain sunscreen ingredients. This is to know and understand the effects of sunscreen on the skin and body as well as the degree of absorbance of sunscreen ingredients. Two of the ingredients proposed by the FDA that are not GRASE are tolamine salicylate and PABA. However, in the U.S., these two ingredients are not legally sold in sunscreens. The FDA is also asking for more safety data regarding 12 ingredients: ensulizole, cinoxate, homosalate, dioxybenzone, meradimate, octisalate, padimate O, octinoxate, sulisobenzone, octocrylene, oxybenzone, and avobenzone. However, the FDA did not warn or ask to abandon the use of sunscreens with these ingredients. Research on four sunscreen ingredients conducted by the FDA concluded that more safety data is necessary due to their absorption in the body. The research did not determine the effects of the sunscreens on a person's health but concluded that more research needs to be conducted to understand the possible risks and dangers [1].

In conclusion, DIY essential-oil sunscreen does not offer enough sun protection and dermatologists should be concerned about the influence of social media on skincare trends. In addition, family doctors, dermatologists, and the relevant healthcare providers should be prepared and open to talk to patients about sun safety and address their concerns regarding sun protection, as this can help reduce the risk of skin cancer in the long run.

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Our Dermatology Online

Review Article

Association of NLRP1 and NLRP3 gene polymorphism with psoriasis

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ABSTRACT

Psoriasis is a global health problem and one of the most frequent chronic, relapsing inflammatory skin diseases. With a prevalence of 1–3% worldwide, it is typically observed in patients 20–30 years old, with a second peak occurring at 55 to 60. Its pathogenesis is unclear but there is strong genetic influence associated. Several genes have been found to be linked with an altered immune system resulting in the development of psoriasis. This literature review is aimed at the current knowledge and the role of inflammasomes in psoriasis with regard to the NLRP1 and NLRP3 gene polymorphism associated with the pathogenesis of the disease.

Key words: NLRP1; NLRP3; psoriasis; gene polymorphism

INTRODUCTION

With a prevalence of 1-3% worldwide, psoriasis is one of the most common, inflammatory cutaneous diseases in genetically predisposed individuals [1]. It is a noncontagious, relapsing condition usually affecting the elbows, knees, lumbosacral areas, intergluteal clefts, and glans penis. Triggered by various factors, such as infections and the environment, psoriasis also affects the joints in 10–30% of patients. The disease has a characteristic bi-modal peak and presents itself between the ages of 20 and 30 and between 55 and 60. Males and females are affected at equal rates with not much variation seen in the prevalence of psoriasis [2]. Although psoriasis affects all races, it has a higher prevalence in Caucasians (2.5%) than in African Americans (1.3%). Psoriasis is a debilitating condition affecting the quality of life even in its early stages and, if present along with other comorbidities, can lead to death [3]. Around 15–20% of patients suffer from moderate to severe cases of the disease and require intensive and vigorous treatment plans that negatively impact their lives. Psoriasis not only presents itself with skin and joint manifestations but is increasingly seen in conjunction with other systems such as cardiovascular disease, chronic obstructive pulmonary disease (COPD), and even gastrointestinal disease. Additionally, due to the social stigma associated with the appearance of psoriatic patients, depression is frequently diagnosed together with unhealthy habits, such as excessive smoking and alcohol intake [4]. Due to these multiple comorbidities, there is a 50% increased risk of mortality among patients with severe cases of the disease as compared to patients with milder psoriasis [3].

Clinically, psoriasis vulgaris can easily be diagnosed by observing the skin lesions with the naked eye. The characteristic erythematous and scaly plaques found over the extensor surfaces on the arms and legs help in distinguishing psoriasis from other scaly, cutaneous diseases. Another important finding is the changes observed in nails usually accompanying skin lesions. The Auspitz's sign—a common indicator appearing as numerous fine bleeding points after the removal of psoriatic scales—can further aid in the differentiation of psoriasis from other scaly-plaque diseases. However, if clinical doubt arises, a biopsy specimen can be taken

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from the site of the lesion and a histological diagnosis can be made.

Prior to the commencement of treatment, it is essential to categorize patients according to the severity of their lesions as this helps provide them with appropriate treatment. To this end, several scoring systems have been employed based on factors such as the extent and location of lesions, the severity of inflammation, treatment responsiveness, and the impact on the quality of life. One example, and probably the gold standard in clinical trials, is the Psoriasis Area and Severity Index (PASI) score [5] invented by Fredriksson and Pettersson in 1978. According to the European S3 Guidelines on the systemic treatment of psoriasis vulgaris, a PASI score of more than 10 is interpreted as a moderate to severe case of psoriasis [6]. However, systemic treatment may also be provided in lower PASI scores if accompanied by visibly affected sites and severe symptoms such as itching. Other scoring systems include the body surface area (BSA) and the Physician Global Assessment (PGA).

With regards to treatment, patients with mild cases of psoriasis are usually administered topical measures. These have fewer side effects and are the most favorable. Depending on severity, patients can be given systemic treatment with steroids and/or biologics along with phototherapy. Topical corticosteroids, with their anti-inflammatory properties and suppression of the immune system, are commonly prescribed and highly favored for use in mild to moderate psoriasis [7]. These are usually prescribed for short-term treatment to control flares. Other topical medications include vitamin A derivatives, such as retinoids, that help control the inflammation. Oral preparations are also available; however, they are accompanied by side effects such as hair loss and birth defects. Vitamin D analogues and calcineurin inhibitors are also commonly prescribed. Moisturizers and creams may help alleviate the symptoms of itching and dryness.

Light therapy or phototherapy can be used alone or in combination with other medications. This involves use of artificial ultraviolet A (UVA) or ultraviolet B (UVB) light. In spite of the potential side effects, such as premature skin aging and the development of skin cancer, phototherapy is still considered one of the safest therapeutic measures for moderate to severe cases of psoriasis. If the skin lesions are of increased severity or even if mild but resistant and with symptoms that do not lessen with topical treatment and phototherapy, systemic drugs—oral or injected—can be considered; these include methotrexate, retinoids, cyclosporine, apremilast, and biologic agents. Due to their strong side effects of renal damage, hepatotoxicity, marrow suppression, and birth defects, caution needs to be exercised in the administration of these drugs and the patient's blood parameters need to be monitored regularly.

Pathogenesis

The etiopathogenesis of psoriasis has been studied for decades. From altered cell cycles and dysfunctional keratinocytes to T-cell involvement, much progress has been made to identify the components responsible for this inflammatory, cutaneous condition. An imbalance has been found between innate and adaptive immunity with subsequent infiltration of leukocytes into psoriatic skin lesions. T cells, constantly stimulated by keratinocytes, and members of the innate immune system play a major role in inflammation accompanying psoriasis [8]. Antigen-presenting cells such as dendritic cells (myeloid and plasmacytoid DCs), Langerhans' cells, neutrophils, macrophages, and cytokines produced by Th1-type cells all contribute to the pathogenesis of psoriasis. The role of IL-23/Th-17 cell lineage likewise proves to have prime importance. Other factors, such as vascular endothelial growth factor (VEGF) and keratinocyte growth factor (KGF), contribute to the angiogenesis and dyskeratosis commonly seen in psoriasis.

Besides the immune system, genetic, epigenetic, and environmental factors, such infection, trauma, obesity, and vitamin D3 deficiency have also been found to contribute to the presentation of the disease, although the exact mechanism is still not fully understood [9,10].

Genetics in psoriasis

Psoriasis can be linked to multiple genes associated with linkage disequilibrium in genetically susceptible individuals. Over the past four decades, with the help of genome-wide association studies (GWASs) with large cohorts, much light has been shed on the role of genetics in the pathogenesis of psoriasis. Besides GWA studies, population-based epidemiological studies, association studies with human leukocyte antigens (HLAs), genome-wide linkage scans, and candidate-gene studies within and outside the major histocompatibility complex (MHC) region have also generated much information to establish

a definitive genetic background of psoriasis [11]. Psoriasis is associated with familial recurrence, and disease concordance is higher in monozygotic than in dizygotic twins [12]. Through genetic studies, more than 60 psoriasis susceptibility loci have been discovered, with PSORS1—located in the major histocompatibility complex (MHC, chromosome 6p21.3)—being the major susceptibility locus for psoriasis vulgaris (PV) [13]. The nine different regions of Psoriasis Susceptibility (PSORS) 1–9 are linked with disease susceptibility [14]. Several HLA alleles have been linked with psoriasis, particularly, HLA-B13, HLA-B37, HLA-B46, HLA-B57, HLA-Cw1, HLA-Cw6, HLA-DR7, and HLA-DQ9 [10]. Many of these alleles are in linkage disequilibrium with HLA-Cw6, which has demonstrated the highest relative risk of psoriasis in Caucasian populations [11]. Besides the MHC, other genes have been studied to determine their role; these include ERAP1, IL12Bp40, IL23Ap19, IL4, IL13, and TNFAIP3 [15]. The genes that have been studied so far and whose role is well established in the pathogenesis of psoriasis may be grouped and related to different immune pathways. These include antigen presentation (HLA-C and ERAP1), innate antiviral signaling (IFIH1, DDX58, TYK2, RNF114), innate immunity (NF-κB), and, being of particular importance, Th17 cell activation [13,16].

Environmental factors

Psoriasis may be triggered by exogenous or endogenous environmental stimuli. These include group A streptococcal pharyngitis, viremia, allergic drug reactions, withdrawal of systemic corticosteroids, local trauma (Köbner phenomenon), and emotional stress [16]. A correlation has been found between streptococcal throat infections and guttate psoriasis, the former preceding any outbreak of the disease [9,17]. In one study [18], 17% of psoriatic patients with newly-developed or aggravated symptoms were associated with staphylococcal super-antigens. Drugs can also trigger the presentation of psoriatic symptoms; those most commonly triggering include lithium, beta-blockers, antimalarials, non-steroidal anti-inflammatory drugs, and tetracyclines [16].

Immunological interactions

In the last 30 years, several important subsets of immune cells have been identified and proven to play a role in the pathogenesis not only of psoriasis but of other autoimmune diseases as well. An imbalance in the regulation of innate immunocytes mediated by antigen-presenting cells (including natural killer T lymphocytes, Langerhans' cells, and neutrophils) and acquired or adaptive immunocytes mediated by mature CD4+ and CD8+ T lymphocytes in the skin are mainly responsible for the characteristic clinical plaques and histological patterns seen in psoriasis [11]. These include Th1, Th2, T-reg, and Th17 cells and their corresponding cytokines, such as IFN-γ, TNF-α, IL-23, and IL-17 [9]. The early phase of psoriatic plaques may be linked to autoinflammation due to its association with neutrophils and cytokines related to the interleukin-1 (IL-1) family, such as IL-1α, IL-1β, and IL-36 [19]. Hence, similarities between psoriasis and other classic autoinflammatory diseases such as pyogenic arthritis, acne, and pyoderma gangrenosum (PAPA) syndrome may be inferred because of the same involvement of cytokines and their pathogenesis resulting from mutations in similar genes that are involved in innate immunity [20,21]. The roles of different cell lineages in both innate and adaptive immunity with respect to psoriasis are described below.

a) Th1 and Th2 cells

Initially, psoriatic plaques are associated with Th1 cells and increased levels of Th1 cytokines such as c-interferon (IFN-c), tumor necrosis factor-a (TNF-a), and interleukin (IL)-12 [22]. Under such influence, dysregulated keratinocytes release a rich source of AMP and IL-1 family cytokines, including IL-1β and IL-18, which are further involved in the differentiation of Th1 and Th17 cells, respectively [23]. This may suggest a role of Th17 cells along with Th1 in the pathogenesis of psoriasis. IL-1β plays an important role in upregulating IL6, IL-8, TNF-α, and hBD2 expression, differentiation of Th17 and Th22 cells, and stimulation of IL-17 and IL-22 secretion [24].

b) Th17 cells

Th17 cells are crucial in the secretion of IL-17, IL-12, IL-22, and IL-9, all of which directly or indirectly promote the inflammatory response of keratinocytes. High levels of IL-17 mRNA have been recorded in psoriatic skin lesions, but not in non-lesional skin, indicating its possible role in the pathogenesis of the disease [16]. IL-17 also leads to an increased release of IL-6 and IL-8 in keratinocytes leading to an inflammatory condition and exaggeration of psoriatic lesions [25]. Dysregulated IL-17 signaling and Th17 cell pathway function, thus, promote the chronic inflammation in psoriasis.

c) IL-23
The role of IL-23 in the pathogenesis of psoriasis has been studied in both humans and mice. In humans, exaggerated levels of IL-23p19 and IL-12p40 (IL-12/23p40) were observed in psoriatic skin lesions, and most of the IL-23p19 was released by mature DCs, monocytes, and monocyte-derived DCs in the papillary dermis [26]. IL-23 is also essential for the development and maintenance of Th17 immune cells.

Several innate immune cells have also been linked with the pathogenesis of psoriasis. Neutrophils, found in the early phase of psoriasis, are present in the stratum corneum of psoriatic skin. Both plasmacytoid DCs and myeloid DCs are accumulated in psoriatic skin lesions and, by producing IFN-α and TNF-α, respectively, play important roles in the development of lesions [10]. Innate immunity is associated with the production of proinflammatory or primary cytokines. The most important of them are IL-1α and TNF-α.

d) TNF
Tumor necrosis factor plays a vital role in the pathogenesis of psoriasis. Besides activation of the nuclear-factor (NF-κB) signal pathway, which affects cell survival, proliferation, and the anti-apoptotic effects of lymphocytes and keratinocytes, it also causes Th17 to produce proinflammatory cytokines through the NF-κB pathway [27,28].

e) Interleukin 1
Due to their role and function in innate immunity, members of the IL-1 family constitute an integral part of the development of several inflammatory diseases. The IL-1 family is comprised of three subfamilies, including IL-1, IL-36, and IL-18 [29]. IL-1 is an essential pro-inflammatory cytokine and is strongly expressed by monocytes, tissue macrophages, and dendritic cells, and also produced by B lymphocytes, NK cells, and epithelial cells [30]. It helps regulate T helper cell polarization and the release of various pro-inflammatory cytokines that engage in apoptosis/pyroptosis, resulting in tissue damage. IL-1α (IL-1F1) and IL-1β (IL-1F2) are the two members of the IL-1 family that were discovered first. The secretion of IL-1β is mediated by various stimuli, such as microbial pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns or endogenous signals, e.g., ATP and uric acid crystals. These stimuli result in the activation of specific inflammasomes such as NLRP3 inflammasomes, which further activate Caspase-1, which, in turn, cleaves pro-IL-1β to produce mature IL-1β [31].

ROLE OF IL-1 IN PSORIASIS

Clinical findings show that the IL-17/IL-1 axis plays an important role in the pathogenesis of psoriasis [30,32]. IL-1 cytokines have been reported to be highly expressed in psoriatic skin lesions, leading to Th17 cell development and a subsequent release of IL-17 [33-35]. IL-1β, together with IL-23 and IL-6, plays a major role in the differentiation of human naïve T cells into IL-17 producing cells [31]. IL-17 further continues the cascade by causing keratinocyte activation and a further release of IL-1–family members. This enhances inflammation and exaggerated immune response, which may lead to psoriatic skin lesions. After the treatment of skin lesions in psoriasis patients, the levels of IL-1β substantially decrease. This may also suggest the role of the IL-1 family in the inflammatory changes exhibited by psoriatic patients [36,37].

NLRP GENE

The NLRP gene is linked with the production of the members of the nucleotide-binding domain and leucine-rich repeat containing (NLR) family of proteins. These NLR proteins are important mediators of the immune system and are mainly found in white blood cells and in cartilage-forming cells. These proteins become activated after being exposed to injury, toxins, or foreign particles, and help in the formation of multimeric protein complexes called inflammasomes that take part in and regulate inflammatory processes [38].

The two most extensively studied NLRP genes are the NLRP1 NLRP3 genes. The NLRP1 gene is located at position 13.2 of the short (p) arm of chromosome 17 (17p13.2) while the NLRP3 gene is located at position 44 of the long (q) arm of chromosome 1 (1q44). The latter specifically provides instructions for making a protein called cryopyrin, one of the members of the NLR protein family that is necessary for the formation of inflammasomes.

What is an inflammasome?
Inflammasomes, first described in 2002, are cytosolic multimeric protein complexes that form an integral part of the innate immune system and are responsible for the activation of inflammatory responses via IL-1b and IL-18 [39]. Excessive inflammasome activation can result in an immunological imbalance, thus causing various autoimmune and metabolic disorders. Hence, it is important to understand the various physiological and pathological mechanisms of inflammasomes [40].

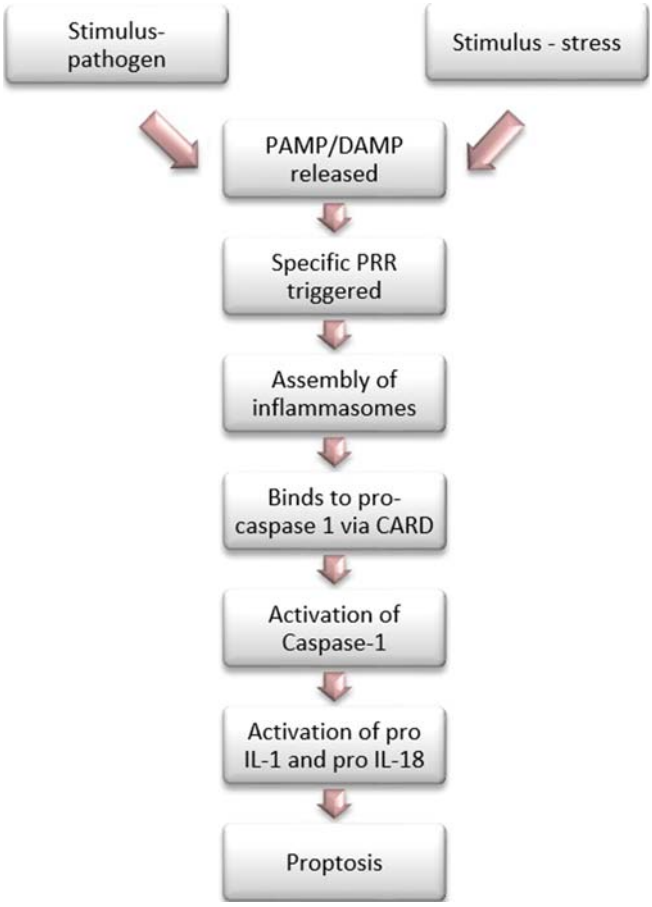

Figure 1: Mechanism of action of an inflammasome in response to both pathogenic and external stimuli.

Structure and components

An inflammasome (Fig. 1) consists of an inflammasome sensor molecule—which is a pattern recognition receptor (PRR)—an adaptor protein ASC, and caspase-1 [41]. There are three types of inflammasome sensors:

a) nucleotide-binding domain–like receptors (NLRs),
b) absent in melanoma 2–like receptors (ALR-AIM2), and
c) pyrin.

Besides a PRR (NLR, ALR, or pyrin) and an enzymatic component (caspase-1), most inflammasomes also use an adaptor molecule known as ASC (apoptosis-associated speck-like protein containing a caspase activation and recruitment domain) [40]. ASC consists of two death-fold domains, one pyrin domain, and one caspase activation and recruitment domain (CARD) [41]. With the help of a CARD, ASC is responsible for the aggregation of monomers of procaspase-1.

Mechanism of action

Depending on the stimuli, inflammasomes detect either PAMPs or DAMPs in the cytosol (Fig. 2). This

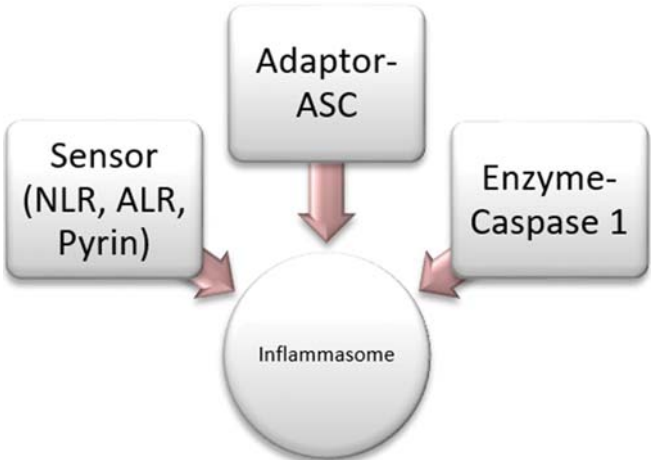

Figure 2: The components of an inflammasome.

triggers the respective inflammasome sensors and leads to the assembly of the PRRs. Proteolytic cleavage of dormant procaspase-1 into active caspase-1 takes place, which results in the conversion of cytokine precursors pro-IL-1β and pro-IL-18 into mature and biologically active IL-1β and IL-18, respectively [42]. Pyroptosis, a form of programmed pro-inflammatory cell death distinct from apoptosis, also manifests itself in inflammasome activation [43].

What are the different types of inflammasomes?

Inflammasomes are identified by the PRR linked and, to date, 6 types are known to form these immune protein complexes: NLRP1, NLRP3, NLRC4, NLRP12, pyrin, and AIM2 [44]. Other members of the NLR and PYHIN family include NLRP6, NLRP7, and IFI16 [45]. Several studies have focused on NLRP1 and NLRP3 as mediators of inflammation, which are under investigation for their possible role in autoimmune and auto-inflammatory diseases [46-48].

