International Journal of Medical Science and Clinical Research Studies

ISSN(print): 2767-8326, ISSN(online): 2767-8342

Volume 03 Issue 08 August 2023

Page No: 1578 -1581

DOI: https://doi.org/10.47191/ijmscrs/v3-i8-24, Impact Factor: 6.597

Use of IgG4 Immunohistochemistry as a Diagnostic Tool for Pemphigus

Rachel P Shaji[1], Nandakumar G[2], Anuja Elizabeth George[3]

[1]Senior Resident, Department of Pathology, Government T D Medical College, Alapuzha-

[2]Professor, Department of Pathology, Government Medical College, Thiruvananthapuram

[3]Professor and Head of Department, Department of Dermatology and Venereology, Government Medical College, Thiruvananthapuram

ABSTRACT

Pemphigus belongs to the group of antibody mediated autoimmune vesiculobullous disease (AIBD) of skin and mucous membranes. Antibodies are directed against the desmosomal adhesion proteins and IgG4 is the predominant subclass. It is a rare disease associated with significant mortality and morbidity. DIF is the gold standard test for AIBD. It requires fresh frozen section which is a major technical issue in most centres. The aim of this research was to study the diagnostic accuracy of IgG4 immunohistochemistry in pemphigus. The study was conducted as a diagnostic test evaluation in a tertiary care hospital. Paraffin blocks of histopathologically diagnosed AIBD were used for doing immunohistochemistry. The findings were compared with DIF findings. Statistical analysis was performed using SPSS, version 20.0. 48 paraffin blocks of AIBD were selected, DIF of all cases were already done from outside centre. There were 21 cases of pemphigus, 25 cases of bullous pemphigoid and one case each of epidermolysis bullosa acquisita and dermatitis herpetiformis. The sensitivity of IgG4 IHC was found to be 85.7%, specificity 96.3%, positive predictive value of 94.7% and negative predictive value 89.7%. So, we conclude that IgG4 IHC done on paraffin sections can be used as a diagnostic tool for pemphigus.

KEYWORDS: Immunohistochemistry, Pemphigus, Immunofluorescence.

ARTICLE DETAILS

Published On:
09 August 2023

Available on:
https://ijmscr.org/

INTRODUCTION

Pemphigus is a group of autoimmune vesiculobullous disorders affecting skin and mucous membranes causing intraepithelial lesions.(1) Pemphigus is a rare disease with an incidence of 0.1 to 3.2 per 100000 person years.(2) But the incidence has increased over time with significantly high mortality and morbidity in the absence of prompt treatment. There are different types of pemphigus according to severity and blister location- pemphigus vulgaris, pemphigus foliaceus and paraneoplastic pemphigus. In pemphigus vulgaris, autoantibodies are against desmoglein 3 on surface of keratinocytes. Here patient presents with flaccid blisters on skin and oral mucosa.(3) In pemphigus foliaceus the antibodies are against desmoglein 1, present on upper layers of skin and is characterized by superficial bullae and erosions.(8)

The diagnosis of pemphigus depends on clinical features, hisopathologically by intraepidermal blister and by demonstrating circulating and bound autoantibodies (IgG) against desmosomal adhesion proteins. IgG4 antibodies are the predominant subclass in patients with active disease. (4)

Direct Immunofluorescence is used to demonstrate the autoantibodies bound to tissue antigens and it is the gold standard test for diagnosis of autoimmune vesiculobullous diseases. But it requires fresh frozen tissue which is a major technical issue in most centres.(5)

Immunohistochemistry for total IgG performed on paraffin sections is of no diagnostic value because of high background stain. But the background level of IgG4 immunostain in paraffin sections is of very low intensity and false positivity is rarely seen.(6) In the present study, we assessed the sensitivity and specificity of IgG4 immunohistochemistry in the diagnosis of pemphigus.

