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BRIEF REPORT

Liquid-based cytology for differentiating two cases of pemphigus vulgaris from oral squamous cell carcinoma

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Abstract

Pemphigus vulgaris (PV) is a rare autoimmune disease characterized by blisters on the skin and mucous membrane. Since it often appears in the oral mucosa first, it may be diagnosed by oral mucosal cytology. Although the cytologic finding is characterized by acantholytic cells, that is, Tzanck cells, it is important to distinguish PV from neoplastic lesions of the oral mucosal epithelium, including differentiation from atypical parabasal/basal cells, which appear in squamous cell carcinoma (SCC). In this study, we examined the cellular findings in two cases of PV and a case of well-differentiated SCC with loss of epithelial cell cohesion. The samples were prepared using liquid-based cytology, which showed small round-shaped and deeply stained atypical, orangeophilic keratinocytes not only in SCC but also in PV, which made differentiation between the two difficult. However, Tzanck cells found in PV differ from the deep atypical parabasal/basal cells of SCC, suggesting that the cell outline is indistinct and small protrusions and brush-like structures are observed. This feature of Tzanck cells may be useful in cytological judgment.

KEYWORDS
liquid-based cytology, oral pemphigus vulgaris, oral squamous cell carcinoma

1 | INTRODUCTION

Pemphigus vulgaris (PV) is a type of autoimmune bullous disease of the skin and mucous membrane. It is often diagnosed by oral mucosal cytology as it first appears in the oral mucosa.[1]

Oral PV is characterized by the formation of the bullous lesion followed by thrush and cytological findings of acantholytic cells, also called Tzanck cells. Cell smear of erosive oral lesions is a sensitive and highly specific method for rapid diagnosis of PV.[2] Fine chromatin patterns and smooth nuclear films seem to be easier to assess in liquid-based PV smears.[3] However, it is sometimes difficult to differentiate Tzanck cells from the atypical parabasal/basal cells that appear in high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC), and it is important to differentiate Tzanck cells from neoplastic lesions of the oral mucosal epithelium for appropriate diagnosis.

In this study, we examined the cytologic features of two cases of oral PV using the liquid-based cytology (LBC) method. Furthermore, we compared their cellular features to those from a well-differentiated SCC case showing loss of epithelial cell cohesion, one of the structural variants of oral epithelial dysplasia (OED) in the WHO 2017 head and neck tumor classification.[4] We found cellular features that could serve as a diagnostic aid for differentiating pemphigus from neoplastic lesions in the oral mucosal epithelium.

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2 | CASE REPORT

2.1 | Case details and histological findings

In the first case, a 74-year-old man had erosive mucosal lesions on the right buccal mucosa and gingiva around the left mandibular molar region for 3.5 years. The cytological diagnosis was HSIL, and the patient was referred to Niigata University Hospital for confirmation of the diagnosis. A biopsy specimen was obtained from the gingiva of the left mandibular molar region. The gingival epithelium was almost entirely detached, leaving the basal cells on the side of the mucosal lamina propria, and an intraepithelial bulla extending from the prickle

