View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Journal of the Portuguese Society of Dermatology and Venereology

Dermatoses com Alterações Histológicas Mínimas: Tornar o Invisível Visível

Katarína Kieselová[1], Luís Soares-de-Almeida[2,3]
[1]Serviço de Dermatologia, Centro Hospitalar de Leiria, Leiria, Portugal
[2]Serviço de Dermatologia, Centro Hospitalar Lisboa Norte, Hospital de Santa Maria, Lisboa, Portugal
[3]Clínica Universitária de Dermatologia de Lisboa, Universidade de Lisboa, Faculdade de Medicina, Lisboa, Portugal

RESUMO – As biópsias cutâneas continuam a ser uma ferramenta indispensável no auxílio de dermatologistas para um diagnóstico e tratamento precisos. Contudo, algumas doenças dermatológicas clinicamente evidentes mostram imagens histológicas normais, quando examinadas após preparação com hematoxilina-eosina (H&E). No sentido de estabelecer um diagnóstico correcto, é essencial a correlação clínico-patológica ou executar outras investigações adicionais, como colorações especiais e técnicas de imuno-histoquímica. Neste artigo, são discutidas as mais relevantes destas dermatoses "invisíveis" em H&E, incluindo a estratégia de abordagem nestes casos.
PALAVRAS-CHAVE – Biopsia; Doenças da Pele/patologia; Pele/patologia.

Dermatoses with Minimal Histological Changes: Making the Invisible Visible

ABSTRACT – *Skin biopsies remain an indispensable tool for aiding dermatologists in accurate diagnosis and treatment. However, some clinically evident skin diseases show histological picture resembling normal skin when examined after preparation with hematoxylin and eosin (H&E). In order to establish the correct diagnosis, clinicopathological correlation is essential, together with further investigations such as special stains and immunohistochemistry techniques. Hereby, we discuss the most relevant of these "invisible" dermatoses on H&E, and include strategy for approaching such cases.*
KEYWORDS – *Biopsy; Skin/pathology; Skin Diseases/pathology.*

INTRODUCTION

Skin biopsies remain an indispensable tool for aiding dermatologists in accurate diagnosis and treatment. However, several inflammatory and non-inflammatory dermatoses show no or only subtle alterations in routine hematoxylin and eosin (H&E) stain, especially on low magnification. This concept of "invisible" dermatoses was first introduced by Brownstein and Rabinowitz in 1983, as clinically evident skin diseases that show a histologic picture resembling normal skin.[1] Diagnosis of such dermatoses require meticulous microscopic observation with recognition of subtle changes, with the aid of special stains and additional techniques. Both clinical and histopathological data are critical for the proper diagnoses. Despite representing a relatively common issue in dermatopathological practice, only few authors systematically reviewed this topic.[1-7] This article aims to review the most relevant microscopically invisible dermatoses and discuss the approach for such diseases.

Diagnostic approach to normally appearing skin biopsy (Fig. 1)

1. Clinicopathological correlation and critical analysis of the available data
2. Performing serial sections and multiple level cuts (e.g. superficial basal cell carcinoma)
3. Comparison with normal skin (e.g. morphea)
4. Special stains:
   a. Periodic acid-Schiff (PAS) stain (e.g. fungal skin infections, Lafora disease)

Correspondência: Katarína Kieselová
Department of Dermatology
Centro Hospitalar de Leiria – CHL
Rua das Olhalvas
2410-197, Leiria, Portugal
E-mail: katarinakieselova@gmail.com
DOI: https://dx.doi.org/10.29021/spdv.78.2.1206

Recebido/*Received* 2020/04/30 | **Aceite/*Accepted*** 2020/05/10 | **Publicado/*Published*** 2020/06/30

© Autor (es) (ou seu (s) empregador (es)) 2020 Revista SPDV. Reutilização permitida de acordo com CC BY-NC. Nenhuma reutilização comercial.
© Author(s) (or their employer(s)) 2020 SPDV Journal. Re-use permitted under CC BY-NC. No commercial re-use.

