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Case Report

Fallacious Diagnosis Based on Cytological Smear from Oral Lesion of Mucous Membranous Pemphigoid: A Case Report and Cytological Comparison with Oral Pemphigus Vulgaris

Deepak Pandiar[1*], Manjusha P[2], Saakshi Gulati[3], Shameena PM[4]
[1]Department of Dentistry, Faculty of Dental Sciences, IMS, BHU, Varanasi, India
[2]Fellow in Head and Neck Pathology, Malabar Cancer Centre, Thalassery, Kerala, India
[3]Department of Oral Medicine and Radiology, Sathyabama Dental College and Hospital, Chennai, Tamilnadu, India
[4]Department of Oral Pathology and Microbiology, Government Dental College, Thrissur, Kerala, India

***Corresponding Author:**
Deepak Pandiar
Email: deepakpandiar1923@yahoo.com

Abstract: Pemphigus vulgaris and mucous membrane pemphigoid are uncommon dermatological disorders which also involves the mucosae of the oral cavity. Both the conditions differ in microscopy and prognosis. Thus the aim of the present paper is to present one case each of pemphigus vulgaris and mucous membrane pemphigoid with oral involvement. Special attention has been made on the clinical tests to evaluate these disorders and cytological pitfalls, which may be helpful for both clinicians and pathologists**.**
Keywords: acantholysis; cytology; pemphigus; pemphigoid.

INTRODUCTION

Mucous membrane pemphigoid (MMP) is a part of the group of autoimmune sub-epithelial bullous disorders, recognized by the term "immune mediated sub-epithelial blistering diseases". Also known as cicatricial pemphigoid, MMP mainly involves oral and ocular mucous membranes along with less frequent involvement of mucous membranes of nasal cavity, pharynx, larynx, esophagus and genitalia. Occasional involvement of skin is also seen. BP180 epidermal antigen is a major antigenic target of IgG auto-antibodies produced by MMP patients [1].

Similarly, Pemphigus vulgaris (PV) is the most common variant of Pemphigus group of lesions. Once considered fatal, PV usually affects oral mucosa and skin causing blisters, erosions and ulcers. IgG antibodies against both desmoglein 1 and 3 are expressed but in oral mucous membrane desmoglein 3 is particularly expressed. The proportion of Dsg1 and Dsg3 antibodies appears to be related to the clinical severity of PV [2].

PV has an approximate annual prevalence of 0.5–3.2 cases per 100,000. It is more frequent among Ashkenazi Jews and, in some rare variants, in South America and affects individuals of age range of 40 to 60 years. MMP is an uncommon disease of elderly females (>50 years) [3].

Although scalpel biopsy is the gold standard for the diagnosis of a disease, role of cytopathology cannot be overlooked. PV shows rather characteristic if not pathognomonic acantholytic cells in cytosmears, as similar cells are also seen in herpetic gingiva-stomatitis, familial pemphigus. These cells contain large nucleoli (often multiple) and may be readily mistaken for cells of an adenocarcinoma. And it is essential to differentiate such cells from normal basal and parabasal cells which are of similar size and thus may lead to erroneous diagnosis and treatment delay. Thus the purpose of present paper is to report cytological aspects of PV and MMP with emphasis on literature review pertaining to cytology.

CASE REPORTS

Case 1

A seventy four years old female patient (Dravidian- linguistically Malayali) was referred from a private clinic to oral medicine unit. The patient complained of red areas and burning sensation of cheek for three months. Patient recalled vesicle formation in oral cavity and on skin with remissions for last three years. On examination raw erosive areas were seen involving entire oral mucosa and were more severe on the hard palate where mild sloughing was also noticed. The lesions were tender and bled even on slight provocation. Nikolsky's sign was negative. Fresh bullae were noticed on the forearm in addition to healing lesions. Recently the patient noticed redness of eyes

which were itchy. On examination the conjunctivae appeared erythematous and severely inflamed with excessive lacrimation (Figure 1).