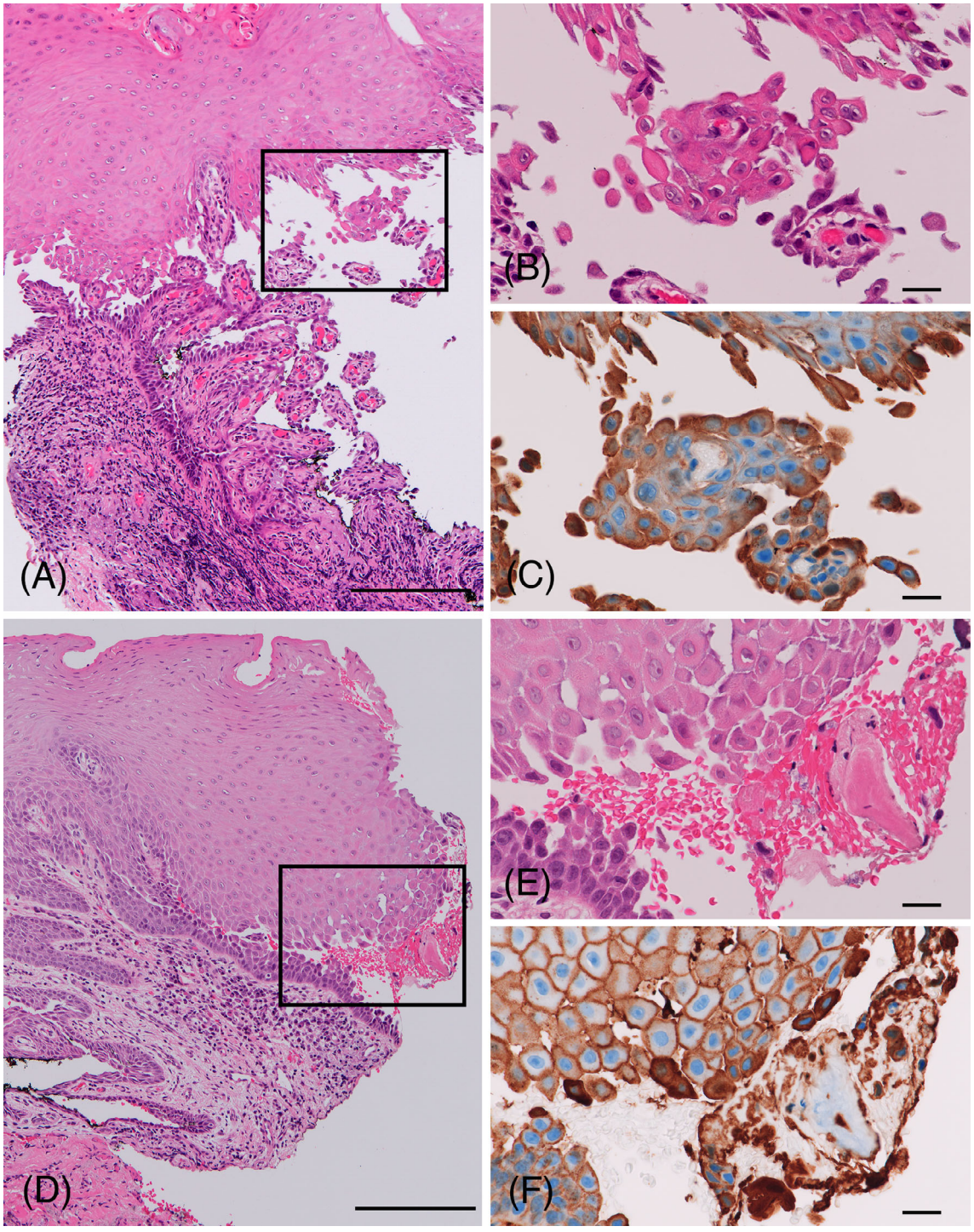

FIGURE 1 Histopathology and immunohistochemical profile of IgG in pemphigus vulgaris (PV). (A–C) Case 1, (D–F) Case 2, (A, D) On the gingival epithelium, intraepithelial bulla was formed from the prickle cell to the parabasal cell layer due to acantholysis, leaving the basal cells on the side of the mucosal lamina propria. (B, E) Tzanck cells, which are rounded, acantholytic epithelial cells were observed within the intraepithelial cleft. (C, F) IgG immunopositivity was found mainly at the interface and/or interepithelial area of the intraepithelial bulla and in a membrane pattern at the outline of the floating epithelial cells (B; black square of A, E; black square of D). Hematoxylin–eosin staining (A, B, D, and E), immunoperoxidase staining for IgG (C, F), Scale bars, 200 μm (A, D), 20 μm (B, C and E, F).

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cell layer to the parabasal cell layer due to acantholysis (Figure 1A). In the intraepithelial bulla, acantholytic cells, also called Tzanck cells, were observed (Figure 1B) and immunostaining for IgG deposition was observed mainly at the interface of the intraepithelial bulla and at the margins of the free epithelial cells (Figure 1C).

