The Significance of Tzanck Smear in Evaluation of Vesiculo Bullous Skin Lesions in Correlation with Clinical Diagnosis - A Cross Sectional Study

Heera KP[1], Anoop TV[2], Ajaya Kumar S[3], Robins K[4], Rajiv S[4]

ABSTRACT

Introduction: Cytology is a diagnostic tool used to investigate the characteristics of individual cells. Tzanck smear is a rapid, simple sensitive cytology technique that can be performed with minimal patient discomfort and cost. This study was thus aimed to establish the significance of Tzanck smear in evaluating the vesiculo bullous disorders in correlation with the clinical diagnosis.

Material and methods: A cross sectional study was conducted to know the significance of Tzanck smear in evaluation of various vesiculo bullous skin lesions in comparison with clinical diagnosis. Fisher's exact test was used for statistical analysis.

Results: Out of the 70 patients studied, majority had viral infections and auto immune vesiculo bullous disorders. All the patients with viral infection showed multi nucleated giant cells on cytology and all the patients with pemphigus group of disorder revealed plenty of acantholytic cells, along with inflammatory infiltrate. All the bullous pemphigoid patients showed signicant number of eosinophils. There was highly significant correlation between the presence of acantholytic cells, multi nucleate giant cells as well as eosinophils with clinical diagnosis.

Conclusion: Although not a substitute for standard histology, Tzanck smear can aid in establishing the clinical diagnosis with ease and rapidity.

Keywords: Tzanck Smear, Cytology, Acantholytic Cell, Multi Nucleated Giant Cell, Vesiculo Bullous Lesions.

INTRODUCTION

Diagnostic cytology is the study of individual cells and their intrinsic characteristics and functions. Cytodiagnosis is a very simple, cheap, rapid and reliable and investigative tool. The various methods of cytodiagnosis include aspiration cytology, imprint smear, exudate smear, skin scraping smear and Tzanck smear. Being rapid and simple, Tzanck smear can be used as a routine investigation in common vesiculobullous disorders, certain cutaneous infections such as chicken pox and also in various geno dermatoses like Hailey-Hailey disease. The sensitivity of Tzanck smear (under magnification of X100) in some vesiculo bullous disorders, are as follows: herpetic infection showing multinucleate giant cells 84.7%, acantholytic cells and cocci in bullous impetigo 92% and acantholytic cells in pemphigus vulgaris 100%.[1]

There is a paucity of Indian studies on the usefulness of Tzanck smear as a diagnostic tool. Being simple and cheap procedure, more studies will help in validating the usefulness of Tzanck smear as a routine investigative procedure in vesiculo bullous disorders. This study was thus aimed to establish the significance of Tzanck smear in evaluating the vesiculo bullous disorders in correlation with the clinical diagnosis.

MATERIAL AND METHODS

All patients with vesiculo bullous disorders attending the department of Dermatology in a tertiary care hospital, during a period of one year were chosen for this hospital based cross sectional study. Patients with any kind of vesiculo bullous disorders were taken as study group, who were not under any medication and were willing to give their written informed consent. Patients getting specific treatment for the underlying vesiculo bullous disorders within a period of two weeks were excluded from the study. The data was collected from patients, as per the above mentioned criteria. Tzanck smear obtained was stained with Giemsa staining method. The findings were recorded along with other essential data and clinical diagnosis. The entire procedure was done in the OPD itself. Each variable in the collected data had an allotted score, to assess the correlation with clinical diagnosis.

Preparation of Tzanck smear

An intact vesicle of bulla was chosen for the smear preparation. The lesion is de-roofed and the base scrapped with the blunt edge of a scalpel blade (no 15). The material is transferred to a glass slide and gently smeared over it. Then it is air dried. The smear is then fixed with methanol. The staining was done using Giemsa stain. The smear is then air dried and the findings were evaluated with a microscope under oil immersion.

