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Utility of IgG4 immunohistochemistry detection in pemphigus diagnosis

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Abstract

Pemphigus includes a group of blistering autoimmune diseases that affect the skin and mucosa, characterized by the formation of epidermal bullous and the presence of antibodies against binding proteins. Pemphigus is classified according to clinical presentation, target molecule, and IgG production as pemphigus vulgaris, foliaceous, IgA-pemphigus, and paraneoplastic pemphigus. Thus, the identification of autoantibodies class and site of deposition is mandatory. The gold standard to identify the immune complex deposition is the direct immunofluorescences technique, performed in fresh tissue; unfortunately, this method is unavailable in the regional hospital at the Mexican provinces. Nevertheless, IgG subclass-4 is the prevalence of immunoglobulin in acantholysis. Therefore, this IgG subclass could be detected using IgG4 immunohistochemistry. Because direct immunofluorescences technique is absent in provinces or patients denied a new biopsy to confirm the diagnosis, this work presented pemphigus vulgaris confirmation using the IgG4 immunohistochemistry technique in patients with clinical lesions suggestive of pemphigus vulgaris and intraepidermal blister manifestation in histopathology.

Keywords

Pemphigus, immunohistochemistry, IgG4 subclass

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Introduction

Pemphigus vulgaris (PV) is a low prevalence, chronic, autoimmune, a bullous disease characterized by the formation of flaccid blisters that break easily; consequently, skin resulted exposed. The presence of denuded dermis favored electrolyte disequilibrium and infections. Thus, PV is considering a severe dermatologic disease that implied a threat to life. The complication risks have been compared to the threat presented in burned patients. Physiopathology development included IgG autoantibody formation, especially IgG4 subclass autoantibodies, which block protein–protein interactions. Though, PV is sighted as IgG4-autoimmune-related disease (IgG-RD), characterized by IgG4-autoantibodies target transmembrane antigens (Desmogleins); IgG4 is directly pathogenic, and it exerts effect without complement or cell-dependent cytotoxicity; patients respond favorably to treatment with rituximab, a B cell depleting agent.[1] Nevertheless, the pathogenesis of PV remains incompletely understood.

According to clinical presentation, target molecule, and IgG production, pemphigus is classified as PV, pemphigus foliaceous (PF), endemic foliaceous pemphigus, IgA-pemphigus, and paraneoplastic pemphigus (PNP). PV and PF are the more frequent variants. PF affects only skin, whereas PV attacks mucous membranes as oral and genital, plus skin. Usually, the first symptoms are presented in mucous membranes. Thus, it is frequently and erroneously diagnosed as herpes simplex or thrush.[2] Overall, 70% of pemphigus group cases corresponded to PV;[3] the reported incidence worldwide is between 0.1 and 3.2 patients in 100,000 persons/year, plus increasing mortality

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is three times higher than the presented in general population.[4] Clinical presentation related to physiopathology thought in PV autoantibodies is produced against desmogleins three (Dsg 3). Whereas in PF, autoantibodies detected have been report directed against desmoglein one (Dsg 1). Production of IgA-autoantibodies against union desmoglein and desmocolin proteins is observed in IgA-pemphigus. Minor frequency variants included herpetiform pemphigus and PNP. The former was associated with desmoglein, transglutaminase, and desmocolin antibodies, whereas in PNP, antibodies against envoplaquins, periplaquins, or BP230 were reported.[5,6] Consequently, identifying autoantibodies class and deposition site is mandatory to distinguish pemphigus from other bullous diseases. Thus, the gold standard to determine the immune complex deposition is the direct immunofluorescences technique (DIF) performed in fresh tissue, unavailable in general at provincial hospitals. Nevertheless, IgG4 is the immunoglobulin prevalent in the acantholysis process and it can be detected by immunohistochemistry. Therefore, we used IgG4 immunohistochemistry stain to confirm PV diagnosis because immunofluorescent was not available. Immunohistochemistry system applied antihuman IgG4 antibody (monoclonal primary mouse antibody, ABCAM) to identify IgG4 expression. The avidin–biotin–peroxidase method procedure was performed using the Lab Vision Secondary Detection Kit to analyze IgG4 presence in different cell structures. The color was observed by incubation with chromogen 3, (3'diaminobenzidine) for 5 min. IgG4 immunostaining positivity was shown by distinctive, concentrated, uninterrupted, brown immunoreactivity limited to the intercellular joints of keratinocyte; otherwise, the staining was reported negative. Each sample was worked in triplicated; one for IgG4 immunohistochemistry, one negative control, and one quality control (white sample) were included. In negative control and quality control, the primary antibody was substituted by other no related antibody and albumin protein, respectively. Diagnosis procedures were according to Helsinki's declaration of human rights. Therefore, written informed consent was collected before the diagnosis procedure and authorization to publish clinical history and pictures was solicited and signed by patients or family members.

