

Role of direct immunofluorescence on Tzanck smear and plucked hair in the diagnosis of pemphigus vulgaris

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Abstract

Objective To determine the diagnostic value of direct immunofluorescence (DIF) findings on Tzanck smear and plucked hair in patients of pemphigus vulgaris and to compare the diagnostic value of both the tests.

Methods Thirty consecutive patients of pemphigus vulgaris confirmed histopathologically were enrolled in the study. Tzanck smear from the blisters/ erosions was performed. Hair were plucked in a similar manner to that of trichogram. Approximately, 4 to 5 selected anagen hair were processed. Both the Tzanck smear and hair were stained with antihuman IgG, IgM, IgA and C3 fluorescein isothiocyanate (FITC) conjugate. DIF findings were recorded and compared.

Results Out of 30 patients, there were 21 (70%) males and 9 (30%) females. DIF was positive on Tzanck smear in 23 (76.7%) patients. Intercellular deposition of IgG was seen in the outer root sheath of anagen hair in 27 out of 30 (90%) patients.

Conclusion DIF on Tzanck smear and plucked hair is a simple, painless and non-invasive test in diagnosing pemphigus vulgaris. DIF on hair is more reliable as compared to DIF on Tzanck smear.

Key words

Direct immunofluorescence, Tzanck smear, pemphigus vulgaris.

Introduction

Pemphigus vulgaris is an autoimmune, fatal, intraepidermal blistering disorder affecting skin and mucosa. It is an immunologically-mediated disease in which autoantibodies are directed against desmoglein 3 and 1 resulting in loss of adhesions between keratinocytes.^{1,2} Clinically, it is characterized by flaccid blisters and erosions that usually start in oral mucosa and then involve rest of the body.^{1,3} Diagnosis of the disease should be established earlier in the course of the disease due to its life-threatening nature.⁴

Suprabasal clefting and acantholysis are histopathological hallmarks of the disease.⁵ Acantholysis also extends in the outer root sheath (ORS) of hair follicle, which is structurally analogous to epidermis. Demonstration of immune deposits has been the gold standard for the diagnosis of pemphigus.^{1,6} Direct immunofluorescence of the perilesional skin demonstrates intercellular deposition of IgG with or without C3 in the epidermis. But this requires expensive and sophisticated equipment and specific expertise, in addition it is not always possible to take biopsy as in mucosa, flexural areas and in children. Moreover, this facility is not available in every setting.^{1,5}

Tzanck smear is a rapid and useful test for the diagnosis of pemphigus vulgaris. The presence of acantholytic cells in the smear indicates but

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cannot confirm the diagnosis so it is not a specific test.⁴ DIF performed on the Tzanck smear indicating immunoglobulin deposits on the acantholytic cells makes it a specific, inexpensive and easy diagnostic test for the diagnosis of pemphigus vulgaris.^{4,7}

ORS of anagen hair is structurally similar to epidermis of skin. The process of acantholysis in pemphigus vulgaris seen in epidermis also involves the wall of hair follicle. Pemphigus specific immunofluorescence pattern found in the skin has also been demonstrated in the ORS of a plucked hair follicle.^{8,9} DIF on hair is easier and less invasive as compared to DIF on perilesional skin.¹

Taking a sample for DIF from oral or skin biopsy is a relatively invasive and unpleasant method for the patient. It is further complicated by the need for repeating the test until positive test results are obtained causing reluctance in many patients to accept it. Therefore, finding a less invasive way for collecting an appropriate substrate would be helpful.⁵

Methods

This cross-sectional study was carried out at the Department of Dermatology, Services Institute of Medical Sciences/ Services Hospital Lahore, during the period from June 2016 to June 2017. Thirty patients of pemphigus vulgaris diagnosed clinically with histopathological confirmation were included in the study. Patients of any age and of either sex having active disease and with any duration of disease were enrolled after taking an informed consent. The disease was considered to be active if patients had new blisters for the last 2 weeks. Patients with other forms of pemphigus and vesiculobullous disorders were excluded from the study. DIF was performed on Tzanck smear and plucked hair of each of the patient.

Tzanck smear was prepared by scraping the base of each blister/ erosion. Material obtained was then smeared on glass slide. Four slides were made and air dried. Smears were stained with fluorescein isothiocyanate (FITC) conjugate antihuman IgG, IgM, IgA and C3 for 30 minutes. These slides were then rinsed in phosphate buffer saline (PBS) solution 3 times for 10 minutes each, mounted in buffered glycerol and examined under fluorescent microscope. Bright green fluorescence at margin of cells in case of individual cells and in intercellular region in case of group of cells was considered as positivity of smear for pemphigus vulgaris.

Hair was plucked from the scalp using rubber-tipped artery forceps. Four anagen hair was selected, placed on glass slide and washed in PBS for 10 minutes. They were then incubated with antihuman IgG, IgM, IgA and C3 FITC conjugates for 1 hour. At the end of procedure, they were washed again with PBS with 3 cycles of 10 minutes each, mounted in buffered glycerol and examined under fluorescent microscope. Intercellular deposition of immunoglobulins in ORS of plucked hair was considered positive for the diagnosis of pemphigus vulgaris.

