Original Article

Comparison of Direct Immunofluorescence of Plucked Hair and Skin for Evaluation of Immunological Remission in Pemphigus

Reena Rai, Manu V. Harikumar

Department of Dermatology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India

Abstract

Background: Pemphigus is a chronic autoimmune bullous disorder characterized by autoantibodies directed against desmoglein 3 and/or 1. Demonstration of intercellular deposition of IgG on the cell surface of keratinocytes by direct immunofluorescence (DIF) of the skin is the gold standard in the diagnosis of pemphigus. Recently, DIF of plucked hair demonstrating intercellular deposition of IgG in the outer root sheath (ORS) has shown to be useful. **Objective:** To compare the DIF of plucked hair and skin for the evaluation of immunological remission in pemphigus vulgaris patients in clinical remission. **Materials and Methods:** A total of 30 patients with pemphigus vulgaris with positive DIF of the skin and hair at baseline were included, and DIF of skin and hair was repeated after 6 months or more of clinical remission (with no new/non-healing lesions). Presence of intercellular deposits of IgG and or C3 in skin and ORS of the hair was considered as positive. **Results:** Of the 30 patients, DIF of skin was positive in 10 patients and hair was positive 14 patients. The findings of hair and skin DIF correlated with each other in 22 patients. In 6 (20%) patients DIF of hair was positive even though the DIF of skin was negative. The sensitivity of hair DIF was 80% and specificity was 70%. **Limitations:** Small sample size. **Conclusion:** DIF of hair is a simple, non-invasive, and cost effective procedure and can be used as an additional procedure for the assessment of immunological remission in patients with pemphigus vulgaris.

Keywords: *Hair immunofluorescence, immunological remission, pemphigus*

Introduction

Pemphigus is a chronic autoimmune bullous disorder characterized by autoantibodies directed against desmoglein (Dsg) 3 and/or 1.[1] Direct immunofluorescence (DIF) of the skin or mucosa is considered as the gold standard in the diagnosis of pemphigus. Systemic steroids alone or in combination with other immunosuppressive agents are the main stay of treatment, and long-term administration of the same is associated with significant adverse effects, morbidity, and mortality.

Therefore, a system is required to monitor disease activity to lower the dosage of the drugs and eventually withdraw treatment.Various methods for assessment of immunological remission include DIF, indirect immunofluorescence, and anti-desmoglein enzyme-linked immunosorbent assay (ELISA) titres.[2-7]

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%.[8-11]

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. We conducted this study to compare the DIF of plucked hair and skin for the evaluation of immunological remission in pemphigus vulgaris patients in clinical remission.

Materials and Methods

After obtaining approval from the institutional ethics committee and consent from the patients, a prospective study was conducted in the Department of Dermatology in the PSG institute of medical sciences and research (PSG IMS&R) over a period of 1 year.

How to cite this article: Rai R, Harikumar MV. Comparison of direct immunofluorescence of plucked hair and skin for evaluation of immunological remission in pemphigus. Indian Dermatol Online J 2017;8:319-22.

Received: July, 2016. **Accepted:** November, 2016.

Address for correspondence:
Dr. Reena Rai, Department of Dermatology, PSG Institute of Medical Sciences and Research, Coimbatore - 641 004, Tamil Nadu, India.
E-mail: drreena_rai@yahoo.com.in

Access this article online

Website: www.idoj.in

DOI: 10.4103/idoj.IDOJ_280_16

Quick Response Code:

A total of 30 patients with pemphigus vulgaris who showed intercellular deposits of IgG antibodies against Dsg3 and/orDsg1 and/or Complement 3 (C3) in the DIF of skin and hair during the active stage of disease fulfilling the following inclusion criteria were included in the study: (a) patients in clinical remission, defined as those who had no new or non-healing skin or mucosal lesions for the past 6 months or more and (b) with or without daily prednisolone dosage equal to or less than 10 mg with or without adjuvant immunosuppressive therapy such as azathioprine 50mg or cyclophosphamide 50 mg daily.

Patients with new or non-healing skin or mucosal lesions in the preceding 6 months and patients with other bullous disorders were excluded from the study.

The presence of lace-like pattern in DIF in the ORS of the hair follicle and in the epidermis was considered as positive [Figure 1].

Skin and hair DIF was performed in all the patients. The skin sample was obtained from the upper trunk. Trichogram was done and anagen hair was collected. The samples were transported in the Michels medium to the Department of Pathology in PSG IMS&R and subjected to DIF staining with fluorescent isothiocyanate (FITC) labeled rabbit anti IgG and C3 antibodies. The samples were washed in PBS three times, each wash taking 10 minutes. Following which they were incubated with FITC labeled anti-IgG and anti-C3 for 1 hour at 37°C and once again washed in PBS three times for 10 minutes each, and then air dried and mounted on slide with PBS-glycerol solution for examination. The PBS contained propidium bromide as a counterstain.

Patients demographic parameters, phenotype of pemphigus vulgaris, history of scalp involvement, duration of disease, duration of remission, and past and current treatment were recorded.