Clinical cases

First case

Male patient aged 57 years, without previous illness, presented with skin lesions that affected the head, thorax, and the four extremities. Skin lesions were characterized by areas of denuded skin; on the face, the patient also showed honey and blood crust (Figure 1(a) and (b)). The patient was referred six months of evolution with current illness (Table 1). In this time, he received initial topic treatment with mupirocin, fusidic acid; then different oral antibiotics, all without improvement. On the contrary, the extension of body lesions increases. Thus, he presented to Veracruz State General Hospital No. 71 Benito Coquet. Therefore, physicians decided to perform a cutaneous biopsy with a presumptive diagnosis of Steven-Johnson syndrome versus PV versus PNP. Hematoxylin-eosin (H&E) stain reveals suprabasal blister (Figure 1(d)), suggesting PV. At this moment, the patient was in critical condition. Thus, to obtain confirmation, IgG4 immunohistochemistry stain was solicited (Figure 1(f)), using paraffin block.

Second case

A 46-year-old male patient from La Piedad, Michoacan, who refer 8-month evolution dermatoses, started with oral lesions, treated as herpes simplex, without improvement. However, 2 months later, he developed bullous skin lesions on the thorax and arms; thus, he consulted a private physician in Guadalajara, who performed a skin biopsy and started topical steroids. However, lesions increased, so he consulted a dermatologist service in Mexico City; after complete physical examination, histopathological analysis (Figure 1(e)), IgG4 immunohistochemistry (Figure 1(g)), standard laboratory test, radiologic and tomographic evaluation, the diagnoses of PV, and diabetes mellitus were made.

Third case

A 62-year-old female diabetic and rheumatic arthritis patient from Chilpancingo (Guerrero States) presented with a painful oral ulcer on the soft paladar. She received deflazacort treatment for the last year with a presumable diagnosis of oral ulcer associated with rheumatic arthritis without recovery. We performed a cutaneous biopsy; the H&E stain result suggests PV. The paraffin block was solicited to perform IgG4 immunohistochemistry. Also, laboratory and imagen studies (including thorax and abdomen computer tomography) were requested to discharge PNP or another associate disease. Results just reported high blood sugar and osteoarticular changes associated with a previous diagnosis of rheumatic arthritis.

Four cases

A female patient aged 36 years from Xochimilco, southern periphery of Mexico City, presented with gingival erythema plus inflammation (Figure 1(c)) and halitosis. She mentioned the previous presence of oral ulcers and referred a 1-year diagnosis of hypothyroidism in treatment with euthyrox. The prior physician diagnosed her as a PV patient, according to clinical evaluation and histopathology study. Thus, she was treated with local steroids for the last 8 months, with partial improvement but not total recovery. However, she refuses a new biopsy to verify the diagnosis and to perform immunofluorescent. Thus, IgG4 immunohistochemistry was solicited to confirm the diagnosis of PV using the previous paraffin block.

