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Research Article

D2-40 and Collagen IV: Effective Diagnostic Markers for Acquired Reactive Perforating Collagenosis

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Abstract

Reactive perforating collagenosis (RPC) is a rare perforating dermatosis characterized by transepidermal elimination of collagen fibers. The disease subsets are mainly divided into acquired reactive perforating collagenosis (ARPC) and inherited reactive perforating collagenosis (IRPC). ARPC is misdiagnosed easily due to its low incidence and similar clinical manifestations to a variety of skin diseases. The purpose of this study was to improve the diagnostic accuracy of ARPC through multiple histochemical methods and to find the specific expression of biomarkers. A deep analysis by immunohistochemical and histochemical methods applied on four ARPC patients in Zhongnan Hospital of Wuhan University in the 2021. We found that the main pathological component in ARPC is collagen type IV, which provides new clues for the pathogenesis of this disease. On the basis of this finding, we observed that D2-40 is an effective marker for the ARPC. Our results illustrate the important role of different histochemical staining techniques in the differential diagnosis of penetrating diseases. In this study, we further confirmed that collagen type IV penetrates the skin surface in APRC and the D2-40 was an effective diagnosis marker for ARPC, which provides new clues for its research and treatment.

INTRODUCTION

Reactive perforating collagenosis (RPC) is a rare perforating dermatosis characterized by transepidermal elimination of collagen fibers [1]. Studies have shown that some cases of RPC may have an autosomal recessive or dominant genetic predisposition, which is called, inherited reactive perforating collagenosis (IRPC) [2]. Cases with no genetic predisposition are called acquired reactive perforating collagenosis (ARPC) [2,3]. IRPC is usually found in children, while ARPC is present in adults [3]. ARPC cases are often accompanied by serious systemic diseases such as chronic renal failure, hypertension, autoimmune disease, diabetes, hepatitis, or malignant tumors [4,5]. The incidence of ARPC is approximately 253/100,000 per year, the ratio of male and female patients is about 1.5:1 [6], and the median age is 56.8 years [6]. ARPC is prone to missed diagnosis and misdiagnosis, mainly due to its low incidence, unclear pathogenesis, and clinical symptoms that are similar to eczema and tuberous itching rash [7]. Therefore, the accurate diagnosis of ARPC is clinically very important. In this study, we found that D2-40 was specifically expressed at the lesion site, which providing a strong morphological clues for diagnosis. And then, we confirmed the specific expression of Collagen type IV was consistent with D2-40, which also provided evidence for diagnosis of ARPC. Therefore, we compared the specificity and accuracy of commonly used histochemical and immunohistochemical methods in the differential diagnosis of a confirmed ARPC case, which provides an effective diagnosis scheme for ARPC.

MATERIALS AND METHODS

Data Screening and HE Staining

We collect 4 cases with ARPC at Zhongnan Hospital of Wuhan University from January 1, 2019 to December 31, 2019. All patients 'biopsies were diagnosed by histopathologic examination. Biopsy tissues were fixed in 10% neutral formalin followed by gradient dehydration, clearing, and paraffin embedding. Tissues were cut into 3-μm-thick sections followed by routine dewaxing and hydration. And then, routine HE staining was performed.

Masson Staining

After dewaxing, the routine 3-μm-thick sections were stained with iron hematoxylin and acid magenta dye for 10 min and then slightly rinsed with running water. Then a solution of phosphomolybdate and the toluidine blue for 5 min separately. After acetic acid and conventional alcohol dehydration was used, the samples were sealed with neutral gum.

Elastic Dyeing

After oxidized potassium permanganate and bleached with oxalic acid, the routine 3-μm-thick sections were slightly washed with 95% alcohol. Then the slices were immersed in elastic fiber dye overnight. The next morning, 1% hydrochloric acid was used for differentiation and applied Van Gieson's dyeing. Then the sections were differentiated with 95% alcohol, dehydrated and sealed with neutral gum.