Diseases caused by NLRP1 mutations

An NLRP1 inflammasome is triggered by muramyl dipeptide (MDP) and anthrax lethal toxin (mouse NLRP1b) [49]. Several NLRP1 gene polymorphisms have been associated with an increased risk of autoimmune disorders and vitiligo.

A study [50] carried out in Jordanian Arab patients revealed two SNPs in the NLRP1 gene to be especially correlated with generalized vitiligo and, marginally, several other SNPs in the NLRP1 region. Another study [51], using association analysis, identified DNA sequence variants in the NLRP1 region to be associated with a risk of vitiligo and several autoimmune and autoinflammatory diseases. Sequence variants in the NLRP1 gene were

found to be in association with Addison's autoimmune disease, type-1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus [52]. Another study [53], conducted to find an association between polymorphisms in the NLRP1 gene and autoimmune thyroid disease, produced promising results. A study [54] in southern Brazil also showed an association between NLRP1-gene polymorphisms and systemic lupus erythematosus, while the same study failed to find any association between NLRP3 and SLE. Single-nucleotide polymorphisms (SNPs) in the NLRP1 and NLRP3 genes are also associated with inflammatory bowel diseases, rheumatoid arthritis, and juvenile idiopathic arthritis [55-58].

Diseases caused by NLRP3 mutations

Several infectious and endogenous ligands have been known to trigger NLRP3 and this is thought to be involved in the pathogenesis of several autoinflammatory diseases, including arthritis, gout, diabetes, obesity, and Alzheimer's disease [46,48,59]. The triggers include pathogen-derived ligands such as microbial cell wall components, nucleic acids, and pore-forming toxins; environmental crystalline pollutants such as silica, asbestos, and alum; and endogenous danger signals such as ATP, serum amyloid A, and uric acid crystals [40]. Because of the diversity in its activators, NLRP3 is very much distinct from the other inflammasomes and makes the possibility of direct interaction with each activator unlikely. NLRP3 inflammasomes, hence, may either interact with another common activator downstream of these triggers or respond to cellular stress stemming from infection or tissue damage [40].

NLRP3 inflammasomes have been associated with hereditary autoinflammatory syndromes—Cryopyrin-Associated Periodic Syndromes (CAPS)—which include:

a) familial cold autoinflammatory syndrome (FCAS),
b) Muckle–Wells syndrome (MWS), and
c) neonatal-onset multisystem inflammatory disease (NOMID).

In order of increasing severity, FCAS, MWS, and NOMID/CINCA represent the mildest, intermediate, and most severe diseases, respectively [60]. Together, they constitute a spectrum of diseases characterized by skin rashes and episodes of fever. Other clinical features include joint and ocular symptoms, amyloidosis, and, in the case of NOMID/CINCA, severe neurological complications [61].

Abnormal NLRP inflammasome activity has also been observed in diseases such as gout, pseudo-gout, silicosis, and asbestosis. Genetic mutation may not be the cause for these diseases as much as chronic exposure to inflammasome triggers such as MSU (causing gout), calcium pyrophosphate dihydrate (CPPD; responsible for pseudo-gout), and inflammation-inducing dust [62]. In one study [63] conducted in a pediatric population in northeastern Brazil, two SNPs in the NLRP3 gene were found to have a predilection for type-1 diabetes and celiac disease. Another study [64] indicated that NLRP3 rs10754558 C/G polymorphism was associated with susceptibility to SLE and with autoimmune and inflammatory diseases in Latin Americans.

Role in psoriasis

The relationship of the NLRP3 inflammasome with The relationship between NLRP3 inflammasomes and psoriasis has been investigated for its association with pro-inflammatory cytokines IL-1β and IL-18, which play a key role in many inflammatory diseases, including psoriasis [65]. In skin lesions of psoriasis patients, IL-1β has been shown to be substantially increased, and effective treatment of psoriasis to lead to a significant decrease in epidermal IL-1β expression, suggesting that the IL-1 subfamily plays a role in the pathogenesis of psoriasis [40]. In psoriatic skin lesions, caspase-1 and caspase-5 have been found to be elevated, leading to IL-1β production aided by NLRP1 and NLRP3 inflammasomes, with or without ASC, and these further prove the role of inflammasomes in psoriasis [66].

Several gene polymorphisms that have been linked with psoriasis include IL23A, IL23R, STAT3, RUNX3, and TYK2. All these genes are associated with the Th17 immune response, thus establishing the importance and role of Th17 immune response in the pathogenesis of psoriasis [67]. Moreover, a link is observed between the Th17 and IL-1b pathways. IL-1b is important for the IL-23–dependent development of Th17 cells and stimulates cytokine maturation and production in these cells [68].

In a mouse model of imiquimod-induced psoriasis, there was increased expression of the transcriptional factors pNF-kB and pSTAT-3 and the proinflammatory cytokines IL-6, IL-1β, and TNF-α I in the skin lesions. Treatment with an inflammasome blocker caused reduced expression of pNF-kB, pSTAT-3, IL-6, and TNF-α [69]. These results demonstrate that NLRP3 inflammasomes play an important role in psoriatic inflammation and that their blockade might present

a promising, novel therapeutic approach to improving psoriatic lesions.

Polymorphisms of NLRP1, NLRP3, and CARD8 were associated with susceptibility to psoriasis [68,70]. In one study conducted in Sweden [70] on 741 psoriatic patients, inflammasomes and their components were reported to play a role in the defective innate immune response and chronic inflammation of psoriasis. The NLRP3 rs10733113G genotype showed a significant p value of 0.015. Another study [71], conducted on a Chinese Han population of 540 patients, showed its significant association with genetic polymorphisms in the NLRP3 gene. Two SNPs, rs3806265 ($p = 0.0451$; OR = 0.791; 95% CI = 0.627–0.998) and rs10754557 ($p = 0.0344$; OR = 1.277; 95% CI = 0.987–1.652), exhibited strong association with psoriasis vulgaris.

However, a study [72] conducted in Denmark on 480 patients assessed 53 SNPs in 37 candidate genes and showed no significant association of psoriasis, cutaneous psoriasis, or psoriatic arthritis with NLRP1 or NLRP3 gene polymorphisms.

CONCLUSION

The pathogenesis of psoriasis is complex and can be ascribed to factors such as genetics, immunology, and environmental triggers. An imbalance between innate and adaptive immunity may be responsible for the cascade of inflammatory events observed in psoriasis. As per the available literature, the importance of inflammasomes as part of innate immunity in the pathogenesis of psoriasis, while controversial, cannot be denied. The NLRP genes that regulate inflammasomes may, in fact, have a bigger role than what is known today, and this can help in the future development of various novel therapeutic measures to control psoriatic flares. With further large-scale studies and research, more light can be shed on the perplexing pathogenesis of psoriasis and the possible role that inflammasomes play in it.

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Our Dermatology Online

Review Article

A new variant of endemic pemphigus foliaceus in Colombia South America

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ABSTRACT

A new variant of endemic pemphigus foliaceus in El Bagre and surrounding municipalities (El Bagre-EPF), also titled pemphigus Abreu-Manu, is a complex disease that has a genetic component and likely environmental triggering factors. The disease is present only in certain high-prevalence areas and affected individuals present in a unique cluster, that represent opportunities to study interactions of the environment and genetics with the immune system. The primary autoantibodies are directed to known cell junctions, but also directed to recently discovered ones that are formed via amalgamation of classic cell junctions. Besides desmoglein 1, main autoantigens are members of the p120 and plakin families, myocardial zonula adherens protein, and the armadillo repeat gene deleted in velo-cardio-facial syndrome. Other autoantibodies are directed to cell junctions in neurovascular bundles and neural receptors. A possible genetic founder effect in Indians may predispose individuals to this disease. El Bagre-EPF prevails in males after their forties and occasionally affects peri-postmenopausal females. Ultraviolet radiation is a main exacerbating factor of the disease, and seborrheic areas are most frequently affected. One third of the patients have a mild form of the disease with minimal clinical findings; one third of the patients show moderate clinical features with alterations in the skin and other organs, and one third of the patients show multiorgan systemic abnormalities, which are manifested clinically, histopathologically and immunologically. Mining, deforestation, environmental pollution and alterations in the ecosystem may partially trigger El Bagre-EPF.

Key words: Endemic pemphigus foliaceus in El Bagre (El Bagre-EPF); Autoimmune blistering diseases; Cell junctions

Abbreviations: Endemic pemphigus foliaceus (EPF), endemic pemphigus foliaceus in El Bagre, Colombia (El Bagre-EPF), fogo selvagem (FS), pemphigus foliaceus (PF), hematoxylin and eosin (H&E), direct immunofluorescence (DIF), indirect immunofluorescence (IIF), immunohistochemistry (IHC), confocal microscopy (CFM), basement membrane zone (BMZ), intercellular staining between keratinocytes (ICS), desmoglein 1 (Dsg1), myocardial zonula adherens protein (MYZAP), fluorescein isothiocyanate (FITC), kilodaltons (kDa), immunoblotting (IB), immunoprecipitation (IP), indirect immune electron microscope (IEM), complex segregation analysis (CSA), armadillo repeat gene deleted in velo-cardio-facial syndrome (ARVCF), bullous pemphigoid antigen I (BP230), desmoplakins I-II (DP I-II)

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INTRODUCTION

One disease that belongs to the autoimmune blistering diseases group [1] is endemic pemphigus foliaceus (EPF), that afflicts rural populations in tropical low-income countries, with high-prevalence areas; affected individuals present in a unique cluster. He disease provides opportunities to study interactions of the environment and genetics with the immune system [2,3]. EPF is found in relatively well-defined regions of South and Central America and Africa [4-6].

A retrospective study performed at the Hospital Universitario San Vicente de Paul in Medellin, Antioquia State, Colombia South America reported that between 1982 and 1986 21 patients were diagnosed with pemphigus foliaceus (PF) similar to the 'fogo selvagem' (FS) type [7]. The patients came from El Bagre and Nechi, rural towns in Colombia (See map upper panel, Figure 1), which were known for gold mining. The authors reported this disease as the first outbreak of South American pemphigus foliaceus (PF) reported in Colombia. The majority of the patients were mestizo men, who worked as farmers, miners or both [7]. These

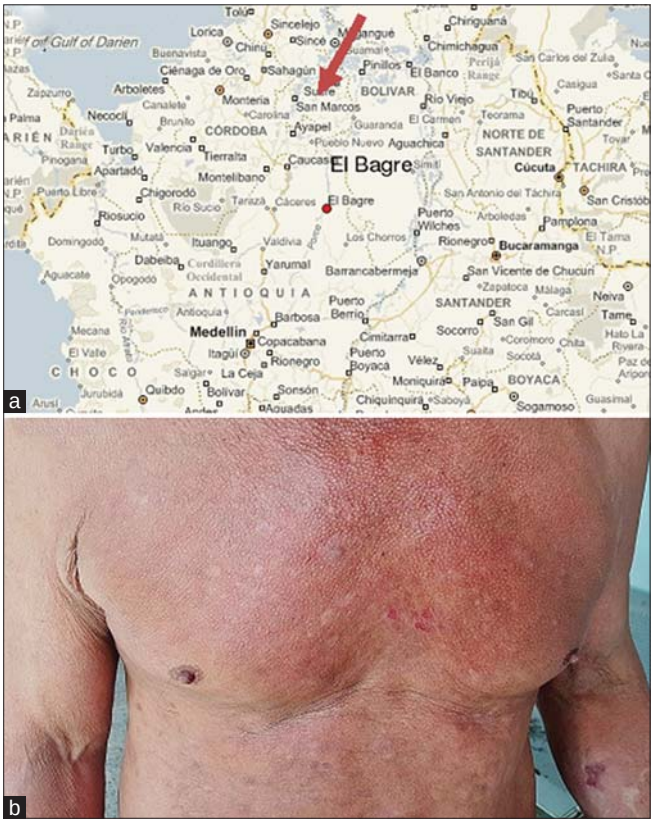

Figure 1: a) A map showing the location of El Bagre municipalities and surrounding areas (red arrow). **b)** A typical presentation of El Bagre-EPF affecting the seborrheic areas of the chest and featuring plaques, blisters, and erythema.

authors never visited the endemic area, and some of the data was retrospectively collected from the hospital files.

One of our authors has visited the endemic area since 1991 and was able to confirm that this disease was indeed a new variant of EPF in El Bagre (El Bagre-EPF). For the discovery of this new disease, we also named it pemphigus Abreu-Manu following the names of the author who discovered the disease and her daughter [8-38]. Patients affected by El Bagre-EPF live in an area rich in gold ore, and environmentally polluted with mercury. The endemic region of El Bagre-EPF is also rich in metals and metalloids [8-38]. Because of illegal mining of gold and the cocaine trade, the endemic area had suffered from ferocity, power struggles and significant fighting among the armed forces, the guerillas, paramilitary and common delinquents for over 50 years. The conditions has made our work difficult at times, but we have been respected by the polarizing groups for our humanitarian help to the patients, their families and the community.

Based on the fact that this malady highly differs from other types of pemphigus or EPF in clinical and epidemiological aspects, we further characterized this new variant of EPF as mentioned above [8-38]. EPF is characterized by the deposition of autoantibodies mainly directed to the ectodomain of desmoglein 1 (Dsg1) [2-8].

The lesions are usually blisters that easily denuded and show typical acantholysis on hematoxylin and eosin (H&E) staining [2-8]. The patient sera immunoprecipitates a 45 kDa ectodomain of Dsg1 and shows intercellular epidermal deposits of IgG4 mostly directed to the stratum granulosum of the epidermis using direct immunofluorescence (DIF) [2-8].

In 1991, we created a humanitarian volunteer program populated by medical researchers and volunteers to help the community and the patients affected by the new variant of EPF in El Bagre, Colombia [8-38]. Since then, we have had the opportunity to study a large cohort of the patients affected with this disease longitudinally for 30 years [8-38]. From the original 8 patients, we have found many other patients, many of whom had died due to complications of the disease and their precarious conditions. We found four to five new patients affected by this disease annually [8-38].

Investment in research in this disease has been extremely limited; unlike in the USA, the public

health agenda for developing countries is not well funded. In addition, in some cases, the funds need to be associated with universities and are not given to the lead authors. The mechanisms and pathogenesis of El Bagre-EPF thus remain largely unknown, which precludes the development of effective prevention and therapeutic strategies. The pathogenesis might combine genetic and environmental factors, which would explain the distribution of the disease in specific geographic hot spots [8,9]. Untreated El Bagre-EPF conveys a very poor prognosis. However, the small local hospital does not have radiology equipment, and, when we visited the endemic areas, we had to carry a portable echocardiogram. The only tests available in the local hospital are studies for malaria and some basic chemical tests, as well as complete blood count (CBC) when the proper reagents are rarely available.

With our work, we demonstrated that in about one third of the patients showed a systemic morbidity not only in the skin but also in other organs. We also demonstrated the presence of autoantibodies against multiple organs using multiple immunologic techniques and case-controlled studies [15-17,20,21].

El Bagre-EPF differs from previously described forms of EPF in several aspects. In addition, this disease shares features with paraneoplastic pemphigus in terms of heterogeneous immunoreactivity, although it is not associated with malignant tumors. El Bagre-EPF clinically simulates Senear-Usher syndrome (pemphigus erythematosus) but occurs endemically.

El Bagre-EPF occurs either as a localized form with stable clinical course, or as a systemic form [15-17,20,21]. Our work has shown that the latter systemic form seems to affect organs and show the presence of autoantibodies to the molecules of the plakin family, which include desmoplakins I, II (DP I, II), periplakin, envoplakin, the 230 kilodaltons (kDa) bullous pemphigoid antigen 1 (BP230) and others to be determined [6-8], including Dsg1. Many of these plakin family molecules are located in most organs and are involved in both inherited and autoimmune diseases that affect the skin, neuronal tissue, and cardiac and skeletal muscle. The plakin family proteins, which are known as cyto-linkers, play a crucial role in orchestrating cellular development and maintaining tissue integrity.

Patients recruited to our comprehensive studies, fulfilled the following diagnostic criteria for El Bagre-EPF: (i) the patient presented the clinical and epidemiological features described for this disease [8-10]; (ii) they lived in the endemic area [8-10]; (iii) their sera displayed staining to epidermal keratinocyte cell surfaces and to the cutaneous basement membrane zone (BMZ) by DIF using fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies to human total IgG or to IgG4, as previously described [8-10]; (iv) their sera were positive for reactivity against Dsg1 and plakin molecules, confirmed by IB performed as previously described [9-11], (v) their sera immunoprecipitated a Concanavalin A affinity-purified bovine tryptic 45 kDa fragment of Dsg1 [9-11]; and (vi) the patient sera yielded a positive result using an ELISA when screening for autoantibodies to PF antigens [12].

All our studies were approved by a human quality assurance review board at the Hospital Nuestra Señora del Carmen in El Bagre. All participants signed informed consent forms, and we conducted multiple case control matched studies comparing El Bagre-EPF patients and healthy controls from the endemic area, matched by age, gender, demographics (including history of malaria, gastrointestinal infections or sexually transmitted diseases, dengue, tuberculosis; cohabitation with domestic animals; exposure to wild animals, living and working activities, distance to rivers; tobacco, marijuana or liquor habits; exposure to agricultural and jungle vegetation; exposure to rodents, mosquitoes, and snakes and other jungle animals during rest or work hours; basic diet, and employment activities). In all our studies we evaluated the cases and controls clinically, and biopsy samples were assessed by hematoxylin and eosin (H&E) staining, by DIF, immunohistochemistry (IHC), confocal microscopy (CFM), ELISA, IB and immunoprecipitation (IP), and indirect inmunoelectron microscopy (IEM), as previously described [8-15]. For DIF, biopsies were taken from perilesional skin on the chest, and control biopsies were also obtained from normal skin on the chest. Indirect immunofluorescence (IIF) used the skin samples obtained from cadaver donors with a proper institutional review board permit.

Epidemiological and Phenotypic Expression

Our work revealed that El Bagre-EPF is endemic in rural areas surrounding El Bagre and surrounding municipalities [8-11]. The disease appeared in 4.7% of middle-aged and older men and postmenopausal women from these rural areas. The long-term outcome from medical charts for the patients in advanced stages is very poor, with 75% mortality at 2 years [8-11]. The phenotypic

expression is variable due to the stages at which the patients were diagnosed. Treatment usually requires systemic corticosteroids, which were administrated according to the patient's weight. Clinically, the disease can manifest with several clinical forms, ranging from a fruste form, pigmentary form, ichthyosis-like form, and generalized form [8-17]. In general, the nature of this disease is chronic, the patients may present episodic relapses, and generalized form exhibits poor prognosis in comparison with the localized form [8-17]. The implementation of a program design only with local treatment decreased the morbidity and mortality significantly at the hospitals in the endemic areas.

Clinical Forms, Relapses, and Cure

El Bagre-EPF differs from previously described forms of EPF. El Bagre-EPF is a chronic inflammatory disease that has unique manifestations, including a fruste form (localized to the skin and resembling Senear-Usher syndrome (see Figure 1 lower panel), a combination of pemphigus and lupus erythematosus with photosensitivity). El Bagre-EPF also presents with relapsing episodes. Both a chronic form and a systemic form exist, and the latter affects multiple organs with a less favorable prognosis [5,6,12-19]. The disease prevalence is 4.7% of middle-aged and older men and postmenopausal women. The endemic focus has not changed during the 30 years we have been visiting the geographic area [8-10]. At this point in our understanding of El Bagre-EPF, we are uncertain why some patients present a stable localized form in the skin, and others present a systemic form with relapses [8-10]. In addition, a few patients may enter clinical remission for years.

Histopathology

Histopathological examination of El Bagre-EPF patient's specimens following H&E staining establishes several patterns depending on the clinical form and the severity of the disease. By H&E, the blisters at the subcorneal epidermis are most commonly observed, although intra-spinous and subepidermal blisters were seen in some cases. Our results presented very diverse histopathologic patterns in non-glabrous skin, which seemed to associate with the clinical features [14]. The most common pattern was typical PF-like pattern, with some lupus erythematosus-like features. A non-specific, chronic dermatitis pattern prevailed in the clinically controlled patients taking daily corticosteroids. In the patients who had the most severe and relapsing disease, early sclerodermatous changes prevailed in their reticular dermis. In addition to the sclerodermoid changes, the reticular dermis showed a paucity of appendageal structures. The skin biopsies of the palms displayed a similar pattern in all cases, including thickening of the stratum corneum, hypergranulosis, edema in the papillary and reticular dermis and a dermal perivascular lymphocytic infiltrate [14].

Importance of the Autoantibodies

Human autoantibodies in autoimmune diseases of the skin and other organs have been instrumental in the identification of important new cell junction molecules and their function in physiologic conditions.

AUTOANTIGENS

Ectodomain of Dsg1

We obtained additional information about this disease by focusing on the characterization of some autoantigen profiles, using a case-control study with sera samples obtained from patients and the controls from the endemic area [10,11]. We were able to resolve the identity of the most significant antigenic moiety, a 45 kDa tryptic fragment which is recognized by all sera from patients with FS, PF and El Bagre-EPF patients, as well as half of PV sera [8-11]. We documented a conformational epitope on the 45 kDa peptide obtained by trypsin digestion of viable bovine epidermis, and glycosylated peptides were partially purified on a concanavalin A (Con-A) affinity column. This column fraction was then used as an antigen source for additional immunoaffinity purification [11]. A PF patient's serum covalently coupled to a *Staphylococcus aureus* protein A column was incubated with the Con-A eluted products and the immuno-isolated antigen was separated by SDS-PAGE, transferred to a membrane, and visualized with Coomassie blue, silver and amido black stains. The 45 kDa band was subjected to amino acid sequence analysis revealing the sequence, EXIKFAAAXREGED, which matched the mature form of the extracellular domain of bovine Dsg1. Our discovery established the biological importance of the ectodomain of Dsg1, as well as the relevance of conformational epitopes in various types of pemphigus [11].