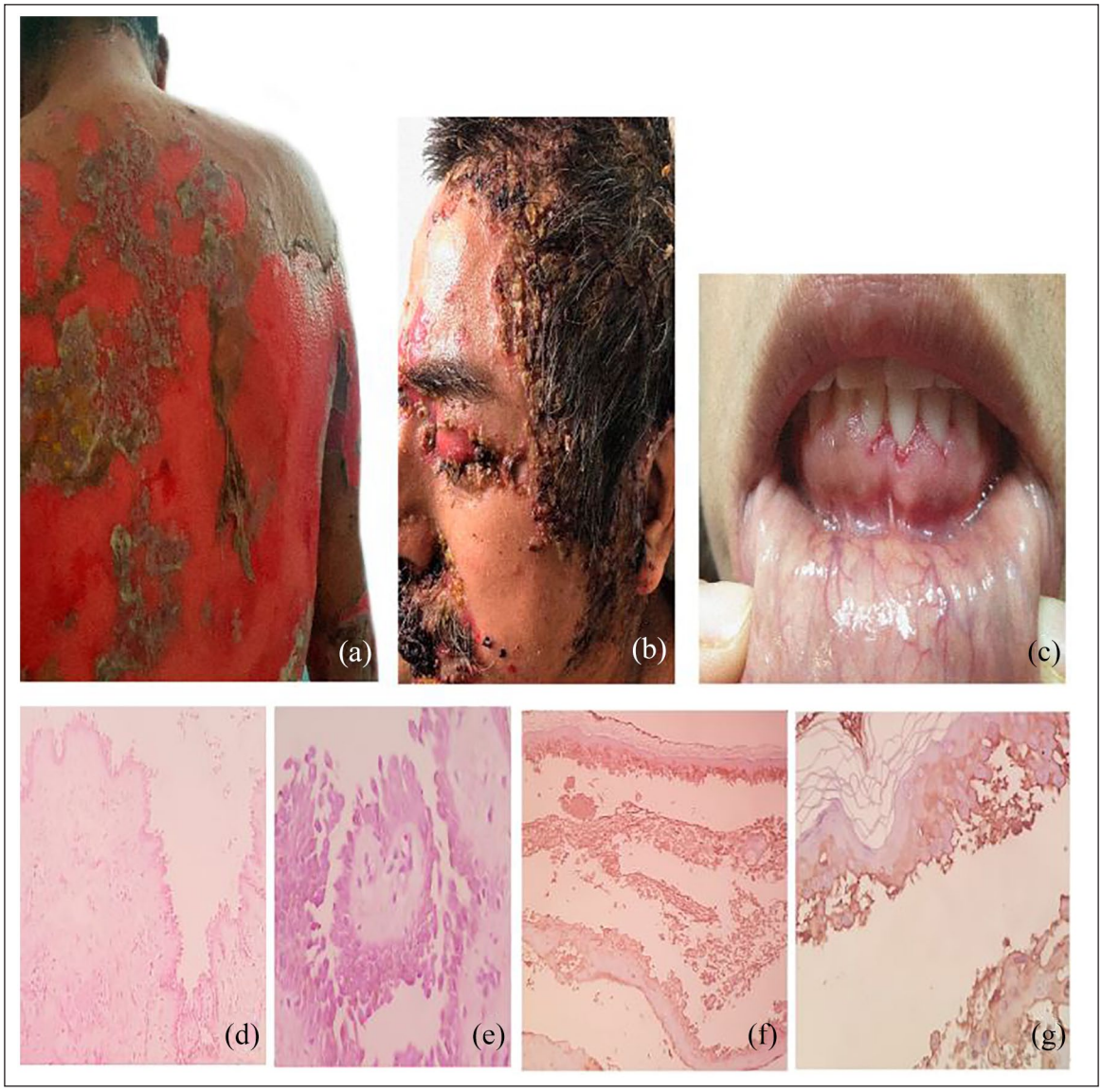

Figure 1. (a) and (b) Clinical imagen Case 1; (c) Clinical imagen Case 3; (d) Histopathology H&E 10X, suprabasal blister, case 1; (e). Histopathology H&E 40X, suprabasal blister, case 2; (f) and (g). Ig4 immunohistochemistry. Observed deposition of immunostaining on the cellular membrane, such as the honeycomb pattern, exhibits in immunofluorescent. 10x and 40x magnification, patients 1 and 2, respectively.

Discussion

Pemphigus included a group of autoimmune, bullous skin, and mucous diseases characterized by the formation of intraepidermal blisters, associated with antibodies deposition on junction proteins. The pemphigus classification depends on the anatomic level of acantholysis, plus antibodies target molecule and type of immunoglobulin presented in the intercellular junctions of keratinocytes.[7,8] PV is characterized by intraepidermal blister, deposition of IgG immunoglobulin, and adhesion to Dsg 3 and Dsg 1 presented in keratinocytes superficies. In addition, patients with actives diseases presented blood autoantibodies and tissue depositions, with the prevalence of IgG4 subclass autoantibodies. The acantholysis process is complex, but in general, it is accepted that blisters are produced by IgG4 autoantibodies action on intercellular union, without the presence of inflammatory cells.[9–12]

Diagnosis is based on clinical presentation and histopathology study. However, confirmation required DIF; nevertheless, this technique is expensive; the average value is 4 times more than immunohistochemistry and 20 times more than the minimum wage per day in Mexico (similar relations are also presented in rest of Latin American countries and many African and some Asian countries). In addition, the immunofluorescence technique needs fresh tissue, rapid process, and well-trained personal to be performed. In general, DIF is not available in regional hospitals or small cities. Thus, IgG4 immunohistochemistry using original paraffin block is a less expensive and easy diagnosis option to confirm PV.