The second case is of a 53-year-old woman, who was diagnosed with pemphigus foliaceus by a dermatologist. She was referred to the Department of Dentistry and Oral and Maxillofacial Surgery due to the appearance of blisters and erosions on the oral mucosa for 1 year. A biopsy sample was collected from the mandibular buccal gingiva. Acantholysis was observed between the basal and prickle cell layers of the mucosal epithelium, and the epithelium was exfoliated and lost, leaving the basal cells on the side of the mucosal lamina propria (Figure 1D). In the intraepithelial bulla, acantholytic cells, also called Tzanck cells, were observed (Figure 1E) and immunostaining for IgG deposition were observed mainly between the interepithelial area of prickle cell and at the margins of the free epithelial cells (Figure 1F).

2.2 | Materials and methods

Cell samples were collected from each lesion by gently scraping the lesion with an Orcellex brush RT (Rovers Medical Devices B.V., Oss, Netherlands). The brush was inserted directly into a BD SurePath collection vial containing a methanol-based preservative liquid fixative solution (SurePath, BD Diagnostics, Franklin Lakes, NJ, USA), and LBCs were processed using the BD SurePath™ system (TriPath) and then Papanicolaou stained. The biopsies were fixed in 10% buffered formalin and processed for routine paraffin wax embedding. Sections of 4 μm in thickness were prepared and stained with hematoxylin–eosin (H&E) and immunohistochemical staining for IgG (Cell Marque™, Sigma-Aldrich, IgG Rabbit Polyclonal Antibody, Concentration-adjusted antibodies). Cytology specimens of oral SCC were obtained from a 75-year-old man, who had SCC of the right mandibular gingiva and were prepared in the same manner as above. Ethical approval was not required for these cases. In addition, patient consent from all the patients were obtained for the publication of this case report.

2.3 | Cytologic findings

In Case 1, the cellular findings of the sample collected for LBC from the left mandibular molar gingival region showed many cells possibly corresponding to small round-shaped and deeply stained atypical, orangeophilic keratinized cells or atypical parabasal/basal cells with mild inflammatory infiltration by neutrophils in the background (Figure 2A). A cytological diagnosis of HSIL was made as atypical keratinocytes are not considered to be present in PV. However, if we focus on the deep atypical parabasal/basal cells, unlike oral SCC, there is no obvious increase in nuclear chromatin density, and the cells