Other Autoantigens

We compared the El Bagre-EPF and the Brazilian-EPF FS by means of IB and baculovirus expression

systems. By IB using normal human epidermal extracts, 38% of El Bagre-EPF sera and 25% Brazilian EPF sera showed IgG antibodies reactive to Dsg1. The sera of both types of EPF also showed protein bands co-migrating with plakin family proteins, particularly periplakin [15-21]. IB analyses also showed positive reactivity of El Bagre-EPF sera with recombinant proteins of various domains of envoplakin, periplakin and bullous pemphigoid antigen I (BP230) [8-10]. These finding indicate that a considerable number of El Bagre-EPF sera reacted with recombinant proteins of periplakin, while only few FS sera reacted with some of the recombinant proteins of any plakins. Enzyme-linked immunosorbent assays (ELISAs) for Dsg1 and Dsg3 showed that Dsg1 was reacted by almost all sera of both types of EPF [8-10]. However, unexpectedly, while none of El Bagre-EPF sera reacted with Dsg3, about half of FS sera reacted with Dsg3. Thus, we conclude that the El Bagre-EPF is basically similar to FS in that the major antigen is Dsg1, but there are significantly different antigen profiles between these two types of EPF [8-10].

We continued the characterization of other El Bagre-EPF antigens and found p0071, armadillo repeat gene deleted in velo-cardio-facial syndrome (ARVCF), plus BP230, periplakin, envoplakin, DP I-II (the 5 plakin family proteins) and myocardial zonula adherens protein, (MYZAP) as possible autoantigens. Other antigens need to further determined. Of interest, these molecules are present not only in the desmosomes but also in other multiple cell-junctions, including gap, tight and adherens junctions, as well as BMZs in several different tissues. Our discoveries present a molecular penetrance paradigm, because most of these molecules are ubiquitously present in the majority of organs [15-20].

Based on these findings, we had been continuously describing several systemic anomalies in the patients affected by El-Bagre-EPF, including alterations in their eyes, sudden death syndrome, kidney problems and others systemic anomalies. Indeed, before the steroid era, the systemic mortality associated with this disease was huge and the patient necropsies showed multiple anomalies [15-20]. We also focused on characterizing other antigens for protein bands, which were seen in the IB studies using different techniques, including radio-immune assay (RIA), enhanced chemiluminescence (ECL) and IP as well as sequential solubilization techniques, using several antigen sources (e.g.: human skin, MCF-12 mammary epithelial cell line (ATCC), cow snouts, rat, mice, veal, and other animal and human antigen sources [15-21].

Autoantibodies in the El Bagre-EPF Relatives

Susceptible individuals living in geographically defined rural areas develop the disease. In addition, their genetic relatives have increased levels of autoantibodies to EPF antigens, compared with controls from the endemic area. In the patient's relatives, these autoantibodies do not seem to be pathogenic [8,9]. Upon leaving the endemic area, patients exhibit a milder clinical outcome or possibly a "clinical cure". However, in most cases the autoantibodies are still detected in their sera [8,9].

Development of a Novel ELISA

We also established a cost-effective ELISA capable of distinguishing the heterogeneous antibody population observed in these El Bagre-EPF patients to confirm patients for epidemiological studies. We used the protein extract obtained from trypsin-digested fresh bovine cow snouts and further purified on a Concanavalin A (Con A) matrix as an antigen [13]. The inclusive sensitivity and specificity of the assay were resolute to be 95% and 72%, respectively, with reproducibility's of 98% (intra-assay) and 95% (inter-assay), permitting testing of multiple serum samples; the assay correlated well with the clinical activity and extent of disease in patients with El Bagre EPF [13].

Cutaneous Test

Atopy is a common cause of an increase in serum IgE levels. We tested for a cutaneous response to different allergens by injecting allergen samples of *Dermatophagoides (D. pteronyssinus* and *D. farinae)*. Approximately 20 to 30 *ul* of these agents (Abello ALK, Madrid) at a concentration of one biologic unit were applied intradermally (1 UI/ml I.D.) in the lateral area of the arm, using an insulin syringe. Positive (histamine chlorhydrate 1:10,000) and negative (saline solution) controls were also used. A positive result was indicated by erythema with a diameter of greater than 5 mm and/or edema. Only one El Bagre-EPF patient was positive for IgE for *D. pteronyssinus* and *D. farinae*, although no statistically significant difference was detected [8,9].

DIF and IIF

DIF for skin biopsies taken from the glabrous and the non-glabrous skin also showed some

differences [8-20]. IIF with the use of human skin sections showed IgG anti-keratinocyte cell surface antibodies. In all, of 120 El Bagre-EPF patients whose skin biopsies were taken from perilesional areas, DIF showed positive results. Furthermore, 70% of the patient sera showed IgG anti–basement membrane zone (BMZ) antibodies by IIF. IIF with monoclonal antibodies to human IgG subclasses showed that 80% of El Bagre-EPF showed IgG4 anti-cell surface antibodies [8,9]. In addition, 22% of sera showed IgG1 anti–cell surface antibodies, but no sera showed either IgG2 or IgG3 antibodies. Some sera also showed IgG4 antibodies to the BMZ.

By DIF of biopsy skin, 90% of the El Bagre-EPF sera tested showed reinforcement of reactivity with the BMZ, resembling in several cases the pattern observed in lupus; mostly depositions of C3c, C1q, IgG, IgM, Kappa light chains, Lambda light chains, fibrinogen and albumin (90%), as well as positive reactivity for anti-human IgG3 mAb. On IIF using rat and human skin and anti-human IgA mAb, cell surface staining was observed mainly in the upper layers of the epidermal keratinocytes.

By IIF, positive staining was observed on rat smooth muscle. Positive staining for IgA, IgG and IgM was also detected in the papillary dermis [8-20]. The basal epithelial layer of the rat bladder was also positive IgA and IgM. These findings were observed in patients with the most active disease. The staining observed in these cases differs from that observed in paraneoplastic pemphigus. Presence of immune staining on cardiac tissue was also evident, mainly located at the intercalary level. We also detected immunoreactivity on gastric and intestine tissues from rat was present in the El Bagre-EPF sera. IIF using IgG2 mAb and human skin revealed immune staining against the intermediate filaments, similar to that produced with mAb directed against keratins. IIF using IgG, IgM and IgA mAbs and rat bladder shows immune staining, mainly in the basal layer of the epithelia. IIF on rat stomach using IgG, IgM, and IgA showed goblet cell staining. The antibodies displayed staining between the gastric glands, similar to that produced on smooth muscle. The pattern suggested that these antibodies were directed against endomysial proteins and reticulins 1 and/or 2. We also showed in this study that the immune response is polyclonal in patients affected by El Bagre-EPF, as previously described [8-20]. We concluded that most of El Bagre-EPF patients have autoantibodies to known and many unknown cell junctions, not only in the skin but also other organs. In summary, the autoimmune response is polyclonal in nature.

Most Common Histopathological Alterations in the Lesional Skin

We had performed a case-control matched study comparing the most frequent histopathologic patterns in non-glabrous skin and in glabrous skin observed and their clinical correlation in El Bagre-EPF. The study was performed on non-glabrous skin biopsies of 100 patients from the dominant clinically affected areas (either on the chest, arms or face). Simultaneously, biopsies from the palms were obtained in 10 randomly chosen patients of the 100 total patients [14]. The specimens were examined by H&E staining. The blisters most commonly observed in the subcorneal areas of the epidermis, although in some cases intra-spinous and subepidermal blisters were visualized. Our results showed a very heterogeneous histopathologic patterns in non-glabrous skin, which seemed to correlate with the clinical features. We conclude that the histopathologic features of this new variant of EPF are complex.

Subclinical Oral Involvement in Patients with El Bagre-EPF

We performed a case-control study for oral changes in 45 patients affected by El Bagre-EPF and 45 controls from the endemic area matched by demographics, oral hygiene habits, comorbidities, smoking habits, place of residence, age, sex, and work activity [21]. Oral biopsies were taken and evaluated via H&E, DIF, IIF, CFM, microarray staining and IHC [21]. We found radicular pieces and loss of teeth in in 43/45 El Bagre-EPF patients and 20 of the 45 controls ($p < 0.001$; confidence interval [CI] 98%). H&E staining showed that 23/45 El Bagre-EPF patients had corneal/subcorneal blistering and lymphohistiocytic infiltrates under the BMZ and around the salivary glands, the periodontal ligament, and the neurovascular bundles in all cell junction structures in the oral cavity; these findings were not seen in the controls ($p < 0.001$) (CI 98%) [21]. The DIF, IIF CFM, and microarray staining displayed autoantibodies to the salivary glands, including their serous acini and the excretory duct cell junctions, the periodontal ligament, the neurovascular bundles and their cell junctions, striated muscle and their cell junctions, neuroreceptors, and connective tissue cell junctions. The autoantibodies were polyclonal. IgA autoantibodies reactive with neuroreceptors in the glands were positive in 41/45 patients and in 3/45

controls. We concluded that the patients affected by El Bagre-EPF have some oral anomalies and an oral immune response, primarily to cell junctions. The intrinsic oral mucosal immune system, including IgA and secretory IgA, may play an important role in this autoimmunity. Our data contradicts the hypothesis that PF does not affects the oral mucosa due to the Dsg1 compensation theory [21].

Palm Tissue Displaying A Polyclonal Autoimmune Response in El Bagre-EPF Patients

On physical examination, the palms and soles of El Bagre-EPF patients revealed an edematous texture and mild hyperkeratosis, in comparison with the non-glabrous skin of the patients where blisters, pustules or other lesions are commonly found. Based on the preceding observation, we tested the palms of 20 El Bagre-EPF cases and 20 controls from the endemic area for any pathological alterations in the samples by DIF for palm skin biopsies [22]. Our DIF demonstrated pathological deposits of fibrinogen and albumin, as well as IgG, IgA, IgM, IgD and C3c at the epidermal basement membrane zone, around isolated areas in the epidermis, within the dermal vessels and nerves, and in areas surrounding dermal neurovascular structures and sweat glands [22]. Specific markers for blood vessels, including intercellular adhesion molecule 1 (ICAM-1/CD54) and junctional adhesion molecule (JAM-A), as well as specific markers for nerves, including glial fibrillary acidic protein (GFAP), and human neuron specific enolase (NSE) co-localized with the patients' autoantibodies. Although no blisters, ulcerations, pustules or erosions were clinically observed on the palms of El Bagre-EPF patients, our DIF detected distinct immunoreactivity in palm tissue [22]. These alterations may contribute to the clinically edematous texture of the palms and the mild clinical hyperkeratosis found in most of these patients. We proposed that normal glabrous skin and non-glabrous skin may be different with regard to the expression of selected molecules, which may vary in number, size or structural organization depending on their anatomical site. Our findings may also partially explain the hyperkeratotic palms that have been clinically well documented in the chronic phase of Brazilian FS [22].

Sweat Glands

We examined the El Bagre-EPF patients, and noted several polymorphic clinical lesions around their axillary areas. Based on our clinical and on previous histopathological studies on the skin of these patients that showed abnormalities in their sweat glands, as well as the presence of mercuric selenides and iodine by autometallographic assays, we studied the sweat glands in these patients on the palms that are rich on sweat glands. DIF, IIF, IHC and H&E stains were also done. We visualized a specific autoreactivity to sweat glands in most of the patients and, using IHC using anti-human monoclonal antibodies to CD3, CD20 and CD68, we also detected deposits around the sweat gland's indicative of specific immune responses *in situ*, and around the sweat glands. No healthy controls yielded positive findings [23]. In some chronic cases, a decrease or sometimes a complete absence of sweat glands and other skin appendices was found. In addition, sclerodermoid changes or early sclerodermatous changes sometimes extended into the adipose tissue as a membranous lipodystrophy [23]. Autoreactivity to the neurovascular components around the sweat glands were also observed. Our data demonstrate for the first time that there is immunoreactivity toward sweat glands in El Bagre-EPF patients that may destroy some of these structures [23].

Human Eyelid Meibomian Glands and Tarsal Muscle are Recognized by Autoantibodies from Patients Affected by El Bagre-EPF

We performed IIF studies using normal-appearing human eyelid skin from routine blepharoplasties as substrate tissue. We tested sera from 12 patients with El Bagre-EPF and ocular lesions, 5 patients with sporadic (nonendemic) PF, and 20 healthy control subjects (10 from the El Bagre-EPF endemic area and 10 from nonendemic areas). We used FITC-conjugated goat antiserum to human total IgG/IgA/IgM as a secondary antibody [24]. In addition, we used FITC-conjugated antibodies to human fibrinogen, albumin, IgG, IgE, C1q, and C3, Texas Red (Rockland Immunochemicals, Inc, Gilbertsville, PA), and Alexa Fluor 555, or Alexa Fluor 594(Invitrogen, Carlsbad, CA). Ki-67 (a cell proliferation marker) was used to determine the cell proliferation rate, and nuclear counterstaining was performed with either 49, 6-diamidino-2-phenylindole or Topro III (Invitrogen) [24]. We observed autoreactivity to multiple eyelid structures, including meibomian glands and tarsal muscle bundles at different levels, and some areas of the epidermis and the dermis close to the isthmus of the eyelids. Tarsal plate autoreactivity was seen in 10 of 12 of the El Bagre-EPF sera and in one control with pemphigus

erythematosus. Furthermore, IP using an eyelid sample as a substrate with 1 mmol/L of sodium orthovanodate showed autoreactivity to several antigens, including some of possible lipid origin [24].

Antibodies to Pilosebaceous Units Along their Neurovascular Supply Routes in Patients Affected by El Bagre-EPF

We tested for El Bagre-EPF patient sera for autoreactivity to pilosebaceous units by utilizing DIF, IIF, CFM, IHC and IEM [25]. H&E staining of skin biopsies revealed that one third of the patients affected by El Bagre-EPF demonstrated some histologic alteration of the pilosebaceous units [25]. By IHC, most El Bagre-EPF biopsies demonstrated evidence of an autoimmune response along the neural and vascular supply routes of the pilosebaceous units. An active immune response was seen with antibodies against human mast cell tryptase, myeloid/histoid antigen, CD8, CD20, CD68, CD117/c-kit, ZAP-70 and vimentin. IEM demonstrated autoantibodies within the hair follicle and at the basement membrane area of the sebaceous glands. El Bagre-EPF patients had autoantibodies to pilosebaceous units and to their surrounding neurovascular packages. Our results may also explain the loss of hair described in severe Brazilian FS before the therapeutic steroid era [25].

Patients with El Bagre-EPF have Autoantibodies against Arrector Pili Muscle, Colocalizing with MYZAP, p0071, Desmoplakins 1 and 2 and ARVCF

We took skin biopsies from 30 patients with El Bagre-EPF and 30 healthy controls (HCs) matched by age, sex and occupation who were all from the endemic area, and tested these using DIF, CFM and IHC tests [26]. We detected that 27/30 patients had autoantibodies to against arrector pili muscle (APM) that colocalized with commercial antibodies to MYZAP, DP I-II, plakophilin 4, and ARVCF ($P < 0.001$, Fisher's exact test). The positive staining also colocalized with JAM-A, a control antibody for gap cell junctions. No samples were positive in IHC studies.

In IB, sera of 27/30 patients that were APM-positive, also displayed colocalization with the protein bands shown by antibodies to MYZAP and ARVCF (Progen) ($p < 0.001$, Fisher's exact test). We concluded that patients affected by El Bagre-EPF have autoantibodies to APM, colocalizing with the antibodies MYZAP, ARVCF, p0071, DP I-II, suggesting that these molecules are El Bagre-EPF antigens [26]. Further, all of these antigens represent components of cell junctions, indicating that the immune response is directed, at least partially, against cell junctions. We also verified that the immune response in patients affected by El Bagre-EPF is polyclonal, and affecting to B and T lymphocytes, mast cells, IgG, IgA, IgM, IgD, IgE, fibrinogen, albumin, complement/C1q, C3c and C4 [26].

HLA-DPDQDR is Expressed in Lesional Skin from Patients Affected by El Bagre-EPF

Human genes responsible for human antigen presentation and transplant rejection functions are located on the short arm of Chromosome 6, and are called the Major Histocompatibility Complex (MHC). Moreover, the primary physiologic function of MHC molecules is to present peptides to T lymphocytes [27]. MHC molecules are integral components of the ligands that most T cells recognize, since the T cell receptor (TCR) has specificity for complexes of foreign antigenic peptides, as well as self-MHC molecules. We investigated the presence of HLA-DPDQDR within lesional skin biopsies from patients affected by El Bagre-EPF, and compared with controls from the endemic areas [27]. Among patients with El Bagre EPF, 23/30 exhibited positive staining in the upper dermal blood vessels and perivascular inflammatory infiltrates with a significance of $p<0.05$. Also, positivity was seen around the neurovascular supply structures of sebaceous glands, and around dermal blood vessels surrounding eccrine ducts. Only two controls from the endemic area displayed positive staining, specifically in some upper dermal perivascular infiltrates ($p<0.05$) [27].

Ribosomal Protein s6-ps240 is Expressed in Lesional Skin from Patients with El Bagre-EPF

In autoimmune skin blistering diseases, autoantibodies seem to trigger several intracellular signaling pathways; we investigated the presence of the phosphorylated form of ribosomal protein S6-pS240 within autoimmune skin blistering diseases biopsies. Therefore, we utilized IHC staining to evaluate the presence of S6-pS240 in lesional skin biopsies of the El Bagre-EPF patients and matched controls from the endemic area [28]. We utilized monoclonal mouse anti-human ribosomal protein antibody S6-pS240; phosphorylation site specific, clone DAK-S6-240, Dako catalog No. M7300, at a dilution of

1:50. We found that the biopsies from the diseased skin showed positive staining for S6-pS240 around lesional blisters, including adjacent areas of the epidermis; in the corneal layer, stratum granulosum, epithelial layers of hair follicles, the sebaceous glands especially at their BMZ, within upper dermal inflammatory infiltrates, and/or mesenchymal-endothelial cell junctions within the dermis. We documented that S6-pS240 is expressed in lesional areas of skin biopsies from patients with El Bagre-EPF disease, as well as on eccrine glands and piloerector muscles [28].

Langerhans Cells, S100, HAM 56, CD68 and CD1a in Lesional Skin from Patients and Controls from the Endemic Area

We noted that populations of epidermal Langerhans cells were significantly decreased in lesional skin, when compared to perilesional skin in El Bagre-EPF patients [29]. In controls from the endemic area, CD1a positive Langerhans cells were quantified as ~1-2 cells/mm^2. HAM56 antibody staining was very strongly positive in the EPF cases, especially around dermal neurovascular packages supplying sebaceous glands (a median of 15-18 cells/ mm^2), compared to normal controls (~1-2 cells/mm^2; $p = 0.001$) [29]. The HAM56 antibody was also positive in the epidermis above the blisters, and in the dermis under the blisters (1-5 cells/mm^2), in comparison to normal skin controls (~1-2 cells/mm^2). In regard to CD68 staining, it was also very strongly positive around dermal eccrine gland coils and ducts, and at the edges of the deep adipose tissue in El Bagre-EPF patients (a median of 15-18 cells/mm^2), in comparison to normal controls (~1- 2 cells/mm^2;($p <= 0.001$) [29].

Autoreactivity to Neural Receptors and Nerves in Patients Affected by El Bagre-EPF

We tested the sera of 20 El Bagre-EPF patients, 20 normal controls from the endemic area, and 20 age- and sex matched normal controls from outside the endemic area for the autoreactivity to various nerve components [15]. Both normal human skin and bovine tail were used as antigens. As a result, we detected autoreactivity to neural structures, mechanoreceptors, nerves, perineural cell layers of the arachnoid envelope around the optic nerve, brain structures, and to neuromuscular spindles; these structures colocalized with several neural markers [15]. The patient antibodies also colocalized with DP I-II, ARVCF and p0071. The study also showed autoreactivity with neurovascular bundles innervating the skin, and IEM using gold conjugated-protein A showed that patient antibodies were positive against the nerve axons [15]. Paucicellularity of the intraepidermal nerve endings and defragmentation of the neural plexus were seen in 70% of the cases and not in the controls from the endemic area ($p<0.005$). Neuropsychological and/ or behavioral symptoms were detected in individuals from the endemic area, including sensorimotor axonal neuropathy. Our findings may explain for the first time the "pose of pemphigus," representing a dorsiflexural posture seen in EPF patient's vis-a-vis the weakness of the extensor nerves, and furthermore, the autoreactivity to nerves in EPF could explain the "burning sensation" encountered in EPF disease [15].

El Bagre-EPF Patients have Autoantibodies against ARVCF

A critical cell junction protein is armadillo repeat gene deleted in velo-cardio-facial syndrome (ARVCF); we found that this molecule is expressed in the skin and colocalizes with autoantibodies of EL Bagre-EPF patients [30].

