Received: 25 May 2017 | Revised: 18 July 2017 | Accepted: 20 July 2017

DOI: 10.1002/dc.23792

BRIEF REPORT

WILEY

Oral pemphigus vulgaris: Liquid-based cytological findings and pitfalls

Seiji Kondo, D.D.S., Ph.D.[1] | **Jiro Kawashima, D.D.S.[1]** | **Katsumi Kobata, C.T.[2]** | **Toshihiro Ohgawara, D.D.S., Ph.D.[3]** | **Shiho Tanaka, D.D.S.[1]** | **Kazuki Nabeshima, M.D., Ph.D.[2]** | **Toshihiro Kikuta, D.D.S., Ph.D.[1]**

[1]Department of Oral and Maxillofacial Surgery, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

[2]Department of Pathology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

[3]Department of Oral and Maxillofacial Surgery, Mitoyo General Hospital, Kagawa, Japan

Correspondence
Seiji Kondo, Department of Oral and Maxillofacial Surgery, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan.
Email: kondo@fukuoka-u.ac.jp

Pemphigus vulgaris (PV) is a chronic autoimmune bullous disease characterized by the formation of suprabasal cleavage and acantholysis. As this disease almost always affects the oral mucosa, conventional cytological smears of oral lesions can be used for the initial diagnosis of PV. We report two cases of PV that were initially diagnosed based on cytological smears of an oral sample. As atypical squamous cells were present even in the liquid-based cytological (LBC) smears of the oral lesion in these two cases, this ultimately led to the misinterpretation of squamous cell carcinoma. These findings demonstrate that cytological mimicry of oral PV can occur in malignant cases when there is an absence of appropriate clinical information.

KEYWORDS
cytological mimicry, liquid-based cytology, oral pemphigus vulgaris

1 | INTRODUCTION

Pemphigus vulgaris (PV) is a rare autoimmune disease that is characterized by acantholysis followed by blistering of the mucosa or skin.[1] Although the initial onset of PV occurs in the oral mucosa (70–90%), it is subsequently observed in other mucosal sites such as the esophagus, pharynx, larynx, and genital or cutaneous lesions during the later stages.[2,3] Thus, the initial step of PV is referred to as the "oral PV" condition.

When initially trying to diagnose PV, cytological evaluations using the oral lesion smears from patients have proven to be a useful and informative tool.[4] However, the final diagnosis of PV needs to be confirmed by a histological examination and immunofluorescence study.[1,5,6] The main characteristic associated with PV cytopathologic findings is the presence of acantholytic cells. The characteristics of these so called Tzanck cells are well known, as they exhibit strong perinuclear acidophilic staining.[5,6]

A widely used alternative to the conventional cytopreparatory methods is liquid-based cytology (LBC).[4] This monolayer preparation method is characterized by the random cell distribution. Thus, after preparing the liquid-based smears collected through the use of a cytobrush, there is a higher specimen resolution that leads to a better cytological morphology. For example, use of LBC during a gynecological diagnosis has helped reduce the number of unsatisfactory specimens, and significantly improved the sensitivity and specificity of these cervical cancer screening tests.[7] Even so, cases of vaginal PV examined by LBC smears of the uterine cervix have been initially misdiagnosed as squamous cell carcinoma (Sq.C.C.) due to the presence of atypical acantholytic cells.[7–10] The cytological features of PV associated with cervical involvement are similar to that seen for PV evaluated by liquid-based oral smears.[6,8] However, there have been few studies that have investigated the identification and interpretation of acantholytic cells observed in the liquid-based smears obtained through the use of scrapings from oral vesicles. This case report presents two cases of oral PV, describes the cytomorphological features associated with both conventional and liquid-based smears, and discusses the under-recognized pitfalls of PV.