Results

A total of 30 patients of pemphigus vulgaris were studied. Among these patients there were 21 (70%) males and 9 (30%) females with mean age of 36 years. There were 11 (37%) patients who were having the disease for greater than 3 months and 19 (63%) patients had the disease for less than or equal to 3 months. Patients with both mucosal and cutaneous involvement were 23 (77%) and those with disease limited to mucosa were 7 (23%). There were 16 (53%) patients of pemphigus vulgaris who had lesions on the scalp while 14 (47%) patients did not

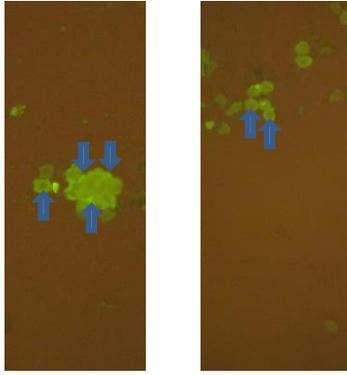


Figure 1 Bright green fluorescence at the cell margins of single cells & in the intercellular region in case of groups of cells.

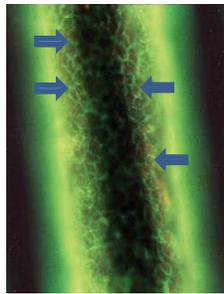


Figure 2 Intercellular deposition of immunoglobulins in ORS of plucked hair.

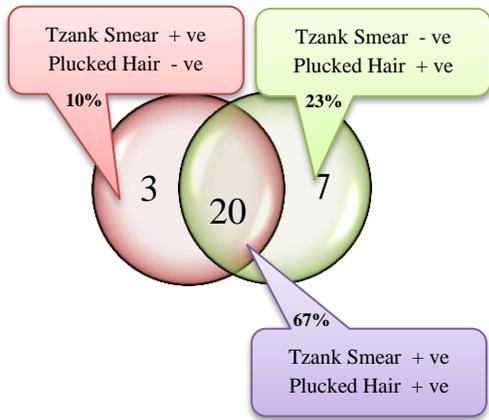


Figure 3 Venn diagram for direct immunofluorescence results.

show any scalp involvement. DIF was positive on Tzanck smear in 23 (76.7%) of patients (**Figure 1**). DIF on plucked hair was positive in 27 (90%) of patients (**Figure 2**). In 20 (67%) of the patients both tests were positive (**Figure 3**).

Chi-square test was used to compare the diagnostic value of both these tests using the

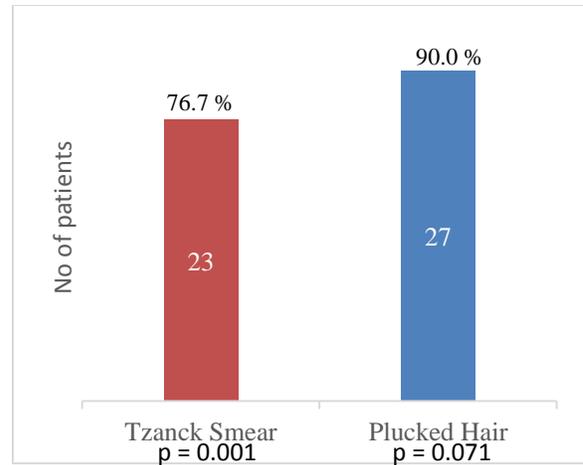


Figure 4 Positive direct immunofluorescence on Tzanck smear and plucked hair.

Table 1 Positive direct immunofluorescence results with relation to duration of disease and site of involvement.

	Duration of disease	
	≤ 3 Months (n=19, 63.3%)	> 3 Months (n=11, 36.7%)
Plucked hair (p=0.702)	17 (89.5%)	10 (90.9%)
	Site of involvement	
	Mucosa only (n=7, 23.3%)	Mucocutaneous (n=23, 76.7%)
Tzanck smear (p=0.556)	3 (42.9%)	20 (87.0%)
Plucked hair (p=0.596)	5 (71.4%)	22 (95.7%)

Fisher exact's method. There was no significant difference between the diagnostic value of these tests suggesting that both tests are useful to diagnose pemphigus vulgaris ($p=0.436$).

In order to determine the reliability of each test, the results of DIF on Tzanck smear and on plucked hair were compared separately against an expected 'n' using chi-square test – Fisher exact method with the hypothesis that the results were positive in all the patients. The result indicated that DIF on plucked hair is more reliable ($p=0.071$) as compared to DIF on Tzanck smear ($p=0.001$) in the diagnosis of pemphigus vulgaris (**Figure 4**).

Patients with duration of disease ≤ 3 months showed DIF positivity on Tzanck smear in 16 (84.2%) and on plucked hair in 17 (89.5%) patients. DIF was positive on Tzanck smear in 7 (63.6%) patients and on plucked hair in 10 (90.9%) patients with disease of greater than 3 months duration. There was no statistically significant difference on DIF positivity in respect of disease duration.