MATERIALS AND METHODS

The study was conducted as a cross sectional diagnostic test evaluation during a period of one year in a tertiary care centre after obtaining clearance from ethical committe. Paraffin blocks of 48 consecutive cases of histopathologically

diagnosed autoimmune vesiculobullous diseases of skin were selected for this study. Amon these 48 cases, the clinical diagnoses were 15 pemphigus vulgaris, 2 pemphigus foliaceus, 17 bullous pemphigoid, 4 epidermolysis bullosa acquisita, 2 lichen planus pemphigoides, 1 paraneoplastic pemphigus, 4 linear IgA disease, 2 dermatitis herpetiformis and 1 pemphigus erythematosis. Direct immunofluorescence was already done in all cases from outside centres and the number of cases according to DIF include 21 pemphigus, 25 bullous pemphigoid, 1 dermatitis herpetiformis and 1 epidermolysis bullosa acquisita. The histopathological diagnoses are 18 pemphigus vulgaris, 3 pemphigus foliaceus, 25 bullous pemphigoid, 1 epidermolysis bullosa acquisita and 1 dermatitis herpetiformis . Immunohistochemistry for IgG4 was performed in all cases in the paraffin blocks.

For immunohistochemistry, 3 to 4 micrometre thickness formalin fixed paraffin embedded tissue sections were taken and kept in incubator for 30 minutes. Following this, deparaffinisation done with xylene (3 changes each for 5 minutes), then hydration in descending grades of alcohol and distilled water. Antigen retrieval was done using PBS at 95 degrees for 15 minutes. The sections were then treated with hydrogen peroxide for 10 minutes. Primary antibody in 1:100 dilution added, subsequently secondary antibody added and incubated for 20 minutes. This was followed by horseradish peroxidase (BAB chromogen- Pathn Situ) for 5 minutes.

Positivity is defined as continuous distinct immunoreactivity localized to the intercellular junction of keratinocytes.[6]

Data were entered into excel sheets and statistical analysis was performed using SPSS version 20.0. For all the statistical interpretations, $p<0.05$ was considered the threshold for statistical significance.

RESULTS

A total of 48 cases were enrolled in this study which included 18 pemphigus vulgaris (Figure 1), 3 pemphigus foliaceus(Figure 2), 25 bullous pemphigoid(Figure3), 1 epidermolysis bullosa acquisita(Figure 4) and 1 dermatitis herpetiformis(Figure 5). In all cases of pemphigus, acantholysis was present and DIF was positive. Two pathologists independently assessed IgG4 immunostained slides using same criteria and achieved a 100 % agreement regarding positive versus negative results for all specimens.

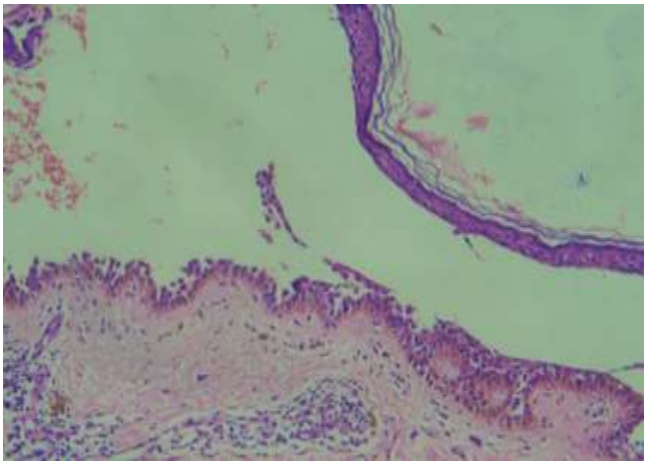

Figure 1- Skin biopsy showing suprabasilar clefting with acantholytic cells in the blister cavity- pemphigus vulgaris (100x magnification)

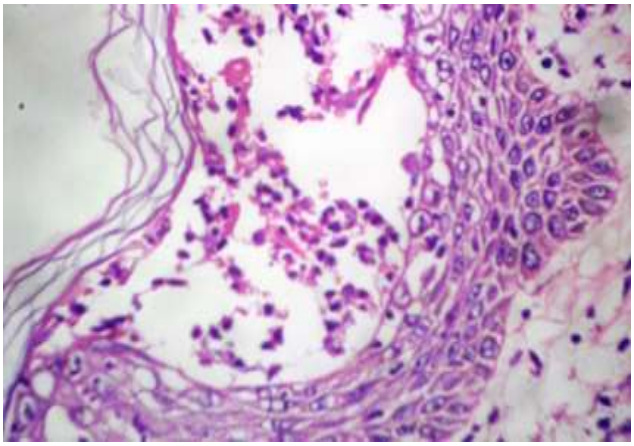

Figure 2- Skin biopsy shows subcorneal blister with acantholytic cells in blister cavity – Pemphigus foliaceus (400x)