2 | CASE DESCRIPTION

After a 77-year-old woman (case 1) and a 66-year-old woman (case 2) presented to a dental surgeon with similar symptoms, which included a

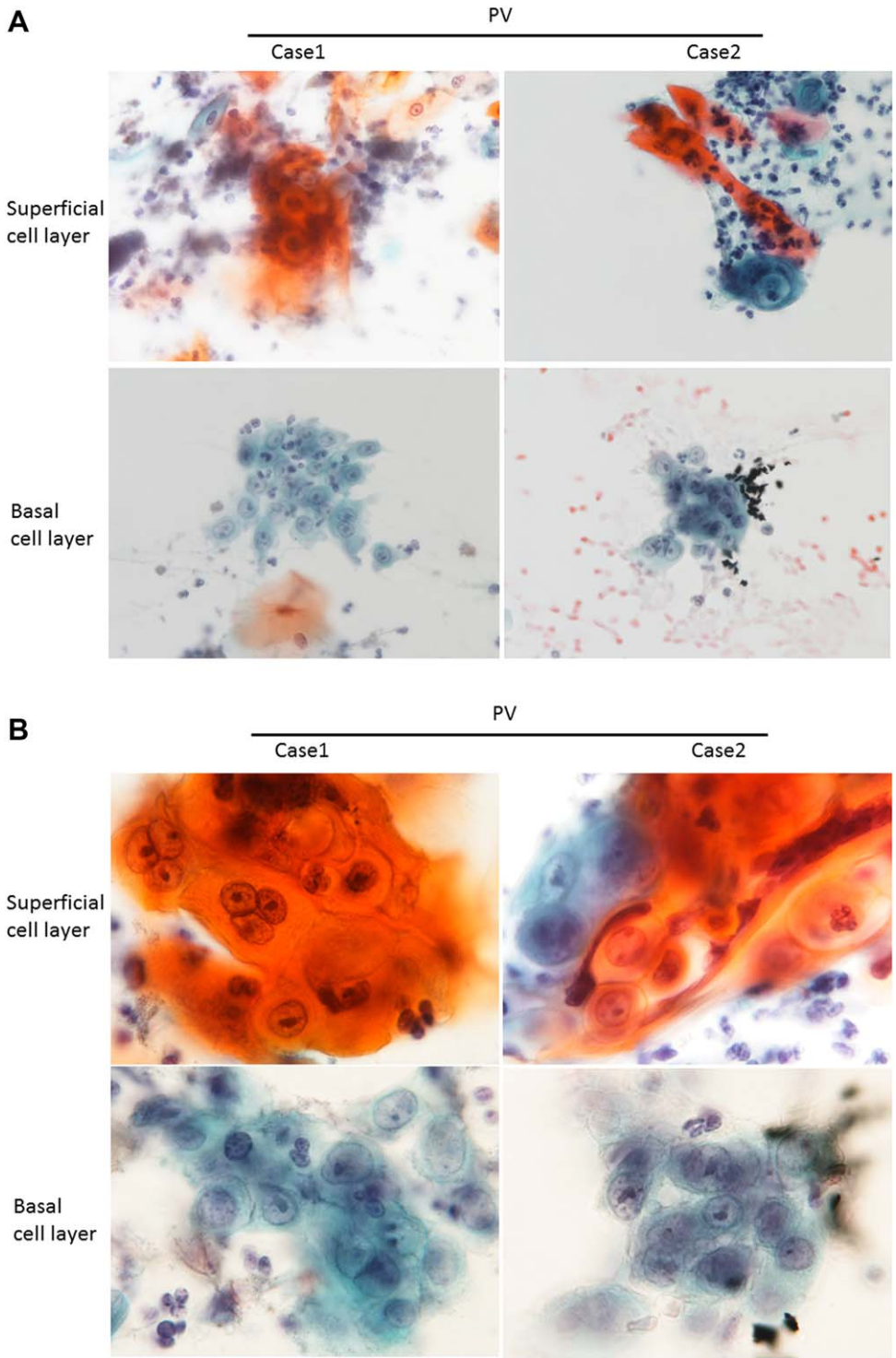

FIGURE 1 (A) Low magnification of the conventional oral smears (Papanicolaou stain, ×400) showed parabasal-sized abnormal cells in loosely cohesive sheets on both the superficial and basal cell layers from both patients (cases 1 and 2). (B) Higher magnification (Papanicolaou stain, ×1 000), especially for the basal cell layer from both patients, demonstrated that the samples were hypercellular with a high nuclear-cytoplasmic ratio and prominent nucleoli [Color figure can be viewed at wileyonlinelibrary.com]

