ROLE OF DIRECT IMMUNOFLUORESCENCE ON TZANCK SMEAR IN PEMPHIGUS VULGARIS

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ABSTRACT

BACKGROUND

The Tzanck smear is a simple, sensitive, and rapid test to diagnose pemphigus vulgaris (PV), a life-threatening autoimmune blistering disorder. The presence of acantholytic cells in cytology is indicative of, but not specific for PV. Hence, a direct Immunofluorescence (DIF) test to demonstrate immunoglobulin deposits on the acantholytic cells would make the Tzanck test more specific, and rapid. The DIF smears were compared with DIF on skin biopsies in the same patient to evaluate the diagnostic efficacy.

Aims and Objectives-To study the expression of IgG and C3 in cytology of pemphigus vulgaris using Tzanck smears and to compare direct immunofluorescence in Tzanck smears with corresponding perilesional skin biopsies.

MATERIALS AND METHODS

Study Design- Diagnostic test evaluation.

Study Population- Study was performed on oral scrape smears procured from clinically diagnosed cases of pemphigus vulgaris attending the dermatology department during the study period. (March 2017-August 2018).

Sample Size- 30.

Sampling Procedure- Continuous sampling.

Study Procedure- DIF for IgG and C3 done on both Tzanck smears and corresponding perilesional skin biopsies.

Analysis-Sensitivity, specificity, positive predictive value and negative predictive value was used to compare immunofluorescence in Tzanck smears with skin biopsies. Non-parametric test (Kendall's tau-b) was used to assess correlation.

RESULTS

Age group of study population ranges from 14 to 74 years with mean age being 49 years. IgG was positive in 80% of Tzanck smears and 83% of skin biopsies. C3 was positive in 60% of Tzanck smears and 70% of skin biopsies. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of Tzanck smears in the investigation of IgG were (%) 92, 80, 95, and 66; and C3 were (%) 76, 77, 88, 58. The correlation coefficient between Tzanck smears and corresponding skin biopsies for IgG and C3 were 0.670 and 0.505 respectively.

CONCLUSION

There is definite correlation between IgG and C3 staining in Tzanck smears compared to skin biopsy with a correlation coefficient of 0.670 and 0.505 respectively. So DIF on Tzanck smears can be used for presumptive diagnosis of pemphigus vulgaris which avoids the need for biopsy and delay in starting therapy.

KEY WORDS

Tzanck Smear, Skin Biopsy, IgG, C3, Direct Immunofluorescence.

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BACKGROUND

Pemphigus is a group of autoimmune, intraepithelial, chronic blistering skin diseases characterised by loss of normal inter-cellular adhesion. It occurs due to the formation of autoantibodies, mainly IgG & or C3 against epithelial adhesion molecules, desmosomes.[1,2]

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There are three major types of pemphigus, which vary in severity-pemphigus vulgaris, pemphigus foliaceus and paraneoplastic pemphigus.[1,2] Pemphigus vulgaris and foliaceus differ in the level of intraepithelial blisters-in vulgaris suprabasal, and in foliaceus subcorneal bullae are formed.[2]

Pemphigus vulgaris (PV) is the most common pemphigus disorder. It affects mainly middle-aged adults, both sexes equally. It is characterised by autoantibodies against desmoglein-3. It shows a geographical predilection-Mediterranean, South Asia and Jewish region are affected more.[2]

Clinical features are cutaneous or mucosal blisters. Oral lesions precede skin lesions in more than 50% of the patients and presents as blisters which rupture rapidly resulting in painful erosions. Buccal mucosa, lips and soft palate are the most commonly affected sites.[2,3,4] Diagnosis of PV is based on clinical, histopathological and immunological correlation.[1,2]

Skin biopsy is done for tissue diagnosis[3]. A proper histopathological examination can form the diagnosis in accordance with the location and morphology of blisters. Demonstration of immunoglobulins in the spinous cell junctions by direct immunofluorescence (DIF), from perilesional biopsy (Within 1 cm of the lesion) is often used for a complete diagnosis of PV.[3]