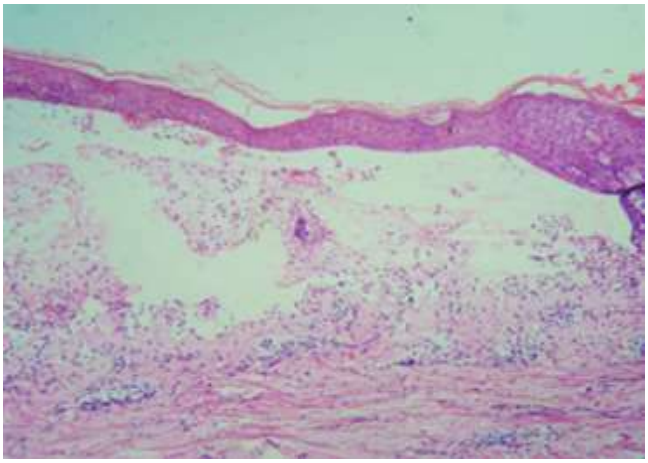

Figure 3- Skin biopsy show subepidermal blister formation with mild inflammatory infiltrates in dermis – Bullous pemphigoid(100x magnification)

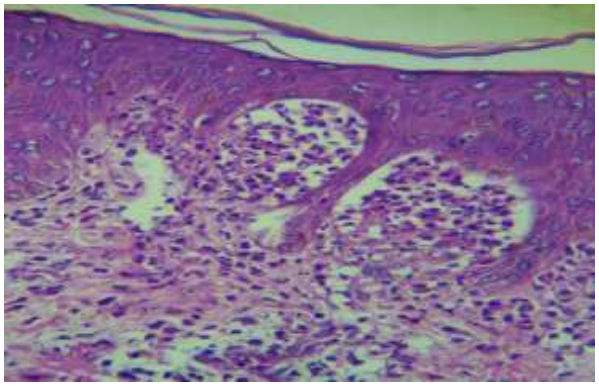

Figure 4- Skin biopsy show subepidermal blister with neutrophilic infiltrates in the bullous cavity- Dermatitis herpetiformis (400x magnification)

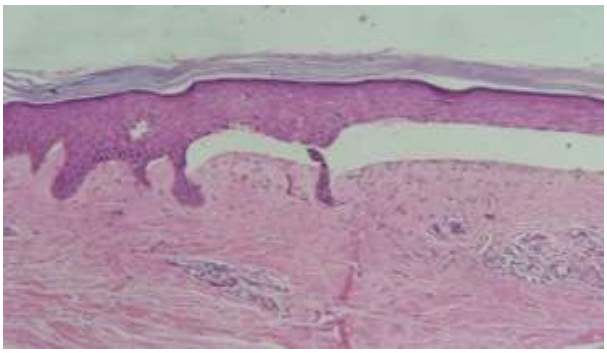

Figure 5- Skin biopsy show subepidermal blister formation – epidermolysis bullosa acquisita (100x magnification)

Out of 18 pemphigus vulgaris, 16 were positive for IgG4 and the positivity was condensed to the intercellular junction of suprabasal keratinocytes (Figure 6). 2out of 3 pemphigus foliaceus were immunoreactive for IgG4 (Figure 7). Among the remaining 27 cases, 1 bullous pemphigoid showed positivity at the basal layer. The sensitivity of IgG4 was 85.7% and specificity was 96.3%. The positive and negative predictive values were 94.7 % and 89.7% respectively. A significant difference was seen among the immunostain result of pemphigus ($p < 0.01$) in comparison with non pemphigus group.