Artigo de Revisão

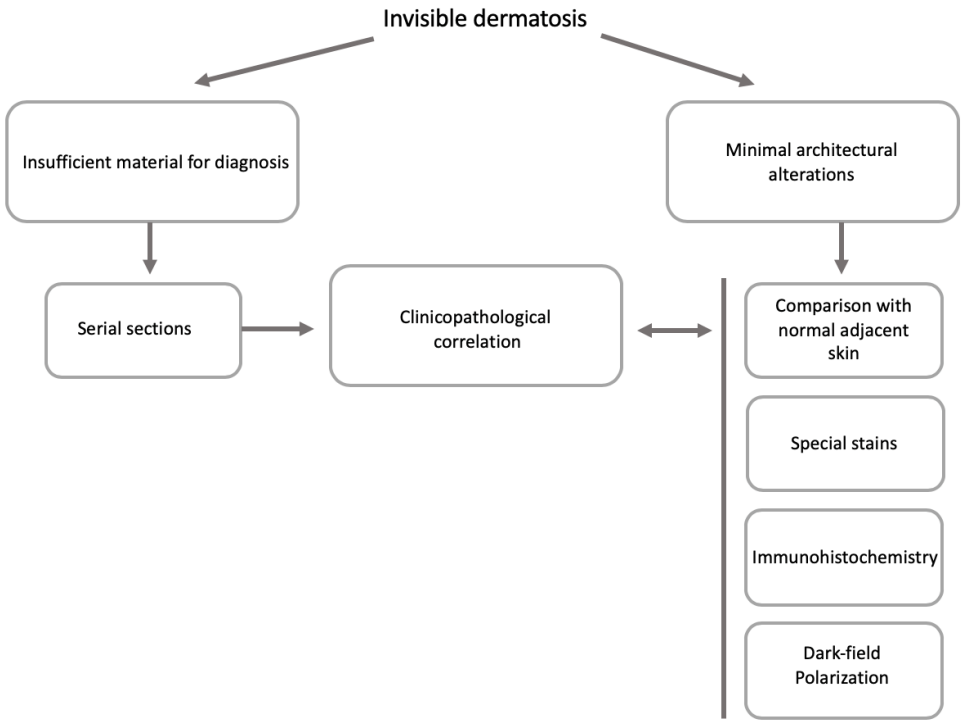

Figure 1 - Diagnostic approach to normally appearing skin biopsy (adapted from Tomasini C[7]).

b. Gram stain (e.g. bacterial skin infection)
c. Perls stain (e.g. stasis dermatitis, Zoon balanitis)
d. Alcian blue (e.g. mucinoses, early lupus erythematosus tumidus)
e. Special stains for amyloid: Congo-red, thioflavin, orcein
f. Giemsa, Leder or Panoptic stain (e.g. telangiectasia macularis eruptiva perstans)
g. Orcein stain (e.g. anetodermia, cutis laxa, Buschke-Ollendorf syndrome)
h. Von Kossa stain (e.g. calciphylaxis)
i. Fontana Masson stain (e.g. vitiligo)
j. Melan-A (e.g. dermal melanocytosis)

5. Dark-field illumination (e.g. argyria, syphilis) and observation under polarized light
6. Biopsy repetition: improper site or insufficient material[2,3]

Systematic examination from the skin surface to depth and the knowledge of subtle clues in each layer of the skin allows recognition of most of these diseases (Table 1).

SUPERFICIAL SKIN INFECTIONS AND INFESTATIONS

The diagnosis of superficial cutaneous infections is usually made clinically. In some cases, examination with Wood lamp, direct microscopic examination of skin scrapings and/or culture is needed to establish or confirm the diagnosis. Histopathological examination is rarely required and there is often a discord between the clinical and histopathological findings and many times clinical appearance can have a poor histopathological correlation.