Immunohistochemical Examination

After fixation with 4% neutral formaldehyde, 5-μm tissue sections were dehydrated and embedded in paraffin. Detailed procedures were according to the kit and instrument standard protocol. Anti-IV collagen and D2-40 rabbit antibody were from Wuhan Zhongji Biotechnology Company (1:200), and IHC detection was conducted by the EnVision two-step method. The black and brown granules in the cells indicated positive reactions; otherwise, they were negative.

RESULTS

Cases and Skin Specialist Examination

Among the 4 patients, there were 2 males and 2 females. The age of the 4 patients ranged from 46 to 65 years, with an average age of 53 years. The patients had multiple skin lesions without obvious cause. The patients were treated with external drugs and oral antiallergic drugs in a hospital. The skin lesions were not progressive, but still itchy. Two patients had a history of hypertension, and the control of blood pressure via drug intervention was acceptable. Three of the patients had a history of diabetes for more than 10 years, with the highest blood glucose level being 29 mm, while, they all could maintain normal glucose levels after insulin injection. Laboratory examination confirmed that routine blood glucose, liver, and kidney function were normal. One of the cases, a skin mass about 8 cm×5 cm was seen in the middle of the back, with a bright red color, high local skin temperature, a clear boundary and obvious tenderness which were the symptoms of acute inflammation caused by scratching. Red papules and crusted ulcers were seen all over the body in all of the cases, especially in the lower extremities. In the center of the lesions, umbilicus pits were seen with concentric yellow horn plugs, which seemed to be firm and difficult to remove; pigmentation was seen around the lesions (Figure 1).

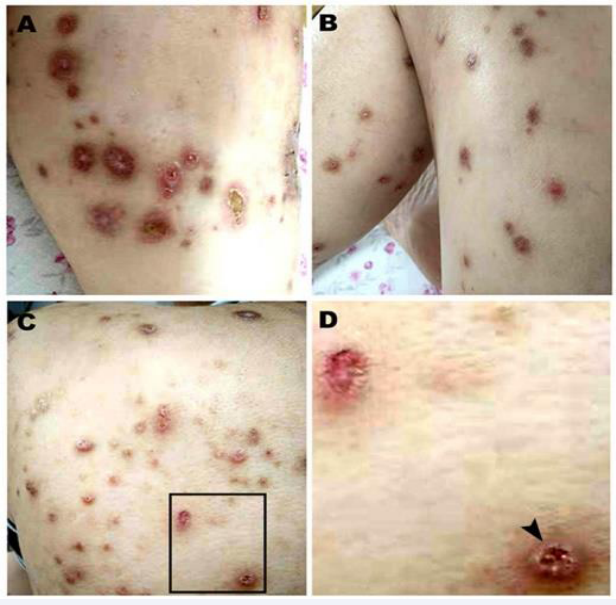

Figure 1 Pigmentation lesions.

Pathological Features in Hematoxylin-Eosin (HE) Staining

This is a squamous hyperplasia with a localized pseudoepitheliomatoid hyperplasia. Cup-shaped pits of the epidermis with hypertrophy or hyperkeratosis of the spines on both sides of the lesions were seen in HE sections. Basophilic collagen fibers and keratinized cell fragments filled in the depression, while degeneration and obvious collagen fractures or curls were observed below the lesions. In the epidermis, the degenerated collagen bundles penetrated vertically, and the degenerated collagen fibers were interlinked with the epidermal ulcer. A few collagen lumps existed in the superficial layer of inflammatory necrosis in the ulcer area. In the superficial layer of the dermis, a few lymphocytes and infiltration of eosinophil cells around the small blood vessels were seen (Figure 2 A and B).

Masson and Elastic Fiber Staining

Masson stain highlighted collagen fibers were clearly seen in the bottom of the ulcer, and blue collagen fibers were visible that penetrated the epidermis according to Masson's staining (Figure 2 C and D). Stretched fibers were dyed blue-black by hydrogen bonding from the elastic fiber dye. Elastic fiber staining showed elastic fiber fractures in skin lesions. The elastic fibers were mixed with collagen fibers throughout the epidermis, but the number of elastic fibers did not increase (Figure 2 E and F). The manifestation of the elastic fibers was a secondary pathological change in this case, but the major pathological changes were key to the differential diagnosis with elastosis perforans serpiginosa (EPS).