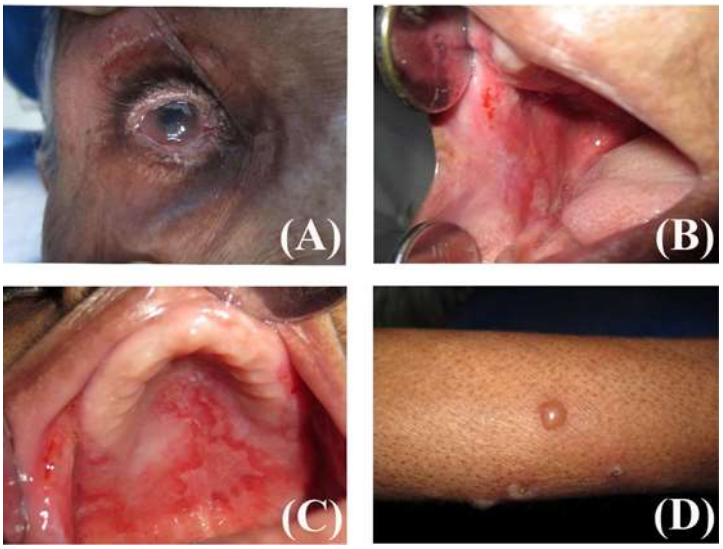

Fig-1: Clinical presentation of case 1 (mucous membrane pemphigoid)

Provisionally the patient was diagnosed as pemphigus vulgaris and a cytosmear was taken and stained. The cytological report suggestive of pemphigus vulgaris, and showed a few clusters of round to ovoid cells with large vesicular basophilic cells resembling acantholytic cells admixed with inflammatory cells and normal desquamated cells in a fibrinous background (Figure 2A-D). Biopsy was advised for further evaluation. The incised specimen was cut into two equal halves. One part was sent for routine histological examination and other part for direct immuno-fluorescence (DIF).

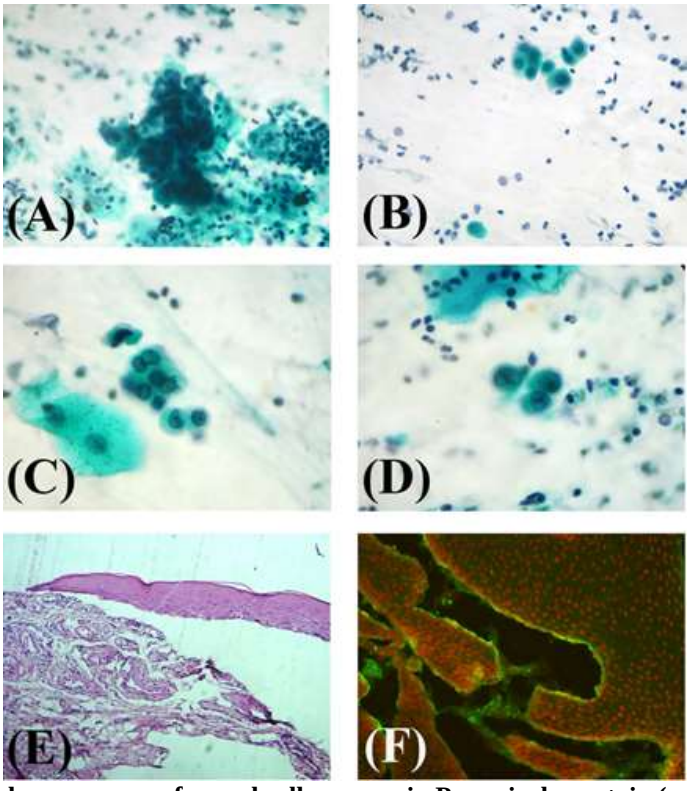

Fig-2: A-D) Cytological appearance of round cells as seen in Papanicolaou stain (cells were actually basal and parabasal cells and can be mistaken for acantholytic cells); E) H&E section showing complete separation of full thickness epithelium from connective tissue (100X); F) MMP confirmed by direct immunofluorescence

H & E section showed a sub-epithelial split. The stratified squamous epithelium was completely detached from the underlying fibrovascular connective tissue (Figure 2E). The histological features were not consistent with cytological report. The cytological slides were carefully re-evaluated and it was noticed that the round to ovoid cells were actually basal and para-basal cells. These cells had vesicular nuclei and cytoplasm had a greenish tinge in contrast to acantholytic cells which are almost of the same size but contain deeply basophilic nuclei and dense cytoplasm. Correlating clinical, cytological and histological features a final diagnosis of mucous membrane pemphigoid was given which was confirmed by DIF. DIF report showed a sub-epithelial separation and focal linear staining of basement membrane zone (BMZ) with IgG and IgA (Figure 2F). The patient was referred to dermatology unit of medical college, Calicut where she was treated with systemic corticosteroids (prednisolone started at 40mg/day and tapered down to 10mm/day over a period of three months).

