CASE REPORT

Clinical variability in dystrophic epidermolysis bullosa and findings with scanning electron microscopy *

Variabilidade clínica em epidermólise bolhosa distrófica e achados de microscopia eletrônica de varredura

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Abstract: In dystrophic epidermolysis bullosa, the genetic defect of anchoring fibrils leads to cleavage beneath the basement membrane and its consequent loss. A 46 year-old female patient presented blisters with a pretibial distribution associated with nail dystrophy. Her two children had hyponychia and anonychia, which affected all toe nails and the thumb, forefinger and middle finger. DNA sequencing identified in exon 75 of COL7A1 gene a pathologic mutation: c.6235G>A (p.Gly2079Arg). Immunomapping of a blister demonstrated collagen IV (basal membrane) in the blister roof and collagen VII in its floor, confirming dystrophic epidermolysis bullosa. Scanning electron microscopy of an inverted blister showed net-forming collagen attached to the blister roof . The variability found in this family has already been reported and confirms, on a clinical basis, the nail subtype as a dystrophic variant.

Keywords: Epidermolysis bullosa dystrophica; Genetic variation; Microscopy, electron, scanning; Nails; Skin diseases, vesiculobullous

Resumo: Na epidermólise bolhosa distrófica, o defeito genético das fibrilas de ancoragem leva à clivagem abaixo da membrana basal com sua consequente perda. Uma paciente de 46 anos apresentava bolhas pré-tibiais associadas à distrofia ungueal. Seus dois filhos apresentavam hipo e anoníquia, afetando todas as unhas dos pododáctilos e dos primeiros, segundos e terceiros quirodáctilos. O sequenciamento de DNA identificou no exon 75 do gene COL7A1 uma mutação patológica: c.6235G>A (p.Gly2079Arg). O imunomapeamento identificou o colágeno IV no teto e colágeno VII no assoalho de uma bolha , confirmando o diagnóstico de epidermólise bolhosa distrófica. A microscopia eletrônica de varredura de um teto invertido de bolha demonstrou rede de colágeno aderida ao mesmo. A variabilidade clínica encontrada nessa família já foi escrita e confirma, que o subtipo ungueal das epidermólises bolhosas é uma forma distrófica.

Palavras-chave: Dermatopatias vesiculobolhosas; Epidermólise bolhosa distrófica; Microscopia eletrônica de varredura; Unhas; Variação genética

INTRODUCTION

The denomination epidermolysis bullosa (EB) was first used in 1886. In 1962, a classification was proposed by Pearson based on the application of transmission electron microscopy. [1]

Epidermolysis bullosa refers to a group of inherited bullous diseases with different modes of inheritance, characterized by the formation of blisters triggered by minimum trauma or skin traction, hence the denomination mechanobullous diseases; nail dystrophy may be an associated feature.[1-4]

The subtypes are classified into three groups according to cleavage level: (Simplex, Junctional and

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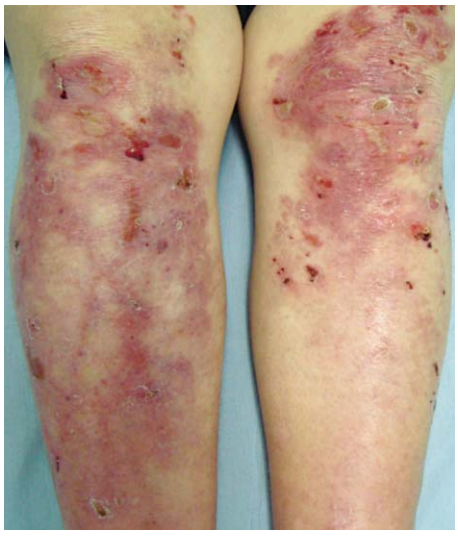

Figure 1: Pretibial blisters and erosions

Dystrophic). Currently, there are more than 20 clinical variants, in several subtypes.[5] Genetic mutations are found in at least seven proteins (Keratin 5 and 14; Laminin 332; Collagen type VII; Collagen XVII; Plectin and Integrin $\alpha 6\beta 2$). [5]