Immunohistochemical Characteristics

Immunohistochemical methods detected specific, dark brown, positive staining of collagen type IV in the center of the lesions. Clearly positive collagen fibers were seen throughout the epidermis and gathered nest-like in the skin surface from the bottom of the lesions. These findings confirmed that the perforating substances from the dermal tissue throughout the epidermis were collagen fibers of type IV(Figure 3 A and B). Sections of ARPC immunochemistry stained with monoclonal antibody D2-40 (Figure 3 C and D). In the basal epidermis, the D2-40 positive staining in the local areas of normal basal layers relatively (Figure 3C, Start). However, the positive D2-40 staining marker were seen throughout the epidermis and gathered in the skin surface such as the expression of the collagen fibers of type IV rather than gathered in the basal layer A.

DISCUSSION

RPC is a rare disease that is mainly divided into ARPC and IRPC [7]. The etiology and pathogenesis of RPC have not been clearly reported [8]. ARPC may be associated with systemic diseases is 75%–100%, such as hypertension (40%–73%), diabetes (30%–80%), chronic renal failure (12%–53%), liver disease (12%–20%) and hyperlipidemia (7%)[6]. Some ARPC patients often develop the disease after the exacerbation of a systemic disease [9]. Studies have shown that the expression of transforming growth factor beta 3 (TGF-β3) is increased in the serum of ARPC patients [10]. TGF-β3 plays an important role in wound repair, which can delay epidermal regeneration, regulate epidermal

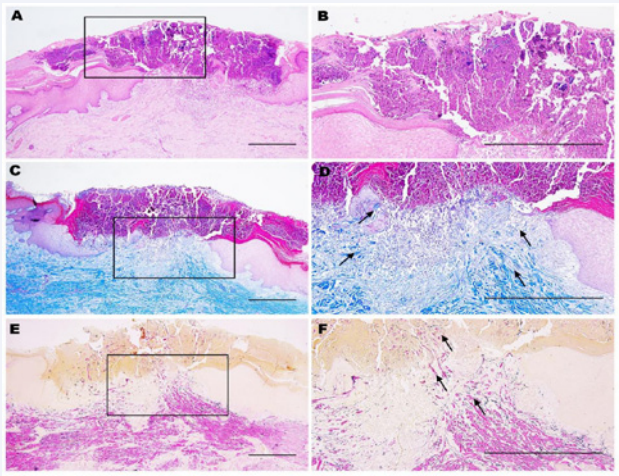

Figure 2 (A-F): Lymphocytes and infiltration of eosinophil cells.

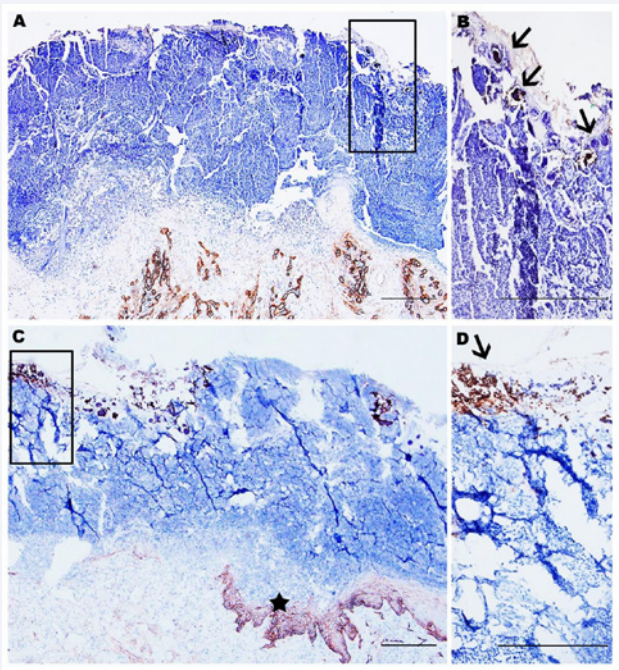

Figure 3 Collagen fibers.

remodeling, and alter the metabolism of extracellular matrix proteins [11]. Some references suggest that increased TGF-β3 has a relationship with the itching and scratching trauma in the early stage of ARPC [11]. Furthermore, itching and scratching trauma can induce the degradation of collagen fibers throughout the epidermis, which secondarily aggravates the skin lesions. The low incidence of ARPC leads to its insufficient understanding by clinicians and pathologists, which makes it easy to misdiagnose. Therefore, the effective detection method becomes the necessary means for ARPC diagnosis.