Figure 6- IgG4 positive in basal layer in pemphigus vulgaris (400x magnification)

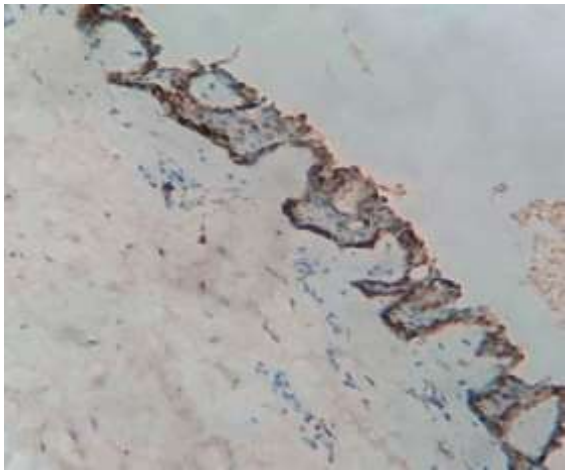

Figure 7 – IgG4 IHC positive in subcorneal layers in pemphigus foliaceus (400x magnification)

DISCUSSION

Pemphigus is a group of grievous autoimmune blistering disease characterized by IgG autoantibodies against desmosomal adhesion proteins. This lead to acantholysis. IgG4 is the predominant subclass in active disease.[7] DIF for IgG4 on frozen tissue is the gold standard test in diagnosing autoimmune blistering diseases. But there may be a hindrance to DIF as frozen section is not available in most centres. So in this study, we used immunohistochemistry for IgG4 performed on formalin fixed paraffin embedded tissue sections as a diagnostic tool.

In our study, the sensitivity of IgG4 immunostain in diagnosing pemphigus is 85.7 % and specificity 96.3%. This is in concordance with a study by Zhang et al,[5] which found that the sensitivity was 72.2% and specificity 97.2%. It is noteworthy to point out that immunohistochemical analysis is less tedious and less time consuming than DIF, but has promising similar diagnostic accuracy in current study setting. This is especially helpful when the histopathologic picture is suspicious of pemphigus and DIF is not available.

Owing to the fact that the study is conducted in a local population , further studies with larger number of patients are required for increasing the validity of this study.

CONCLUSION

Pemphigus is a group of chronic autoimmune potentially fatal vesiculobullous disease of skin and mucous membranes. The classic clinical presentation and histopathology usually lead to straightforward diagnosis of pemphigus. However in equivocal cases, where histopathology is suspicious of pemphigus and when DIF is not available, immunohistochemistry for IgG4 can be used as a diagnostic tool for pemphigus.

CONFLICT OF INTEREST

There is no conflict of interest for this study.

REFERENCES

I. Black M, Mignogna MD, Scully C. Number II pemphigus vulgaris. Oral Dis. 2005;11(3):119–30.

II. Langan SM, Smeeth L, Hubbard R, Fleming KM, Smith CJ, West J, et al. Bullous pemphigoid and pemphigus vulgaris – Incidence and mortality in the UK: Population based cohort study. BMJ. 2008;337:a180.

III. Sitaru C, Mihai S, Zillikens D. The relevance of the IgG subclass of autoantibodies for blister induction in autoimmune bullous skin diseases. Arch Dermatol Res. 2007;299:1–8.

IV. Heidarpour M, Rajabi P, Pour EB, Fayyazi E. Immunohistochemistry for Immunoglobulin G4 in the Diagnosis of Pemphigus. Indian J Dermatol. 2019 Jul-Aug;64(4):338.
doi: 10.4103/ijd.IJD_87_18. PMID: 31516156; PMCID: PMC6714200.

V. Zhang X, Hyjek E, Soltani K, Petronic-Rosic V, Shea CR. Immunohistochemistry for immunoglobulin G4 on paraffin sections for the diagnosis of pemphigus. Arch Pathol Lab Med. 2012;136(11):1402–7.

VI. Kwon EJ, Yamagami J, Nishikawa T, Amagai M. Anti-desmoglein IgG autoantibodies in patients with pemphigus in remission. J Eur Acad Dermatol Venereol. 2008;22:1070–5.

VII. Rock B, Martins CR, Theofilopoulos AN, Balderas RS, Anhalt GJ, Labib RS, et al. The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem) *N Engl J Med.* 1989;320:1463–9.

VIII. Kusudo E, Endo Y, Kitayama N, Ishida Y, Fujisawa A, Dainichi T, et al. Pemphigus foliaceus developed in a patient with long-term erosion on the genital skin. *J Dermatol.* 2017;44:e126–7.