prolonged history over several months of painful oral erosions in the soft palate, buccal mucosa, lower lip, and tongue, they were selected for routine cytological tests. Atypical squamous cells of an uncertain significance were found in both cases. As we clinically found that the formation of the erosion including the intraepithelial blisters occurred over all of the oral mucous membranes but not over any of the local regions, we suspected bullous disease. Because of this diagnostic dilemma, we performed blood examinations that included the autoantibodies, anti-desmoglein (Dsg)1 and anti-Dsg3 instead of carrying out a direct immunofluorescence study. We additionally performed biopsies

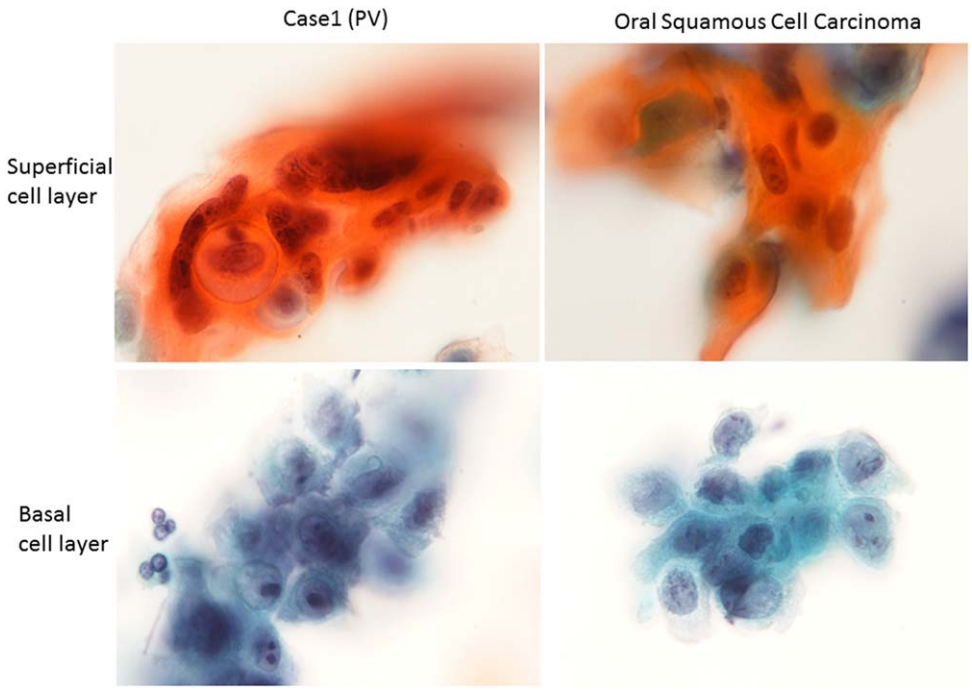

FIGURE 2 The LBC smears demonstrated adequate squamous cellularity and clusters of atypical parabasal type cells from the PV (case 1) and the oral Sq.C.C. patients, with the superficial and basal cell layers exhibiting a clear background (Papanicolaou stain, magnification ×1 000). [Color figure can be viewed at wileyonlinelibrary.com]

in order to ensure that there was a correct diagnosis, and to exclude the possible coexistence of malignant conditions. Based on these findings, both of these cases were finally diagnosed as PV.

3 | MATERIALS AND METHODS

Each of the complete transepithelial samples, including the basal layer were prepared from suspicious lesions on the buccal or soft palate by gently scraping with a cytobrush. For the conventional method, the sample was smeared onto a microscope slide, and fixed in ethanol for Papanicolaou staining. All stainings were performed manually, with the cytobrush directly inserted into a single vial containing a liquid-based fixation medium, CytoRich^{TM}Red (TriPath, Burlington, NC) followed by the LBC processing using the BD SurePath^{TM} system (TriPath). The liquid-based smears of the oral Sq.C.C. were obtained from a 57-year-old man suffering from left tongue Sq.C.C. and were prepared using the same method described above. No ethics approval was required for these cases. The patient's permissions to publish this case report were obtained.

