Original Research Paper

Volume-8 | Issue-3 | March-2018 | PRINT ISSN No 2249-555X

Dermatology

EVALUATION OF DIRECT IMMUNOFLUORESCENCE (DIF) OF SKIN AND HAIR IN PEMPHIGUS VULGARIS

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ABSTRACT Pemphigus is a group of autoimmune bullous diseases, in which anti-desmoglein autoantibodies induce the loss of keratinocyte adhesion, leading to blisters. Evaluation of the direct immunofluorescence of plucked hair and skin was done in pemphigus vulgaris patients in clinical remission. On direct immunofluorescence test of hair (IgG), among the 20 patients, 14 showed intercellular IgG deposition in Outer Root Sheath of hair follicle. Six cases showed no such IgG deposition in ORS of hair follicle. On DIF test of skin (IgG), among the 20 patients, 14 showed lace like deposition of IgG in squamous intercellular spaces of epidermis. Similarly 6 cases showed no such IgG deposits. Fourteen patients were positive in both hair and skin DIF tests. There was one case with false positive test as ORS of hair follicle was positive for DIF but skin DIF was negative. One case showed false negative result as ORS of hair follicle was negative but skin was positive on DIF. This present study shows the sensitivity of 92.8%, however, the specificity is 85.7%.

KEYWORDS : DIF, Hair follicle and Pemphigus vulgaris.

Introduction:

Pemphigus is a group of autoimmune bullous diseases, in which anti-desmoglein autoantibodies induce the loss of keratinocyte adhesion, leading to blister formation. Two main types of pemphigus may be distinguished: Pemphigus Vulgaris (PV) and Pemphigus Foliaceus (PF). Desmoglein3 is considered to be the main autoantigen in pemphigus vulgaris. Additionally, anti-desmoglein1 autoantibodies may be detected in more than half of patients with pemphigus vulgaris.[1] Desmoglein1 is the main autoantigen of pemphigus foliaceus. Mucosal lesions with or without skin lesions are observed in pemphigus vulgaris, whereas in pemphigus foliaceus only cutaneous involvement is observed. The diagnosis of pemphigus is based on the clinical picture, direct and indirect immunofluorescence, histopathology as well as anti-desmoglein 1 and 3 enzyme-linked immunoassay. DIF of the skin or mucosa is an invasive and expensive procedure and the patient may not be willing for the same. Recently, pemphigus-specific immunofluorescence pattern has been demonstrated in the outer root sheath (ORS) of hair follicles, which is structurally analogous to epidermal keratinocytes, with a sensitivity ranging from 85–100%.[2-5] Hence, DIF of hair may be an ideal substrate for assessment of immunological remission because it is a simple, non-invasive and cost effective procedure. Aim of this study was to evaluate direct immunofluorescence of plucked hair and skin in pemphigus vulgaris patients in clinical remission.

Material and Methods:

This present study was carried out on patients diagnosed as cases of pemphigus vulgaris, attending the outpatient clinic in the department of Dermatology, Command Hospital (Southern Command), affiliated teaching hospital of Armed Forces Medical College, Pune during the period from March 2013 to November 2014.After obtaining approval from the institutional ethics committee and consent from all the patients, a prospective study was conducted in Department of Dermatology of Command Hospital(Southern Command). A total of twenty (20) patients with pemphigus vulgaris who showed intercellular deposits of IgG antibodies against Dsg3 and/or Dsg1 and/or Complement 3 (C3) in the DIF of skin and hair during the remission stage of disease and fulfilling the following exclusion/ inclusion criteria were included in the study:

EXCLUSION CRITERIA

Patients with new or non healing skin or mucosal lesions in the preceding 6 months and Patients with other bullous disorders.

INCLUSION CRITERIA

Patients with no new or non-healing skin or mucosal lesions in the past 6 months or more and who were on daily prednisolone dosage equal to or less than 10mg and/or on adjuvant immunosuppressive therapy like azathioprine 50mg or cyclophosphamide 50mg.