STATISTICAL ANALYSIS

Statistical method used was Fisher's exact test. P-value < 0.05 was considered significant. All statistics were done with the help of SPSS version.

RESULT

The present study regarding the significance of Tzanck smear included a study group of 70 patients with vesiculo bullous skin lesions, who satisfied both the inclusion and exclusion criteria. Most of the cases included in the study was constituted by viral infections, followed by autoimmune vesiculo bullous disorders. Other dermatological conditions presented with vesiculo bullous skin lesions includes irritant contact dermatitis, blister

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beetle dermatitis, bullous fixed drug eruption, toxic epidermal necrolysis, bullous impetigo and tinea cruris. A single case of tinea cruris also presented with vesicles which was treated with topical steroids from periphery.

The major group among the study population was comprised of viral infections (55.7%). Among the same group varicella (46%) and Herpes zoster (30.7%) constituted the major group. The rest of the cases were comprised of herpes labialis, herpes genitalis and a single case of cow pox. All the cases revealed presence of multinucleated giant cells (MNG) in the cytology, while only 18% of cases among the viral infections showed intra nuclear inclusion bodies. A case of herpes genitalis was presented with Stevens Johnson syndrome, which showed presence of multinucleated giant cells in smear from genitalia and absence of the same with degenerated keratinocytes and neutropihils in smear taken from the lip as well as cutaneous erosions (Figure 1).

Among the study group, 11cases were pemhigus group and all the cases revealed presence of plenty of acantholytic cells among study group smears. Even though there are few other conditions showing acantholytic cells in the Tzanck smear, only the pemhigus disorders showed acantholytic cells in plenty (Figure 2).

Among 4 cases of bullous pemphigoid, all of them showed sparse number of epithelial cells and inflammatory cells and showed significant number of eosinophils along with neutrophils. Thus the sensitivity of Tzanck smear was 100% in bullous pemphigoid. But other cases such as blister beetle dermatitis also showed presence of eosinophils in the Tzanck smear along with neutrophils, rendering the Tzanck smear findings alone becomes less specific in case of bullous pemphigoid. Among eosinophil positive smears, all the cases of bullous pemphigoid has shown eosinophils in plenty, along with few percentage of other cases such as toxic epidermal necrolysis (TEN), contact dermatitis and blister beetle dermatitis (Figure 3).

Other findings include presence of neutrophils, degenerated keratinocytes and bacteiae in a single case of bullous impetigo. On applying Fisher's exact test to the data, there was highly significant correlation (0.000) between the presence of acantholytic cells, MNGs as well as eosinophils with the clinical diagnosis (Table 1).

DISCUSSION

Among the 70 cases studied, 39 were viral infections, which constituted the major group. Among viral infections, the major group was constituted by herpes simplex and varicella zoster virus infections, and showed both sensitivity and specificity of 100%, in smears taken from vesicles. This findings are comparable to Durbu[2] et al which states a sensitivity of 100%, 69.2% and 59.7% for vesicular, pustular and erosive skin lesions respectively and a specificity of 100% for multinucleated giant cells, in herpes simplex as well as varicella zoster virus infections. Intra nuclear inclusion bodies are characteristic of herpetic infections, but are difficult to find.[3] In this study

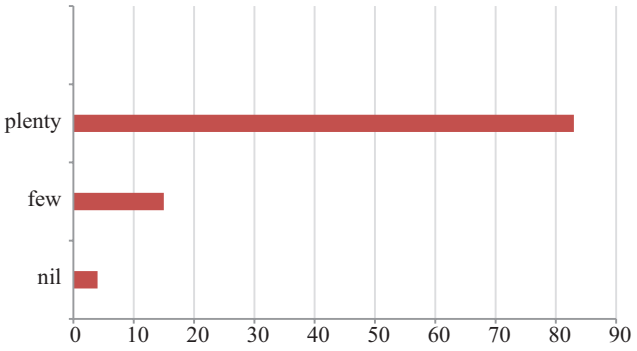

Figure-1: Distribution of multinucleated giant cells in Tzanck smears of viral infections