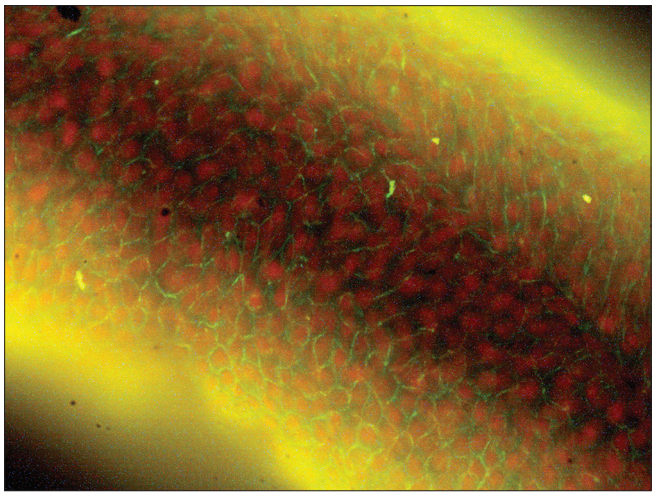

Figure 1: Fish net-like pattern of immunofluorescence in the outer root sheath of hair on DIF

Results

Patient characteristics and hair and skin DIF findings are shown in Tables 1 and 2, respectively. The age of patients in our study ranged from 17–69 years, and majority (33.3%) of the patients were in the age group of 41–50 years. All the 30 patients had a history of scalp involvement. Of the 30 patients, DIF of skin was positive in 10 patients and hair was positive 14 patients. The findings of hair and skin DIF correlated with each other in 22 patients. In 6 patients, DIF of hair was positive and skin was negative. Whereas in 2 patients, DIF of the skin was positive and hair was negative, as seen in Table 2.

The skin DIF was considered as the gold standard, and the sensitivity and specificity of hair DIF was calculated. The sensitivity (true positive/true positive + false negative) of hair DIF was 80% and specificity (true negative/true negative + false positive) was 70%.There was a statistically significant association between skin and hair DIF positivity with a *P* value of 0.010 [Table 2]. Positive predictive value of hair DIF (true positive/true positive + false positive), which refers to the percentage of patients with a positive test who actually have the disease was 57.14%, and negative predictive value (true negative/true negative + false negative), which refers to the percentage of patients with a negative test who do not have the disease was 87.5%.

A total of 18 patients were undergoing treatment in our study. In these patients, the hair and skin DIF was negative in 10 and 13 patients, respectively, which was higher than that in patients not undergoing treatment. However, the findings were not statistically significant. There was no

Table 1:Patient characteristics

Total Number of patients (*n*)	30
Age (years) Mean±SD	44.83±13.27
Sex – female:male	19:11
Phenotype of disease Mucocutaneous (*n*)	30
History of scalp involvement (*n*)	30
Duration of disease (months) Mean±SD	45±16.87
Duration of remission (months) Mean±SD	32.512±23.61
DIF positive with either substrate	16
Number of patients on treatment	18
Number of patients not on treatment	12
Number of positive hair DIF	14
Number of positive skin DIF	10

Table 2: Hair and skin DIF skin findings in pemphigus patients in clinical remission

Hair test	Skin test		*P* (Chi square test)
	Positive	Negative	
Positive	8(57.1%)	6(42.9%)	0.010 Significant
Negative	2(12.5%)	14(87.5%)	

significant association between hair DIF and duration of disease, remission, and treatment.

Discussion

Desmogleins are the main target antigens in pemphigus. These desmogleins are calcium dependent transmembrane adhesion proteins belonging to the cadherin family. In the epidermis and hair, the distribution of desmogleins correlates with the degree of differentiation. Desmoglein 1 is expressed in the suprabasal cells of the epidermis and inner root sheath as well as the innermost layers of the ORS of the hair follicle.[12] Desmoglein 3 is present in the basal and suprabasal 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 its confined to the basal layer of ORS.[12] Desmoglein 3 is also responsible for anchoring the telogen hair in the follicle.[13]

Wilson *et al*. in 1991 demonstrated that the human hair follicle is rich in the target antigens of pemphigus.[14] In 2003, Schaerer and Trueb first reported positive DIF findings in the ORS and the matrix of hair follicle in 100% of pemphigus patients.[8] Subsequently, Rao *et al*. reported 80% positivity and Daneshpazhooh *et al*. reported 91% positivity of DIF in the anagen hair.[9,10] 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.[11,15]

David *et al*. in 1989, based on their study suggested that repeated negative DIF in pemphigus patients on clinical remission could be a sign of immunological remission.[16] Similar findings were also reported by Balighi *et al*. and Ratnam *et al*.[6,17]

Ratnam *et al*. also noted that the patients with positive DIF findings during clinical remission had a significantly higher relapse rate after the discontinuation of treatment.[17]

Various studies have shown that the rate of relapse was approximately 44–100% in patients with positive DIF findings during remission and 13–27% in patients with negative DIF.[16,18]

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.[19]

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.[20]

In their study, the sensitivity and specificity of hair DIF was 79% and 48%, respectively. Similarly, our study had a sensitivity of 80%, however, the specificity was 70% which is comparatively higher.