Mast Cells, Mast/stem Cell Growth Factor Receptor (C-KIT/CD117) and IgE may be Integral to the Pathogenesis of EPF

Forty-four skin biopsies from EPF patients (30 patients from El Bagre, Colombia, and 14 from the northeastern region of São Paulo State, Brazil), 48 control biopsies from Colombian and Brazilian endemic areas, and additional control biopsies from non-endemic areas in Colombia and the USA were studied. IHC was performed to evaluate skin biopsies with anti-mast cell tryptase (MCT), anti-c-kit and anti-IgE antibodies [31]. We also searched for serum IgE in 30 EPF and 30 non-atopic controls from the El Bagre region by ELISA. In our El Bagre patients and controls, we also searched for IgE in skin samples by DIF. All EPF biopsies showed MCT, c-kit and IgE expressions stronger than control biopsies, especially in the inflammatory infiltrates around upper dermal blood vessels and dermal eccrine glands. IgE staining was positive along the BMZ in some EPF skin samples [31]. Increased IgE serum levels were also noted in EPF patients relative to controls. We concluded that, in patients with EPF, the increased expression of MCT, c-kit and IgE in lesional skin is associated with higher serum IgE levels and may indicate IgE participation in the antigenic response [31]

Cyclooxygenase 2 (COX-2) in Lesional Skin in Patients affected by El Bagre-EPF; Quantitative Digital Morphometry and IHC Staining

We tested skin of 40 patients and 40 controls for the presence of COX-2 [32]. The staining intensity of the antibodies was also evaluated in a semiquantitative mode by an automated computer image analysis system, designed to quantify IHC staining in hematoxylin-counterstained histopathological sections. Slides were scanned with a ScanScope CS system, utilizing brightfield imaging. For IHC, we utilized a Dako monoclonal mouse anti-human COX-2 antibody, clone CX-294[32]. We noted that 26/30 patients with El Bagre-EPF were positive for COX-2 in the epidermis, particularly in spot areas of the corneal layers, around the neurovascular areas of eccrine and hair follicles. Only 2 controls from the endemic area showed some reactivity in the corneal layer ($p<0.05$) [32].

Proteinase and Proteinase Inhibitors in Patients and Controls

We also evaluated lesional skin of patients and matched controls from the endemic area to study for evidence of tissue damage, regeneration and/or modification using IHC. We tested lesional skin for α-1-antitrypsin, human matrix metalloproteinase 9 (MMP9), human tissue inhibitor of metalloproteinases 1 (TIMP-1), metallothionein and urokinase type plasminogen activator receptor (uPAR). uPAR and MMP9 were basically negative on lesional skin, and only 3 patients with chronic El Bagre-EPF were positive to MMP9 in proximity to telocytes in the dermis [33]. TIMP-1 and metallothionein were positive in several skin appendices in the dermal, inflamed blood vessel inflammation and dermal mesenchymal-epithelial cell junctions. All these findings led us to conclude that the immune response, at least *in situ*, seems to be more complex, and directed not only to the desmosomes or hemidesmosomes but also to the skin appendices and the junctions of the vessels and the dermal tissue [33].

C5b-9 in Skin Biopsies from Patients and Controls

We also performed a case-control study for the presence of complement/C5b-9 in lesional skin in 43 patients affected by El Bagre-EPF, as well as 43 matched healthy controls from the endemic area using IHC stains. We detected complement/C5b-9 in all cases of the patients affected by El Bagre-EPF, but not in the controls from the endemic area ($p < 0.001$) [34]. The patients' IgG and IgM autoantibody titers in IIF were correlated to the intensity of complement/C5-b9 staining ($p < 0.001$). We concluded that patients affected by El Bagre-EPF have lesional deposition of complement/C5b-9, which links with disease severity and previously established serologies [34].

Rouleaux and Autoagglutination of Erythrocytes Associated with a Pink Material Resembling Fibrin-like Aggregates in Skin Biopsies from Patients Affected by El Bagre-EPF

We documented that rouleaux and the pinkish aggregates are present in within biopsies taken from lesional skin in the majority of patients with El Bagre-EPF; we speculated that this may be as result of the exocytosis of antibodies from inflammatory cells, that form when the cells exposed to the extracellular matrix in the edematous diseased skin. In addition, red blood cells in the presence of plasma proteins or other macromolecules may form aggregates [35]. Rouleaux, a type of autoagglutination of erythrocytes, along with a pink material that resembles fibrinoid aggregates, was observed in 24/30 biopsies from El Bagre-EPF. Skin biopsies from none of the 30 controls from the endemic area showed this positivity. The rouleaux, the aggregated erythrocytes and the pink material were uniformly seen under the blisters [35].

In situ Immune Response Evaluation by IHC in Skin Biopsies from Patients Affected by El Bagre-EPF

We tested by IHC for CD4, CD8, CD19, CD20, CD45, CD56/NCAM, PAX-5, granzyme B, myeloperoxidase, neutrophil elastase, LAT and ZAP-70 in 30 patients affected by El Bagre-EPF, in 15 controls from the endemic area, and 15 biopsies from healthy controls from the USA [36]. We found a predominantly CD8 positive/CD45 positive T cell infiltrate in El Bagre-EPF. El Bagre-EPF patients biopsies displayed negative staining for CD4 and B cell markers, and natural killer cell markers were also rarely seen. ZAP-70 and LAT were frequently detected. In El Bagre-EPF, a significant fragmentation of T cells in lesional skin was noted, as well as autoreactivity to lymph nodes [36]. The documented T cell and myeloperoxidase staining are indicative of the role of T lymphocytes and neutrophils in lesional biopsies in these patients in addition to previously documented deposition of B cells, immunoglobulins

and complement *in situ*. In El Bagre-EPF, T cells could also target lymph nodes [36].

SYSTEMIC AUTOIMMUNITY IN PATIENTS AFFECTED BY EL BAGRE-EPF

Autoantibodies to Full Body Vascular Cell Junctions Colocalize with MYZAP, ARVCF, DP I-II and p0071

We investigated autoreactivity to vessels in all the organs/systems of the body. We compared 57 patients and 57 matched controls from the endemic area. We performed DIF, IIF, CFM, IHC, CFM, IEM, IB, and autometallographic studies [18]. We performed ultrasonography on large patient arteries to investigate vascular anomalies. In addition, we reviewed autopsies on seven patients who died of El Bagre-EPF. We immunoadsorbed positive vessel immunofluorescence with Dsg1 to investigate for new autoantigens. 57/57 patients affected by El Bagre-EPF displayed autoantibodies to vessels in all the organs/systems of the body by all methods ($p < 0.01$). The autoreactivity was polyclonal, and the patient's antibodies colocalized with commercial antibodies to DP I-II, p0071, ARVCF, and MYZAP (all from Progen Biotechnik, Germany; $p < 0.01$; all present at cell junctions) (Fig. 2). Immunoadsorption with Dsg1 on positive vessel immunofluorescence showed that the immune response against the vessels was directed against non-Dsg1 antigen(s). Autometallographic studies showed deposits of metals and metalloids in vessel cell junctions and in erythrocytes of 85% of patients ($p < 0.01$). We concluded that the immune response to these vascular antigens is likely altering endothelial cells and vessel shapes, thus disturbing hemodynamic flow [18]. The flow alterations likely lead to inflammation and may play a role in the atherogenesis often seen in these patients.

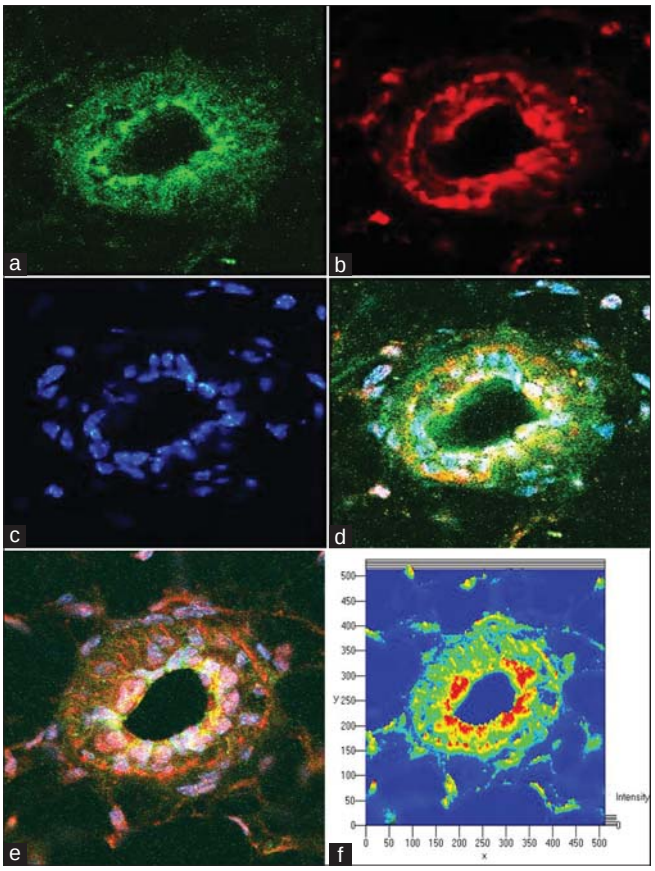

Figure 2: a) A DIF-confocal image from a large vessel from the skin of one of the patients affected by El Bagre-EPF. Positive staining in the cell junctions of the vessels using anti-human IgG-FITC conjugated (green staining, 1000X). **b)** Positive staining with ARVCF (red staining, 1000X). **c)** Nuclei of the vessels are positively counterstained with Dapi (blue staining, 1000X). **d)** Colocalization of a patient's autoantibodies with ARVCF (1000X). **e)** Colocalization of the patient's autoantibodies and the p0071 antibody (1000X) **f)** Diagram of the intensity and colocalization with El Bagre-EPF patient antibodies and p0071 and AVCF antibodies (1000X).

Cardiac Rhythm and Pacemaking Abnormalities in Patients Affected by El Bagre-EPF

We investigated rhythm disturbances with the presence of autoantibodies and correlated them with ECG changes in these patients [19]. We performed a study comparing 30 patients and 30 matched controls from the endemic area. ECG as well as DIF, IIF IHC, CFM studies focusing on cardiac node abnormalities were also studied at autopsies of 7 patients. The main ECG abnormalities seen in the El Bagre-EPF patients were sinus bradycardia (in one-half), followed by left bundle branch block, left posterior fascicular block, and left anterior fascicular block compared with the controls. One-third of the patients displayed polyclonal autoantibodies against the sinoatrial and/or AV nodes, and the His bundle correlating with rhythm anomalies was delayed in the cardiac conduction system ($p < .001$) [19]. The patient antibodies colocalized with commercial antibodies to DP I-II, p0071, ARVCF, and MYZAP (the last one from Progen Biotechnik) ($p < .01$). We concluded that one-third of the patients affected by El Bagre-EPF have rhythm abnormalities that slow the conduction of impulses in cardiac nodes and the cardiac conduction system. These abnormalities likely occur as a result of deposition of autoantibodies, complement, and other inflammatory molecules. We demonstrated for the first time that MYZAP is present in cardiac nodes [19].

Autoreactivity to the Heart, Nodes, Conducting System

For autoreactivity to heart, IIF, CFM, IEM and IB were performed utilizing heart extracts as antigens. We found that El Bagre-patients had a polyclonal immune response to cell junctions of the heart, often colocalizing with known markers [18]. These colocalizing markers included the area compositae of the heart, such as DP I-II; markers for gap junctions, such as connexin 43, markers for tight junctions, such as ezrin and junctional adhesion molecule A, and markers for adherens junctions, such as pan-cadherin. We also detected colocalization of the patient antibodies with the blood vessels and with the cardiac sarcomeres [18]. The strongest patient serum autoreactivity was observed against the transverse tubule system of the heart. Reactivity to some nerves and the Purkinje fibers was also noted. We demonstrated that El Bagre-EPF patients display autoreactivity to multiple cardiac epitopes, and, further, that the cardiac pathophysiology of this disorder warrants further evaluation [18]. We also have shown preliminary studies on kidney, and had shown also autoantibodies to the proximal and distal collecting tubes of the kidney as well as against membranes of some mitochondria and cell junctions of these organs.

Patients Affected by El Bagre-EPF have Autoantibodies Colocalizing with MYZAP, p0071, DP I-II and ARVCF, which cause Renal Damage

We detected a systemic pathologic alteration in one third of El Bagre-EPF patients. In the current study, we focused on autoreactivity to the kidney and its pathologic correlations [17]. We investigated patients with El Bagre-EPF for renal compromise. We performed a case control study with 57 patients with El Bagre- EPF and 57 matched controls from the endemic area. We took skin and renal biopsies, performed DIF, IIF IHC, CFM, IB, direct and indirect IEM, and tested kidney function in all living patients. We also used IHC to study seven kidney autopsy samples [17]. Of the 57 patients, 19 had autoantibodies to kidney, with polyclonal reactivity ($p < 0.01$). Most cases were positive along the basement membrane of the proximal tubules, but in some cases, there was also positivity against the glomeruli and/or mixed patterns. Fifteen patients had increased serum urea and creatinine compared with controls ($p < 0.01$). The autoantibodies colocalized with DP I-II, p0071, ARCVF and MYZAP ($p < 0.01$). All of the kidney disease autopsies showed pathologic alterations, mostly in the vessels. We demonstrated for the first time that one third of patients with El Bagre-EPF have polyclonal autoantibodies to kidney. The kidneys showed a mixed histopathological pattern resembling lupus nephritis, with a diffuse proliferative Class IV (G) global diffuse pattern in active lesions, and additional interposition of membranoproliferativ glomerulonephritis [17].

Patients Affected by El Bagre-EPF Exhibit Autoantibodies to Optic Nerve Sheath Envelope Cell Junctions

The majority of the patients with El Bagre EPF experienced vision problems, and we have previously reported several ocular abnormalities. To investigate reactivity to optic nerves in these patients, we utilized bovine, rat and mouse optic nerves, and performed IIF and CFM to test optical nerve autoreactivity in 45 patients and 45 matched controls [37]. Overall, 37 of the 45 patient sera reacted to the optic nerve envelope that is composed of leptomeninges, and the reactivity was polyclonal and present mostly at the cell junctions ($P < 0.001$). The immune response was directed against optic nerve sheath cell junctions and the vessels inside it, as well as other molecules inside the nerve. No controls were positive. Of interest, all the patient autoantibodies co-localized with commercial antibodies to DP I–II, MYZAP, ARVCF, and plakophilin-4 (p0071) from Progen Biotechnik ($p < 0.001$) [37]. We conclude that the majority of the patients have autoantibodies to optic nerve sheath envelope cell junctions. These antibodies also co-localize with armadillo repeat gene deleted in ARVCF, p0071 and DP I–II. The clinical significance of our findings remains unknown [37].

Environmental Factors as Putative Triggers of the Disease

The main exacerbation elements are ultraviolet radiation, extreme heat, humidity, stress and well as decreased food intake. The disease has unique autoimmune response due to environmental and genetic factors with photosensitivity [38-42]. Colombia is the fourth largest producer of gold in South America, and is among the 20 major producers in the global ranking [38-45]. The exploitation of metallic and non-metallic minerals can be carried out in soil, sub-soil, or even in riverbeds. In regard to etiologic factors, the focus of El Bagre-EPF is located in a rural mining community that is exposed to high environmental levels of minerals, mercuric sulfides/

selenides, metalloids and trace elements (e.g., quartz, rutile, granite, copper, lead, magnetite, and almenite). In the El Bagre area and near areas, municipal alterations of the environment and addition of toxic elements such mercury and cyanide are used to improve of gold extraction [38-45]. The El Bagre area is rich in elements including gold, quartz, micas (biotite and muscovite), potassium, Cu, Pb, Mn, Ni, Zn, Mo, Pu, Au, quartz, rutile, granite, magnetite, almenite, biotite, sulfurs and minerals such as pyrite, chalcopyrite and galena. El Bagre is also rich in metals, metalloids and trace elements based on geological studies that show igneous rocks of the Batholitic of Segovia (Jurassic) are in contact with shales belonging to the Cajamarca Complex (Paleozoic) [38-42]. Quartz veins with gold mineralization have been found embedded in igneous rocks, with grades up to 48 g/ton. Mining in alluvial deposits occurs and the endemic area is rich in quartz, potassium, feldspar (which gives it the pink color in some sectors) and plagioclase, what allows classifying the rock as a Gneiss Quartz-Feldspathic. Micas (biotite and muscovite), potassium feldspar sulfurs and minerals such as pyrite, chalcopyrite and galena are present, which were observed in major quantities in the cretacean sediments. The Bagre-Nechí mining district is known historically by its exploitations of gold in placer deposits associated with Neogene terraces and alluvial deposits formed by the Nechí River [38-42]. Currently, exploration campaigns are being carried out in lode gold manifestations. Two zones within the district will be referred, a northern zone, in the surroundings of the Nechí municipality, where quartz vein structures with thickness over 2m and strike persistence over 2 km, hosted by quartz-diorite intrusive rocks, migmatites and quartz-feldspar gneisses [38-42]. Towards the south zone of the district in El Bagre and Zaragoza municipalities, two vein structures are known, La Ye and El Carmen veins, characterized for presenting thicknesses between 1 and 3 m, they are hosted by intrusive rocks that locally present important igneous facies variations from diorites to granodiorites, intruded in some sectors by aphanitic dykes from basic to intermediate composition. Mineralizations are structurally controlled and this work suggests a direct association to shear zones parallel to the possible main stress responsible of the ancient dextral dynamic along Otú fault, supporting the idea that mineralization's are of the Orogenic Gold Deposit type [38-42]. The endemic area has a high-grade metamorphic rock and although at this latitude the Otú fault is covered under the hazogen sediments, the rocks on the right bank of the Cauca River in front of Nechí are tentatively attributed to the Chibcha terrain. They are characterized by the dominant presence of quartz-feldespetic gneises with hornblenda and biotite [38-42].

The endemic area is rich in gold. Mercury is commonly used to amalgamate gold. The people in this rural mining community are exposed to high environmental levels of mercury, used for gold extraction, as well as other minerals, metalloids, and trace elements (e.g., quartz, rutile, granite, magnetite, and almenite) and ultraviolet radiation (UV). Metalloids, minerals, and trace elements (e.g., quartz, rutile, granite, magnetite, and almenite) are ubiquitous in El Bagre [38-42].

Mercury has been demonstrated to trigger autoimmune phenomena in rats and mice that possess the proper genetic background.We previously examined fifty control subjects and fifty El Bagre-EPF patients in the testing for the presence of mercury in skin biopsies and hair, using autometallographic and mass spectroscopic analyses, respectively. Simultaneously, serum levels of IgE were measured using and ELISA assay, and we also tested for skin hypersensitivity reactions [43]. We used autometallography; mercuric sulfides/selenides were detected in 14 of 51 skin biopsies distributed similarly in the control and patient groups. However, significantly higher serum IgE levels and mercury concentrations in hair, urine, and nails were found in patients compared with controls. Microscopic scrutiny exposed mercuric sulfides/selenides focused within and around the sweat gland epithelium, as well as in dendritic cells [43]. Five skin biopsies from El Bagre-EPF patients and five from controls that tested positive for the presence of mercuric sulfides/selenides by autometallography were arbitrarily selected for electron microscopic (EM) analysis [42]. This analysis revealed a mixed electron-dense and electron-light material closely associated with desmosomes in patients. Moreover, there were intracellular vesicles containing an amalgam of electron-dense and electron-light materials only in the El Bagre-EPF patients. Thus, El Bagre-EPF patients are exposed to high levels of environmental mercuric sulfides/selenides and other elements [43]. We reported mercuric sulfides/selenides in skin biopsies from people living in a focus of EPF, and we believe based on our evidence, that these compounds may play a role in the pathogenesis of this autoimmune disease.

Active Mine Exploitation Represents Challenging Environments for Microorganisms

The direct consequences of mining in the endemic area create pollution in water bodies, a decrease in biologic

diversity, and health problems then emerge in the local communities. Areas with active mining represent challenging environments for microorganisms, due to their extreme conditions such as high pressure and temperature, elevated salt concentrations, a diverse range of acid and alkaline soils/water, and many others abiotic factors. However, a remarkable diversity of microorganisms has been found in these types of habitats including those associated with gold mining activity, where communities of *Phylum Proteobacteria, Firmicutes and Actinobacteria*, are predominant. Despite the fact that the most profuse organisms are bacteria, representatives of the *Archea and Eukarya* domains have also been found. The plentiful metabolic capacity of the organisms present in these endemic areas is obvious, and their capability to act as Fe, S, NH3, and CH4 oxidants and SO4 2− reducers has been proven [44]. We have to take into account that multiple microorganisms are present in the endemic area and have metabolic capacities, and we do not know if these can be part of the putative triggering factors. For example, it has been established that some of them accelerate the process of sulfur oxidation and, as such, can be used in biomining and bioleaching. Furthermore, acidophilic microorganisms have allowed the growth of different strategies for the remediation of important contamination problems in the exploitation of minerals; for example, the regulation and management of pH in the precipitation of iron in acid mine drainage. Also, microorganisms, especially bacteria, have great potential in terms of the immobilization and accumulation of heavy metals such as Cu, Pb, Cr, and Fe among others [44].

Genetic

Our studies indicate the model of inheritance in this disease to be mixed, with multifactorial effects within a recessive genotype and strong ancestral Amerindian tribes (such as the Zenues and Embera-Catios) that have historically lived in closed communities, and practiced interracial outbreeding due to the presence of guerilla, paramilitary and military warfare in the endemic area for least in the last forty years. We performed pilot genetic studies and reported that they are some possible models of inheritance in this disease using Complex Segregation Analysis (CSA) and short tandem repeats to distinguish between environmental and/or genetic factors in this illness. The CSA analysis was carried out according to the unified model, implemented using the transmission probabilities implemented in the computer program POINTER, and assessed by using a software package for population genetic data scrutiny, Arlequin [45]. We did pedigree analyses by using Cyrillic 2.1 software, with a total of 30 families with 50 probands (47 males and 3 females) tested. In parallel to the CSA, we tested for the occurrence of short tandem repeats from HLA class II, DQ alpha 1, linking the gene locus D6S291 by using the Hardy-Weinberg-Castle law. Our results show that the best model of inheritance in this disease seem to be a diverse model, with multifactorial effects within a recessive genotype [45]. Two types of conceivable segregation patterns were found; one with robust recessive penetrance in families whose phenotype is more Amerindian-like, and another of likely somatic mutations. We conclude that the penetrance of 10% or less in female patients 60 years of age or older directs that hormones could protect younger females. The highest risk factor for men being affected by the disorder was the NN genotype [45]. These findings were probably due to somatic mutations, and/or strong environmental effects. We also found a protective role for two genetic loci (D6S1019 AND D6S439) in the control group [45].