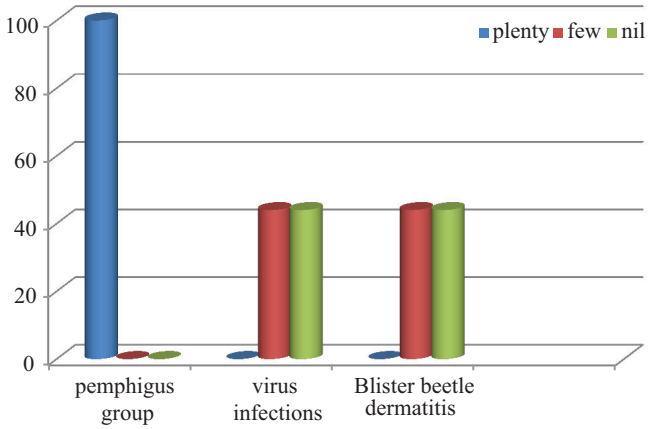

Figure-2: Distribution of acantholytic cells among the Tzanck smear positive cases.

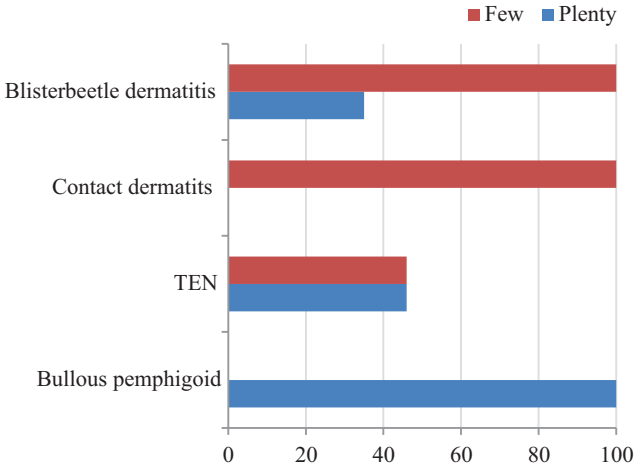

Figure-3: Distribution of eosinophils among positive smears

Correlation between clinical diagnosis and Tzanck smear	Fisher's exact test (P value)	Significance
Acantholytic cell and clinical diagnosis	0.000	Highly significant
MNG and clinical diagnosis	0.000	Highly significant
Eosinophils and clinical diagnosis	0.000	Highly significant
Bacteria and clinical diagnosis	0.325	Not significant
Degenerated keratinocytes and clinical diagnosis	0.016	Significant
Intra nuclear inclusion bodies and clinical diagnosis	0.729	not significant

Table-1: Correlation between clinical diagnosis and Tzanck smear, applying Fisher's exact test.

inclusion bodies were identified only in 18% of specimens stained by Giemsa stain, with the help of pathologist. According to Vincezo Roucco,[4] Solomon A.R[5] et al, the application of Tzanck smear helps less in differentating between the varicella virus infections and herpes infections and this study also goes in support with the same observation. Though not included in the study, Papinicolou stain done on the smears of viral infections provided a better visualisation of intra nuclear inclusion bodies. A single case of cowpox also showed multinucleated giant cells, but failed to demonstrate Guarneri bodies, when compared to other studies. [3,6] This study, as the above mentioned studies, shows a very high significant correlation (0.000), between the clinical diagnosis and presence of multinucleated giant cells, in the Tzanck smears taken from the skin lesions. Though the results were not comparable to observations in other studies in case of bullous impetigo, as there was only a single case included in the study as per inclusion and exclusion criteria, showed presence of sparse acantholytic cells and cocci in clumps showing gram positivity performed on a separate smear, as in many of other studies.[3,7]