Table 1. Epidemiology and clinical characteristics of pemphigus patients.

Cases	Sex	Age (years)	Clinical presentation	First physician diagnosis	Time of evolution before diagnosis in month	Initial treatment by general physicians	Treatment after correct diagnosis	Complementary studies	Associated disease	Current state
1	M	57	Oral ulcer, ampullas, denuded skin	Herpes simplex	6	Topical antibiotics Later oral antibiotics	Prednisone Azathioprine Ceftazidime Linezolid	Complete blood count, Chemical blood profile, Thorax and abdomen radiograph imaging. Results reported high blood sugar, infection, and other alterations during clinical evolution. Tumor markers as markers tumors alphabet 1.3, antigen carcinoembryonic 3.87, CA 19 were negative.	Diabetes	Died from infectious complications
2	M	46	Oral ulcer, ampullas, denuded skin	Herpes simplex	8	Acyclovir Topic and oral antibiotics	Prednisone azathioprine Metformin Vitamin D	Complete blood count, thorax, and abdomen radiograph imaging reporting normal. Chemical blood profile reporting high blood sugar.	Diabetes	In good control and in process of reduction of prednisone. Actual treatment prednisone 20 mg/day and azathioprine 50 mg/day Metformine 500 mg before each meal
3	F	62	Oral ulcer, gingivitis, halitosis	Oral ulcers associated with rheumatic arthritis	12	Acemetacin Leflunomide Indomethacin/ betamethasone Deflazacort (low doses) Tramadol/ paracetamol	Prednisone Hydroxychloroquine Methotrexate Metformin	Complete blood count and chemical blood profile during a period of 7 years follow presented anemia, hypercholesterolemia, and hypertriglyceridemia. Thorax and abdomen radiograph imaging. Thorax X-rays presented chance associates with age as osteoporosis.	Diabetes Rheumatic arthritis Blood high pressure	In remission
4	F	36	Oral ulcer	Herpes simplex	10	Acyclovir Topic and oral antibiotics Topic steroids	Prednisone azathioprine	Complete blood count, Chemical blood profile, thorax and abdomen radiograph imaging reporting normal.	Hypothyroidism	In good control and in process of reduction of prednisone. Actual treatment prednisone 10 mg/day and azathioprine 50 mg/day Euthyrox

Zhang et al.[13] reported IgG4 subclass immunochemistry in biopsies from PV, PF, pemphigoid, and other no bullous disease and concluded that this technique is highly sensitive and specific for PV diagnosis. More recently Al-Shenawy HA, confirmed the utility of this technique to differentiate PV and pemphigus foliaceus from bullous pemphigoids, pemphigoid gestationis, and linear IgA. In PV and pemphigus foliaceus, IgG4 immunohistochemistry exhibits distinct continuous positivity for IgG4 at the intercellular junctions of the keratinocytes along the epidermis, like observed in our patients. Whereas in bullous pemphigoids, patients exhibit linear deposition of IgG4 at intercellular junctions of the keratinocytes only in basal layer. However, pemphigoid gestationis and linear IgA show negative expression for IgG4. They also analyzed complemented expression by immunohistochemistry of C3d, found negative in pemphigus group and linear IgA, but positive in bullous pemphigoids and pemphigoids gestationis. Thus, these results suggest that the use of both stains may be more useful to differentiate between these blister diseases, than IgG4 or C3d alone.[14]

These cases have also exposed the difficulties of general physicians to perform PV diagnoses. This situation delays correct management and contributes to diseases progression. In addition, rituximab is a standard treatment in rich countries;[15–18] however, the economic conditions of most patients in Latin America and the lack of medical insurance force the Latin American dermatologist to continue the management with oral prednisone as a based drug; the patients presented were treated with prednisone 1 mg/kg/day or prednisone plus azathioprine. The first patient was in poor general conditions at the time of diagnosis and died from associated complications (sepsis), whereas the other three improved and eventually reduced the prednisone dosages.

Conclusion

In our experience, IgG4 immunohistochemistry is a practical, more accessible, and less expensive technique to confirm the clinical diagnosis of PV. Thus, we recommend when specific tests as DIF are not available.

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