Immunofluorescence is a histochemical laboratory staining technique used for demonstrating the presence of antibodies bound to antigens in tissues or circulating body fluids. This technique is used to supplement clinical and histopathological findings in PV and other vesiculobullous diseases. They help in the early diagnosis, treatment and monitoring of the disease activity in PV.[4]

Pemphigus vulgaris has a relentless course, unless timely identified and its immunological progression checked, it can lead to a fatal outcome. So early diagnosis of the condition is imperative to prevent complications. DIF can be done both on tissue biopsies and in cytology smears for confirming the diagnosis.[5]

Tzanck smear combined with DIF is used as an effective and simple tool, in the rapid diagnosis of PV.[5,6,7] The Tzanck smear has the advantages of being easy to perform, inexpensive, not requiring a specialised laboratory, and causing negligible trauma and discomfort to the patient.[7]

Objectives of The Study

To assess the role of direct immunofluorescence (DIF) on Tzanck smear taken from mucosal or cutaneous lesions in pemphigus vulgaris and compare it with DIF done on skin biopsy.

MATERIALS AND METHODS

Study Design

Diagnostic test evaluation.

Study Period

18 months (March 2017-August 2018).

Study Setting

Department of Pathology, Govt. Medical College, Kottayam.

Sample Size:[5]

Sample size $N = \frac{Z\alpha^2 \times \text{sensitivity}\ (1-\text{sensitivity})}{d^2 \times P}$

$Z\alpha = 1.96$ at 95% CI

p= prevalence of pemphigus vulgaris in India = 1.8

d= precision/ allowable error

So, sensitivity of imprint smear in previous study=40%

Taking allowable error as 10%.

Sample size, $N = \frac{Z\alpha^2 \text{sensitivity}\ (1-\text{sensitivity})}{d^2 \times P} = \frac{(1.96)^2 \times 40 \times 60}{100 \times 1.8} = 51.22$

Taking sample size as 51.

Calculated sample size is 51. As the annual number of patients newly diagnosed as pemphigus per year is below 30 in the Department of Dermatology, Government Medical College Kottayam, this study included all newly diagnosed cases of pemphigus.

Inclusion Criteria

All clinically diagnosed cases of pemphigus vulgaris were included following a histopathological confirmation.

Exclusion Criteria

1. Previously treated pemphigus vulgaris patients.
2. Other pemphigus group of diseases.
3. Patients who are not willing to take part in the study.

Clinically suspected cases of PV attending the skin OPD were evaluated. Study was performed from oral scrape smears procured from clinically diagnosed cases of pemphigus vulgaris attending the dermatology department during the study period. Two scrape smears were taken from oral erosions and air dried and sent immediately to the Department of Pathology. One was stained with May-Grunwald–Giemsa (MGG) stain and detailed cytological analysis done. If oral/mucosal lesions were not present skin erosions are scraped, smeared and evaluated.

The other air-dried smear for DIF staining was stained with fluorescein conjugated rabbit antihuman IgG and with C3 (Dako) for 30 min. Then the smear was rinsed in PBS solution three times for 5 min each, mounted in buffered glycerol and examined immediately under the immunofluorescence microscope. If the smears were positive for immunofluorescence the pattern of staining by the acantholytic cells were noted. The skin biopsy specimens of these patients were received in 10% formalin solution and DIF on skin biopsy was done. Results of DIF on Tzanck smear were correlated with DIF on biopsy, which is the gold standard. Histological diagnosis was made and correlated with Tzanck smear findings.

Written informed consent from each patient was taken prior to the procedures.

Data Management and Analysis

The data was entered in Microsoft excel and further statistical analysis was done using SPSS software (version 20).

Statistical Methods

1. Sensitivity, specificity, positive predictive value and negative predictive value of Tzanck smears in the assessment of IgG and C3 immunofluorescence was compared with the same in histopathology.
2. Non-parametric test (Kendall tau b) for-
   - Correlation of IgG fluorescence in Tzanck smear as compared to skin biopsy.
   - Correlation of C3 fluorescence in Tzanck smears as compared to skin biopsy.