Bacterial infections

Erythrasma is a superficial bacterial infection caused by excessive proliferation of *Corynebacterium minutissimum*, a Gram positive bacillus, within the stratum corneum. Minute microorganisms can be seen in the orthokeratotic stratum corneum using Gram or PAS stain.[8] Pitted keratolysis affects palmo-plantar skin and is caused by various Gram positive bacteria that degrade keratin in stratum corneum, creating well-defined craters. Gram, PAS or Gomori methenamine silver reveal small cocci with or without an admixture of filamentous form in the walls and at the bottom of the craters.[3,8]

Mycoses

Dermatophyte infections can show a wide range of histological changes such as psoriasiform hyperplasia, presence of neutrophils in the stratum corneum, compact orthokeratosis, papillary dermal edema and the presence of fungal hyphae between two layers of cornified cells, so called the "sandwich" sign (Fig. 2A).[9] However, sometimes these changes are very subtle especially in the setting of long term topical steroid application (*tinea incognito*). Dermatophyte septate hyphae are difficult to visualize on H&E staining, but they are typically highlighted with the PAS stain (Fig. 2B). In candidiasis, neutrophils in stratum corneum may be the

Tabe 1 - A strategy for revealing subtle alterations in invisible dermatoses[2,3]

	LOOK FOR	EXAMPLES
STRATUM CORNEUM	bacteria	erythrasma, pitted keratolysis
	fungi	pityriasis versicolor, tinea nigra
	parasites	scabies
	cornoid lamella	porokeratosis
	irregular cornification	granular parakeratosis
	loss of stratum corneum	circumscribed acral hypokeratosis
	pigment	foreign pigment, subcorneal hemorrhage
EPIDERMIS	absence of granular layer	ichtyosis vulgaris
	absence of pigment and melanocytes	vitiligo, hypomelanosis guttata
	necrotic keratinocytes	notalgia paresthetica, UV induced
	atypical lymphocytes	early mycosis fungoides
	elongation of rete ridges with flat bottom	Becker´s nevus
PAPILLARY DERMIS	mast cells	telangiectasia macularis eruptiva perstans
	erythrocytes and siderophages	pigmented purpuric dermatosis
	melanophages	postinflammatory hyperpigmentation or hypopigmentation
	pigment	ochronosis amiodarone/minocycline pigmentation
	elastic fibers changes	papillary dermal elastolysis
	hyaline deposition	amyloid
	parasites	microfilaria
RETICULAR DERMIS	eosinophils	urticaria, drug eruption
	mast cells	urticaria pigmentosa
	dermal melanocytes	mongolian spot
	vessels	calciphylaxis, livedo reticularis
	collagen fibers changes	scar, morphea
	elastic fibers changes	mid-dermal elastolysis, anetoderma
	mucin deposition	mucinoses and scleredema lupus tumidus
	pigment	tattoo
HYPODERMIS	subcutaneous adipose tissue	lipoatrophy
ADNEXA	sweat glands	anhidrotic ectodermal displasia
	hair follicles	alopecia
	inclusion bodies	Lafora´s disease
	pigment	argyria, aurantiasis, mercury pigmentation

only alteration observed. Fungal elements, spores and filaments, are usually sparse, best visualized with the PAS stain. That is why it has been proposed performing PAS staining for biopsy specimens of all superficial inflammatory dermatoses, in order to increase the diagnostic accuracy.[8] Unlike dermatophytes, *Malassezia* fungal forms, causing pityriasis versicolor, are readily seen on H&E sections. In the stratum corneum, there are numerous round budding yeasts and short septate hyphae giving a so-called "spaghetti and meatballs" appearance.[8] An important differential diagnosis of acral lentiginous melanoma, *tinea nigra*, may show no epidermal alterations at low power magnification, however, on closer view, thick brown or yellow hyphae in the stratum corneum may be recognized.