Case 2

A thirty two years old male patient (Dravidian-linguistically Malayali) presented with a chief complaint of ulcers in mouth of one month duration. Patient noticed ulcers in the left and right cheek region since one month. The ulcers were painful especially while having spicy food and hot beverages. Initially there was increased salivation which later decreased significantly. The saliva was thick and pasty. Two weeks from the onset of cheek ulcers he noticed painful ulcers on tongue, roof of mouth, and lips. Patient was treated by a general practitioner by systemic acyclovir but it did not bring relief.

On intraoral examination, multiple ill-defined, irregularly shaped ulcers were present bilaterally on buccal mucosa extending from the commissures of mouth anteriorly to the molar area posteriorly; and extending to the buccal vestibules supero-inferiorly. The ulcers were tender on palpation. Also, irregular tender erosive areas were noticed on the dorsum of the tongue extending on to the ventral surface. Mucosa of hard and soft palate was also involved. Large multiple ulcers were also present on the lower lip with well defined margins (Figure 3). Floor of the ulcer appears yellowish with erythematous halo. The ulcers are tender on palpation, non-indurated. Surrounding areas appear inflamed without any discharge. Marginal Nikolsky's sign was positive. No lesions were present on upper lip and gingivae.

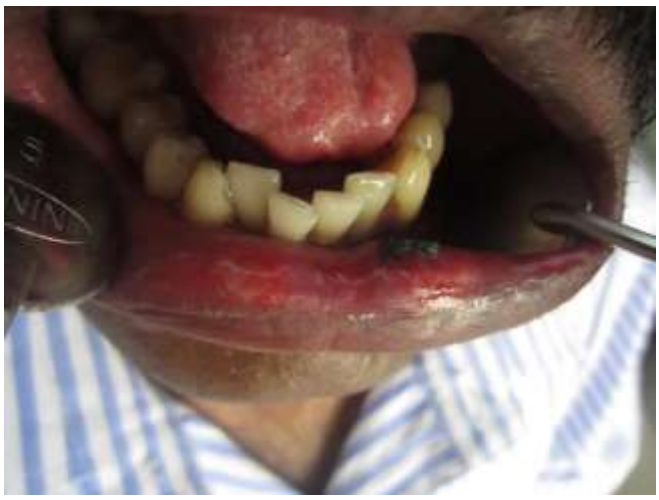

Fig-3: Clinical presentation of case 2 (Pemphigus vulgaris) on inferior lip and tongue

Provisionally the patient was diagnosed as pemphigus, herpetic lesions, pemphigoid lesions, Hailey-Hailey Disease**.** A Tzanck smear was prepared and stained with routine Papanicolaou procedure which showed a few clusters of round to ovoid cells with basophilic nuclei and deeply eosinophilic cytoplasm (Acantholytic cells) and normal desquamated epithelial cells with few inflammatory cells in a fibrinous background, suggestive of Pemphigus vulgaris (Figure 4A-B).

