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Role of Tzanck Smear microscopy for detection of vesiculobullous skin lesions and active genital herpes- a pilot study in tertiary care hospital, Patna, Bihar

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ABSTRACT

Background: Cytology is a diagnostic technique used to evaluate the properties of individual cells. Tzanck smear is a quick, simple, sensitive cytology procedure that can be done with little patient discomfort and expense.

Objective: The purpose of this study was to determine the value of Tzanck smear in evaluating vesiculobullous illnesses and active genital herpes in relation to clinical diagnosis.

Methods: A cross-sectional study was carried out to determine the value of Tzanck smear in evaluating various vesiculobullous skin lesions in comparison to clinical diagnosis. For statistical analysis, the Fisher's exact test was applied.

Results: The majority of the 70 patients evaluated had viral infections or auto immune vesiculobullous diseases. On cytology, all patients with viral infection had multinucleated giant cells, and all patients with pemphigus had a large number of acantholytic cells as well as an inflammatory infiltrate. All of the patients with bullous pemphigoid had a significant number of eosinophils. The presence of acantholytic cells, multinucleate large cells, and eosinophils had a highly significant connection with clinical diagnosis.

Conclusion: Tzanck smear, while not a replacement for traditional histology, can aid in establishing the clinical diagnosis with ease and speed.

Key Words: Tzanck Smear, Cytology, Acantholytic Cell, Multi Nucleated Giant Cell, Vesiculobullous Lesions**.**

INTRODUCTION

Vesiculobullous diseases are a diverse group of dermatoses with a wide range of symptoms. The examination of clinical, histological, and immunofluorescence findings is required for the appropriate diagnosis of bullous illnesses of the skin and mucous membrane [1]. Cytology can be used to diagnose a variety of dermatological diseases. It allows for speedy diagnosis at a minimal cost, as well as prompt referral of the patient for appropriate therapy. The patient tolerates the operations well, and problems are uncommon. In addition to aiding in disease diagnosis, cytology can be used to track disease progression and detect relapses [2, 3]. Clinical information accompanied by cytodiagnosis is useful in the examination of numerous infectious illnesses in developing countries. Furthermore, in instances where biopsy is not an option, cytology can provide extremely trustworthy information about a variety of skin tumours. When compared to biopsy, cytological sampling procedures cause less tissue damage [3]. Cancer

cytology may become the preferred diagnostic approach as the adoption of novel, non-invasive topical treatment options for non-melanoma skin grows. A thorough understanding of the cytological characteristics of primary skin neoplasms is especially crucial in determining the precise origin of a skin tumour and distinguishing it from metastasis from another site.
Tzanck smears, due to their speed and simplicity, can be utilised as regular investigations in common vesiculobullous illnesses, certain cutaneous infections like as chicken pox, and certain genetic dermatoses such as Hailey-Hailey disease [4, 5]. Tzanck smear sensitivity in several vesiculobullous illnesses is as follows: herpetic infection displaying multinucleate giant cells 84.7 percent, bullous impetigo showing acantholytic cells and cocci 92 percent, and pemphigus vulgaris showing acantholytic cells 100 percent [6, 7, 8]. There is a scarcity of Indian research on the utility of the Tzanck smear as a diagnostic tool. Because the Tzanck smear is a straightforward and inexpensive procedure, more research will be needed to validate its utility as a routine investigative procedure in vesiculobullous illnesses.The purpose of this study was to determine the value of Tzanck smear in evaluating vesiculobullous illnessesand active genital herpes in relation to clinical diagnosis.

MATERIALS AND METHODS

This hospital-based cross-sectional study included all patients with vesiculobullous illnesses who attended the department of Dermatology in a tertiary care hospital over a two-year period Jan 2019- Jan 2021. Patients with any type of vesiculobullous illness who were not taking any medication and were prepared to offer their written informed consent were included in the study. Patients who received particular treatment for the underlying vesiculobullous illnesses within two weeks of enrolling in the research were excluded. The information was gathered from patients using the above-mentioned criteria. The Tzanck smear was stained using the Giemsa staining procedure.The observations were documented, together with other important data and a clinical diagnosis. The entire surgery was carried out in the OPD. To examine the association with clinical diagnosis, each variable in the collected data was assigned a score.