Infestations

The diagnosis of scabies is usually based on the clinical presentation, biopsy is rarely performed. In atypical cases, where the clinical diagnosis is not straightforward, biopsy may reveal the presence of mites, ova, feces or curled pink structures resembling "pigtails" in the stratum corneum. Usually, multiple sections are needed to identify the burrow

Artigo de Revisão

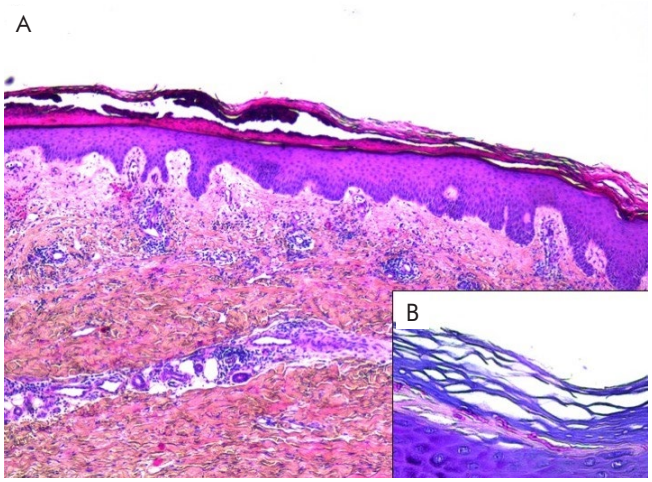

Figure 2 - (A) Dermatophyte infection. Orthokeratosis and layering of stratum corneum ("sandwich sign") and intracorneal neutrophils (H&E; x100), **(B)** presence of fungal hyphae in stratum corneum (PAS; x400).

with mite structures.[3,8] On the contrary, in Norwegian scabies, abundant mite elements can be visualized in the hyperkeratotic cornified layer covering psoriasiform epidermis.[10]

PIGMENTARY DISORDERS

Vitiligo

Vitiligo is a common acquired disorder clinically characterized by depigmented macules and patches of various sizes as a result of selective destruction of melanocytes. The number of melanocytes is decreased in vitiligo, which may be difficult to demonstrate in early lesions, unless adjacent, normal skin is examined (Fig. 3A), thus performing biopsy at the border of the lesion is imperative.[11] Fontana–Masson stain (Fig. 3B) and Melan-A helps to unveil subtle changes in early vitiligo lesions and facilitates the differentiation from other disorders such as early stage of cutaneous mycosis fungoides.[2,12]

Pigment deposits

Diverse exogeneous and endogenous pigment deposits may be present in skin layers. The use of Perls stain helps to better visualize hemosiderin deposits in Zoon balanitis, hemochromatosis, the patch stage of Kaposi sarcoma, stasis dermatitis of the lower legs and also hyperpigmentation due to amiodarone or minocycline.

Pigmented purpuric dermatoses are a group of chronic skin disorders characterized by variable pigmentation resulting from the deposition of hemosiderin as a consequence of erythrocyte extravasation from capillaries in the papillary dermis. To date, six different variants have been described. In the most frequent form, Schamberg disease, hemosiderin deposits are only subtle, therefore Perls stain can help to identify hemosiderin-laden macrophages (Fig. 4).[12,13]

In argyria, multiple small dark brown granules along the basement layer of sweat glands can be observed, more easily visualized on dark-field examination.[2,3]

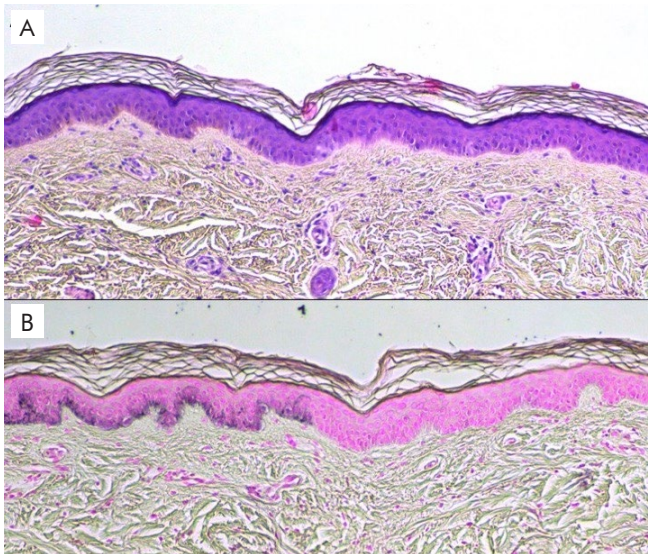

Figure 3 - (A) Vitiligo. Normally appearing skin (H&E; x100), (B) Fontana-Masson stain highlights the intraepidermal melanin loss in the right half of the biopsy specimen (FM; x100).

