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Direct immunofluorescence of skin and hair in pemphigus

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Abstract

Background: The outer root sheath of the anagen and telogen hair is structurally similar to the epidermal layer of the skin. Hence Direct Immunofluorescence(DIF) demonstrating Antibodies to Desmoglein 1 and 3 in Pemphigus group of autoimmune bullous diseases would show a similar pattern in skin & hair.

Aim: To compare DIF of skin and telogen hair in pemphigus group of patients in clinically active cases and those in remission.

Method: 30 consecutive diagnosed cases of Pemphigus vulgaris (PV) (both active cases and remission) were selected for the prospective study. Other blistering diseases –Dermatitis herpitiformis, Chronic Bullous Dermatosis of childhood and vasulitis were excluded. Skin biopsy and telogen hair obtained by combing were subjected to Direct Immunoflurescence. (Fluorescein Isothyocyanate) FITC –conjugated rabbit antihuman IgG was used. The samples were viewed under fluorescence microscope using a blue filter (490 nm). Green fluorescence was observed. The pattern of staining & intensity of staining were compared.

Results: 30 cases of Pemphigus included 22 cases of Pemphigus vulgaris(PV), 5 cases of Pemphigus Foliaceous(PF) and 3 of pemphigus erythematosus(PE). Pemphigus Vulgaris and Pemphigus Foliaceous patients with active disease showed strong ICS(Intercellular substance) deposits in both skin and hair. 37.5% and 50% of Pemphigus Vugaris and Pemphigus Foliaceous in remission showed weak deposits in hair only. All cases of Pemphigus Eryhtematosus showed only ICS pattern with no (BMZ) Basement membrane zone deposits.

Conclusion: Direct Immunoflurescence of skin and hair are comparable in pattern and intensity. Telogen hair is a useful tool in remission period as it is easily accessible and picks up potential cases likely to develop relapse and therefore requiring close follow up.

Keywords: DIF, Telogen hair, Skin, Pemphigus, Disease activity

Introduction

Demonstration of immune deposits has been the gold standard for the diagnosis of Pemphigus – an autoimmune bullous disease of the skin and mucosa. The nature of the deposits as seen by Direct immunofluorescence (DIF) in the perilesional skin points to the diagnosis in conjunction with histopathological(HP) and clinical findings (CL). Similar to the epidermis of the skin, is the hair with the presence of desmogleins which are transmembrane glycoproteins responsible for maintaining cell to cell contact[1]. So the pattern of DIF seen in Pemphigus group of blistering diseases in skin is comparable to hair since the autoantibodies in this disorder is directed against Desmoglein. In this study we compare the intensity of staining by DIF in skin with that of telogen hair in Pemphigus patients (diagnosed by HP, CL and IF) with active disease and those in remission to see if telogen hair can replace a skin biopsy to follow up the patients with Pemphigus.

Material and Methods

Thirty consecutive Pemphigus patients (diagnosed by HP, CL and IF) both with active disease and those in remission were included in our prospective study study. Two non-pemphigus group of autoimmune diseases-Bullous Pemphigoid and Discoid lupus erythematosus were included for comparison. Other blistering diseases –Dermatitis herpitiformis, Chronic Bullous Dermatosis of childhood and vasculitis were excluded. The clinical status of patients with new skin lesions was considered active and those with no skin lesions for a period of 6 months was considered to be in remission. The intensity of DIF staining was graded as negative, weak, strong. Perilesional skin biopsy and telogen hair were collected in every case. Telogen hair was obtained by collecting loose strands of hair from the comb after combing. The study was conducted in the departments of Pathology and Dermatology at PSGIMS&R from 2009to 2011.

No transport Medium was used as the samples were immediately transported and processed. The skin biopsy for DIF was frozen in OCT medium; 4 micron thick sections were cut in cryostat[2]. One slide was taken for each case. The sections were washed with PBS (Phosphate buffered saline) at pH 7.4 for 10 min before covering the sections with FITC –conjugated rabbit antihuman IgG, (dil 1: 10). The slides were incubated in a moist chamber for one hour. The sections were again washed with PBS for 10 min, mounted in glycerine and viewed under a fluorescence microscope using a blue filter (490 nm). Green fluorescence was observed[2]. Telogen hair samples were subjected to DIF by the same procedure as described above. Hair strands were not cut but were placed on glass slide and stained with fluorescent dye and examined. Skin biopsy and hair from normal individuals were used as negative controls.

DIF report indicated the location (skin biopsy-epidermis, hair- outer root sheath in bulb, hair shaft) and intensity (weak, strong) of the intercellular deposits (ICS).