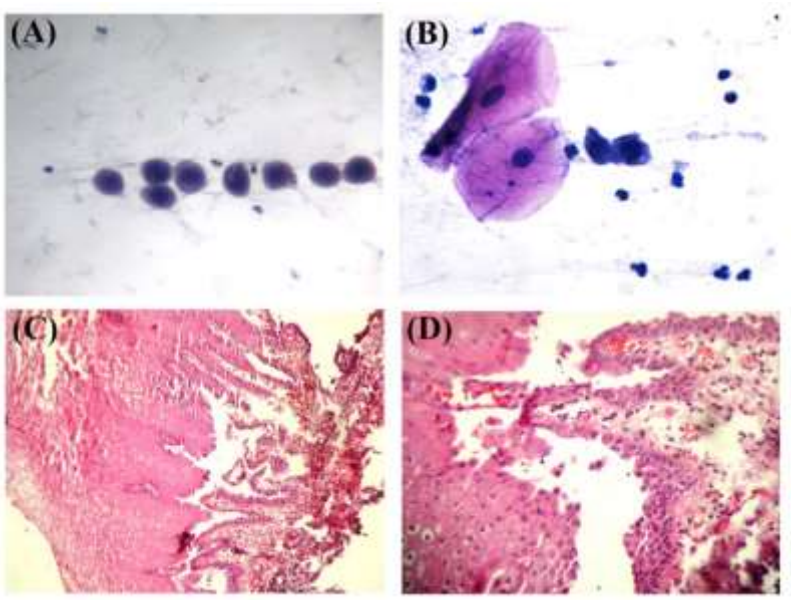

Fig-4: A-B) Acantholytic cells seen in Papanicolaou stain (100X); C) H&E Section showing suprabasilar split (40X) and D) H&E section showing Acantholysis of cells and tomb stone appearance of basal cells (100X)

The confirmatory diagnosis was made by scalpel biopsy from lower lip which showed bit of tissue covered by hyperplastic parakeratinized stratified squamous epithelium showing supra-basilar split just above the basal layer at one or two areas. In this areas a few round to ovoid cells were seen with scantly deeply eosinophilic cytoplasm and large basophilic nucleus (acantholytic cells). At one area the epithelium was completely stripped away leaving behind just the basal layer (tomb stone appearance) (Figure 4C-D). The underlying connective tissue was fibro-vascular which was mildly infiltrated by chronic inflammatory cells and contained endothelium lined vascular spaces filled with RBCs. Areas of haemorrhage were also seen. The patient responded well to topical 0.1% triamcinolone acetonide and lignocaine.

DISCUSSION

The term acantholysis refers to the loss of coherence between epidermal cells due to the breakdown of intercellular bridges [4]. In simple terms, the intercellular attachment mechanism including desmosomal cadherins (responsible for taut appearance of the cell) are disintegrated resulting in loss of prickly appearance. The cells tend to become round with resultant false appearance of large nuclei. This can actually be misinterpreted as dysplasia in the absence of clinical details or by the learners. This can be better demonstrated as shown in the figure using a balloon (Figure 5).

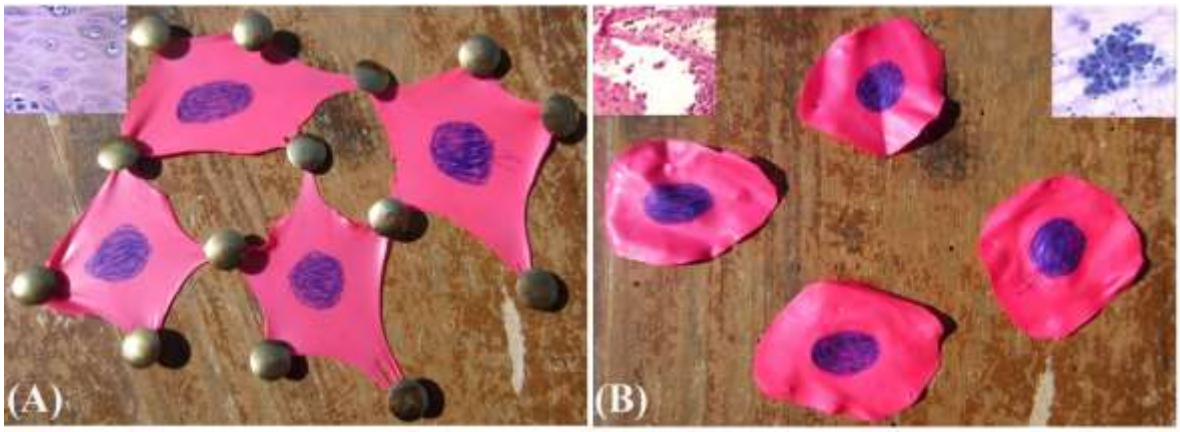

Fig-5: Demonstration of acantholysis: A) Cell junctions as represented by paper pins are responsible for the prickly appearance of cells (inset shows analogous H&E section showing prickled appearance of cells); B) When the antibodies are directed against theses junctions, the cells lose their prickly appearance to acquire a more rounded shape known as acantholytic cells (inset shows acantholytic cells in H&E section on left and PAP smear on right corner)