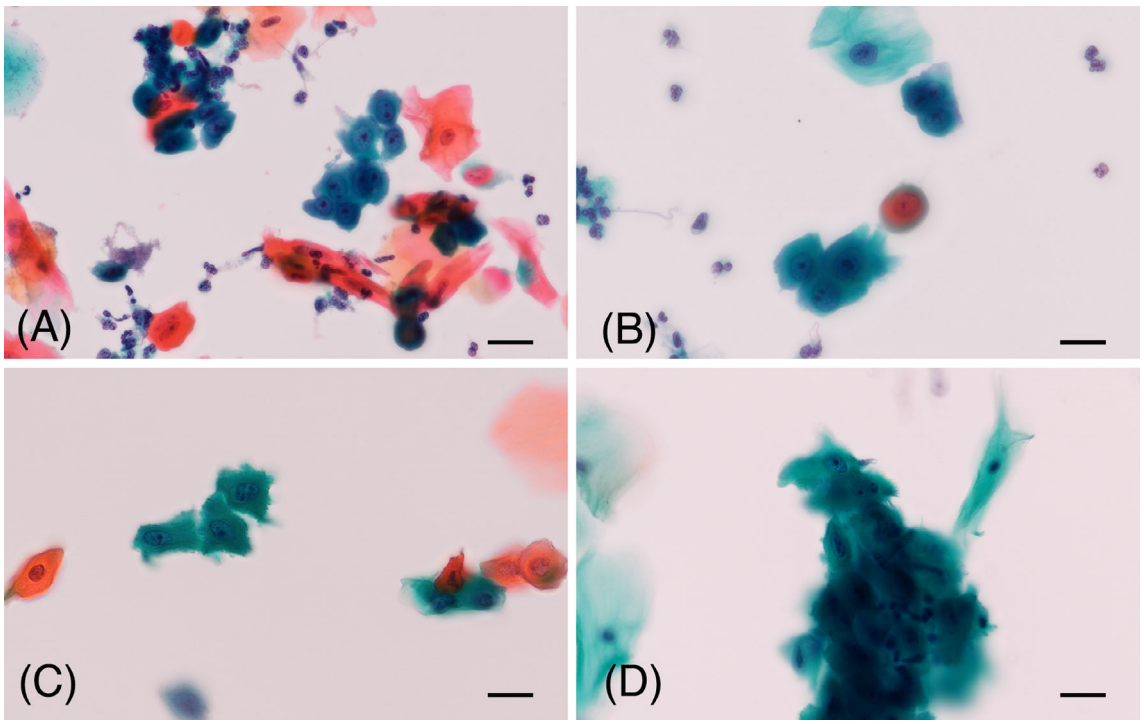

FIGURE 2 Cytology of PV. (A, B) Case 1, (C, D) Case 2. (A) Small, round-shaped, and deeply stained atypical, orangeophilic keratinized cells and atypical parabasal/basal cells are observed with mild inflammatory infiltration by neutrophils in the background. The outlines of atypical parabasal/basal cells are obscured, with spike-like elongation (A) and multiple small projections (B). Strong perinuclear acid affinity staining characteristic of Tzanck cells was also observed (B). (C, D) Small, round-shaped, and deeply stained atypical, orangeophilic keratinized cells and atypical parabasal/basal cells are seen on a clear background. At the margins of atypical parabasal/basal cells with large, rounded nucleoli, loss of cell adhesion (C) and brush-like cell morphology (D) are observed. Papanicolaou stain (A–E), Scale bars, 20 μm (A–D).

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show small protruding morphology with obscuration of the cell margins (Figure 2A). The cellular findings of LBC for a sample collected from the right buccal region also showed deep atypical parabasal/basal cells with obscured margins and multiple small protrusions (Figure 2B). In addition, strong perinuclear acid affinity staining characteristic of Tzanck cells was also observed (Figure 2B).

In case 2, atypical parabasal/basal cells were mainly observed in the cytological specimen of the left lower buccal gingival region collected by the LBC method. Next, cellular findings of LBC method for the sample collected from the lingual gingival region of the same area showed small, round-shaped, and deeply stained atypical, orangeophilic keratinized cells and atypical parabasal/basal cells (Figure 2C). The cells from the lingual gingiva do not show increased nuclear chromatin, but showed obscuration of cell outlines and loss of cell cohesion. Another cluster of atypical parabasal/basal cells with brush-like margins was also seen (Figure 2D). Large, rounded nucleoli were found in the acantholytic cells with basophilic cytoplasm, but the perinuclear halo was not clear (Figure 2C,D).

2.4 | Comparison of cytologic findings with a case of squamous cell carcinoma with acantholysis

We compared the findings of Tzanck cells with those of a case of well-differentiated SCC with loss of epithelial cell cohesion, one of the diagnostic criteria for OED in the WHO 2017 head and neck tumor classification (Figure 3A).[4] Numerous orange G-preferred keratinocyte surface cells and light green-preferred keratinocyte surface cells were seen in small to scattered clumps in a background of a mild inflammatory infiltrate composed mainly of neutrophils (Figure 3B). Owing to the presence of small, round-shaped, and deeply stained atypical, orangeophilic keratinized cells and atypical parabasal/basal cells, a diagnosis of SCC was made (Figure 3B,C). Unlike Tzanck cells, atypical parabasal/basal cells had distinct cell outlines (Figure 3D) and some cells showed nuclear shape irregularities (Figure 3E).