Even though skin or mucosal DIF is considered as the ideal substrate for the assessment of immunological remission, 6 patients in our study had positive hair DIF even though skin DIF was negative, as seen in Table 1. Thus indicating that these patients were not yet in immunological remission. Had the clinician relied only on the DIF of skin, this finding could have been missed. The sensitivity of hair DIF in our study was not high enough to suggest it as an alternative to skin DIF for the assessment of immunological remission. However, hair DIF, being a non-invasive procedure can be used initially for patients in clinical remission to assess immunological status and if it is positive then the DIF of skin could be avoided.

Conclusion

Hence, DIF of hair is a simple, non-invasive, and cost effective procedure, and can be used as an additional procedure for assessment of immunological remission in pemphigus vulgaris. However, further studies are needed to validate the same.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Absaq C, Mouquet H, Gilbert D, Tron F, Grassi V, Musette P, *et al*. ELISA testing of anti-desmoglein 1 and 3 antibodies in the management of pemphigus. Arch Dermatol 2009;145:585-7.
2. Cheng SW, Amagai M, Nishikawa T. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3. Br J Dermatol 2002;147:261-5.
3. Beutner EH, Jordon RE, Chorzelski TP. The immunopathology of pemphigus and bullous pemphigoid. J Invest Dermatol 1968;51:63-80.
4. Sams WM, Jordan RE. Correlation of pemphigoid and pemphigus antibody titres with activity of disease.Br J Dermatol 1971;84:7-13.
5. Chorzelski TP, Von Weiss JF, Lever WF. Clinical significance of autoantibodies in pemphigus. Arch Dermatol 1966;93:570-6.
6. O'Loughlin S, Goldman GC, Provost TT. Fate of pemphigus antibody following successful therapy. Preliminary evaluation of pemphigus antibody determinations to regulate therapy. Arch Dermatol1978;114:1769-72.
7. Beutner EH, Chorzelski TP, Jablonska S. Immunofluorescence tests. Clinical significance of sera and skin in bullous diseases. Int J Dermatol 1985;24:405-21.
8. Schaerer L, Trüeb RM. Direct immunofluorescence of plucked hair in pemphigus. Arch Dermatol 2003;139:228-9.
9. Rao R, Shenoi SD, Balachandran C. Demonstration of pemphigus specific immunofluorescence pattern by direct immunofluorescence of plucked hair. J Am Acad Dermatol 2008;58:AB85.

10. Daneshpazhooh M, Asgari M, Naraghi ZS, Barzgar MR, Akhyani M, Balighi K, *et al*. A study on plucked hair as a substrate for direct immunofluorescence in pemphigus vulgaris. J Eur Acad Dermatol Venereol 2009;23:129-31.
11. Kumaresan M, Rai R, Sandhya V. Immunofluorescence of the outer root sheath in anagen and telogen hair: An aid to diagnosis in pemphigus. Int J Trichol 2009;1:138-9.
12. Wu H, Stanley JR, Cotsarelis G. Desmoglein isotype expression in the hair follicle and its cysts correlates with type of keratinization and degree of differentiation. J Invest Dermatol 2003;120:1052-7.
13. Koch PJ, Mahoney MG, Cotsarelis G, Rothenberger K, Lavker RM, Stanley JR. Desmoglein 3 anchors telogen hair in the follicle. J Cell Sci 1998;111:2529-37.
14. Wilson CL, Dean D, Wojnarowska F. Pemphigus and the terminal hair follicle.J Cutan Pathol 1991;18:428-31.
15. Tanasilovic S, Medenica L, Popadic S. Direct immunofluorescence of the outer root sheath in anagen and telogen hair in pemphigus vulgaris and pemphigus foliaceus. Australas J Dermatol2014;55:74-6.
16. David M, Weissman-Katzenelson V, Ben-Chetrit A, Hazaz B, Ingber A, Sandbank M. The usefulness of immunofluorescent tests in pemphigus patients in clinical remission. Br J Dermatol 1989;120:391-5.
17. Balighi K, Taheri A, Mansoori P, Chams C. Value of direct immunofluorescence in predicting remission in pemphigus vulgaris. Int J Dermatol 2006;45:1308-11.
18. Ratnam KV, Pang BK. Pemphigus in remission: Value of negative direct immunofluorescence in management. J Am Acad Dermatol 1994;30:547-50.
19. Rao R, Dasari K, Shenoi SD, Balachandran C, Dinesh P. Monitoring the disease activity in pemphigus by direct immunofluorescence of plucked hair: Apilot study. Indian J Dermatol 2013;58:164.
20. Daneshpazhooh M, Naraghi ZS, Ramezani A, Ghanadan A, Esmaili N, Chams-Davatchi C. Direct immunofluorescence of plucked hair for evaluation of immunologic remission in pemphigus vulgaris. JAm Acad Dermatol 2011;65:173-7.