In case 2 of our report, vesiculo-bullous lesions provisionally included in the diagnosis were pemphigus vulgaris, herpes and pemphigoid group. PV cytologically is characterized by clusters or singly present rounded keratinocytes, nuclei appear hypertrophic and cytoplasm is eosinophilic to basophilic. Cytoplasm tends to condense at the periphery near the cell membrane which appears more basophilic (mourning edge) and results in peri-nuclear halo [5]. The background appears clean and lack significant surrounding inflammatory cells. Cytologically, one can misinterpret as herpetic lesions which show tremendously enlarged, multinucleated keratinocytes ('ballooning' or 'pregnant' cells) with plenty of neutrophils. The cytoplasm is hyper-basophilic. The nuclei are large lacking chromatin network but may contain inclusion bodies due to the presence of reproducing viral units [6]. The acantholysis in herpetic lesions is termed as secondary acantholysis implying that keratinocytes are first injured followed by dissociation of desmosomes. This is in contrast to primary acantholysis of pemphigus. Cells in Hailey-Hailey disease (uncommon oral lesions) show similar features as in PV. By using immunocyto-fluorescence microscopy and treating the smears with fluoresceine labelled anti-IgG serum, pemphigus Tzanck cells show a typical greenish fluorescence on the cell membrane whereas Hailey–Hailey acantholytic cells do not fluoresce at all. Other differential diagnosis include bullous pemphigoid which shows absence of acantholytic cells, scarcity of keratinocytes, abundance of leucocytes (eosinophils in prevalence) and often showing cell adherence (streptocytes).

One should not pose any difficulty in cytological differential diagnosis of PV and oral squamous cell carcinoma (OSCC). Cells in OSCC contain coarse chromatin (fine chromatin in PV), anisonucleosis, and disorganized arrangement with nuclear contours are irregular (smooth nuclear contour in PV). The cytoplasm of pemphigus cells tends to be denser than squamous carcinoma cells [7]. In case 1, cytological picture was erroneously diagnosed as PV. But careful reevaluation could lead to correct identification of the cells. The cells were actually parabasal cells with open phase nuclei, prominent single nucleoli and crisp cytoplasm with greenish tinge. Correct diagnosis could be made by scalpel biopsy and DIF.

Clinical test are also helpful in the diagnosis of these vesiculo-bullous diseases. Nikolsky's sign is considered as positive if there is extension of the blister and/or removal of epidermis in the area of application of tangential pressure. Nikolsky's sign is usually positive in diseases with epidermal acantholysis and typically negative in diseases with dermo-epidermal separation [8]. Thus, this test cannot be elucidated in BP. Further, Nikolsky's sign is considered to be 'wet' as in PV (moist glistening base) and dry as in pemphigus foliacous (higher level of blistering; re-epithelialization). Sheklakov's sign, also termed as false Nikolsky's sign, is due to necrosis of epidermal cells rather than acantholysis and is seen to be positive in Stevens-Johnson syndrome and toxic epidermal necrolysis. Asboe-Hansen sign is elicited by application of pressure on the roof of intact bulla which leads to its enlargement. In PV, the blister extension has a sharp angle, whereas in BP, the advanced border is rounded [9].

Thus, all rounded cells smaller than normal desquamated cells should not be labeled as acantholytic cells. Each slide should be carefully scanned for shape and size of cells, outline number and chromatin characteristics of nuclei, staining characteristics of cytoplasm, and background. These all can collectively lead to a correct diagnosis making cytology an important and interesting diagnostic tool.

CONCLUSION

Cytology plays an important role in diagnosis. Correct identification of the disease can prevent unnecessary invasive scalpel biopsy. Sometimes, basal and parabasal cells can appear in the smears of MMP which are not to be confused with acantholytic cells as PV respond well to conservative topical corticosteroid therapy in contrast to systemic therapy of MMP.

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