4 | CYTOLOGIC FINDINGS

Conventional oral smears, especially from the basal cell layer of both patients were hypercellular with a high nuclear-cytoplasmic ratio that showed cellular alterations similar to those found in Sq.C.C. (Figure 1, magnification 400×, 1,000×). Because the chromatin pattern and nuclear outline can be more easily evaluated when using a liquid-based smear versus a conventional oral smear, all of the cytological assessments were performed by liquid-based smears. In case 1 (PV), the findings for the liquid-based smear (Figure 2) demonstrated that there were vesicular- and/or hypo-chromatic nuclei with single to multiple small nucleoli along with slightly irregular nuclear membranes on both the superficial and basal cell layers. When compared to the oral Sq.C.C. samples, similar cytomorphological features were found, with a dense cytoplasm and cytoplasmic edges characterized by a wispy appearance observed. Furthermore, cell polarity appeared to be relatively maintained in both case 1 (PV) and in the oral Sq.C.C. Tzanck cells, which are normally used for confirming the presence of PV, were not seen in either of the PV cases, even when

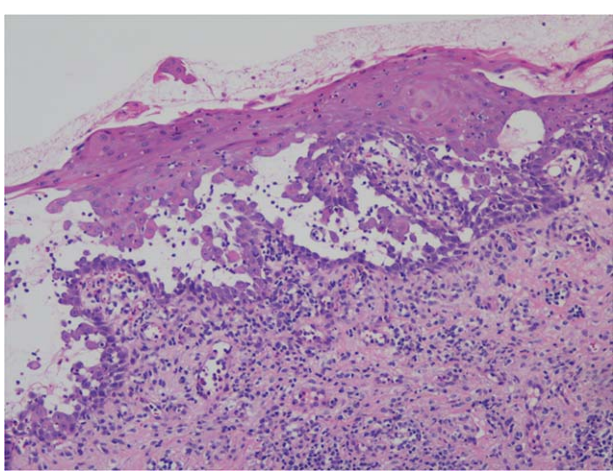

FIGURE 3 Histological section of the buccal mucosa of the oral PV (case 1) showed suprabasal acantholytic bulla with an acantholytic cells with mild atypia (hematoxylin and eosin stain, ×100). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Liquid-based cytology comparison of the cytomorphological features between pemphigus and oral squamous cell carcinoma present in two patient cases

	Pemphigus	Oral squamous cell carcinoma
Nuclear feature		
Chromatin pattern	Vesicular and/or hypo-chromatic nuclei	Vesicular and/or hypo-chromatic nuclei
Nucleoli	Multiple	Multiple
Nuclear contour	Slightly irregular	Slightly irregular
Cytoplasm		
Concentration	Dense	Dense
Margin	Wispy	Wispy
Cell arrangement		
Polarity	Relatively maintained	Relatively maintained

using liquid-based smears. Because the cellular specificity that distinguishes between the benign and malignant chromatin pattern was not observed, it was difficult to identify the disease.

5 | HISTOLOGICAL FINDINGS AND BLOOD EXAMINATIONS

The typical suprabasal bulla was formed immediately above the basal layer in case 1 (Figure 3). Acantholytic cells were scattered within the bulla, which is a characteristic feature of PV. Furthermore, both Dsg1 and Dsg3 were over-expressed in the serum of case 1 (i.e., 34 and 379 U mL^{-1}).