PREPARATION OF TZANCK SMEAR

For the smear preparation, an entire bulla vesicle was chosen. The lesion is de-roofed and the base scraped with a scalpel blade's blunt edge. The substance is applied on a glass slide and gently smeared across it. It is then air dried. Methanol is then used to repair the smear. Giemsa stain was used for the staining. The smear was then air dried before being examined under an oil immersion microscope.

STATISTICAL ANALYSIS

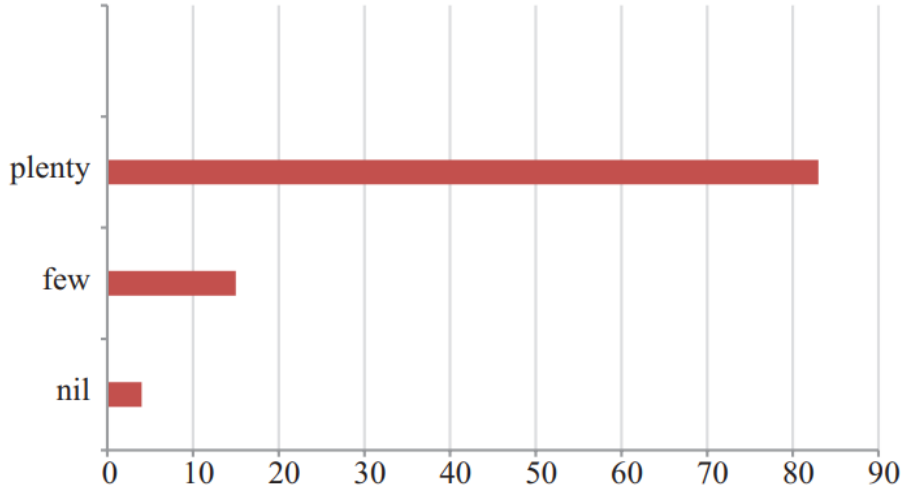
Fisher's exact test was utilised as a statistical method. P-values less than 0.05 were considered significant. All statistics were generated using the SPSS version.

RESULTS

The current investigation on the importance of Tzanck smear comprised 70 individuals with vesiculobullous skin lesions who met both the inclusion and exclusion criteria. The majority of the patients in the study were caused by viral infections, followed by autoimmune vesiculobullous illnesses. Irritating contact dermatitis, blister beetle dermatitis, bullous fixed drug eruption, toxic epidermal necrolysis, bullous impetigo, and tinea cruris are some of the other dermatological disorders associated with vesiculobullous skin lesions. A solitary case of tinea cruris was also found to have vesicles that were treated with topical steroids from the periphery.
Viral infections made up the majority of the research population (55.7 percent). Herpes zoster (30.7 percent) and Varicella (46 percent) were the most common within the same group. The