Results

Thirty cases of Pemphigus included 22 (73.3%) cases of Pemphigus vulgaris PV, 5(16.6%) cases of pemphigus foliaceous (PF), 3 (10%) cases of Pemphigus erythematosus (PE). One case each of Bullous Pemphigoid (BP) and Discoid lupus erythematosus (DLE) were included in the study for comparison.

Age of patients in the study ranged from 28ys-50yrs; 22 were females and 8 were male. The clinical status and intensity of the immune deposits in skin and hair are shown in Table 1.

14/22 cases (63.6%) of PV had active disease. The telogen hair in all these cases showed strong ICS deposits in the outer root sheath (ORS) in bulb and suprabulbar region.(Fig. 1A, B, C) The perilesional skin showed ICS pattern in the lower epidermis(Fig. 1D). In one of the active cases hair showed strong staining but the skin a weak intensity. 3/8 PV cases(37.5%) in remission showed weak ICS deposits only in the bulb while no deposits were seen in the skin.

Of the five cases of PF, three were active and two were in remission. The active cases showed strong ICS staining in both skin and hair. In the skin the ICS was prominent in the upper epidermis(Fig. 2A). In the telogen hair no such distinction with respect to location could be made. One of the cases in remission (50%) showed weak ICS deposits in hair, skin being negative.

All the three cases of PE showed only ICS deposits in hair but DIF of skin revealed characteristic bimodal pattern of both ICS and BMZ (basement membrane zone deposits (Fig. 2B). DIF of the skin showed linear BMZ and granular BMZ deposits respectively in BP and DLE (Fig. 2C, D) Telogen hair did not show any immune deposits by DIF.

Table 1: Shows clinical status and intensity of the immune deposits in skin and hair in Pemphigus

Diagnosis	Clinical status	No. of cases	Pattern of staining	Intensity of staining	No. of cases with deposits in	
					Skin	Hair
Pemphigus Vulgaris (22 Cases)	Active	14	**ICS**	Strong	13	14
				Weak	1	Nil
				Negative	Nil	Nil
	Remission	8		Strong	Nil	Nil
				Weak	Nil	3
				Negative	5	Nil
Pemphigus Foliaceous (5 cases)	Active	3	**ICS**	Strong	3	3
				Weak	Nil	Nil
				Negative	Nil	Nil
	Remission	2		Strong	Nil	Nil
				Weak	Nil	1
				Negative	2	Nil
Pemphigus erythematosus (3 cases)	Active	3	**Both ICS and BMZ in skin AND ICS in Hair**	Strong	3	3
				Weak	Nil	Nil
				Negative	Nil	Nill
	Remission	Nil		---	---	---

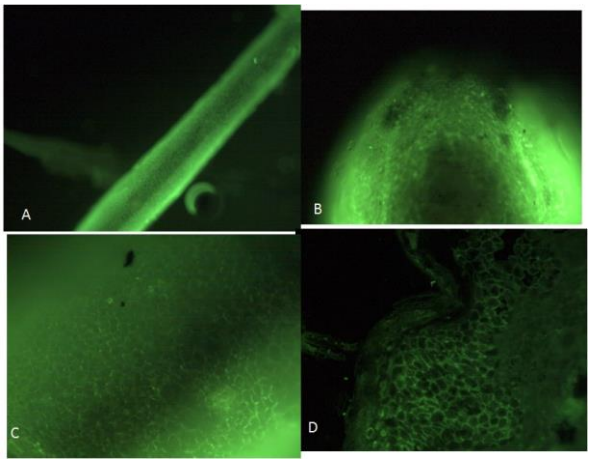

Fig. 1A: Shows strong ICS pattern in outer root sheath of hair shaft of PV x100, B in bulb x100, C shows ICS pattern extending into medulla(x400), D shows ICS pattern in epidermis(x400)

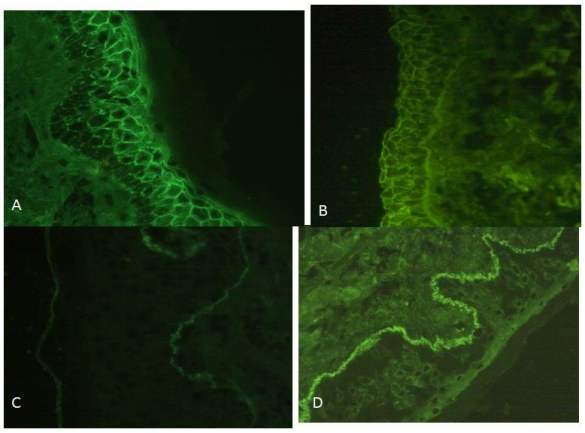

Fig. 2 A: Shows strong ICS pattern in PF(x400), B both ICS and BMZ pattern in PE(x400), C linear BMZ in BP (x400), D granular BMZ in DLE

Discussion

Pemphigus is a disease characterised by antibodies against Desmogleins, Desmoglein 3 being the target in PV and Desmoglein 1 in PF. The outer root sheath of the hair is structurally analogous to epidermis. The three subtypes of desmogleins -1, 2, 3 are expressed in hair, their expression determined by the differentiation of cells within the hair follicle and keratinisation[1,3].