INFLAMMATORY DERMATOSES AND CUTANEOUS DEPOSITS

Urticaria

In most cases the clinical diagnosis of urticaria is straightforward, however, if urticarial vasculitis is suspected, a punch biopsy of the lesion should be performed. Histopathologic findings of urticaria are usually mild, including sparse perivascular and interstitial mixed inflammatory infiltrate and upper dermal edema. The presence of eosinophils and neutrophils in the interstitium may help to confirm the diagnosis.[3,14]

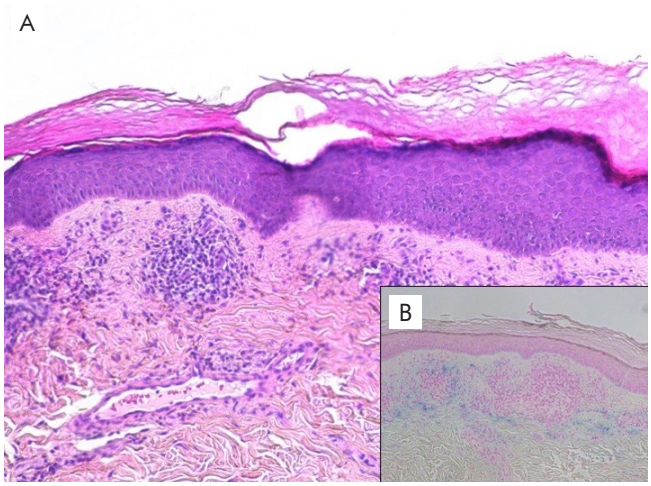

Figure 4 - (A) Purpuric pigmented dermatosis. Lymphocytic cuffing of vessels and dilated capillaries in the papillary dermis, sparse extravasated erythrocytes (H&E; x100), **(B)** interstitial hemosiderin (blue) deposits (Perls stain; x100).

Mastocytosis

Cutaneous mastocytosis is characterized by abnormal mast cell infiltration in the dermis, however the degree of tissue infiltration may vary widely. Telangiectasia macularis eruptiva perstans is a variant with a very subtle increase of mast cells in dermis, posing a challenge for the dermatopathologist, especially in the absence of clinical correlation. Additionally, mast cells may be spindle-shaped and thus indistinguishable from histiocytes. Special stains such as Giemsa, Toluidine blue, Panoptic or Leder stain and immunohistochemistry for CD117 (c-kit) help to highlight the presence of mast cells (Fig. 5).[1,3,15]