Treatment/Management

We treated the patients with topical and oral corticosteroids according to their weight, since most biologic therapies were not available due to their high costs. If the patients had a systemic disease and needed more than 40 milligrams of prednisone per day, we hospitalized the patients and added mycophenolate mofetil. We also used antiparasitic medications regularly, as well as calcium and various vitamins, including vitamin D oral supplements.

CONCLUSION

EL Bagre-EPF presents an excellent disease model to study the interactions between among environmental and genetic factors and autoimmunity, and the intricacy of the immune response in this disease is more multifaceted than considered before.

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Clinical Image

Chronic leg ulcer: a complication of sickle cell disease not to be ignored

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The presence of a chronic leg ulcer in a young person is often suggestive of an infectious or traumatic cause. However, it is also a common complication of sickle cell disease and can sometimes be its indication.

We report a case of a 31-year-old hospitalized in our department for a trailing leg ulcer evolving for 6 months and associated with SS sickle cell disease. A dermatological examination showed an ulcer in the right leg, next to the malleolar region, dragging without healing, painful, without a necrosis zone, with irregular and geographical contours. Hemoglobin electrophoresis confirmed the presence of a homozygous SS form. An infectious balance and an ultrasound have eliminated an infectious and vascular cause. A leg ulcer is a dermatological complication rarely reported in homozygous sickle cell disease. It is a consequence of cutaneous pain caused by repeated vaso-occlusive disorders (Fig. 1) [1].

The presence of trailing malleolar ulcers in a young patient should be suggestive of sickle cell disease and hemoglobin electrophoresis must be conducted. The treatment of leg ulcers in sickle cell disease remains poorly codified. The acceleration of healing rates by the addition of trinitrin during local care has been proven in the series of M. Boustani [2].

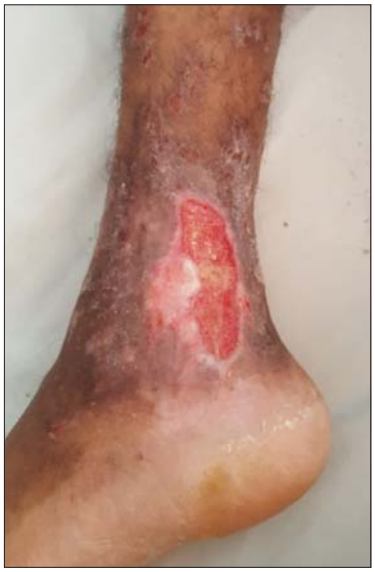

Figure 1: An ulcer in the left perimalleolar region, painful, without a necrosis zone, clean bottom, geographical and irregular contours.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Clinical Image

Subcutaneous nodules as an initial presentation of metastatic gastric carcinoma

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This 83-year-old man presented with a four month history of multiple, hard subcutaneous nodules (Fig. 1) on a background of fatigue and weight loss. He denied any gastrointestinal symptoms. His full blood count showed a haemoglobin of 8.3g/dL (normal, 13-16g/dL) and white cell count $28x10^9$/L (normal, 4-11x10^9/L). Histology from a skin biopsy showed an invasive, poorly differentiated neoplasm infiltrating the dermis but not connected to the overlying epidermis, while histology of his bone marrow also demonstrated a poorly differentiated neoplasm. The tumour cells were positive for pancytokeratin (AE1/AE13) confirming the tumour was a carcinoma and epithelial in origin. Further staining showed positivity for CDX2 (Fig. 2) suggesting a gastrointestinal origin. A gastroscopy showed a large black irregular mass in his stomach (Fig. 3). The patient died five weeks later from metastatic gastric cancer. Less than 1% of gastric cancers present with skin metastasis [1,2].

Figure 1: Multiple subcutaneous nodules.

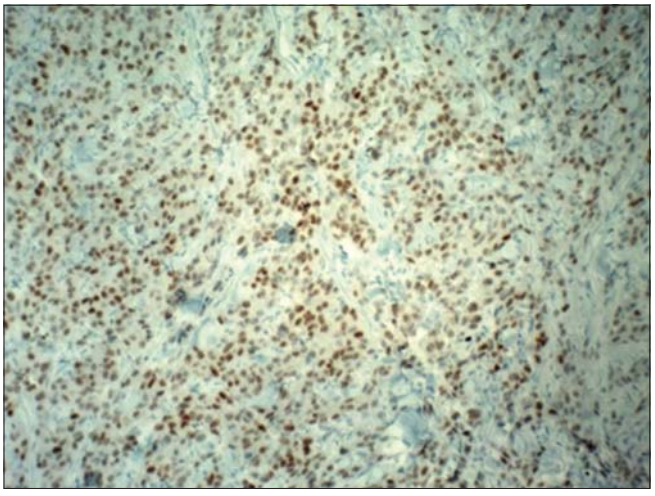

Figure 2: Tumour cells showing scattered positivity with CDX2.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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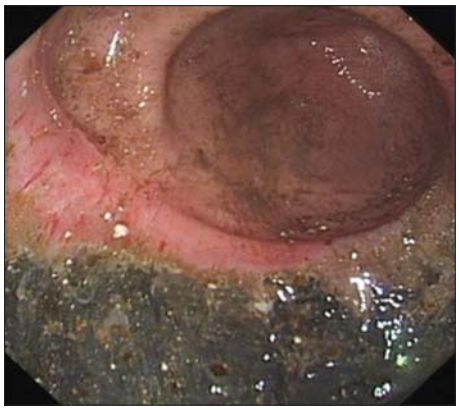

Figure 3: Gatroscopy showing gastric carcinoma.

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Clinical Image

An case report of gluteal erysipelas

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Erysipelas is an acute infection of the upper dermis layer of the skin and the most frequent infection of soft tissues caused by group A β-hemolytic streptococci or Staphylococcus aureus [1]. Gluteal erysipelas is rarely described and not well characterized [2], reported in only 0.6% of all patients with erysipelas [3].

We report a case of a 61-year-old woman with no history of pathology or major surgery. A sudden development of a large area of erythema was observed in the left buttock. After two days, the erythema had spread to the abdomen and to the other buttock.

A general examination finds an apyretic patient in good condition, with a warm erythematous and edematous left buttock and half of the right buttock, and arriving at the left flank (Figs. 1 and 2). The size of the erythematous area was approx. 25–30 cm, and the border of the erythema was slightly elevated and clearly demarcated. Observed was also disseminated intergluteal (Fig. 3), submammary, inguinal, umbilical intertrigo and satellite lymphadenopathy. The rest of the somatic examination was unremarkable.

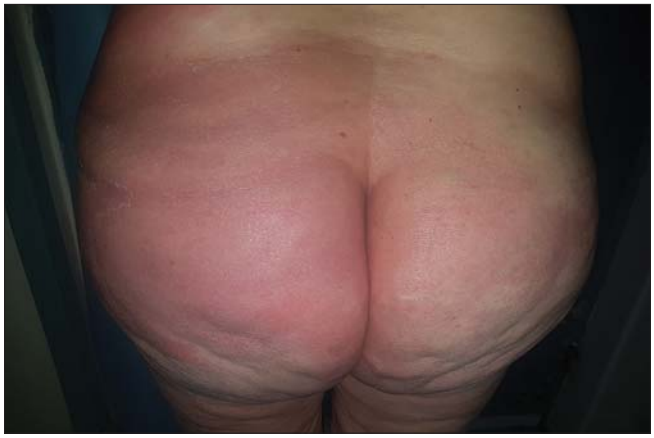

Figure 1: Warm erythematous and edematous left buttock and half of the right buttock.

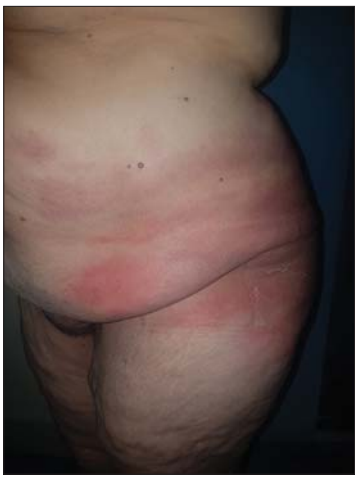

Figure 2: Erythematous edema arriving at the left flank.

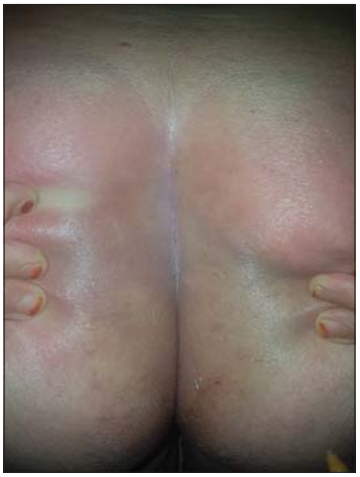

Figure 3: Intergluteal intertrigo.

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We clinically diagnosed these episodes as erysipelas because of the characteristic appearance of the erythema, even if this location, limited to the buttocks, is uncommon.

The patient's white blood cell count was 9200/mL, and her C-reactive protein concentration was 81 mg/L. The erythema disappeared after 3 days of intravenous curative antibiotic therapy continued by oral antibiotics. The erysipelas was healed with the standardization of the infectious balance.

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients have given consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed.

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Clinical Image

Foot pseudo-melanonychia

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A change in the color of the nails is referred to as nail dyschromia, discoloration of the nails, or chromonychia [1]. Black nail discoloration is called melanonychia.

We report a healthy 33-year-old female patient exhibiting onychopathy located in the nail plate of the third toe of the left foot consisting of a blackish coloration on the surface of and under the nail and on the second toe, slightly overlapping the third (Figs. 1a–1c). On dermatoscopy, the coloration is seen as blackish (Fig. 2a), and the microscope shows a linear, blackish-brown coloration (Fig. 2b). Scraping and cutting of the onychopathy are performed. Under the microscope, it is observed as a linear blackish-brown coloration, and the sample is refractive in polarized light (Fig. 2c). The surface of the nails after scraping and cutting can be seen in Figs. 3a–3b.

The results of the rest of the physical exam fall within normal ranges. There is no significant family or personal history.

The patient reports that to have noticed the nail color change some time ago but cannot specify exactly when; she consults for fear of having nail melanoma.

A clinical diagnosis of pseudo-melanonychia onychopathy [2] was conducted favored by the overlap of the second toe.

Deformities of the toes can cause alterations in the nails due to the pressure that they bear and can manifest themselves in ways such as platonychia, frictional hematoma, frictional pseudo-hematoma, onycholysis, onychodystrophy, koilonychia, onychocryptosis, onychogryphosis, and pseudo-melanonychia [3].

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients have given

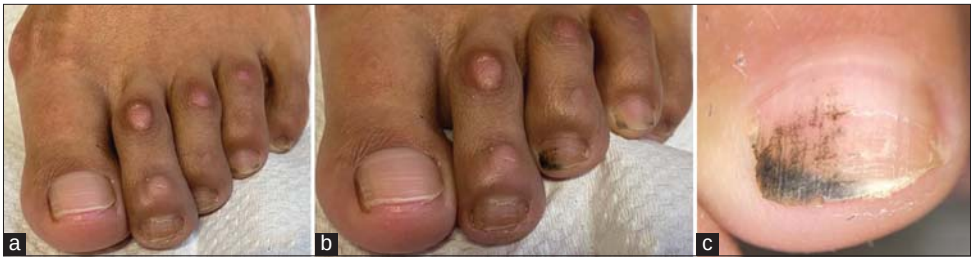

Figure 1: (a) Panoramic aspect of the lesion with an overlap of the second left ear. (b) Blackish coloration of the nail plate surface and underneath. (c) Dyschromia dermatoscopy of the nail plate surface.

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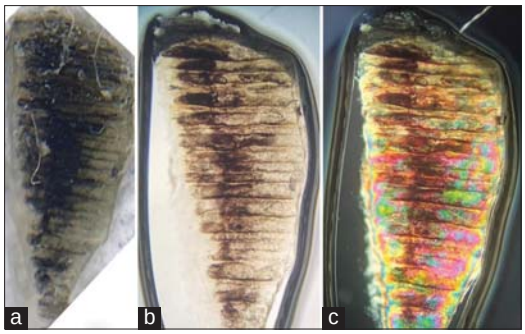

Figure 2: (a) Dermatoscopy of the nail plate sample. (b) Linear microscopy of the blackish lesion. (c) Birefringence of the nail with polarized light.

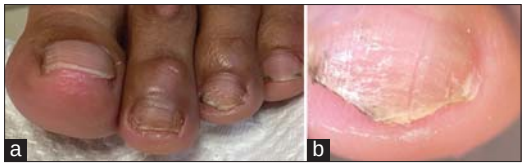

Figure 3: (a-b) Before and after nail curettage.

consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed.

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Letter to the Editor

Can periocular hypermelanosis be treated with platelet-rich plasma (PRP) intradermal injections?

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Sir,

Can periocular hypermelanosis be treated with platelet-rich plasma (PRP) intradermal injections?

Periocular hypermelanosis, also known as periorbital hyperpigmentation, is a common, worldwide condition caused by multiple etiological factors. Here, genetic (hereditary) post-inflammatory endocrinopathy, drug ingestion, hormonal imbalance, excessive hypervascularity, and secondary eyelid edema are common causative agents [1].

Atopic and allergic contact dermatitis are common in the eyelids and, since they are accompanied by significant chronicity and inflammation, they can be involved in numerous cases of periorbital hyperpigmentation [2]. Sometimes, it is likely that infraorbital dark circles are caused by the visibility of prominent veins; in other words, venous congestion may be responsible for the dark pigmentation [3].

From October 15, 2015, to October 15, 2016, 15 female patients with idiopathic periocular hypermelanosis, clinically and photographically documented by two separate dermatologists, were included in the following clinical study after giving consent for participation. They did not report any underlying medically-significant diseases in their histories nor any specific drug ingestion. All possibility of uncooperative behavior or unusual expectation was eliminated from the study. Because a confirmation by histologic examination is rarely necessary, we refused to take biopsies of the patients' skin, even more so as they did not consent to this. The photographs of the patients were taken under standard optical conditions.

The design of the clinical study consisted of three sessions of autologous platelet-rich plasma (PRP) injections into periorbital skin with two months in between the injections. They were followed up to one year, in other words, up to 6 months after the end of the treatment.

Therapeutic outcomes were evaluated by standardized imaging and, then, judged by two independent dermatologists blinded to the study and the patients' satisfaction rates. Three patients refused to continue the study as they were in the process of moving to another city. After 12 months, the final results were as follows: One patient (8%) reported a significant improvement, 3 patients (25%) moderate improvement, and 6 patients (50%) poor if any response to the dark circles. Two patients (17%) reported an exacerbation and dissatisfaction from the trial due to unmet expectations. The only objectively measured side effects were swelling and bruises around the areas of injection, which were transitory in nature and did not need intervention.

In our opinion, due to divergent etiologies and differing patterns, no single treatment can yield miraculous results. For this reason, PRP is not considered, at the moment, an exceedingly helpful treatment for periocular hypermelanosis, although this is in opposition to Mehryan et al., who believe that the use of PRP yields a statistically significant improvement in infraorbital color homogeneity [4]. The differing results may be due to the

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DOI:10.7241/ourd.20203.19

variation of design and technique in the two studies. Very few controlled studies mention a possible therapeutic role of PRP injections in periocular hypermelanosis.

According to Ranu et al., lack of sleep, stress, alcohol abuse, and smoking, although not clinically substantiated, may aggravate the hyperpigmentation of the eyelids. Changes in lifestyle are an important part of preservative therapy [5,6].

There is some concern about the role of needle trauma during PRP injections in inducing post-inflammatory pigmentary changes, especially in dark-skinned individuals, in whom the problem is more common.

Nofal E. et al. believe that PRP injections are a promising treatment option for periorbital hyperpigmentation, but, because of its multifactorial etiology, are not an ideal solution [7]. Medication intolerance, stressful circumstances, and the need for multiple intradermal injections are the challenges that the physicians faced during the PRP injection procedure.

We believe that further studies, with larger sample sizes or different methods and treatment modalities, should be considered for proper periocular hypermelanosis treatment.

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

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Letter to the Editor

Skin cancer and "Dr. YouTube." Using PEMAT Scoring to Determine Understandability and Actionability of Audiovisual Patient Education Material

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Sir,

In an era of widespread social media access, patients may attempt self-diagnosis or self-treatment based on information found online. YouTube is a popular social media source of information for many patients, including information on skin cancers. Much of the YouTube information patients receive on skin cancer or skin disease is provided by consumer videos, rather than medical professionals [1]. Studies examining melanoma screening tools have shown videos submitted by medical or government professionals were more likely to have accurate content, better educational quality and avoid jargon [2,3]. Other studies have shown that many consumer YouTube videos on various health conditions are of poor quality in terms of information delivery, video layout and the use of explanatory pictures or other visual aids [3-5]. However, there seems to be a lack of studies, determining if videos made by medical professionals were made in such a way in which patients could process and use, regardless of educational background.

We sought to use the Patient Education Materials Assessment Tool (PEMAT) scoring system to assess the quality of medical educational YouTube videos specifically relating to melanoma and basal cell carcinoma (BCC). A YouTube search was performed using the descriptive terms "melanoma, medicine" and "BCC medicine." The keyword "medicine" was added as an attempt to get a better quality of videos from reliable reputable sources. Studies regarding user behavior on the internet have indicated that the majority of the users will click on the results within the first few pages, thus each search was limited to the first 35 results [4]. Videos were sorted by relevance. Videos that were less than 1 minute or greater than 15 minutes in length, not from the United States, or not in English, were excluded. One examiner independently examined videos using PEMAT scoring for audiovisual materials [5]. The PEMAT deems patient education materials "understandable" when viewers of various backgrounds and literacy can process key messages. It deems videos "actionable" when viewers can clearly understand what they can do with the material presented in the videos [4,5]. The PEMAT uses 13 questions which pertain to content, word choice and style, organization, layout and design, and the use of visual aids. The "actionability" part of the scoring system consists of four criteria: whether the video addresses the viewer directly, the action the viewer can take and how they can take the action, and whether a concrete tool is provided for how the viewer can take the action [5]. A final score is then calculated using the PEMAT. A score below 70% classifies the video as poorly understandable or not actionable.

Video characteristics were noted and summarized by understandability and actionability. A total of 50 videos were viewed and PEMAT was calculated for each. The majority of the videos were targeted toward patients and published by a hospital or practice (Tables 1 and 2).

The intent of this preliminary study was to examine videos made by medical professionals and determine if the videos adequately explained key concepts regarding BCC and melanoma to the viewer/patient. Although social media has increased communication of health information, there is a general lack of quality control in

How to cite this article: Henning A. Skin cancer and "Dr. YouTube." Using PEMAT Scoring to Determine Understandability and Actionability of Audiovisual Patient Education Material. Our Dermatol Online. 2020;11(3):309-311.