Within the epidermis, desmoglein 1 is present in the upper epidermis while demoglein 3 is seen in lower epidermis and mucous membrane. Hence the ICS pattern involving the upper layer of epidermis in PF and lower epidermis in PV. Similarly in the hair, desmoglein3 is found in the bulb, the suprabulbar region of hair shaft and medulla while demoglein 1 is predominantly seen in the IRS(inner root sheath)[1,4].

In the present study, we compared the ICS deposits in skin and hair in the pemphigus group. Telogen hair was used rather than anagen hair as it is easily obtained by combing. Plucking of anagen hair could cause inconvenience to the patient. Kumeresan and others have also used the same technique in their studies[5]. In all, but one of the clinically active cases of PV, DIF of both skin and hair revealed strong ICS deposits. In the hair, the bulb and suprabulbar shaft showed strong ICS pattern. This is in accordance with the distribution of demoglein 3 –the target antigen of destruction in PV as observed by Wu et al[1]. The single case wherein the hair showed stronger expression than skin probably resulted due to antigen destruction as the biopsy included the lesional skin also. 37.5% of the cases in remission showed weak ICS pattern in the hair bulb. The explanation for these weak deposits in hair during remission is that the hair follicle with its ORS has the highest density of PV antigens and therefore antidemoglein antibodies remain there for a longer period as compared to skin[6]. These are the cases likely to relapse once the steroids are withdrawn as observed by Rao et al[6]. In our study also these cases developed new lesions in a period of 3 months after withdrawal of steroids.

PF patients with active disease showed ICS pattern of staining in both hair and skin. Literature review shows very few studies wherein DIF of hair in PF has been analysed[4]. By Immunofluorescence of hair alone PV and PF cannot be distinguished. Desmoglein 1, the principal Antigen in PF is in the innermost layer of ORS and this distribution cannot be perceived as the hair shafts were directly mounted on the slide without sectioning. Kumaresan & others have also reported similar findings[5]. DIF positivity in 50% of PF cases in remission once again highlights the persistence of antidesmoglein antibodies in hair compared to skin. Relapses were seen in these cases as in PV. Concordant findings have been reported by Rao et al[6].

PE cases failed to show BMZ deposits in hair in addition to ICS pattern unlike in skin same as other studies[5]. Detachment of BM of hair follicle could have been the cause of lack of BMZ deposits.

The prime findings of the present study have been compared with other studies in Table 2.

Table 2: Comparison of our study with other studies

Present study	Other studies
Remission cases with immune deposits in hair but not skin was seen in 37.5% of cases	Remission cases with immune deposits in hair but not skin seen in 50% of cases by Rao et al [6]
Differentiation between Pemphigus vulgaris and Pemphigus foliaceous not possible by DIF of hair alone	Correlates with studies by Kumaresan et al[5]
Only ICS pattern seen in hair in Pemphigus erythematosus	Correlates with studies by Kumaresan et al[5]
Telogen hair can replace	Kumaresan et al[5] also

skin biopsy to follow-up pemphigus patients	found telogen hair DIF as an useful tool in follow –up Rao et al found it useful to monitor disease activity.

No deposits were seen in the hair samples from BP and DLE as the antibodies against the target antigen, desmoglein is lacking in these diseases.

DIF of skin biopsy performed during periods of clinical remission is valuable in the management of the disease as negative DIF has been viewed as a state of immunological remission[7]. Patients in remission are reluctant to undergo repeated skin biopsy. DIF of hair is an ideal choice as it is a non-invasive method and further as seen from the present study a positive DIF of hair during clinical remission could indicate more frequent relapse. These patients need to be closely followed up and steroid therapy closely monitored.

Immunofluorescence of hair has its limitations. Pemphigus Vulgaris cannot be differentiated from Pemphigus Foliaceous. This is possible by the use of monoclonal antibodies to detect desmogleins.[1]

Thus Direct Immunofluorescence of telogen hair correlates well with that of skin and the disease activity of the patient and hence is an ideal choice and can replace a skin biopsy to follow up the patients with Pemphigus.

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