The level of significance was indicated by correlation coefficient (Between 0 and 1).

RESULTS

Diagnostic test evaluation was done on 30 cases of pemphigus vulgaris presented to Department of Pathology, Government medical college, Kottayam during the study period of 18 months (March 2017-August 2018).

- DIF for IgG and C3 were performed on the Tzanck smears from these cases and the results were compared with the DIF for IgG and C3 done on perilesional skin biopsies.
- The mean age of the present study population was 49 and minimum age was
  14 years and maximum was 74 years.
- 53% of the study population were females.
- Majority of study population (70%) had duration of illness not exceeding 6 months.
- Oral mucosa was the initial site involved in majority of cases (70%) followed by skin (7%).
- Initial lesions were erosions in 67% of patients and vesicle in the remaining cases (33%).
- The disease process was generalized in 63% of cases and localized in 37% cases.
- Skin lesions were present in 80% of the cases, with predominant trunk involvement.
- Erosions were predominant lesion (80%) followed by vesicles (20%).
- Oral mucosa was involved in 96% of cases, genital mucosa in 40%, nasal mucosa in 30%.
- Tzanck smear cytology showed acantholytic cells in all the cases with neutrophils as the predominant inflammatory cell.
- Histopathological evaluation of skin biopsies showed suprabasal clefting in all the cases with many showing acantholytic cells and row of tombstone appearance.
- DIF on perilesional skin biopsies showed fishnet positivity for IgG in 83% of cases and for C3 in 70% of cases.
- DIF on Tzanck smears showed fishnet positivity in epithelial keratinocytes for IgG in 80% of cases and for C3 in 60% of cases.
- Sensitivity for IgG was 92% and for C3 was 76%, on Tzanck smear.
- Specificity for IgG was 80% and for C3 was 77%, on Tzanck smear.
- Positive predictive value for IgG was 95% and for C3 was 88%, on Tzanck smear.
- Negative predictive value for IgG was 66% and for C3 was 58%, on Tzanck smear.
- The correlation coefficient between the expression of IgG and C3 on Tzanck smear and that on the skin biopsy was 0.670 for IgG and 0.505 for C3.
- P value for IgG was <0.0004 and for C3 was<0.008, which is statistically significant.

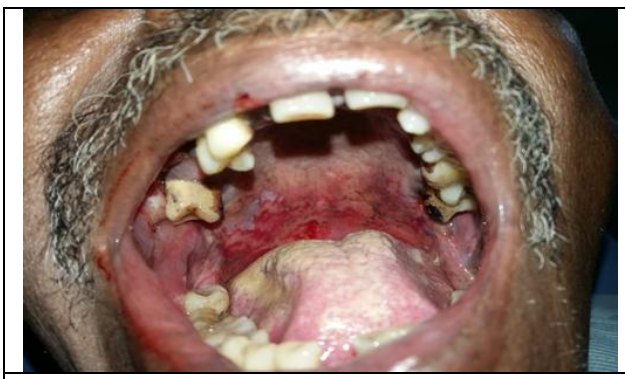

Figure 1. Oral Lesions-Pemphigus Vulgaris

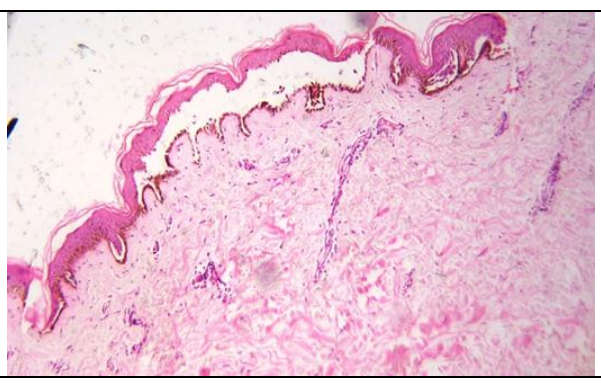

Figure 2. Histopathology-Suprabasal Bullae Showing Tombstone Appearance- H&E- 100X