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Table 1: PEMAT understandability and actionability scores by video characteristics for melanoma videos

Melanoma: PEMAT Score summarized by video characteristics						
		Overall (n=25)	Understandable (n=13)	Not understandable (n=12)	Actionable (n=7)	Not actionable (n=18)
		n(%)	n(%)	n(%)	n(%)	n(%)
Video Length	Mean	4:19				
Video Views	Mean	19,253				
Type of publisher	Hospital/Practice	18 (72)	10 (77)	8 (67)	5 (71.4)	13 (72.2)
	Individual	3 (12)	2 (15)	1 (8.3)	1 (14.2)	2 (11.1)
	Industry	2 (8)	0 (0.0)	2 (16.7)	0 (0.0)	2 (11.1)
	Unknown	2 (8)	1 (7.6)	1 (8.3)	1 (14.2)	1 (5.5)
Video Type	Expert Testimonial	11 (44)	5 (38)	6 (50)	3 (42.8)	8 (44.4)
	Patient Testimonial	5 (20)	2 (15)	3 (25)	0 (0.)	5 (27.7)
	Educational	8 (32)	6 (46)	2 (16.7)	2 (28.5)	6 (33.3)
	Other	1 (4)	0 (0.0)	1 (8.3)	0 (0.0)	1 (5.5)
Graphics	Photographs	4 (16)	3 (23)	1 (8.3)	1 (14.2)	3 (16.6)
	Illustration/ Graphics	6 (24)	4 (30.7)	2 (16.7)	2 (28.5)	4 (22.2)
	None	11 (44)	3 (23)	8 (67)	3 (42.8)	8 (44.4)
	Live Action	1 (4)	0 (0.)	1 (8.3)	0 (0.0)	1 (5.5)
	Animation	0 (0.0)	0 (0.)	0 (0.0)	0 (0.0)	0 (0.0)
	Other/Multiple	3 (12)	3 (23)	0 (0.0)	2 (28.5)	1 (5.5)
Audience Type	Patients	16 (64)	10 (77)	6 (50)	6 (85.7)	10 (55.5)
	Physicians/nurses	4 (16)	3 (23)	1 (8.3)	0 (0.0)	4 (22.2)
	Students	2 (8)	0 (0.0)	2 (16.7)	1 (14.2)	1 (5.5)
	Unknown	3 (12)	0 (0.0)	3 (25)	0 (0.0)	3 (16.6)

Table 2: PEMAT understandability and actionability scores by video characteristics for basal cell carcinoma videos

Basal Cell Carcinoma: PEMAT Score summarized by video characteristics						
		Overall (n=25)	Understandable (n=11)	Not understandable (n=14)	Actionable (n=2)	Not actionable n=23)
		n(%)	n(%)	n(%)	n(%)	n(%)
Video Length	Mean	3:44				
Video Views	Mean	32,193				
Type of publisher	Hospital/Practice	9 (36)	5 (45.5)	4 (28.6)	2 (100)	7 (30.4)
	Individual	2 (8)	1 (9.1)	1 (7.1)	1 (50)	1 (4.3)
	Industry	8 (32)	5 (45.5)	3(21.4)	2 (100)	6 (26.1)
	Unknown	6 (24)	1 (9.1)	5 (35.7)	0 (0.0)	6 (26.1)
Video Type	Expert Testimonial	6 (24)	3 (27.3)	3 (21.4)	3 (150)	3 (13.0)
	Patient Testimonial	4 (16)	1 (9.1)	3 (21.4)	0 (0.0)	4 (17.4)
	Educational	10 (40)	7 (63.6)	3 (21.4)	0 (0.0)	10 (43.5)
	Other	4 (16)	1 (9.1)	4 (28.6)	0 (0.0)	5 (21.7)
Graphics	Photographs	9 (36)	3 (27.3)	6 (42.9)	0 (0.0)	9 (39.1)
	Illustration/ Graphics	1 (4)	0 (0.0)	1 (7.1)	0 (0.0)	1 (4.3)
	None	7 (28)	2 (18.2)	5 (35.7)	1 (50)	2 (8.7)
	Live Action	5 (20)	4 (36.4)	1 (7.1)	1 (50)	4 (17.4)
	Animation	1 (4)	1 (9.1)	0 (0.0)	0 (0.0)	1 (4.3)
	Other/Multiple	2 (8)	2 (18.2)	0 (0.0)	1 (50)	1 (4.3)
Audience Type	Patients	16 (64)	8 (72.7)	8 (57.1)	3 (150)	13 (56.5)
	Physicians/nurses	2 (8)	2 (18.2)	0 (0.0)	0 (0.0)	2 (8.7)
	Students	3 (12)	2 (18.2)	1 (7.1)	0 (0.0)	3 (13.0)
	Unknown	4 (16)	1 (9.1)	3(21.4)	0 (0.0)	4 (17.4)

terms of how information is relayed and what the patient is expected to do once informed. Most videos were not "actionable" even if the video came from a credible medical source, such as a hospital or clinic. Many videos lacked visual aids or cues that might aid in understanding. Medical professionals should consider using previously developed educational or informational tools in order to develop better quality educational videos for patients.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their

names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Letter to the Editor

Deep skin and soft tissue infection of the neck – first sign of unrecognized diabetes mellitus

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Sir,

Skin and soft tissue infections (SSTIs) are common infectious disorders, while complicated SSTI (cSSTIs) representing the more severe subtype developing deep-seated infection, a requirement for surgical intervention, the presence of systemic signs of infection, and the presence of complicating comorbidities [1]. These disorders require initiation of appropriate empiric broad-spectrum antimicrobial therapy, early aggressive surgical intervention for drainage of abscesses and debridement, and identification of responsible bacteria and appropriate de-escalation of antimicrobial therapy [2]. Carbuncles and cSSTIs may be a symptom of unrecognized diabetes mellitus [3].

A 38 year-old male patient presented with a carbuncle on his neck worsening during 7 days of ambulatory oral antibiosis with ampicillin/sulbactam. His medical history was unremarkable despite a type-I-allergy against and dog and cat epithelia. After a couple of days, he developed fever and malaise. Therefore, he was admitted to the Department of Dermatology and Allergology.

On examination we observed a tense and very painful erythematous swelling on his neck about 10 cm in diameter with central pustulations and putride discharge (Fig. 1). Neck lymph nodes were swollen and painful. Or working diagnosis was cSSTI following a neck carbuncle.

Laboratory investigations: Leukocytosis of 20.75 Gpt/l (normal range 3.8 – 11.00), thrombocytosis of 414.0 Gpt/l, neutrophila of 18.4 Gpt/l (1.8 – 7.6), C-reactive protein 277 mg/l (<5), HbA1C (IFFC) 92 mmol/ml (20-42), glucose up to 16.9 mmol/l (4.1 – 5.9).

Urine: glucosuria > 1000 mg/dl (<50), proteinuria 50 mg/dl (<10), ketone >150 mg/dl (<5).

Microbiology: Staphylococcus aureus, sensible to ampicillin/sulbactam.

Histology: Extensive perifollicular putrid and absceding inflammation with perforations to the skin surface.

Imaging techniques: Computerized tomography of neck and chest with extensive inflammatory infiltration of subcutaneous adipose tissue of the neck, multiple inflammatory-reactive lymph nodes (Figs. 2a and 2b). Abdominal sonography remained unremarkable.

Ophthalmologic investigation: Non-proliferative diabetic retinopathy.

Treatment: We started with a dose escalation of 3 g ampicillin/sulbactam i.v. and metronidazole 500 mg i.v. every 8 hours and added oral metamizole 3 x 1 g/d. Aggressive surgical drainage of the abscess was performed. The wounds were irrigated repetitively using Ringer's solution, and framycetin sulfate powder was applied. Systemic antibiosis was adapted to microbiological findings, dose was escalated.

Within 7 days, the fever ceased, leukocytosis, neutrophilia, thrombocytosis, and C-reactive protein normalized.

Drug therapy of diabetes mellitus was initialized with the diabetologist using a combination of short and long acting insulins: Actrapid HM® (Novo Nordisk) and Toujeo® (Insulin Glargin 300 I.E/ml; Sanofi). After release from the hospital the patient was referred to a diabetologist for further treatment and monitoring.

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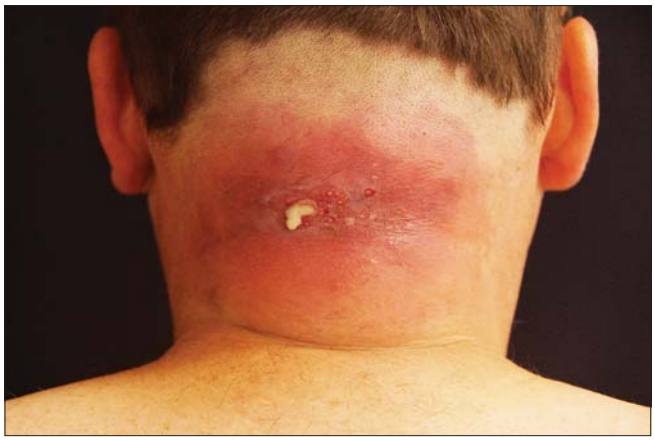

Figure 1: Carbuncle of the neck with putride discharge. The erythema and swelling in the surrounding tissue indicate deep skin and soft tissue infection more extensive than the initial carbuncle.

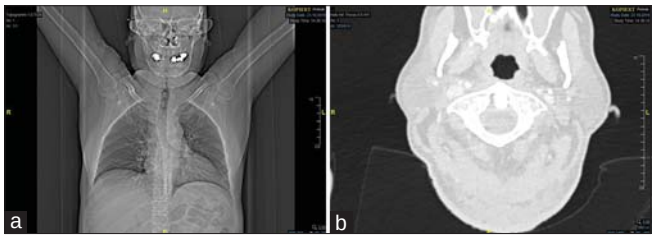

Figure 2: (a) CT-Imaging excludes mediastinal or pulmonary spread of the skin and soft tissue infection. (b) Deep infection of the neck.

DISCUSSION

cSSTI are an extreme of the common SSTIs. They often are associated to significant comorbidities, sometimes unrecognized as in the present case. Analysis of comorbidities in patients with deep neck infections demonstrated diabetes mellitus in 19.0% to 20.5% [4,5]. In a meta-analysis of deep neck infections, diabetes mellitus was associated with multispacer spread of infection (Relative risk [RR] 1.96) and complications (RR 2.42) [6].

The most common bacteria responsible for carbuncles and cSSTI of the neck include anerobic *Peptostreptococcus*, *Streptococcus aureus*, *Streptococcus viridans*, *Streptococcus pyogenes*, but in diabetics *Klebsiella pneumoniae* acquires also importance [5,6].

Other skin diseases that warrant the exclusion of unrecognized diabetes mellitus are necrobiosis lipoidica [7], eruptive xanthomas [8], acne keloidalis nuchae [9], and secondary phimosis [10].

In conclusion, carbuncles and cSSTis can be a symptom of unrecognized and uncontrolled diabetes. Early recognition and a combined approach with aggressive surgery and specific antibiosis is essential to prevent complications such as mediastinitis, pneumonia or sepsis (in case of neck localization). Diabetes and other comorbidities need an adequate treatment in a multidisciplinary approach.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Letter to the Editor

Localized skin-colored nodules and tumors on the trunk

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Sir,

We report a 79-year-old man without a significant medical history referred to our department for multiple nodules and tumors on the left side of his trunk, which had appeared progressively for many years. Despite him having no family history of similar lesions, they had increased in size and number over the same area. A dermatological examination revealed multiple soft to firm skin-colored nontender nodules and pedunculated tumors varying in size from 1 to 7 cm in diameter localized on the left side of the trunk without crossing the midline (Fig. 1). Some of the cutaneous nodules showed a positive buttonhole sign. There were no similar lesions elsewhere. Café-au-lait macules, axillary and inguinal freckling, plexiform neurofibromas, and kyphoscoliosis were absent in the patient. There were no Lisch nodules on ophthalmological examination. A neurological examination was normal. Computerized tomography of the brain, chest, and abdomen was normal and tumor markers were negative. A skin biopsy of the skin-colored nodule was performed revealing well-circumscribed unencapsulated interlacing bundles of elongated cells with darkly stained nuclei (Figs. 2a and 2b). The tumor cells showed positive S100-protein immunoreactivity (Fig. 2c). Combining clinical and histopathological findings allowed us to confirm the diagnosis of late-onset segmental neurofibromatosis. The patient was scheduled for surgical removal of his cutaneous tumors for aesthetic reasons. Regular medical follow-up was also recommended.

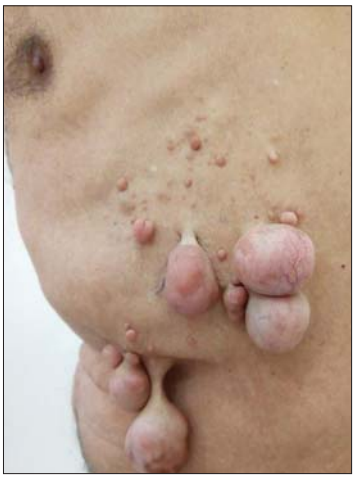

Figure 1: Multiple skin-colored nontender nodules and tumors located on the left lateral side of the trunk without crossing the midline.

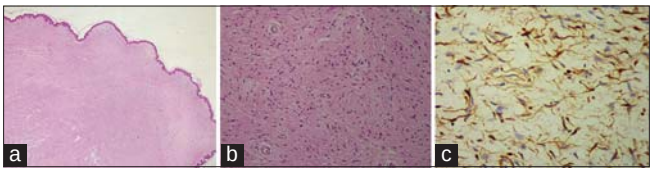

Figure 2: (a) A nonencapsulated circumscribed mass of the dermis (HEx40). (b) A dermal proliferation of spindle cells with wavy nuclei interspersed in a stroma composed of fibrillar collagen with no evidence of nuclear polymorphism, mitoses, or hypercellularity (HEx100). (c) Tumor cells with positive S100 protein (HEx100).

Herein, we report a case of late-onset segmental neurofibromatosis (SNF) in an old patient. The cutaneous nodules had appeared spontaneously and progressively and had been slowly increasing in size. It is a rare variant of neurofibromatosis and its prevalence in the general population is estimated at 1 in 36,000–40,000 individuals [1].

Riccardi established the clinical features of segmental neurofibromatosis [2]. In fact, SNF is considered a noninherited form of neurofibromatosis and results

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from a postzygotic mutation in the NF1 gene leading to somatic mosaicism [3]. The neurofibromas are limited to one body region and the cutaneous features of neurofibromatosis type I are absent. Ophthalmological and neurological complications are rare in SNF [2]. The latter is usually unilateral, as in our case, but could also be bilateral [4] or even multiple [5]. Differential diagnosis includes soft fibromas, cutaneous schwannomas, cutaneous metastases, nevus lipomatosus cutaneous superficialis, cutaneous leiomyomas, trichoepitheliomas, xanthomas, and lymphomas. Histopathology is crucial for diagnosis, showing a well-circumscribed mass composed of spindle cells with elongated wavy nuclei in the dermis consistent with the diagnosis of neurofibroma [3]. In this case, there was no systemic involvement and no evidence of an internal malignancy. In fact, according to Dang et al. [6], segmental neurofibromatosis could be a paraneoplastic manifestation. Malignancies that could be associated with SNF include malignant peripheral nerve sheath tumors, malignant melanomas, breast cancer, gastric cancer, and colon cancer, which were absent in our patient [4,6]. SNF is an often-overlooked disorder since patients are usually asymptomatic and seek medical attention only for cosmetic reasons [7]. Hence, surgical removal of tumors is usually recommended, as performed in our patient. Finally, accurate diagnosis of this rare variant of neurofibromatosis is important since it gives different prognoses than neurofibromatosis type I. It is also important to reassure the patient and to provide proper genetic counseling [2].

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients have given consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed·

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Letter to the Editor

Seborrheic keratoses and concomitant malignant tumors: the controversy

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Sir,

Seborrheic keratosis (SK) is the most common benign epidermal tumor [1,2]. Considering its prevalence, reports of concurrent malignant tumors within SKs are rare, but have added to the debate on the possibility of the development of these neoplasms from SK [3-5]. We briefly describe two clinical cases illustrative of the association of SK with concomitant malignant neoplasms and analyze the controversy over whether they are of incidental coexistence or represent malignant transformation.

The first case concerns a 75-year-old Caucasian female with no relevant dermatological background who presented an asymptomatic violaceus erythematous papule arising adjacent to a larger (35x20 mm), well-demarcated, elevated, flat, brownish plaque on the right frontal area (Fig. 1). The patient noticed the brownish plaque many years prior to the examination while the violaceus erythematous papule developed much later. Dermoscopy revealed a brown lesion with comedo-like openings and, peripherally, a shiny, brownish-red area with arborizing vessels, superficial fine telangiectasia, ulceration, and bluish-gray globules (Fig. 2). The newly developed papule was excised. A histopathological analysis of the papule and the brownish plaque revealed a basal cell carcinoma with large nests of basaloid cells and superficial components in collision with an acanthotic SK, respectively (Fig. 3).

The second case concerns a 66-year-old female who presented with recent changes in a long-lasting, slightly elevated, 80x40 mm, brownish plaque on the left flank. A previous incisional biopsy of the lesion revealed the presence of an SK. However, over the past year, the central area 15 mm in diameter had become persistently erythematous, pruritic, and hemorrhagic after rubbing (Fig. 4).

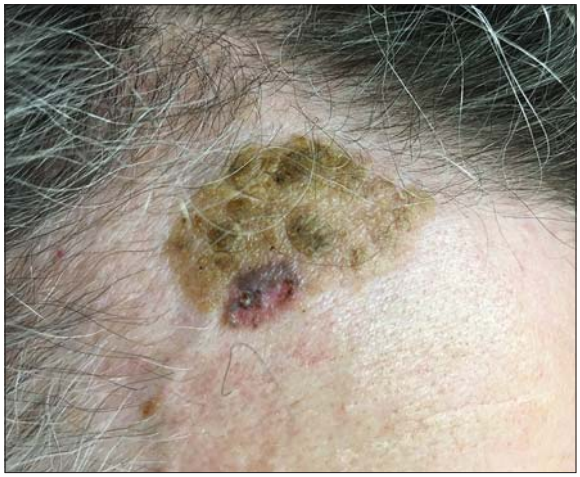

Figure 1: Clinical view of the lesion: a violaceus erythematous papule arising adjacent to a larger, brownish plaque on the right frontal area.

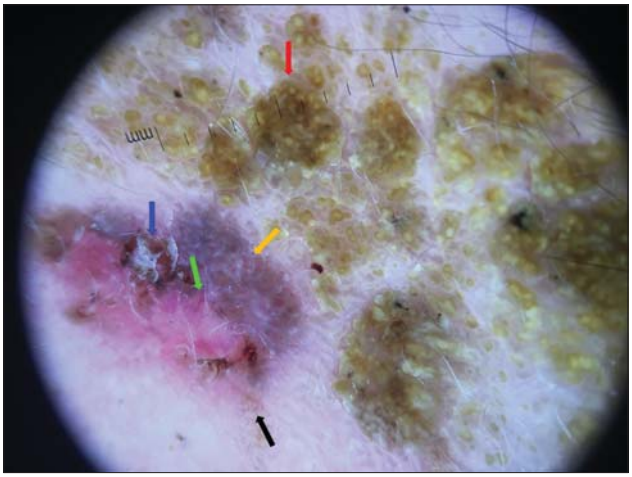

Figure 2: Dermoscopic view: a brown lesion with comedo-like openings (red arrow) and a peripheral shiny brownish-red area with arborizing vessels (black arrow), fine telangiectasia (green arrow), ulceration (blue arrow), and bluish-gray globules (yellow arrow).

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An incisional biopsy of this area was performed revealing a well-differentiated in situ squamous cell carcinoma (Fig. 5). A wider excision was performed, removing the recently developed part of the lesion completely and showing the same histopathologic findings contiguous with an SK.

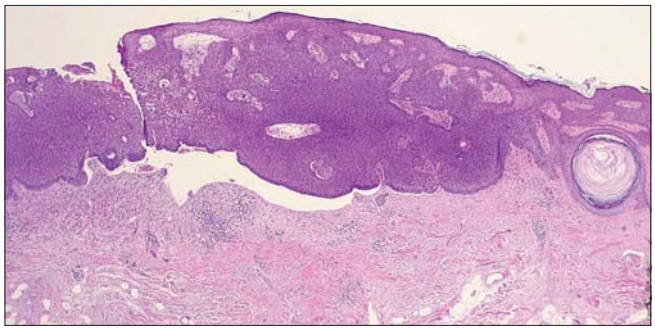

Figure 3: Histologic examination: nests of basaloid cells in collision with SK (hematoxylin and eosin, x40).

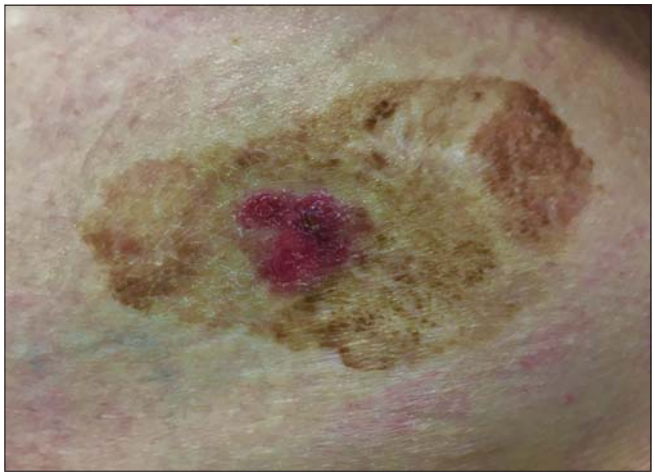

Figure 4: Clinical view of the lesion: a recently developed central erythematous area in the previously biopsied SK.

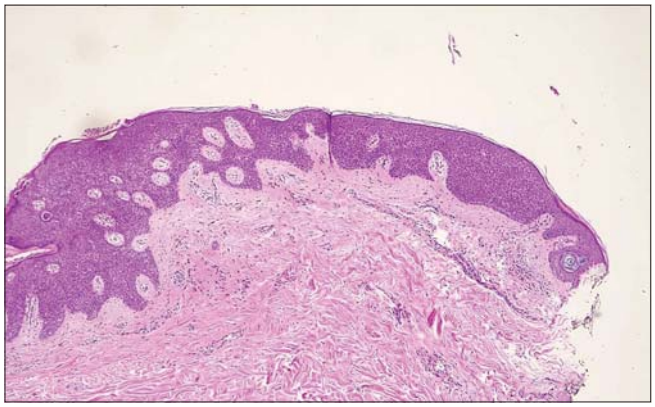

Figure 5: Histologic examination: a well-differentiated *in situ* squamous cell carcinoma (hematoxylin and eosin, x40).

A malignant tumor within an SK is an extremely rare condition [2,6] and the histological subtype involved is usually acanthotic SK [7]. Although the most commonly developed type of malignant tumor is basal cell carcinoma—mainly its superficial type [6,7]—squamous cell carcinoma, eccrine porocarcinoma, and malignant melanoma have also been reported [3]. The relationship between these tumors and SK is still a matter of controversy, and the debate as to whether this exceedingly rare association is incidental or generated by tumor progression continues [2]. Some authors maintain that it is a matter of chance, doubting the existence of any pathogenic relationship between malignant tumors and SK and defending the incidental cohabitation of the two entities [5]. The term collision tumor is applied to the coexistence of two or more different neoplasms, benign or malignant, in the same cutaneous lesion, while the term compound tumor refers to two distinctive neoplasms either directly contiguous or immediately adjacent to each other [3].