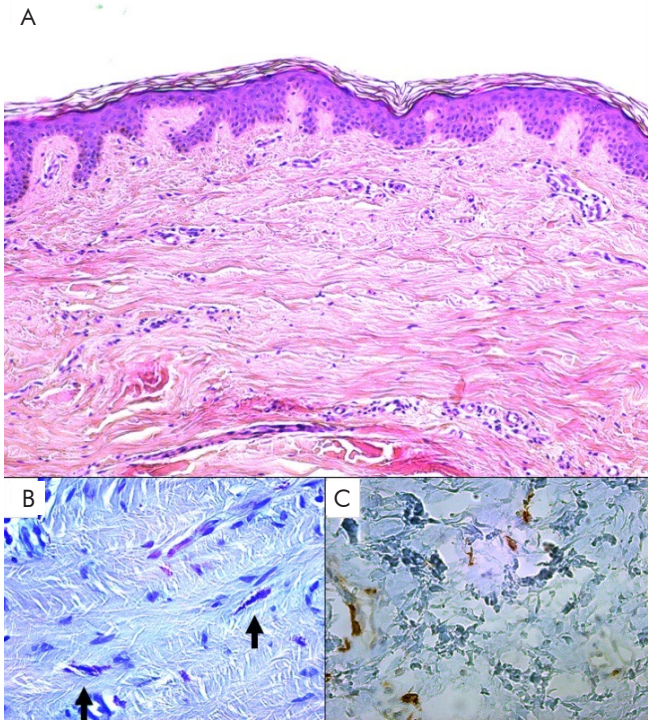

Figure 5 - (A) Telangiectasia macularis eruptiva perstans. Dilated vessels in the superficial dermis (H&E; x100), **(B)** sparse fusiform mast cells (arrow) highlighted by Panoptic stain (x400), **(C)** and positive for CD117 (x400).

Mucinoses

Cutaneous mucinoses are characterized by an increase in the normal dermal mucin either focally or diffusely. On H&E staining, this increase in the dermal mucin may not be evident due to processing of paraffin biopsy creating a dermis picture with separated collagen fibers. To uncover mucin deposits, Alcian blue at pH 2,5 and the colloidal iron stain are commonly used.[2,3]

The demonstration of stromal mucins may be a clue for the diagnosis of several other dermatoses such as cutaneous lupus erythematosus or dermatomyositis.

Amyloidosis

Amyloid is a fibrillar protein that tends to aggregate extracellularly in the form of eosinophilic amorphous material. In macular amyloidosis, inconspicuous amphophilic globules may be observed in papillary dermis. The diagnosis may be confirmed by special stains such as Congo red, crystal-violet and orcein.[1,16]

DISORDERS OF DERMAL CONNECTIVE TISSUE

Alteration of elastic fibers

Special stains for elastic fibers such as orcein or Verhoeff--van Gieson allow the unveiling of elastic tissue abnormalities. Focal loss of papillary or mid-dermal elastic fibers leads to papillary dermal or mid-dermal elastolysis (Fig. 6). In anetoderma, full-thickness disappearance of elastic fibers is observed. In addition, a comparison between lesional and uninvolved skin is crucial to establish the diagnosis. Altered elastic fibers in the reticular dermis together with calcium deposits (highlighted by von Kossa staining) are highly suggestive of pseudoxanthoma elasticum.[6] Additionally, elastic tissue stains may help to differentiate between morphea, particularly in the superficial guttate form, and lichen sclerosus, with elastic fibers preserved in papillary dermis in the former and diminished in the latter.

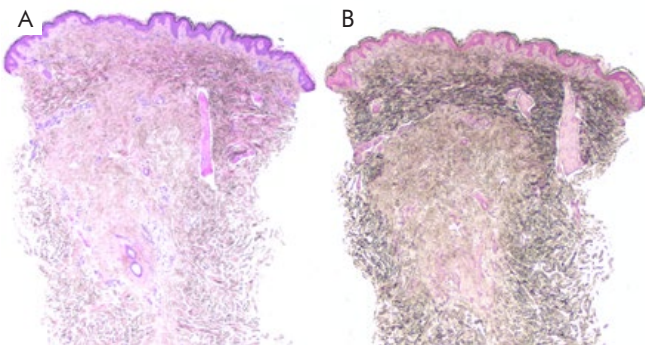

Figure 6 - (A) Mid-dermal elastolysis (H&E; x25), **(B)** Loss of elastic fiber in the mid dermis (Orcein stain; x25).

Alteration of collagen fibers

Sclerosing dermatoses result in thickening and hyalinization of collagen fibers of deep dermis, with extension to subcutis, therefore a deep incisional biopsy is indicated if scleroderma is suspected. If just a punch biopsy is performed, at scanning magnification, tissue sections show notably straight, parallel edges, so called "square biopsy". The dermis may appear normal and one must decide if it is thickened, comparing lesional skin with normal, adjacent skin. In the advanced stages, it is not possible to differentiate between different sclerodermic diseases, therefore clinicopathological correlation is essential to establish the diagnosis.[3,17]

CONCLUSION

To allow the dermatopathologist to correctly interpret the histological findings, clinicopathological correlation as well as selection of an adequate biopsy technique and a suitable

Artigo de Revisão

biopsy site are essential. This is even more true in case of a biopsy with minimal histological changes. The ability to analyze systematically all layers of the skin and recognize subtle histological features is fundamental to achieve the final diagnosis.