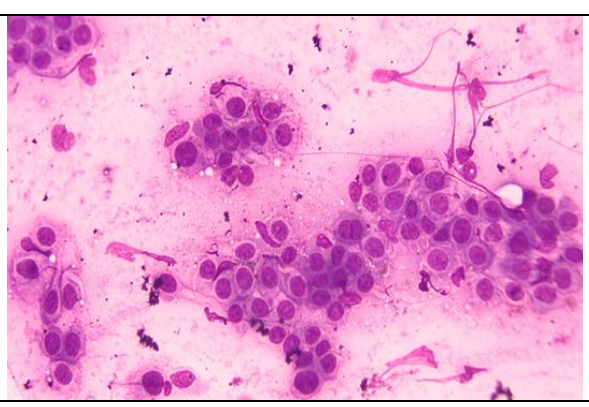

Figure 3. Oral Tzanck Smear Showing Acantholytic Cells- Giemsa- 400X

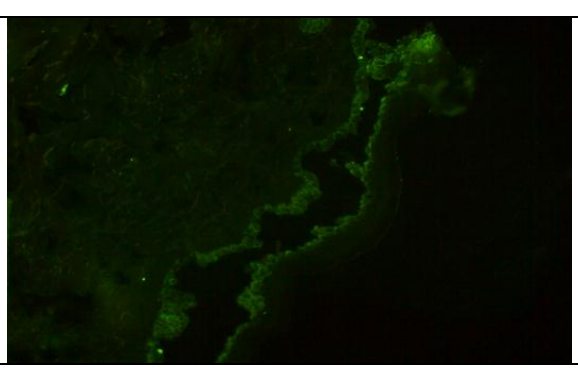

Figure 4. DIF Skin Biopsy- Fishnet Positivity for IgG (200X)