Other authors defend the hypothesis of malignant transformation, arguing that SKs are composed of several cell types and the transformation to a variety of epithelial tumors derives from these individual cells. Therefore, basal cell carcinomas could arise from basaloid cells, squamous cell carcinomas from squamous cells, and melanomas from melanocytes, all within an SK [1]. The histological evidence of a direct connection between the two coexisting lesions might be indicative of a malignant transformation of the SK. It has been proposed that, when malignant lesions are not merely adjacent to or merging with an SK, but are found within, the association may be more than coincidental, supporting the view for potential dysplastic or malignant change [7].

We report two cases illustrative of the unsettled controversy of the coexistence of SKs and malignant tumors: incidental coexistence vs. subtle malignant transformation. In the first case studied, the apparent coexistence of two adjacent, but histologically distinct, tumor components opposes the development of a malignant lesion within the SK examined in the second case, which, according to some authors, is indicative of the possibility of malignant transformation in so-called benign lesions. We also intend to underscore the importance of a low threshold for biopsies of suspicious SK lesions if there exists a modification of macroscopic characteristics and in the presence of atypical clinical

symptoms such as erythema, ulceration, bleeding, and crusting, as they may herald a malignant tumor.

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients have given consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed.

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Letter to the Editor

Erythroderma induced by dermatophytes

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Sir,

Erythroderma is a chronic condition presenting with generalized erythema occupying over 90% of the body surface. The causes of secondary erythroderma are various, such as eczema, contact dermatitis, psoriasis, pityriasis rubra pilaris, pemphigus foliaceus, cutaneous T-cell lymphoma, and drug eruption; however, erythroderma induced by tinea corporis is rare. We herein describe a rare case of erythroderma induced by dermatophytes.

A 66-year-old male visited out department, complaining of extensive erythema on the trunk. He was otherwise healthy and not on medication. He stated that itchy eruption appeared five years previously, and gradually spread in spite of being treated with topical ointment at a nearby clinic. He self-discontinued therapy and thereafter his skin rash gradually exacerbated. Physical examination showed diffuse, coalesced scaly erythemas on the trunk (Fig. 1). Potassium hydroxide (KOH) examination revealed a number of fungi (Fig. 2). Similar erythemas were observed on the genital areas, groins, buttocks, upper and lower extremities, and toe nails. Laboratory examination showed a white blood cell count of 8600/μl, with a differential count of 34% eosinophils. The IgE level exceeded 5,000 IU/ml, and thymus and activation-regulated chemokine (TARC) was also elevated (882 pg/ml; normal <450). Liver and renal function was normal, and human immunodeficiency virus antibody was negative. He was treated with oral terbinafine (100 mg/day), and skin lesions were improved seven weeks later with decreased eosinophil percentage (2%). Evaluation of pruritus with visual analogue scale score showed 89/100 before therapy, which decreased to 0/100 after successful therapy.

Secondary erythroderma is induced by various inflammatory skin diseases such as eczema and psoriasis. By contrast, cases of erythroderma due to dermatophyte are few [1]. In the present case, secondary fungal infection was excluded, because the patient recovered from generalized erythema only by anti-fungal therapy. KOH examination promptly made the correct diagnosis, and biopsy was avoided. Unfortunately, fungal culture was not carried out.

In a case series of erythroderma, cutaneous dermatophytosis was observed in 3 out of 103 cases (2.9%) [2]. In our department, over 70 patients were identified as having erythroderma in these 10 years, among whom only the present case was caused by fungal infection. Previous studies showed a significantly impaired permeability barrier function and reduced stratum corneum hydration in the lesional skin of tinea corporis [3]. In the present case, such profound changes in skin barrier structure and function i nduced

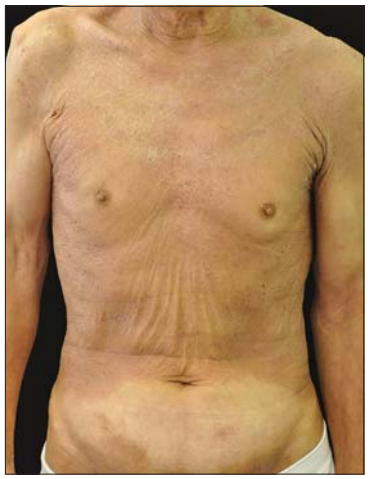

Figure 1: Diffuse erythema with scales on the trunk

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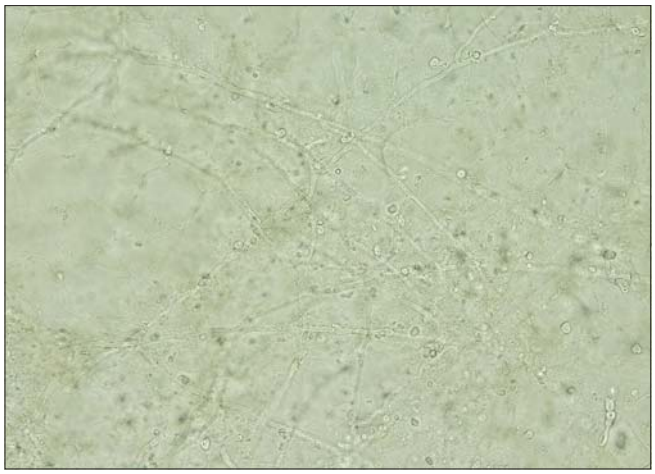

Figure 2: KOH examination revealed a number of hyphae

by diffuse superficial dermatophytosis may have led to erythrodermic condition, via sweat dysfunction. We excluded the possibility that our patient had suffered from erythroderma for a long time, which secondarily developed superficial fungal infection, because his erythroderma was dramatically improved by oral terbinafine in a short period. In conclusion, it should be kept in mind that dermatophytosis is one of the causative factors of erythroderma.

Consent

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Letter to the Editor

Two cases of sporotrichoid leischmaniasis induced by antimonyate infiltration

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Sir,

Sporotrichoid leishmaniasis is a sporadic form of cutaneous leishmaniasis characterized by staged subcutaneous nodules aligned on a lymphatic path. Its onset directly suggests underlying immunosuppression, but its installation after the initiation of local antimonial treatment remains enigmatic. We present two cases illustrating this phenomenon.

Case No. 1: A 30-year-old man, with a suspicion of an endemic area of leishmaniasis, consulted for an ulcerated nodule on the back of the left foot evolving for the past two months. A parasitological examination indicated cutaneous leishmaniasis. The patient received five antimony infiltrations. The evolution was marked by the appearance of three similar lesions staged along a hardened cord of the lower left limb (Fig. 1). A diagnosis of sporotrichoid leishmaniasis was accepted. The patient received systemic treatment with antimoniates with a favorable evolution.

Case No. 2: A 30-year-old man, with a suspicion of an endemic area of cutaneous leishmaniasis four months prior, consulted for two scaly erythematous lesions 4 cm in diameter, one on the right leg and one on the left wrist, evolving for the past two months. A diagnosis of cutaneous leishmaniasis was confirmed by parasitological examination and the patient received seven infiltrations of antimoniates. The evolution was marked by a partial improvement of the initial lesions and the appearance of seven firm subcutaneous nodules of 0.5 to 1 cm in diameter aligned along a lymphatic path of the left forearm (Fig. 2). Venous Doppler echo eliminated thrombophlebitis, and the histology of the nodule was nonspecific. A search for underlying immunosuppression was negative. The evolution was favorable after systemic treatment with antimoniates.

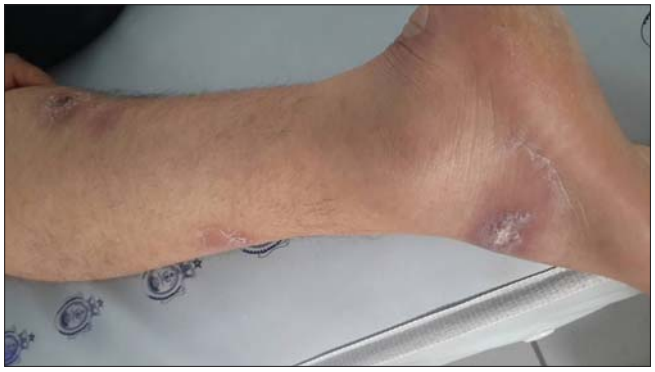

Figure 1: Sporotrichoid leishmaniasis of the left leg.

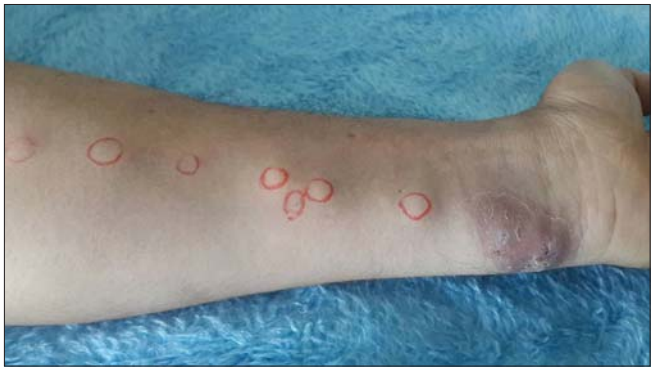

Figure 2: Sporotrichoid leishmaniasis of the left forearm.

Cutaneous leishmaniasis is very common in this context, characterized by its large clinical polymorphism (large simulator). Sporotrichoid forms, on the other hand, are rare. The two main promoting factors of sporotrichoid leishmaniasis are the patient's immune status and the species of leishmania.

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Histology of nodules is often nonspecific and a search for Leishman's bodies is rarely or very rarely positive. Treatment employs antimonates systemically, and resistant forms can be managed with Itraconazole or Amphotericin B. This form does not seem to be associated with a poor prognosis. However, the pathophysiological mechanisms of this dissemination after infiltration and negativation of biopsies remain enigmatic [1-3].

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients have given consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed.

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Letter to the Editor

Dermatologic manifestations of vascular access steal syndrome

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Sir,

A 32-year old right-handed female with past medical history significant for end stage renal disease undergoing hemodialysis and type 2 diabetes mellitus presented to our dermatology clinic with crusted ulcerations of her first, second and fifth digits of her right upper extremity. The patient endorsed associated cramping and pain of her right-hand during dialysis, while performing chores or even after prolonged typing or writing. She noted that lesions began after initiating dialysis, however she was unsure how long they had been present. The patient had tried using hydrogel dressings as well as triamcinolone 0.1% ointment without significant improvement. Of note, she denied any rashes or history of rashes over other locations on her body.

Skin exam of RUE (Right Upper Extremity): Ulceration with central crusting and surrounding hyperpigmentation overlying the PIP (Proximal Interphalangeal) joints of the 2nd and 5th right digit and DIP (Distal Interphalangeal) joint of the thumb (Fig. 1).

Cardiovascular exam: LUE (Left Upper Extremity): Radial pulses +2. RUE: Radial pulses (not palpable). Ulnar pulses weak. Improved with occlusion of arteriovenous (AV) fistula.

Shave biopsy showed epidermal ulceration with transepidermal elimination of collagen and necrotic debris.

Dialysis fistulogram demonstrated findings of profound steal (Fig. 2). The underlying arteries were generally intact. There was no flow in the forearm arteries prior to fistula compression. Following fistula compression there is robust flow in the radial, ulnar and interosseous arteries. Labs were consistent with a patient undergoing dialysis.

Patient had her AV fistula revised and experienced marked improvement of the ulcerations and associated symptoms.

Vascular access "steal syndrome" occurs due to decreased blood flow to the distal extremity as a result of the shunting of arterial blood into a fistula. The presence of radiographic steal syndrome in patients with surgically-inserted arteriovenous ("AV") fistulas can be as high as 73%. However, studies have shown that approximately only 10% of patients with steal syndrome become symptomatic [1].

The time to presentation of this syndrome varies and depends on whether an AV fistula or graft is used. Patients with grafts typically display symptoms approximately two days after placement, whereas patients with AV fistulas notice symptoms closer to 165 days on average after surgery [1].

Steal syndrome is a clinical diagnosis. Patients present with ulcerations, pain, hand stiffness, and/or paresthesia in the affected extremity. On physical exam, there may be decreased sensation and absent radial/ulnar pulses. Skin biopsy results will be non-specific. When the shunt is compressed, there is often alleviation of symptoms as well as a return of the pulses to the radial and/or ulnar arteries [2].

The workup for steal syndrome should start with a duplex ultrasound that can identify arterial stenosis or retrograde flow. If this test is unremarkable and the patient remains symptomatic, arteriography should be

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Figure 1: Ulcerations with central crusting and surrounding hyperpigmentation overlying the PIP joints of the right 2nd and 5th digits.

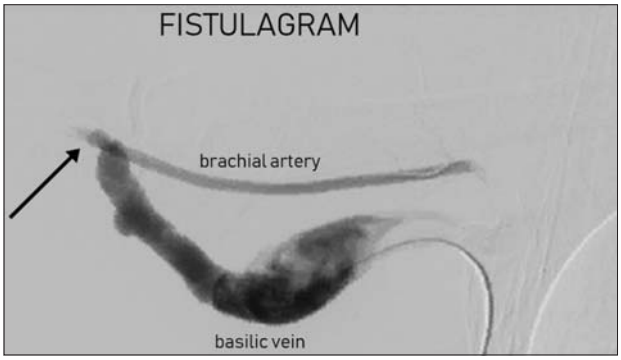

Figure 2: A fistula arteriogram ("fistulagram") showing predominant flow through the basilic vein shunt yet near absent flow to the right upper extremity.

performed. Treatment is typically revascularization in conjunction with ligation of the AV fistula.

This patient was treated by an interventional radiologist who revised her arteriovenous fistula. She experienced marked resolution of her symptoms as well as healing of her ulcerations within weeks following the procedure. However, all-cause mortality after any procedure for severe steal syndrome is high, and the particular intervention for management of steal must account for anatomic, patient, and disease related considerations [3].

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Source of Support: Nil, **Conflict of Interest:** None declared.

Letter to the Editor

A case of erysipelas during secukinumab therapy in a patient with HTLV-1-positive psoriatic arthritis

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Sir,

A 55-year-old male had suffered from joint pain for several years, with psoriatic plaques developed one and a half years before. Physical examination revealed keratotic erythematous plaques on the scalp, trunk, and extremities (Fig. 1a). The Psoriasis Area and Severity Index (PASI) score was 8.6. Furthermore, dactylitis was observed in the left third finger (Fig. 1b). A biopsy specimen taken from the scaly erythema on the elbow revealed hyperkeratosis with parakeratosis, a regular elongation of the epidermis, and mononuclear cell infiltration around the dilated blood vessels in the papillary layer (Fig. 1c). Laboratory examination showed a human T-cell lymphoma virus type 1 (HTLV-1) infection (99.82 S/CO by CLIA), which was confirmed by a Western blot. An examination of the HTLV-1 provirus in the skin was negative. Therapy with subcutaneous secukinumab injections into the abdomen was introduced (300 mg at weeks 0, 1, 2, 3, 4; and every 4 weeks thereafter) under careful supervision. Four months after the initial administration, a clear PASI score was achieved, and joint pain was in remission with an improvement in dactylitis (Fig. 1d). After the ninth injection, the patient wished to reduce the dose of secukinumab to 150 mg because of economic concerns. One month after the first injection of 150 mg secukinumab, the patient complained of painful redness in the left ear. Physical examination revealed that the erythema had spread and coalesced diffusely in the left ear (Fig. 2). Laboratory examination showed an elevated concentration of white blood cells (9,100/μL with 70% neutrophil, 18% lymphocyte, 11% monocyte, and 1% basophil), C-reactive protein (4.88 mg/dL), and antistreptolysin O (195 IU/mL), whereas renal and liver functions were normal. Secukinumab was stopped, and oral amoxicillin trihydrate-potassium clavulanate was administered instead (250 mg/day for 5 days). After an improvement in erysipelas, secukinumab was restarted. Thereafter, the patient has been well-controlled without a recurrence of erysipelas.

Biologics should be administered carefully in immunosuppressive patients with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus infection [1]. Our patient was HTLV-1–positive, but the HTLV-1 provirus was not detected in the psoriatic skin by a Southern blot analysis. To date, there have only been a few reports of the use of biologics in HTLV-1 carrier patients with psoriasis [2,3]. In the case studied, four months after starting secukinumab, a clear PASI score was achieved. In addition, dactylitis of the fingers significantly improved and joint pain subsided. Because of economic concerns, however, the dose of secukinumab was reduced. Although skin and joint manifestations remained well-controlled, the patient developed erysipelas in the left ear. To date, there have been several cases of erysipelas during biologic treatment, but only a handful of cases of erysipelas during secukinumab therapy have been reported [4].

IL-17 recruits leukocytes, such as neutrophils, to infection sites, and aids defense mechanisms in the fight against extracellular bacteria. Neutropenia was reported infrequently in patients receiving secukinumab, and most cases were grade 1 or 2, and transient [5]. Among patients treated with 300 mg and 150 mg secukinumab, there was no significant variation in the occurrence of neutropenia of grade 2 or greater [6]. Serial infections were infrequent. Although this patient developed a mild infection, HTLV-1 positivity may be relevant to the induction of erysipelas. Caution may, thus, be

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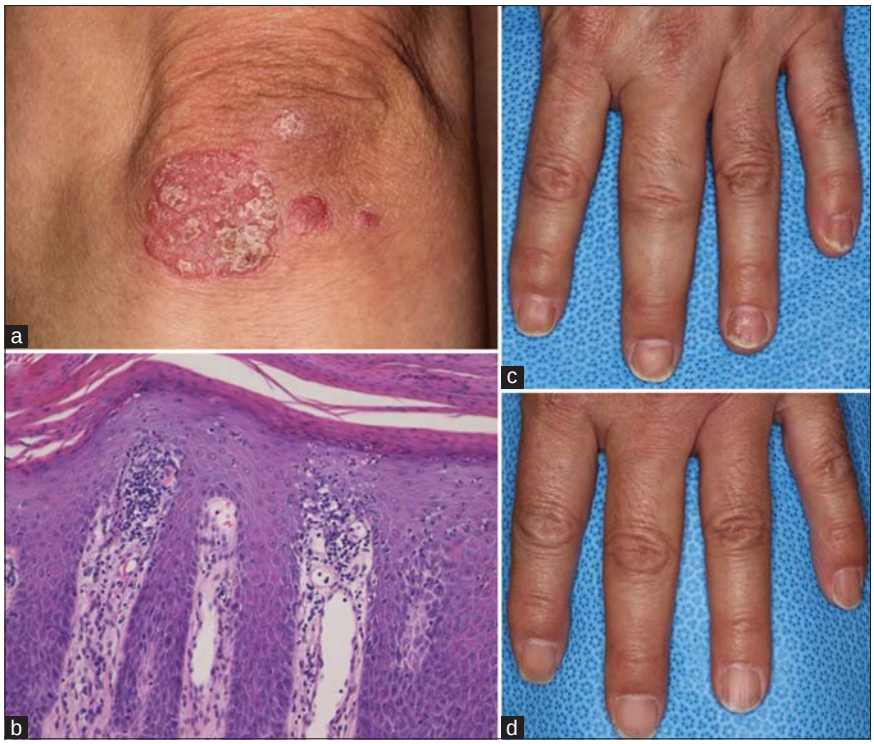

Figure 1: (a) Clinical view of psoriasis on the knee. (b) Dactylitis prominent in the third finger. (c) Histopathological features with an elongation of the epidermis, parakeratosis, Munro's microabscess, and mononuclear infiltration around the dilated capillaries (H-E stain, original magnification × 200). (d) A marked improvement in dactylitis after secukinumab therapy.

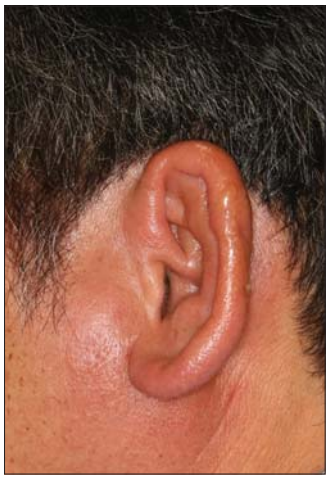

Figure 2: Redness and swelling of the left ear.

necessary in the administration of biologics in patients with HTLV-1.

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients have given consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed.

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Letter to the Editor

An unusual case of Ramsay Hunt syndrome: The pathology yet unknown

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Sir,

The clinical presentation of Ramsay Hunt syndrome includes a vesicular rash in the ear (herpes zoster oticus) or in the oral mucosa, accompanied by acute peripheral facial nerve paralysis.

We present an atypical Ramsay Hunt syndrome case of a 45-year-old female without neuron facial palsy.

The 45-year-old female presented at the emergency department of dermatology complaining of severe pain in the right hemiface, described as electric discharges, with dermatologic lesions that appeared one week before.

She had a history of 20 years of smoking.

An examination indicated a vesicular rash and swollen papules affecting the right tragus, cheek, mental region, and ipsilateral hard palate (Figs. 1a–1c).

Initial treatment included 1000 mg valacyclovir three times a day for seven days.

Two days later, the patient expressed a progressing deterioration: dysphagia to solid foods, hearing loss, and headache without evident dropping eyes or erasing nasolabial folds.

A clinical diagnosis of Ramsay Hunt syndrome (RHS) was made.

The patient was started on treatment with prednisone 60 mg/day for seven days with tramadol 50 mg two times a day.

One week later, the patient showed significant improvement in the hearing loss and headache, and returned to eating foods as normal.