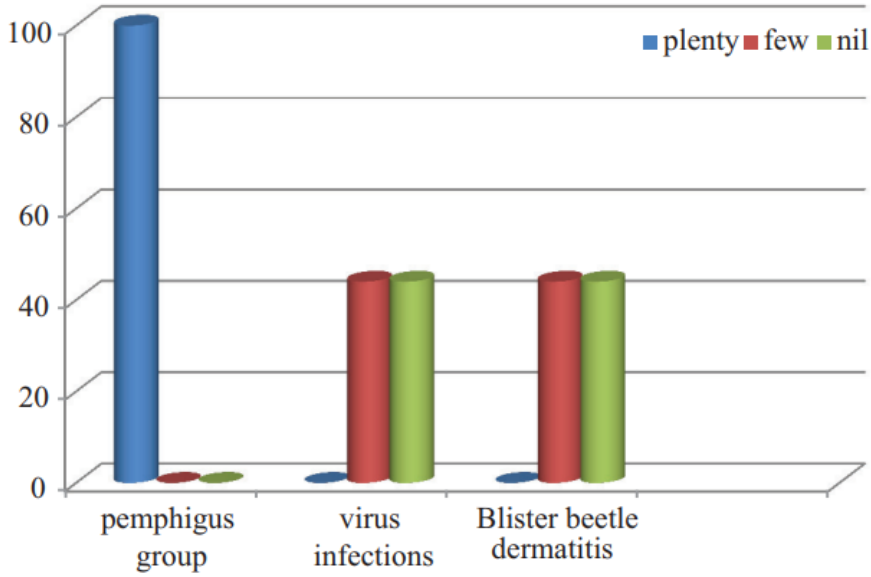
remaining instances were herpes labialis, herpes genitalis, and a solitary incidence of cow pox. In the cytology, all of the patients had multinucleated giant cells (MNG), but only 18% of the viral infections had intra nuclear inclusion bodies. A case of herpes genitalis was given with Stevens Johnson syndrome, demonstrating the presence of multinucleated giant cells in genital smears and the absence of the same with deteriorated keratinocytes and neutropihils in lip smears, as well as cutaneous erosions (Figure 1).

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



There were 11 individuals in the study group who had pemhigus, and all of them had a lot of acantholytic cells in their smears. Even though there are few other ailments that reveal acantholytic cells in the Tzanck smear, only the pemhigus disorders showed a large number of acantholytic cells (Figure 2).

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



All four cases of bullous pemphigoid had a low amount of epithelial and inflammatory cells, as well as a high number of eosinophils and neutrophils. Tzanck smear sensitivity was thus 100 percent in bullous pemphigoid. However, in some situations, such as blister beetle dermatitis, eosinophils were found in the Tzanck smear alongside neutrophils, making the Tzanck smear findings less specific in the case of bullous pemphigoid. All instances of bullous pemphigoid had eosinophils in abundance, as did a small number of other cases such as toxic epidermal necrolysis (TEN), contact dermatitis, and blister beetle dermatitis.In a single episode of bullous impetigo, researchers discovered neutrophils, degraded keratinocytes, and bacteiae. When the data was subjected to Fisher's exact test, there was a very significant correlation (0.000)

between the presence of acantholytic cells, MNGs, and eosinophils and the clinical diagnosis (Table 1 and Table 2).

Table 1: Description of patient's data

		No. = 70
Age	Mean ± SD	35.14 ± 19.76
	Range	3-78
Sex	Male	35
	Female	35
Family history	Positive	37
	Negative	33
Risk Factors	Positive	41
	Negative	29

Table 2: Correlation between Tzanck smear and clinical diagnosis, using Fisher's exact test

Correlation between Tzanck smear and clinical diagnosis	p-value	Significance
MNG and clinical diagnosis	0.000	Highly significant
Bacteria and clinical diagnosis	0.325	Not significant
Intra nuclear inclusion bodies and clinical diagnosis	0.729	Not significant
Acantholytic cell and clinical diagnosis	0.000	Highly significant
Eosinophils and clinical diagnosis	0.000	Highly significant
Degenerated keratinocytes and clinical diagnosis	0.016	Significant

DISCUSSION

The majority of the cases evaluated (39 out of 70) were viral infections. Herpes simplex and varicella zoster virus infections were the most common among viral infections, with sensitivity and specificity of 100 percent in vesicle smears [9]. Durbu et al. [10] found a sensitivity of 100 percent, 69.2 percent, and 59.7 percent for vesicular, pustular, and erosive skin lesions, and a specificity of 100 percent for multinucleated giant cells in herpes simplex and varicella zoster virus infections. Intra nuclear inclusion bodies are a hallmark of herpetic infections, although they are difficult to detect [11, 12, 13].