Results and Discussion:

A total of 20 patients, clinically, histopathologically and immunologically diagnosed cases of pemphigus vulgaris in clinical remission were included in this study. Scalp hair biopsy and perilesional skin biopsy was performed and DIF test was done on biopsy specimens for comparision. A slight preponderance of male patients was observed in the study with male to female ratio of 12:8. The age distribution of patients varied between 20 to 74 years with mean age of 46.83±10.32 years. On direct immunofluorescence test of hair (IgG), among the 20 patients, 14 showed intercellular IgG deposition in Outer Root Sheath of hair follicle. Six cases showed no such IgG deposition in ORS of hair follicle. On DIF test of skin (IgG), among the 20 patients, 14 showed lace like deposition of IgG in squamous intercellular spaces of epidermis. Similarly 06 cases showed no such IgG deposits. Fourteen patients were positive in both hair and skin DIF tests. There was one case with false positive test as DIF of ORS of hair follicle was positive but skin DIF was negative. One case showed false negative result as DIF of ORS of hair follicle was negative but skin DIF was positive, as seen in Table 1.

Table 1: Comparison between DIF of hair and DIF of skin in pemphigus vulgaris patients in clinical remission (n=20)

DIF of hair	DIF of skin	
	Positive	Negative
Positive	13	1
Negative	1	6

Based on the above data, we derived the following parameters;
a. Sensitivity: True positive/true positive + FN = 92.8%
b. Specificity: True negative/FP + true negative = 85.7%

The goal of therapy in pemphigus is to achieve clinical and immunological remission. Clinical remission is achieved in most patients with immunosuppressive agents. The most difficult management decision is how to maintain remission with the least number of medications. In the epidermis and hair, the distribution of desmogleins correlates with the degree of differentiation. Desmoglein1 is expressed in the supra basal cells of the epidermis and inner root sheath as well as in the innermost layer of the Outer Root Sheath of the hair follicle.[6] Desmoglein3 is present in the basal and supra basal cells of the epidermis and throughout the ORS of the hair follicle except in the areas of epidermal-like keratinization such as in the infundibulum, where it is confined to the basal layer of ORS.[6] Desmoglein 3 is also responsible for anchoring the telogen hair in the follicle.[7] Findings of this study differ from that of Rao et. al and Daneshpazhooh et. al[8] where mean age was around 45 and females were more than males. This difference may be due to small sample size. Wilson *et al*. in 1991 demonstrated that the human hair follicle is rich in the target antigens

of pemphigus.[9] In 2003, Schaerer and Trueb first reported positive DIF findings in the ORS and the matrix of hair follicle in100% of pemphigus patients.[2] Subsequently, Rao*et al*. reported 80% positivity and Daneshpazhooh *et al*. reported91% positivity of DIF in the anagen hair.[3,4] Kumaresan*et al*. and Tanasilovic *et al*. also demonstrated positive DIF findings in 100% of their patients with active pemphigus in ORS of both anagen and telogen hair.[5,10] David *et al*. in 1989, based on their study suggested that repeated negative DIF in pemphigus patients in clinical remission could be a sign of immunological remission.[11] Similar findings were also reported by Balighi *et al*. And Ratnam *et al*.[12,13] Ratnam *et al*. also noted that the patients with positive DIF findings during clinical remission had significantly higher relapse rates after the discontinuation of treatment.[13] Various studies have shown that the relapse rate is approximately 44–100% in patients with positive DIF findings during remission and 13–27% in patients with negative DIF.[11,14] Rao *et al*. in 2012 conducted a study to assess the role of hair DIF in monitoring the disease activity in pemphigus; they suggested that, in patients in clinical remission, DIF of hair could be an ideal substrate for assessment of immunological remission.[15] Only limited studies are available till date to analyze the role of hair DIF for the assessment of immunological remission. Apart from the study published by Rao *et al*., one study by Daneshpazhooh *et al*. in 2013 demonstrated the usefulness of hair DIF in the assessment of immunological remission in pemphigus patients.[16] In their study, the sensitivity and specificity of hair DIF was 79% and 48% respectively. Similarly our study shows the sensitivity of 92.8%, however the specificity is85.7% which is comparatively higher.

Conclusion:

This present study proved the value of plucked hair as an appropriate substrate for DIF for the purpose of monitoring of Pemphigus Vulgaris patients. DIF of hair is a simple, non-invasive, cost effective procedure and can be used as an additional procedure for assessment of immunological remission in Pemphigus Vulgaris.

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