Conflitos de interesse: Os autores declaram a inexistência de conflitos de interesse na realização do presente trabalho.
Fontes de financiamento: Não existiram fontes externas de financiamento para a realização deste artigo.
Proveniência e revisão por pares: Não comissionado; revisão externa por pares.

Conflicts of interest: *The authors have no conflicts of interest to declare.*
Financing support: *This work has not received any contribution, grant or scholarship.*
Provenance and peer review: *Not commissioned; externally peer reviewed*

ORCID
Katarína Kieselová
https://orcid.org/0000-0002-0064-7571
Luís Soares-de-Almeida
https://orcid.org/0000-0003-4026-6105

REFERENCES

1. Brownstein MH, Rabinowitz A. The invisible dermatoses. J Am Acad Dermatol. 1983;8:579-88.
2. Requena L, Kutzner H. Invisible dermatosis. Pathologe. 2002;23:54-64.
3. Tomasini C. Invisible dermatoses from the perspective of the dermatopathologist: new observations. G Ital Dermatol Venereol.. 2017;152:500-15.
4. Requena L, Sánchez Yus E. Invisible dermatoses. Additional findings. Int J Dermatol. 1991; 30:552-7.
5. Mysore V. Invisible dermatoses. Indian J Dermatol Venereol Leprol. 2010;76:239-48.
6. Carlotti A. Invisible dermatoses. Ann Dermatol Venereol. 2009;136:152-9.
7. Tomasini C, Michelerio A. Invisible dermatoses: clues and pitfalls to diagnosis. Diagnostic Histopathol. 2018;24:320-37.
8. Cardoso JC, Veraitch O, Gianotti R, Ferrara G, Tomasini CF, Singh M,et al. "Hints" in the horn: diagnostic clues in the stratum corneum. J Cutan Pathol. 2017;44:256-78. doi: 10.1111/cup.12839.
9. Park YW, Kim DY, Yoon SY, Park GY, Park HS, Yoon HS, et al. "Clues" for the histological diagnosis of tinea: how reliable are they? Ann Dermatol. 2014;26:286-8. doi: 10.5021/ad.2014.26.2.286.
10. Fernandez N, Torres A, Ackerman AB. Pathologic Findings in Human Scabies. Arch Dermatol. 1977;113:320-4.
11. Kim YC, Kim YJ, Kang HY, Sohn S, Lee ES. Histopathologic features in vitiligo. Am J Dermatopathol. 2008;30:112-6.
12. Alhumidi A, Alshamlan N, Alfaraidi M, Mohajer K. Invisible dermatosis, diagnostic discrepancy between the general pathologist and dermatopathologist. J Cutan Pathol. 2019;46:905912.
13. Johnston RB. 8 - Vasculopathic Reaction Pattern. In: Johnston RB, editor. Weedon's Skin Pathology Essentials2nd ed. Amsterdam: Elsevier; 2017.p.163-94.
14. Antia C, Baquerizo K, Korman A, Bernstein JA, Alikhan A. Urticaria: A comprehensive review: Epidemiology, diagnosis, and work-up. J Am Acad Dermatol. 2018;79:599-614. doi: 10.1016/j.jaad.2018.01.020.
15. Severino M, Chandesris M-O, Barete S, Tournier E, Sans B, Laurent C,et al. Telangiectasia macularis eruptiva perstans (TMEP): A form of cutaneous mastocytosis with potential systemic involvement. J Am Acad Dermatol. 2016;74:885-91.e1. doi: 10.1016/j.jaad.2015.10.050.
16. Brownstein MH, Hashimoto K. Macular amyloidosis. Arch Dermatol. 1972;106:50-57.
17. Walters R, Pulitzer M, Kamino H. Elastic fiber pattern in scleroderma/morphea. J Cutan Pathol. 2009;36:952-7.