6 | DISCUSSION

Unlike the uterine cervical cytology, conventional oral cytology has proven to be of little value because of the high false rates, which can exceed 30% due to fibrotic tissue that prevents exfoliation of the dysplastic cells to the surface of the epithelium.[4,10] To overcome these issues, a new LBC method was developed in which the oral smears were collected through the use of a cytobrush. This method resulted in a significant improvement in the cell distribution and smear thickness, leading to a significantly lower percentage of unsatisfactory specimens.[4]

Other previous reports have also described the oral cytological findings for PV in detail.[2,8] Study findings included hyperchromatic nuclei with an increased nuclear-cytoplasmic ratio and scanty cytoplasm with dense and dark staining in the periphery. Unlike the unique cytomorphological features for PV described above, vesicular- and/or hypo-chromatin appeared to be seen in both of the oral PV cases. On the other hand, the chromatin seen in the oral Sq.C.C. from the liquid-based smear was often more transparent than that for the so-called "coarse clumping" that was seen in the smear from the cervical Sq.C.C. (Figure 2, right panel). This suggests that the cell specificity appears to be ambiguous when trying to distinguish the oral PV from an unexpected diagnosis of a malignancy, even with the liquid-based smears (Figure 2 and Table 1). Although Onuma et al.[8] have reported that a careful search for fine chromatin, regular nuclear contour, and preserved polarity are useful criteria that can be used to avoid overdiagnosing malignancies in the vaginal liquid-based pap test, differential diagnosis may be difficult when cells from PV made up of atypical squamous alterations overlap the criteria for malignancy, as was seen in our current cases.

The accurate diagnosis of PV depends on three independent sets of criteria: clinical features, histology, and immunological tests.[1] Through the use of these diagnostic processes, especially the clinical-based first step, it is feasible to perform a simple, rapid, inexpensive, and noninvasive diagnostic test on cytological smears from oral lesions. However, while these simple tests can be used to make a definitive diagnosis of PV, it should not be overlooked that atypical acantholytic cells can be seen in some of the oral smears of patients with oral PV. In conclusion, paying careful attention to the cytological features in oral PV should help prevent the misdiagnosis of suspect oral lesions.

ORCID

Seiji Kondo http://orcid.org/0000-0001-9341-5069

REFERENCES

[1] Bystryn JC, Rudolph JL. Pemphigus. *Lancet*. 2005;366:61–73.

[2] Madak H, Burlakow P, McGrew EA, Tiecke R. The cytology of vesicular conditions affecting the oral mucosa: *Pemphigus vulgaris*. *Acta Cytol*. 1970;14:11–21.

[3] Choudhary N, Choudhary V, Goswami GK, Pathak AN. Oral pemphigus vulgaris: a case which was misdiagnosed as stomatitis. *J Infect Dis Ther*. 2014;2:2.

[4] Mehrotra R. The role of cytology in oral lesions: a review of recent improvements. *Diagn Cytopathol*. 2011;40:73–83.

[5] Mokhtari M, Rasolmali R, Kumar PV. Pemphigus vulgaris of skin: cytologyical findings and pitfalls. *Acta Cytol*. 2012;56:310–314.

[6] Aytekin S, Göktay F, Yaşar Ş, Bostan S, Güneş P, Aker F. Atypical tzanck smear findings in *Pemphigus vulgaris*. *Cytopathology*. 2016;27:499–511.

[7] Akamatsu S, Kodama S, Himeji Y, Ikuta N, Shimagaki N. A comparison of liquid-based cytology with conventional cytology in cervical cancer screening. *Acta Cytol*. 2012;56:370–374.

[8] Onuma K, Kanbour-Shakir A, Modery J, Kanbour A. *Pemphigus vulgaris* of the vagina-its cytomorphologic features on liquid-based cytology and pitfalls:case report and cytologyical differential diagnosis. *Diagn Cytopathol*. 2009;37:832–835.

[9] Wright C, Pipingas A, Grayson W, Leiman G. *Pemphigus vulgaris* of the uterine cervix revisited: case report and review of the literature. *Diagn Cytopathol*. 2000;22:304–307.

[10] Carvalho Tavares L, Stefanello Bublitz G, Loos B, Carvalho Costa L, Fronza Júnior H. *Pemphigus vulgaris* of the cervix: diagnostic difficulties associated with the pap test. *Diagn Cytopathol*. 2015;43:635–637.

How to cite this article: Kondo S, Kawashima J, Kobata K, et al. Oral pemphigus vulgaris: Liquid-based cytological findings and pitfalls. *Diagnostic Cytopathology*. 2018;46:63-66. https://doi.org/10.1002/dc.23792