In dystrophic forms (DEB), the genetic defect of anchoring fibrils, collagen VII, leads to cleavage beneath the basement membrane and its consequent loss. Many clinical variants are described such as the pretibial, the albopapuloid, the pruriginous and the nail types.[1,2,5-10]

In recent years, monoclonal antibodies have been used to classify EBs in the so-called immunomapping. In the dystrophic form, the identification of the basement membrane - collagen IV - on the blister roof confirms the diagnosis.[11]

The pretibial DEB is a rare clinical variant, which is clinically differentiated from other types of DEB for presenting more localized lesions, with blisters and erosions, scarring, milia, frequent itching and nail dystrophy.[2,4]

We report here a family with DEB and the ultrastructural findings under scanning electron microscopy of an inverted blister.

CASE REPORT

A 46 year-old female patient was examined in our outpatient clinic and informed that blisters had occurred in her pretibial area since childhood. Dermatologic examination showed blisters and erosions with a pretibial distribution, some blisters with hemorrhagic content. Besides the blisters, dystrophy was observed in all toe nails and nine finger nails (Figure 1). Only the left fifth finger nail was spared (Figure 2). Her two children were also examined and had only ungual dystrophy, which affected all toe nails and the thumb, forefinger and middle finger, characterized by hyponychia and anonychia (Figure 3).

DNA sequencing identified in exon 75 of COL7A1 gene a pathologic mutation: c.6235G>A (p.Gly2079Arg). Immunomapping of on of the mother's blisters demonstrated collagen IV (basal membrane) in the blister roof and collagen VII in its floor, establishing the diagnosis for DEB (Figure 4A and 4B).

Scanning electron microscopy of an inverted blister showed net-forming collagen attached to the blister roof (Figure 5). This net is composed of intertwined fibers of different sizes.

DISCUSSION

Dystrophic Epidermolysis Bullosa is a well-defined group of EB, with loss of the basement membrane resulting from inherited dysfunction of collagen VII, the protein that participates in the adherence of the basal membrane to the dermis.[6-8]

Cases with exclusive nail dystrophy are classified as a DEB subtype.[5] The clinical variability found in this family has already been reported in individuals with the same mutations and confirms the nail subtype as a DEB variant. The autosomal dominant mode

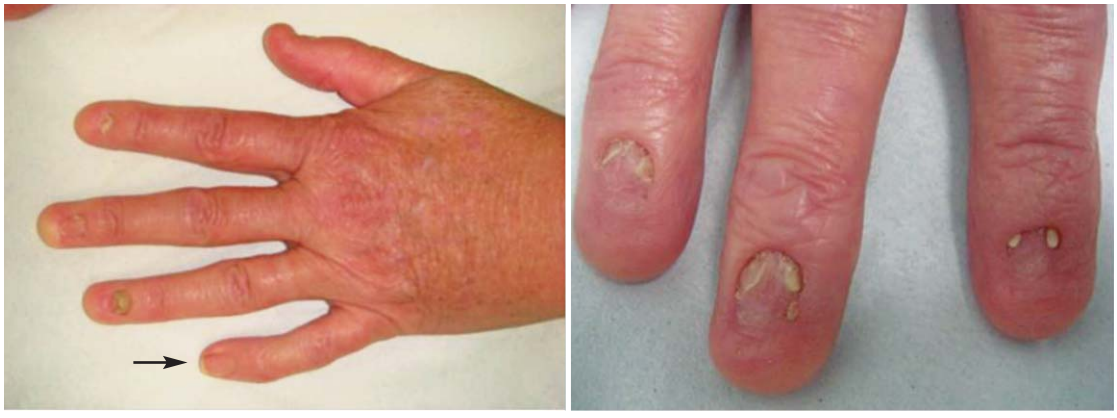

Figure 2: Mother's nail involvement, with secondary hyponychia. Note that the nail of the fifth finger was spared (arrow)