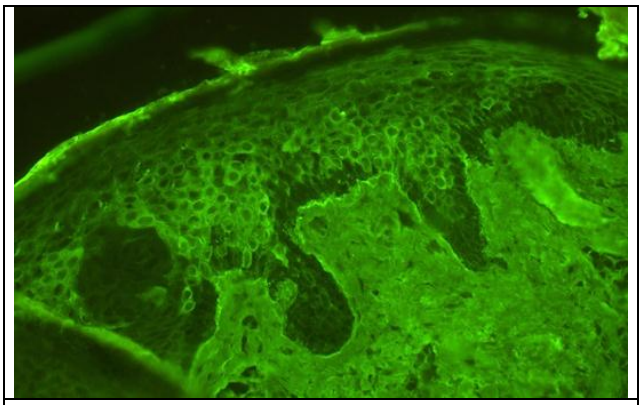

Figure 5. DIF Skin- Fishnet Positivity for C3- 200X

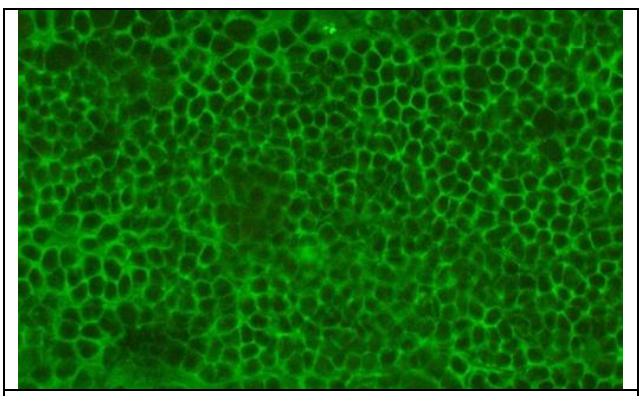

Figure 6. DIF Tzanck Smear- Fishnet Positivity for C3- 200X

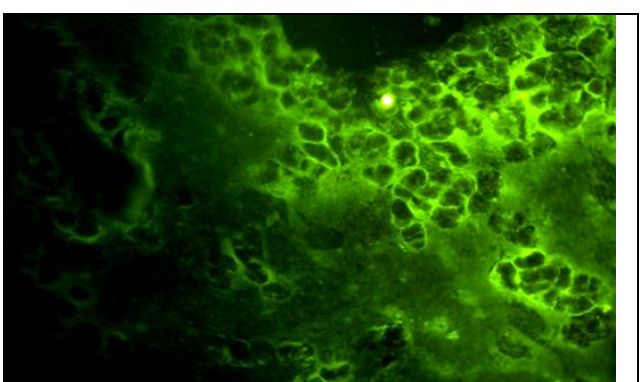

Figure 7. DIF Tzanck Smear- Fishnet Positivity for IgG- 200X

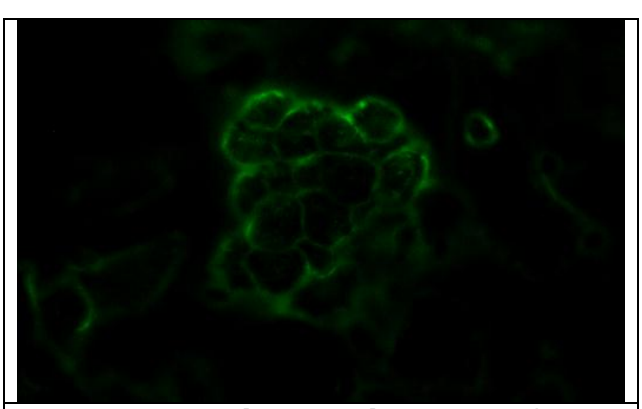

Figure 8. DIF Tzanck Smear-Fishnet Positivity for IgG- 400X

Study	Mean Age (Years)
Present Study	49
Aithal et al[5]	47.35
Yaeen et al[6]	35.1

Table 1. Comparison of Mean Age with Other Studies

In the present study the study population consisted of 16 females (53.33%) and 14 males (46.67%). The slight female preponderance is comparable to study conducted by Srinath et al[1]. Study conducted by Aithal et al[5] showed equal distribution among both sexes.

Study	Males (%)	Females (%)
Present Study	46.67	53.33
Srinath et al[1]	46.67	53.33
Aithal et al[5]	50	50

Table 2. Comparison of Gender Distribution with Other Studies

In the present study 70% of cases presented within 6 months of onset of disease. This is comparable to the study conducted by Aithal et al[5] in which 75% of cases presented within 3 months of onset of disease.

In the present study 70% of cases had oral mucosa as their initial site of involvement. This is comparable to the study conducted by Suliman et al[2] in which 57% of cases had oral mucosa as their initial site of involvement.

Skin was involved in 80% of cases in the present study. This is comparable to studies conducted by Srinath et al.[1] Suliman et al[2] and Aithal et al,[5] which reported skin involvement in 86%, 85% and 75% of cases, respectively.

Study	Skin Involvement (%)
Present Study	80
Srinath et al[1]	86.67
Suliman et al[2]	85.71
Aithal et al[5]	75

Table 3. Comparison of Skin Involvement with Other Studies

The predilection of various site involvement in the present study were as follows-Trunk (73%), extremities (66 %), face (63 %), scalp (60 %).

This is comparable to study conducted by Srinath et al[1] which showed involvement as follows- trunk (73%), extremities (46%), face (53%) and scalp (33%).

This is also comparable to a study conducted by Suliman et al[2] which reported extremities and trunk as the most common sites of involvement followed by scalp.

Study	Trunk	extremities	face	Scalp
Present Study	73	66	63	60
Srinath et al[1]	73	46	53	33

Table 4. Comparison of Various Site Involvement with Different Studies (%)

Erosions (80%) were predominant lesions in the present study followed by vesicles, bullae and pustules. This is comparable to study conducted by Suliman et al[2] which reported erosions as the predominant lesions followed by ulcers and vesicles.

Oral mucosa was the most common site involved in the present study with 96% of the patients having oral lesions. This is comparable to studies conducted by Srinath et al[1] and Suliman et al[2] which reported oral mucosal involvement in 100% and 90% of cases, respectively.