3 | DISCUSSION

In this study, we examined the cytology specimens generated by the LBC method in two cases of PV. Basically, LBCs appear to show the same cell morphology as conventional smears, but chromatin patterns and smooth nuclear membranes appear to be more easily appreciated in LBC smears. Another advantage of LBC smears is that they have a cleaner background than conventional smears. On the other hand, however, it has been reported that the LBC method does not confirm the strong perinuclear acid affinity staining characteristic of Tzanck cells.[3,5] We discovered that small, round-shaped, and deeply stained atypical, orangeophilic, keratinized cells can be found not only in SCC but also in pemphigus (Figure 2A,C and Table 1). Although in Hara's

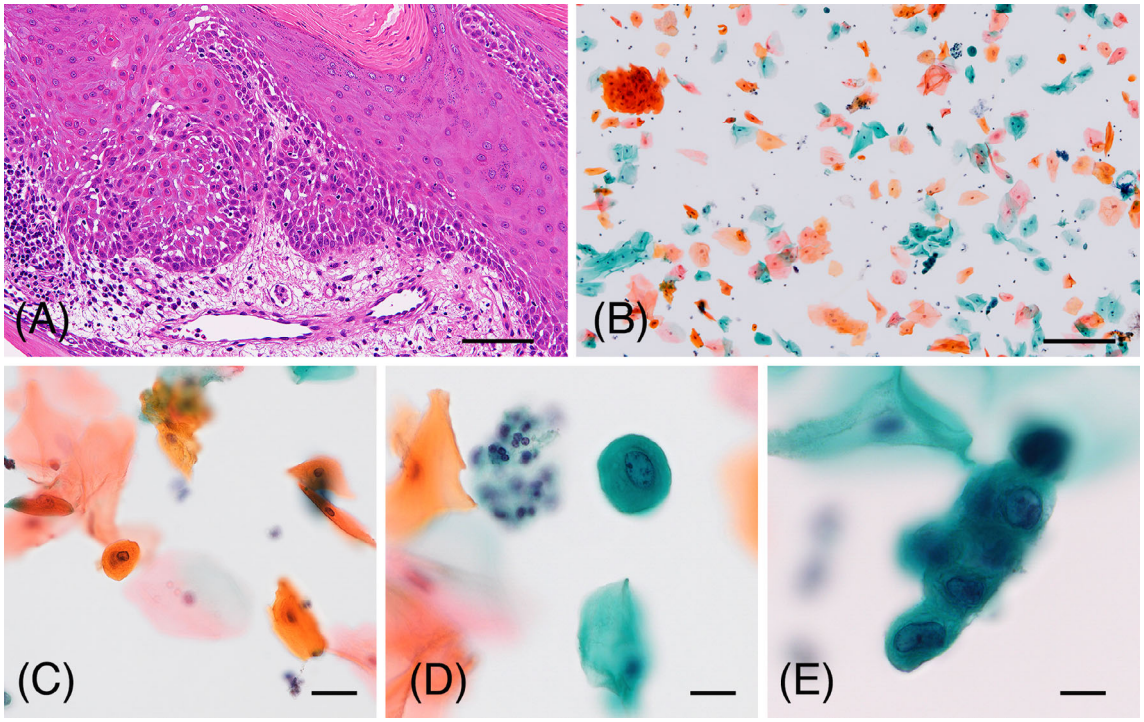

FIGURE 3 Histopathology and Cytology in a case of squamous cell carcinoma with loss of epithelial cell cohesion. (A) Loss of epithelial cell cohesion and expansion of intercellular bridges are seen in the prickle cell layer. (B) Numerous orange G-preferred keratinocyte surface cells and light green-preferred keratinocyte surface cells are seen in small clumps to sporadically. (C–E) Small, round-shaped, and deeply stained atypical, orangeophilic keratinized cells are seen (C), and atypical parabasal/basal cells have distinct cell margins (D), unlike Tzanck cells, and some cells have irregular nuclear shape (E). Hematoxylin–eosin staining (A), Papanicolaou staining (B–E), Scale bars, 100 μm (A), 200 μm (B), 20 μm (C–E).

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TABLE 1 Differences in cytologic picture of pemphigus vulgaris and squamous cell carcinoma.