In the previous studies, sensitivity of acantholytic cells in Tzanck smear test in cases with pemphigus has been reported between 93.3% and 100%,[8,9] where as in the current study the sensitivity is 100% and specificity is only 73% and showed a highly significant correlation with a p value of 0.000 in between the clinical diagnosis and presence of multinucleated giant cells. In addition to typical acantholytic cells, Sertoli rosette cells (consists of aggregates with an epithelial cell at the centre surrounded by neutrophils) and streptocytes (chains of white blood cells) may also be observed in cases of pemhigus.[8] Acantholytic cells were observed in all of the studied cases with pemphigus but streptocytes or Sertoli rosette cells were not observed in any of the cases.

A study conducted by Blank and Burgoon [8] suggests unique features of epithelial cells in pemphigus disorders, which helps in differeniating the same from other types of vesiculobullous conditions; there is a profusion of epithelial cells with relatively few inflammatory cells in pemphigus vulgaris, many of these cells are small and have a round shape, the nuclei are large in relation to the cytoplasm and many of the nuclei are well preserved, with easily distinguished nuclei, the cytoplasm usually condense as a basophilic zone at the periphery and these changes are found a major group of epithelial cells in the smear, when compares to other conditions that shows occasional presence of few acantholytic cells. This study also showed a similar observation especially with profusion of epithelial cells, majority of which showed acantholytic changes, when compared to other vesiculo bullous conditions that showed presence of acantholytic cells occasionally.

According to previous studies,[6,4,3,10,7] Tzanck smear in case of bullous pemphigoid is non specific and basically meant to differentiate the same with pemphigus group of disorders. The cytological findings in bullous pemphigoid show a scarcity of epithelial cells, eosinophil inflammatory infiltrate. But our study showed a significant correlation between the clinical diagnosis of bullous pemphigoid and presence of eosinophil predominant inflammatory infiltrate in Tzanck smear, along with sparse number of epithelial cells.

Most of the previous studies concludes that, Tzanck smear in toxic epidermal necrolysis is to differentiate from staphylococcal scalded skin syndrome, when though rarely, the later occurs in adults, especially in those with chronic immunodeficiency.[4,11] In toxic epidermal necrolysis, the smear shows necrotic basal cells, leukocytes and scattered fibroblasts. [11] In our study, two cases of toxic epidermal necrolysis were included and Tzanck smear taken from the bullous lesion showed degenerated keratinocytes and a mixed type of inflammatory infiltrate with no acantholytic cells.

The Tzanck smear findings are non specific in vesiculo bullous lesions of bullous fixed drug eruption, allergic contact dermatitis and irritant contact dermatitis.[6,8] A case of bullous fixed drug eruption showed necrotic karetinocytes, eosinophils and neutrophils in our study. Smears taken from blister beetle dermatitis showed acantholytic cells along with inflammatory infiltrate and degenerated keratinocytes. On applying Fishers exact test our study showed a highly significant correlation between the clinical diagnosis and presence of acantholytic cells, multinucleated giant cells and eosinophils.

CONCLUSION

The study emphasizes on the well known fact that the usefulness of Tzanck smear as an immediate aid in helping to establish a precise clinical diagnosis in vesiculo bullous skin lesions and despite the development of many sophisticated techniques in the field of diagnostic methods, it carries its own importance in the field of diagnostic cytopathology. More over taking a Tzanck smear hardly cause any trauma or discomfort to the patient and therefore can be easily performed and repeated even in most timorous individuals, in children and in difficult to biopsy sites such as lips, eyelids or genitals.

Although not a substitute for standard histology, with consistent and careful practice, Tzanck smear can aid in establishing the clinical diagnosis with ease and rapidity and can serve as an adjunct to the gold standard techniques in diagnosing vesiculo bullous diseses. This study may be taken as reinforcement to other studies that have already established the utility of Tzanck smear as a cytodiagnostic technique in the field of dermatology.

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