Study	Oral Involvement
Present Study	96%
Srinath et al[1]	100%
Suliman et al[2]	90%

Table 5. Comparison of Oral Involvement with Various Studies

The characteristic cytological finding in Tzanck smear in cases of pemphigus vulgaris are the presence of acantholytic cells. Cytological examination of Tzanck smears, by Giemsa stain is in itself a very sensitive and rapid test to diagnose PV. It is also an easier technique when compared with biopsy, to sample multiple sites as well as poorly accessible sites like the retro molar area, but the findings on Tzanck alone are not pathognomonic for PV, because the characteristic acantholytic cells could also be observed in other subtypes of pemphigus.

In the present study acantholytic cells were present in Tzanck smears in all 30 cases (100%). This was comparable to the studies conducted by Srinath et al[1] and Durdu et al[8], both of which reported 100% positivity for acantholytic cells, but the study done by Shailaja et al[7] showed only 50% positivity for acantholytic cells.

Study	Presence of Acantholytic Cells
Present Study	100%
Srinath et al[1]	100%
Durdu et al[8]	100%
Shailaja et al[7]	50%

Table 6. Comparison of Cytological Positivity of Tzanck Smears

In the present study neutrophils were the predominant inflammatory component in Tzanck smears. This is comparable to a study conducted by Aithal et al[5] which also reported neutrophils as the predominant inflammatory component in Tzanck smears.

Histopathological examination of skin biopsy revealed suprabasal cleft in 100% of cases and acantholytic cells in 90% of cases in the present study. This is comparable to studies conducted by Srinath et al[1] and Kabir et al[9] which showed suprabasal cleft with acantholytic cells in 100% and 87 % of cases, respectively. Also comparable to study conducted by Suliman et al[2] which showed suprabasal cleft in 90% of cases.

Study	Suprabasal Cleft (%)	Acantholytic Cell (%)
Present Study	100	90
Srinath et al[1]	100	100
Kabir et al[9]	87	87
Suliman et al[2]	90	

Table 7. Comparison of Histopathological Findings with Other Studies

Even though the Tzanck smears are highly sensitive, they are not specific for pemphigus. To improve the specificity, DIF is performed on Tzanck smears which makes it a useful diagnostic tool in the early diagnosis of pemphigus. Present study showed DIF positivity for IgG in 24/30 Tzanck smears studied (80%). In a study done by Kabir e tal[9] DIF on Tzanck smears showed positivity for IgG in 13/15 cases (86%). Study done by Durdu et al[8] showed IgG positivity in 14/14 Tzanck smears (100%). Proportion of clinically diagnosed cases of pemphigus vulgaris showing positivity for IgG by DIF on Tzanck smears in studies conducted by Acosta et al[10] and Varma et al[11] were 76% and 77% respectively.

Study	IgG Positivity
Present Study	80%
Kabir et al[9]	86%
Durdu et al[8]	100%
Acosta et al[10]	76%
Varma et al[11]	77%

Table 8. Comparison of DIF Positivity for IgG On Tzanck Smears

DISCUSSION

The present study was conducted on 30 cases of Pemphigus Vulgaris patients who presented in the Department of dermatology and whose Tzanck smear samples and perilesional skin biopsies were concurrently received in the Department of Pathology Government medical college Kottayam during the period from March 2017-August 18. DIF was done on Tzanck smear for IgG and C3 and it was compared with DIF done on their corresponding histopathology sections.

The mean age of the present study population was 49. Minimum age was 14 years and maximum was 74 years. Majority belonged to age groups of 30-40 and 40-50 with 6 patients, i.e., 20% each. Mean age is comparable to study conducted by Aithal et al[5] and Yaeen et al.[6]

CONCLUSION

1. DIF on Tzanck smear from mucosal or skin lesion showed a positivity of 80% for IgG and 60% for C3.
2. The corresponding perilesional skin biopsies on DIF showed a positivity of 83% for IgG and 70% for C3.
3. Hence the correlation coefficient between the expression of IgG and C3 on Tzanck smear and that on the skin biopsy was assessed and was found to be 0.670 for IgG and 0.505 for C3.

Based on the correlation coefficient, DIF on Tzanck smear can be considered a reasonably good supportive diagnostic test for pemphigus vulgaris and may be recommended after larger series of similar studies are performed.

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