A four-month follow-up did not reveal residual symptoms.

Although Ramsay Hunt syndrome is traditionally defined as zoster oticus and lower motor neuron facial palsy, Hunt enumerated other common symptoms, such as tinnitus, hearing loss, nausea, vomiting, vertigo, and nystagmus, and explained these nervous symptoms by the proximity of the geniculate ganglion to the vestibulocochlear nerve within the bony facial canal.

Based on clinical presentations that indicated the involvement of more than one ganglion, Hunt surmised that the gasserian, geniculate, petrous, accessory, jugular, plexiform, and second and third cervical dorsal root ganglia comprise a chain in which inflammation of a single ganglion can extend to nearby ganglia. This hypothesis explains cases of unilateral facial palsy accompanied by contiguous cranial neuropathies associated with vesicles usually in the mouth, tongue, hard palate, and ears [1].

The largest retrospective Ramsay Hunt syndrome treatment study showed a statistically significant improvement in patients treated with prednisone and acyclovir within 3 days of onset [2].

Data from collective case reports and retrospective reviews suggest that both prednisone and acyclovir, if given early, improve the overall prognosis [3].

Patients who were diagnosed early and received appropriate treatment showed an improvement in the

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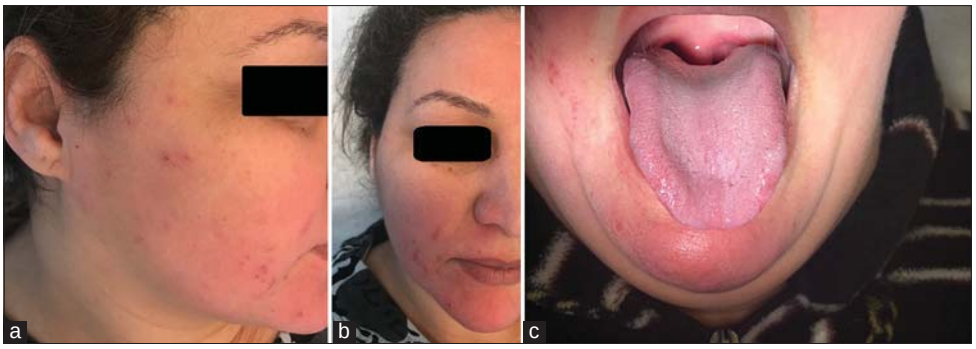

Figure 1: (a and b) Vesicular rash and swollen papules affecting the tragus, cheek, and mental region on the right side of the face. (c) Erythematous macules of the right hard palate.

damaged nerves and achieved a complete recovery of facial nerve function [3,4].

A diagnosis of Ramsay Hunt syndrome can be difficult to reach because the specific symptoms—otalgia, facial paralysis, and the distinctive rash—do not always develop simultaneously.

Our case is significant in that it establishes that early diagnosis leads to early treatment and prevents serious complications, such as permanent hearing loss and facial weakness, eye damage, and postherpetic neuralgia.

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients have given consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed.

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Letter to the Editor

Anaplastic transformation in a classic Kaposi's sarcoma

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A classic Kaposi's sarcoma (KS) is a low-grade vascular tumor with a benign course that affects mostly the skin and oral mucosa. Uncommonly, KS can have a more aggressive evolution leading to local deep invasion and systemic involvement. This particular form is called anaplastic KS. We report a case of an anaplastic transformation in a classic KS in an immunocompetent patient.

A 50-year-old man presented with a large, painful tumefaction of the left foot evolving for over 6 years and resulting in a complete deformation of the forefoot and toes and painful ulcerations. Physical examination revealed an enlarged left foot with multiple violaceous nodules resulting in a massive enlargement and deformation of the whole foot and destruction of the toes. The patient also presented multiple painful ulcerations with permanent serous exudate (Figs. 1a and 1b). The rest of the examination revealed ipsilateral inguinal lymphadenopathies.

A skin biopsy of a nodular lesion was performed. Histological examination revealed a dermal proliferation of spindle cells exhibiting moderate to marked cytonuclear atypia with a high mitotic rate. Immunohistochemical staining for HHV8 was positive, confirming the diagnosis of Kaposi's sarcoma (Fig. 2). An HIV test was negative. An MRI of the affected foot showed deep infiltration and massive bone destruction (Fig. 3). A CT scan revealed micronodules in the lungs, a hypodense lesion in the liver, and lomboaortic and pelvic lymphadenopathies. The patient had been on liposomal doxorubicin for 6 months, which resulted in a reduction in the size of the foot, a complete healing of the ulcerations, and a decrease in the size and number of the nodules.

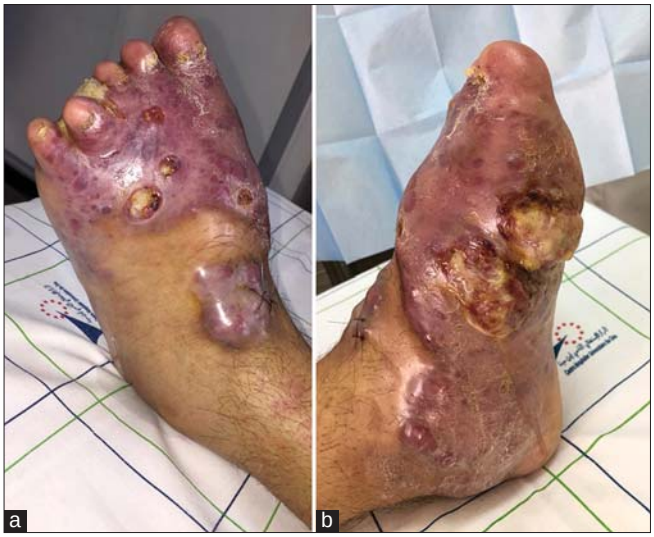

Figure 1: (a and b) An enlargement and deformation of the left foot with multiple confluent violaceous nodules and ulcerations.

Kaposi's sarcoma (KS) was first described in 1872 by Moritz Kaposi as an «idiopathic, multipigmented sarcoma of the skin » [1]. Four epidemiologic variants of KS are known: classic, endemic (African), iatrogenic, and AIDS-associated.

First described in 1959 by Cox and Helwig, a malignant transformation of Kaposi's sarcoma (KS) is characterized by an increase in the number of mitotic figures and marked cellular pleomorphism [2]. Since then, anaplastic transformations have more frequently been observed in African KSs, and rarely in classic KSs.

Clinically, anaplastic KS is characterized by its high local aggressiveness and invasive capacity, along with its metastatic potential. It generally presents itself as rapidly growing nodules or tumors with ulcerations usually associated with lymphedema [3].

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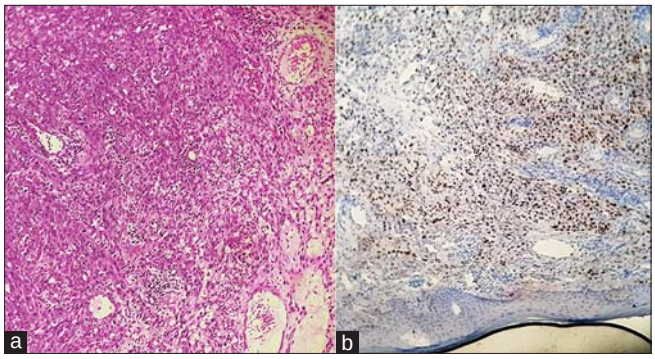

Figure 2: (a) A histological examination (hematoxylin-eosin stain × 100) revealing a dermal proliferation of spindle cells exhibiting moderate to marked cytonuclear atypia with a high mitotic rate. (b) A positive immunohistochemical reaction for HHV8.

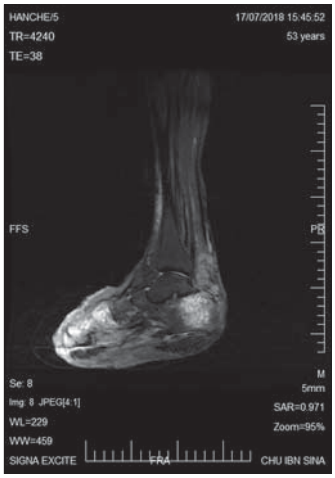

Figure 3: An MRI image showing a deep infiltration and massive bone destruction of the left foot.

Less frequent manifestations include subcutaneous tumors and exophytic lesions. Deep tissue invasion is usually observed, sometimes with bone infiltration. However, none of these clinical aspects is specific to anaplastic KS [3,4].

Histopathologically, anaplastic KS is characterized by a dense proliferation of endothelial, epithelioid, and spindle cells with a significantly greater degree of nuclear and cellular pleomorphism, an increased mitotic index (5–20 mitoses per 10 high-power fields), and atypical mitoses. Necrosis is occasionally observed [5]. Identification of HHV8 by immunohistochemistry is important to the distinction between anaplastic KS and other malignant vascular tumors, especially angiosarcoma.

The causes of an anaplastic transformation of KS are unclear, and different potential inducers, such as a long course of the disease, lymphedema, chemotherapy, and immunological defects in AIDS-related KS, have been proposed [3,4].

Given the extreme rarity of this clinical form, there is no consensual treatment regimen. Paclitaxel and liposomal doxorubicin seem to be the most effective chemotherapeutical agents according to some authors. Others suggest that a more aggressive approach involving nonconservative surgical treatment with systemic chemotherapy is the most appropriate for the treatment of anaplastic KS [4].

Consent

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Letter to the Editor

Ulcerative sarcoidosis on the leg and scalp

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Sir,

We report herein two cases of ulcerative sarcoidosis, one of which involved a rare site, scalp.

Case 1: An 80-year-old woman was referred to the dermatology clinic, complaining of erythematous eruptions of the lower legs which had appeared nearly 30 years previously. When she underwent cataract operation, ophthalmological sarcoidosis was suspected, and she was referred to us. Physical examination showed large and small infiltrative erythematous plaques scattered on the anterior aspects of the bilateral lower legs. Biopsy revealed non-caseating granulomas with epithelioid cells in the dermis (Fig. 1a). Serum levels of angiotensin-converting enzyme (ACE) were normal (15.4 IU/L, normal: 7-25); however, serum blood urine nitrogen (27.0 mg/dl, normal: 8-22), creatinine (1.57 mg/dl, normal: 0.4-0.7), and calcium (10.4 mg/dl, normal: 8.7-10.3) levels were increased, suggesting renal sarcoidosis. Chest computed tomography (CT) scan and Gallium scintigraphy excluded bilateral hilar lymphadenopathy. During the follow-up period, she developed ischemic heart failure. Treatment with topical corticosteroid ointment was started; however, during the course, the plaque ulcerated without trauma (Fig. 1b). Thereafter, ulceration was epithelized by topical therapy without systemic prednisolone.

Case 2: A 73-year-old woman complained of shortness of breath, and examination revealed a complete atrioventricular block on electro-cardiogram and left ventricular systolic dysfunction on echocardiography. During hospitalization, she was referred to our department, complaining of skin lesions on the face, scalp and lower extremities. Physical examination revealed a large ulcer partially covered with crusts on the scalp (Fig. 2). Furthermore, brown-reddish infiltrative plaques were scattered on the face and lower legs. Laboratory examination revealed increased levels of CRP (1.42 mg/dl) and ACE (26.1 U/L), and elevated soluble IL-2R (1290 U/ml, normal; 124-466). CT revealed bilateral hilar and mediastinum lymphadenopathy. Ophthalmological examination revealed ocular sarcoidosis, and endomyocardial biopsy revealed fibrosis without sarcoidal granulomas. A skin biopsy taken from the scalp revealed lack of epidermis, infiltration of inflammatory cells in the upper dermis, and non-caseating epithelioid cell granulomas in the dermis (Figs. 3a and 3b); however, granulomatous vasculitis was not observed. Another biopsy from the lower leg showed non-caseating epithelial cell granuloma in the dermis. The ulcers were treated with topical gentamicin sulfate ointment, which resulted in epithelization 5 months later.

Ulceration in cutaneous sarcoidosis is rarely seen, and commonly arises from pre-existing lesions. The legs are the most common sites, and a recent review collected 34 cases, 85% (29/34) of which involved the legs along with other locations including the face, arms, trunk, and genital area. Another survey of 22 Japanese patients revealed that 16 had leg ulcers, 2 had head ulcers, 1 had a buttock ulcer, and 2 had ulcers in multiple locations (unknown:1) [1]. Patients with ulcerative sarcoidosis tend to have lung and ocular lesions. Both of our patients developed ocular, lung, and cardiac sarcoidosis. Furthermore, renal sarcoidosis was suspected in Case 1. It has been reported that ulcerative sarcoidal lesions are associated with severe and active sarcoidosis. Ulcerative sarcoidosis involving the scalp is rare. Patients with scalp sarcoidosis usually have cutaneous sarcoid lesions also on sites other than the scalp [2-4].

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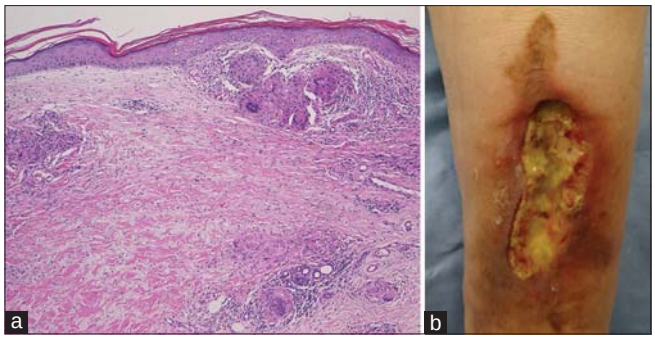

Figure 1: (a) Histological features showing epithelioid granuloma in the dermis (Hematoxylin-eosin, ×100). (b) Ulceration and surrounding plaques on the shin.

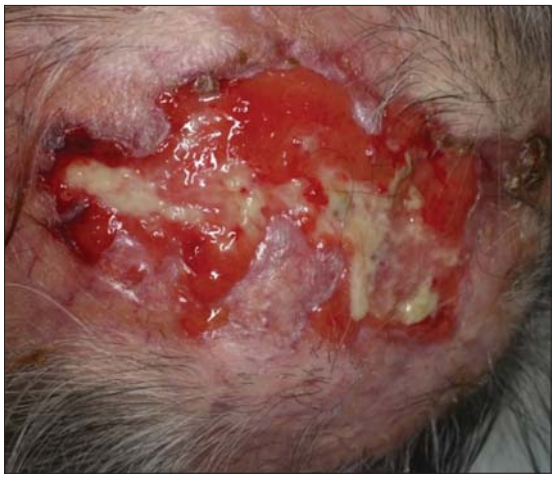

Figure 2: A large ulceration with alopecia on the scalp.

In the majority of cases, scalp sarcoidosis is seen in patients with active systemic sarcoidosis. In this report, Case 2 developed cutaneous sarcoidosis on not only the scalp but also the face and lower legs. This is consistent with previously reported cases of scalp sarcoidosis.

Although previous studies suggested that granulomatous vasculitis, necrotizing granulomas, and hyaline degeneration were the possible causes of ulceration in sarcoidosis, the mechanism of ulceration in sarcoidosis remains uncertain. In our cases, fibrosis was prominent but vasculitis was not detected in the biopsied specimens, which may be due to the biopsied site. We

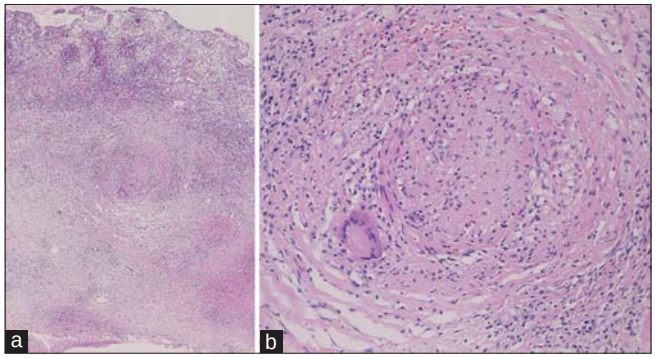

Figure 3: (a) Histological examination revealed a lack of epidermis, inflammatory cell infiltration in the upper dermis, and sarcoidal granulomas in the mid-dermis (×40). (b) Non-caseating epithelioid granulomas in the dermis (×400).

speculate that ischemic conditions may be relevant to the ulceration of sarcoidal plaques.

Consent

The examination of the patient was conducted according to the Declaration of Helsinki principles.

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

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Letter to the Editor

Psoriasis and vitiligo – one therapy for two diseases

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Sir,

Vitiligo is the most common depigmenting disorder, with a world prevalence of approx. 0.5–2% [1]. Its exact pathogenesis is not clear but an autoimmune hypothesis has been supported by both experimental data and clinical association with autoimmune diseases [2]. Psoriasis is, likewise, a dermatotic condition relatively common in the general population, and the literature provides several reports of its coexistence with vitiligo. Some authors consider this simply to be a matter of coincidence, as both are relatively common dermatological diseases. Others have suggested a common pathogenic relationship between the two diseases, although the mechanism has not yet been fully elucidated [3].

We report the case of a 43-year-old female with plaque psoriasis present since the age of 6. At 36, she was diagnosed with vitiligo, and the locations of the individual lesions of both diseases were not coincident. Presenting at our department, the patient had already been on topical therapies and UVB phototherapy. Psoriatic plaques showed a good response to phototherapy and the hypopigmented patches of vitiligo exhibited some repigmentation initially, although only temporarily. A recrudescence of psoriatic lesions during these therapies motivated the institution of methotrexate, to which psoriasis showed some response, but vitiligo patches remained unaltered. However, 17 months later, the patient noticed a worsening of the psoriatic lesions, and methotrexate was discontinued (Figs. 1a–1c). At this point, laboratory tests, including complete blood count, routine blood chemistry, and tests for thyroid function and autoimmunity, fell within normal ranges, and the patient was started on etanercept 50 mg weekly. Over the subsequent weeks, the hypopigmented patches had started to undergo repigmentation and, 4 months later, the vitiligo lesions in the upper and lower limbs displayed a very favorable evolution, with evident repigmentation, which the patient noticed even earlier than the response of the psoriatic plaques, which, this time, were less scaly and infiltrative (Figs. 2a–2c). No adverse side effects related to the administration of etanercept were noted. The patient was kept on the biological agent, with good control of the psoriatic plaques and a steady repigmentation of the vitiligo patches.

The hypothesis of a common pathogenesis of psoriasis and vitiligo has been studied, and tumor necrosis factor alpha (TNF-α) has been considered a plausible intervener in this setting [3]. It has been shown that vitiligo patients have increased tissue and serum levels of proinflammatory soluble mediators. In particular, TNF-α has been shown to inhibit melanogenesis and promote melanocyte apoptosis, at least in vitro: the levels of TNF-α in vitiligo lesions not only become higher than in nonlesional skin, but are also closely related to disease activity [4]. According to some authors, higher tissue levels of TNF are, apparently, correlated with a higher vitiligo activity score, which leads them to consider the intensity of TNF staining in vitiligo lesions as a biomarker for potentially successful anti-TNF treatment in cases refractory to conventional treatment [4]. There have been reports of vitiligo patients receiving TNF inhibitors—including initially for other comorbidities, such as psoriasis, as in our case—and showing repigmentation or at least improvement in vitiligo lesions. However, other reports of TNF inhibitors used in treatment of vitiligo have shown conflicting results [5,6], and some studies have suggested that anti-TNF therapy may induce de novo vitiligo in patients with other autoimmune diseases [1].

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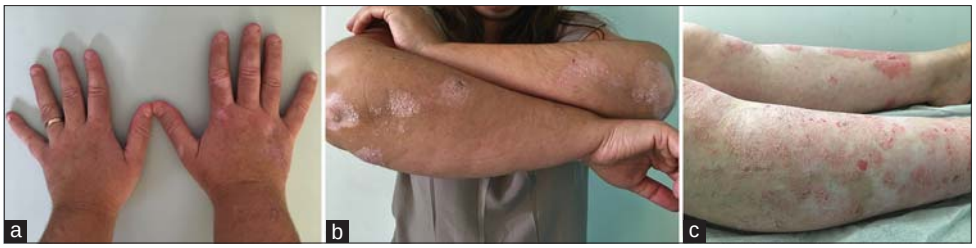

Figure 1: (a-c) Relatively diffuse psoriasis plaques and vitiligo patches in noncoincident locations.

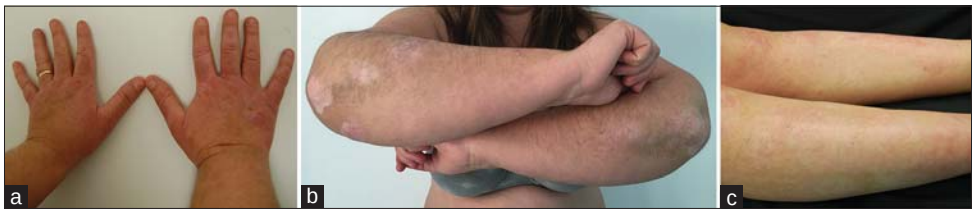

Figure 2: (a-c) Extensive repigmentation of the vitiligo patches at a 4-month follow-up.

Our case corroborates the literature about the likely role of TNF-α in the pathogenesis of vitiligo and emphasizes anti-TNF therapy as a potential therapeutic tool in selected vitiligo patients. The full applicability of TNF inhibitors in vitiligo is far from established and, despite its potential as a viable therapeutic option, further long-term studies investigating its efficacy in vitiligo and/or in combination with other therapeutic agents are necessary.

Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients have given consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed.

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O u r D e r m a t o l o g y O n l i n e

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