Cell type	PV	SCC
Small, round-shaped, and deeply stained atypical, orangeophilic, keratinized cells	+/−	+
Atypical parabasal/basal cells		
Intercellular binding	Loose	Tight
Perinuclear halo	−	−
Perinuclear acid affinity	+	−
Nuclear shape irregularity	−	+
Outline	Small protrusions and brush-like	Clear
Large nucleolus	+	+

Abbreviations: PV, pemphigus vulgaris; SCC, squamous cell carcinoma.

paper, these cell characteristics are shown as one of the five practical items specific to oral squamous cell carcinoma, caution is required in the diagnosis of PV, since it has been reported that the diagnosis is difficult to make when small, round-shaped, and deeply stained atypical, orangeophilic keratinized cells are found in comparison of LBC cytology of cells from PV and SCC.[6,7] In fact, two cases of PV have been reported in which LBC smears of oral lesions showed atypical squamous cells, which were ultimately misdiagnosed as SCC.[5] Another report compared the characteristics of SCC cells with those of intravaginal PV, and noticed that it is difficult to distinguish PV from SCC of the vagina.[3]

Tzanck cells have a high nuclear-to-cytoplasmic (N/C) ratio and are seen as atypical parabasal/basal cells. Tzanck cells have a lacy, serrated or small protruding cytoplasmic limbus, whereas atypical parabasal/basal cells seen in SCC have a distinct limbus and some cells have an irregular nuclear shape (Figures 2, 3 and Table 1). The obscuration of the margins of atypical parabasal/basal cells, which was common in the two cases of PV, was seen due to the binding of IgG autoantibodies to the epidermal intercellular adhesion molecule, desmoglein, resulting in loss of its adhesive function. Regarding the cell morphology of Tzanck cells, it has been reported, similar to our study, that typical acantholytic cells and cells with amoeboid-like cytoplasmic projections were found; but at the same time, although not a detailed comparison by lesion appearance time, in older lesions of PV, the atypical features of parabasal-type cells and the presence of single cells due to discohesion were seen.[7]

A paper on Direct immunofluorescence (DIF) in the diagnosis of PV reported that the sensitivity and specificity of DIF analysis with intraoral Tzanck smears were 87.80% and 100%, respectively.[8] DIF studies on lichenoid lesions have shown that both PV and oral lichen planus (OLP) have high positivity rates.[9] Regarding the cellular findings, reports state that epithelial cells in the oral mucosa of OLP patients show a decreased N/C ratio compared to the mucosa.[10] It is suggested that the lack of deep cell collection could be a possible differentiating factor. Regarding the incidence of acantholytic cells, 80.5% (33/41) of the oral erosive lesions in PV cases were found to be acantholytic, while in the control group, Lichen planus, the incidence was 0% (0/12) in H&E-stained smears and 18.1% (2/11) in Giemsa-stained smears.[2] The sensitivity of acantholytic cells in Tzanck smear of erosive oral lesions of PV cases was 80.5% (for both Giemsa and H&E staining), whereas specificity values of Giemsa and H&E staining were 84.6% and 96.3%, respectively.[2] However, Papanicolaou stain is usually used for cytological evaluation in oral mucosal diseases because it is useful in differentiating squamous cell lesions due to its color tone that reflects the degree of squamous cell differentiation, and we used Papanicolaou stain again for this evaluation.

4 | CONCLUSION

Careful observation of the difference in the morphology of the atypical parabasal/basal cell outlines may be helpful in diagnosis. In addition to the characteristics of cellular morphology, oral mucosal lesions are directly visible, so confirming intraoral findings and clinical information, such as the presence or absence of blister formation, is helpful to the differential diagnosis of pemphigus and neoplastic lesions of the oral mucosal epithelium.

AUTHOR CONTRIBUTIONS

Satoshi Maruyama: manuscript preparation, diagnosis and data collection. **Manabu Yamazaki**: diagnosis and manuscript correction. **Tatsuya Abé**: data collection and manuscript review. **Yusuke Kato**, **Hiroyuki Kano**, **Yoshimasa Sumita** and **Kei Tomihara**: clinical workup of patient and treatment. **Jun-ichi Tanuma**: diagnosis and manuscript review.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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