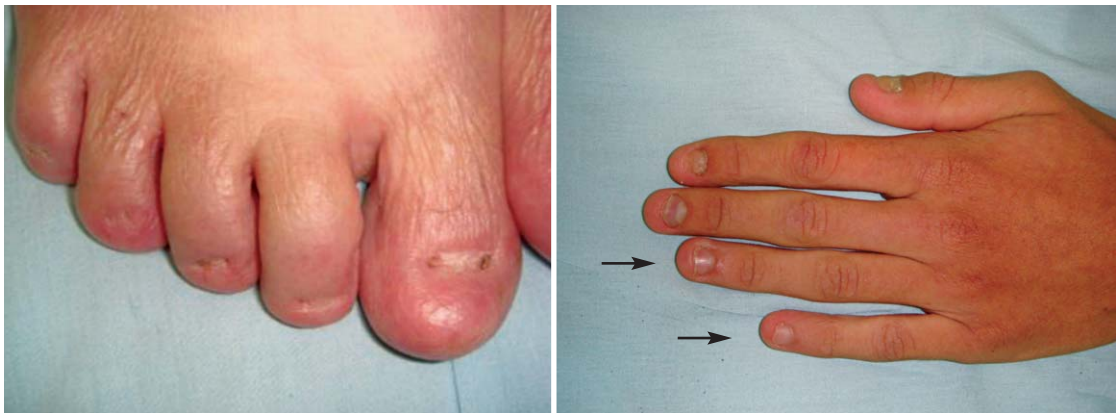

Figure 3: Son's nail involvement. Secondary anonychia in the toes. Nails of the forth and fifth fingers are spared (arrows)

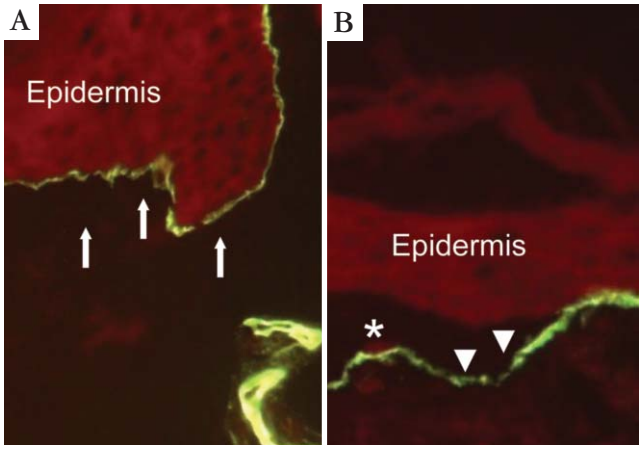

Figure 4: Immunomapping. a. antibody against collagen IV shows the basement membrane in the roof (arrows). b. antibody against collagen VII has positive staining on blister's base (arrowheads). Asterisks show the cleavage

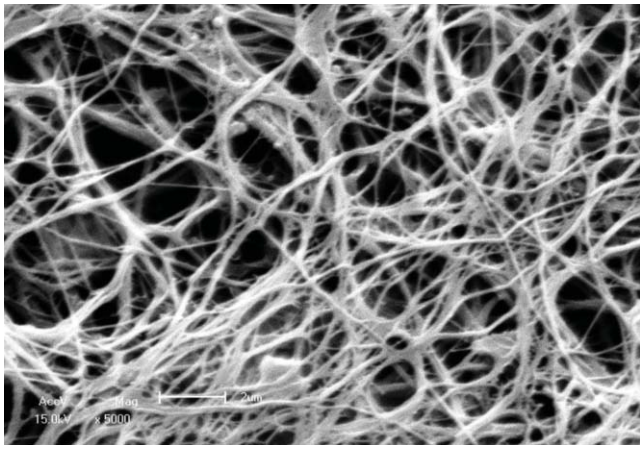

Figure 5: Scanning electron microscopy of an inverted blister's roof shows a collagen net (6,000 X)

of transmission was also reported, as found in this family.[4,6]

The involvement of all toenails and of only the fingernails caused by labor activities in younger patients demonstrates the role of trauma in triggering nail lesions, characterized in this family by hypo and anonychia. [3]

No reports were found in the literature on the use of scanning electron microscopy for DEB. The examination of an inverted roof of a blister with this technique identified a collagen net, attached to the epidermis, similar to immunomapping findings, which also demonstrated collagen IV in the epidermal side of the blister.