With the assistance of a pathologist, inclusion bodies were recognised in only 18% of the specimens stained with Giemsa stain in this investigation [14, 15]. According to Vincezo Roucco [16], Shaheen et al [17], the use of Tzanck smears assists less in differentiating between varicella virus infections and herpes infections, and this study supports the same observation. Though not included in the study, Papinicolou staining of viral infection smears improved visualisation of intra nuclear inclusion bodies [18]. When compared to other research, a single instance of cowpox showed multinucleated giant cells but did not show Guarneri bodies.

This study, like the previous ones, finds a highly significant correlation (0.000) between clinical diagnosis and the presence of multinucleated giant cells in Tzanck smears collected from skin lesions [6, 8, 10, 15, 17]. Though the results were not comparable to observations in other studies in the case of bullous impetigo, as only one case was included in the study according to inclusion and exclusion criteria, they did show the presence of sparse acantholytic

cells and cocci in clumps showing gramme positivity performed on a separate smear, as in many other studies.

Previous studies reported sensitivity of acantholytic cells in Tzanck smear test in cases with pemphigus ranging from 93.3 percent to 100 percent [19, 20, 21]. Whereas, in the current study, sensitivity is 100 percent and specificity are only 73 percent, with a highly significant correlation with a p value of 0.000 between clinical diagnosis and presence of multinucleated giant cells. In addition to typical acantholytic cells, Sertoli rosette cells (aggregates with an epithelial cell at the centre surrounded by neutrophils) and streptocytes (chains of white blood cells) may be seen in pemhigus patients. Acantholytic cells were seen in all of the pemphigus cases investigated, but no streptocytes or Sertoli rosette cells were found.

The study conducted by Blank and Burgoon [8] reveals that epithelial cells in pemphigus illnesses have distinct properties that help distinguish them from other types of vesiculobullous ailments. In pemphigus vulgaris, epithelial cells proliferate but inflammatory cells are few. Many of these cells are tiny and spherical in shape, with large nuclei in comparison to the cytoplasm, and many of the nuclei are well maintained, with plainly distinguishable nuclei. When compared to other situations that reveal the existence of a few acantholytic cells, the cytoplasm usually condenses as a basophilic zone at the periphery and these modifications are detected in a large group of epithelial cells in the smear.When compared to other vesiculobullous circumstances that demonstrated the presence of acantholytic cells on occasion, this study showed a similar observation, particularly with profusion of epithelial cells, the majority of which showed acantholytic alterations.

According to previous research, the Tzanck smear in the case of bullous pemphigoid is nonspecific and is primarily used to differentiate the condition from the pemphigus group of disorders [19, 20]. Cytological studies in bullous pemphigoid demonstrate a shortage of epithelial cells and an inflammatory infiltration of eosinophils. However, our investigation found a link between the clinical diagnosis of bullous pemphigoid and the presence of an eosinophil-dominated inflammatory infiltration in the Tzanck smear, as well as a low number of epithelial cells.

Most of the previous studies concludes that, Tzanck smear intoxic 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 nonspecific in vesiculobullous 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 emphasises the well-known fact that the Tzanck smear is useful as an immediate aid in assisting in the establishment of a precise clinical diagnosis in vesiculobullous skin lesions, and despite the development of many sophisticated techniques in the field of diagnostic methods, it retains its own importance in the field of diagnostic cytopathology. Furthermore, taking a Tzanck smear causes no harm or discomfort to the patient and can thus be readily performed and repeated even in the most apprehensive individuals, youngsters, and difficult to biopsy locations like as lips, eyelids, or genitals. Although not a replacement for standard

histology, 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 vesiculobullous diseases with consistent and careful practise. This study adds to previous research that has established the utility of the Tzanck smear as a cytodiagnostic technique in the field of dermatology.

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