Patients with mucocutaneous involvement had higher DIF positivity on Tzanck smear and plucked hair as compared to those with only mucosal involvement. But this difference was not found to be statistically significant (**Table 1**). Patients with scalp involvement showed higher DIF positivity on plucked hair 15 (55.6%) as compared to the patients who did not have any scalp lesion 12 (44.4%), (**Table 1**).

Discussion

Pemphigus vulgaris is a chronic autoimmune disease characterized by antibodies against desmoglein 3 and 1.¹⁰ It often starts from oral mucosa and could progress to other body parts and may lead to death due to complications. Therefore, a timely diagnosis is mandatory in order to give appropriate treatment.⁴

DIF test on Tzanck smear and hair is a simple alternative to skin biopsy and direct and indirect immunofluorescence for diagnosing pemphigus. These tests are user-friendly diagnostic tools causing trivial pain and discomfort to the patient compared with a biopsy.^{1,4,5,7}

In the present study, we enrolled 30 patients of pemphigus vulgaris and DIF was performed on Tzanck smear and plucked hair of each patient. Mean age of the patients in our study was 36 years (i.e. fourth decade) which is comparable to the studies done by Sayedatun *et al.*⁵ and Hafeez¹¹ where the main age group involved was

also fourth decade. However, the patients suffering from pemphigus vulgaris in the studies conducted by Aithal *et al.*⁴ and Rai *et al.*¹² mainly belonged to the fifth decade. Pemphigus vulgaris can occur at any age but is often seen between the fourth and sixth decade of life.¹³ However, in some countries of Subcontinent including Pakistan, patients of pemphigus have a relatively low age of onset.¹³

The number of male patients in our study (70%) was higher as compared to female, which is similar to that of Sayedatun *et al.*⁵ but is different from the study done by Sandhya *et al.*¹⁴ where the female patients were higher. Pemphigus generally affects both genders equally, however, male preponderance in our study may be due to genetic and ethnic difference of our study population with that of other studies.

In our study, patients who presented within three months of disease duration were higher. This is comparable with the study done by Aithal *et al.*⁴ where most of the patients reported within three months of onset of disease. In present study, patients with mucocutaneous involvement were higher than the patients where only mucosa was involved which shows that pemphigus generally spreads to involve all body parts. This is comparable to the study of Aithal *et al.*⁴ and Rao *et al.*¹

DIF on Tzanck smear was positive in 76.7% of the patients, which is comparable to the study done by Verma *et al.*⁷ where DIF on Tzanck smear was positive in 77.8% of patients. Study done by Aithal *et al.*⁴ showed 40% positivity of DIF in Tzanck smear. This lower percentage of DIF positivity found in the study of Aithal *et al.*⁴ is due to the fact that only one antibody, IgG, was used for staining the smears.

Intercellular deposition of IgG was seen in the outer root sheath of plucked hair in 90% of the patients in our study, which is comparable to the studies conducted by Rao *et al.*¹ and Badran *et al.*¹⁵ where it was positive in 85% and 93.3% of the patients, respectively. The higher positivity may be due to the fact that diameter of terminal hair of scalp is almost twice that of vellus hair in other body areas, hence, the total volume of desmosomal structure is greater per unit area of scalp than in any other epidermal site.^{1,9}

It is also statistically proven in our study that DIF on plucked hair is more reliable as compared to DIF on Tzanck smear. To the best of our knowledge, no study has been performed to compare the diagnostic value of these two tests in pemphigus vulgaris.

Patients with mucocutaneous involvement showed higher positivity of direct immunofluorescence on Tzanck smear and plucked hair as compared to those with only mucosal involvement. These results are in accordance to the studies of Aithal *et al.*⁴ and Rao *et al.*¹ There was no statistically significant impact of duration of disease on DIF positivity on Tzanck smear and plucked hair. This may be due to the fact that the patients enrolled in our study were having active disease at the time of presentation. Aithal *et al.*⁴ showed similar observations about the effect of duration of disease on DIF of Tzanck smear.

A scalp lesion is not a prerequisite for performing direct immunofluorescence on hair as the test may be positive even in the absence of scalp involvement. Intercellular deposition of IgG in the outer root sheath of hair was seen in 15 patients with scalp involvement and also 12 patients who did not have any scalp lesions. Similar observations were made by Schaerer *et al.*⁸ and Rao *et al.*¹

DIF on plucked hair is an ideal choice of substrate for the diagnosis of pemphigus vulgaris. It alleviates the need of repeated skin biopsies during diagnosis and in the follow-up after clinical remission to confirm immunological remission as well in patients of pemphigus.

Conclusion

Direct immunofluorescence on Tzanck smear and plucked hair is a simple, painless and non-invasive test in diagnosing pemphigus vulgaris. DIF on hair is more reliable as compared to DIF on Tzanck smear. Hence, It is recommended that DIF on plucked hair should be used for the routine diagnosis of pemphigus vulgaris.

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