This collagen forms the basal membrane, which is classified as a net-forming collagen.[12,13] These findings provide, due to collagen VII dysfunction, a unique documentation on the morphology of this important dermoepidermal junction structure, which was reported only under artificial conditions, as induced epidermal loss in oral mucosa. [14]

In spite of being available only in few research centers, the scanning electron microscopy could be used as an alternative technique for diagnosis of dystrophic forms of EB, with the identification of the basal membrane on the blister roof. Besides, the tissue can be obtained in a non-traumatic way for the examination, which costs less than the immunomapping technique. ❑

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REFERENCES

1. Souza MJS, Pires CAA, Vieira KKS, Miranda MFR, Unger DAA. Epidermólise bolhosa distrófica pruriginosa: relato de caso. An Bras Dermatol. 2005;80:S370-2.
2. Horn HM, Tidman MJ. The clinical spectrum of dystrophic epidermolysis bullosa. Br J Dermatol. 2002;146:267-74.
3. Tosti A, de Farias DC, Murrell DF. Nail involvement in epidermolysis bullosa. Dermatol Clin. 2010;28:153-7.
4. Lee JY, Chen HC, Lin SJ. Pretibial epidermolysis bullosa: a clinicopathologic study. J Am Acad Dermatol. 1993;29:974-81.
5. Fine JD, Eady RAJ, Bauer EA, Bauer JW, Bruckner-Tuderman L, Heagerty A, et al. The classification of inherited epidermolysis bullosa (EB): Report of the Third International Consensus Meeting on Diagnosis and Classification of EB. J Am Acad Dermatol. 2008;58:931-50.
6. Nakamura H, Sawamura D, Goto M, Sato-Matsumura KC, LaDuca J, Lee JY, et al. The G2028R glycine substitution mutation in COL7A1 leads to marked inter-familiar clinical heterogeneity in dominant dystrophic epidermolysis bullosa. Dermatol Sci. 2004;34:195-200.
7. Dang N, Murrell DF. Mutation analysis and characterization of COL7A1 mutations in dystrophic epidermolysis bullosa. Exp Dermatol. 2008;17:553-68.
8. Jonkmam MF, Moreno G, Rouan F, Oranje AP, Pulkkinem L, Uitto J. Dominant Dystrophic Epidermolysis Bullosa (Pasini) caused by a novel glycine substitution mutation in the type VII collagen gen (COL7A1). J Invest Dermatol. 1999;112: 815-7.
9. Gardella R, Castiglia D, Posteraro P, Bernardini S, Zoppi N, Paradisi M, et al. Genotype-phenotype correlation in Italian patients with dystrophic epidermolysis bullosa. J Invest Dermatol. 2002;119:1456-62.
10. Spinelli LP, Orofino RR, Kac BK, Sodré C, Azulay RD. Epidermólise bolhosa albopapulóide (variante de Pasini). An Bras Dermatol. 2003;78:459-63.
11. Oliveira ZNP, Périgo AM, Fukumori MI, Aoki V. Imunomapeamento nas epidermólises bolhosas hereditárias An Bras Dermatol. 2010;85:856-61.
12. Gordon MK , Hahn RA. Collagens Cell Tissue Res. 2010;339:247-57.
13. Shoulders MD, Raines RT. Collagen structure and stability. Annu Rev Biochem. 2009;78:929-58.
14. Abe M, Osawa T The structure of the interstitial surfaces of the epithelial basement membranes of mouse oral mucosa, gingiva and tongue. Arch Oral Biol. 1999;44:587-94.

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