The typical characteristic of APRC is keratinized papules or nodules, and papules with central keratinization that adhere tightly[12]. Typical ARPC skin examination shows a rash containing papules with a central depression with good keratinocyte adhesion that is difficult to peel off [12]. The rash subsides spontaneously and leaves temporary hypopigmentation and atrophic scars. The skin lesion manifestation of ARPC should be differentiated from tuberculous pruritus, eczema, and papous necrotizing tuberculous rash [13]. Typical patients of tuberculous pruritus will have nodular itchy rashes that are relatively large, about 0.5–1.0 cm in diameter, that are hard and break easily after scratching [14]. The scabs are easy to peel off after the skin is dry. The shape of an eczema rash is diverse, with an exudative itchy rash mainly in the acute phase, while the surface of the rash will be mossy in the chronic phase [15]. Papous necrotizing tuberculous rashes tend to be distributed with the limbs symmetrically. They usually form pustules at the top. Concave necrosis and ulceration can be seen after the rash has dried up.

The typical histopathology of ARPC is that the epidermis shows cup-shaped damage with basophilic collagen fibers and incomplete cell fragments in the depressions. In this study, vertical perforating collagen fibers were visible in the epidermis, but the number of elastic fibers in the dermis did not increase. There were a few histocytes and lymphocytes that infiltrated in the dermis, and there were hypertrophy and hyperkeratosis on both sides of the cup-shaped lesions in the epidermis. According to the different perforating excretions through the epidermis and different histochemical staining methods, colorful excreted materials can be distinguished from several other penetrating dermatoses histologically. The collagen fibers were stained blue by Masson's stain, caused by the different molecular weights, negative charges, and the different properties of the tissues. Elastic fibers were dyed blue-black because of the hydrogen bonds formed between the elastic fibers and phenolic groups in the elastin stain.

The clinical manifestations of EPS are reddish hyperkeratosis papules that are linear, annular, or arc-shaped and spread prostrate [16]. Histopathology shows increased degenerate elastic fibers that are excreted through the epidermis from the superficial dermis. Elastic fiber staining primarily shows elastic fibers bursting out from the dermis to the epidermis, which is also identified with ARPC [16]. Kyrle disease (KD) is a rare skin condition classified as a subtype of acquired perforating dermatosis [17]. The clinical manifestation of KD is sporadic brownish-red papules and brownish conical keratosis embolis [17]. The lesions of KD can be fused into patches with or without hair follicle involvement. The histopathological manifestations of KD are localized epidermal depressions, which are characterized by incomplete keratosis or hyperkeratosis. The keratin can penetrate the whole lesions from the dermis to epidermis. There is granulomatous inflammation in the base of the lesions, but inelastic fiber degeneration. The clinical manifestation of perforating folicullitis (PF) is keratosis papules of hair follicles with a white cork in the center [18]. The histopathological features of PF are hair follicle dilatation and keratosis, which show a mixture of degenerative elastic fibers, collagen, inflammatory cells, and curly hair [19].

CONCLUSION

In summary, in this study we used Masson's staining, elastic fiber staining, and immunohistochemical staining in the case

of APRC. The main objective of the present pilot study was to improve the relative sensitivities of D2-40 and collagen type IV immunostaining for the detection of ARPC. In contrast with Traditional histochemical techniques, D2-40 and collagen type IV immunostaining, ARPC was detected clearer and easier. Our results illustrate the important role of different histochemical staining techniques in the differential diagnosis of penetrating diseases. In this study, we further confirmed that D2-40 and collagen type IV penetrates the skin surface in APRC, which provides new clues for its research and treatment.

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