Cytological features of a lymphoepithelial cyst of the salivary gland with application of the second edition of Milan System for Reporting Salivary Gland Cytopathology

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Abstract. Lymphoepithelial cysts (LECs) of the salivary glands are relatively rare, benign cystic lesions. Characteristic histopathological features of LEC include presence of well-circumscribed unilocular cysts surrounded by dense lymphoid tissue with lymphoid follicles. These cysts are lined by a combination of squamous, ciliated, columnar and mucous epithelia. Fine-needle aspiration (FNA) cytology is the standard preoperative diagnostic procedure for salivary gland lesions. Although the cytological diagnosis of cystic salivary gland lesions is difficult, the use of Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) in the cytodiagnosis of cystic salivary gland lesions has been reported. However, only a few studies have described the cytological features of LEC. To the best of our knowledge, the present study reviewed the cytological features of a case series of LEC and evaluated the application of MSRSGC for the first time. This retrospective study included 13 patients with LEC of the salivary glands who underwent pre-operative FNA followed by surgical resection of the cyst. All the lesions were present in the parotid gland. Cytological analysis revealed no epithelial cell component in eight patients (62.5%) along with a proteinaceous background containing lymphocytes and/or foamy cells. Non-keratinising squamous epithelium was observed in three patients. Amylase crystalloids were noted in two patients. None of the patients were cytodiagnosed with LEC. Eight, three, one and one patients were categorised as MSRSGC I, II, III, and IVa, respectively. The results of the present study demonstrated that cytodiagnosis of LEC was difficult due to the absence of epithelial component in 62.5% of the specimens. However, evaluation of its benignity was not difficult. Thus, it can be summarized that MSRSGC may be useful for cytological evaluation of LECs.

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Abbreviations: AUS, atypia of undetermined significance; FNA, fine-needle aspiration; HIV, human immunodeficiency virus; LEC, lymphoepithelial cyst; MSRSGC, Milan System for Reporting Salivary Gland Cytopathology; ROM, risk of malignancy; SUMP, salivary gland neoplasm of uncertain malignant potential

Key words: lymphoepithelial cyst, salivary gland, fine-needle aspiration cytology, Milan System, cytological reporting, cytodiagnosis

Introduction

Lymphoepithelial cysts (LECs) are relatively rare benign cystic lesions that occur in the salivary glands (1,2). This cyst is also referred to as branchial cleft cyst. LECs are presumed to originate from salivary gland inclusions within peri salivary lymph nodes; however, several theories have been proposed to explain the origin of these cysts (1,2). This cyst is found in increasing numbers in patients infected with human immunodeficiency virus (HIV) and is well known as a common cause of neck enlargement in patients with HIV (1). LECs usually arise in adults as a painless and gradually enlarging mass (1). The characteristic histopathological features of LECs include presence of well-circumscribed unilocular cysts containing serous fluid or mucoid contents surrounded by dense lymphoid tissues composed of small lymphocytes and lymphoid follicles with germinal centres (1,2). These cysts are usually lined by squamous epithelium. However, a variable combination of ciliated, columnar, and mucous epithelia has also been noted (1,2).

Fine-needle aspiration (FNA) cytological examination is a standard and useful pre-operative diagnostic method for salivary gland tumours (3,4). Various reporting systems for cytological diagnosis of salivary gland tumours have been proposed. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was created as a standardised and reproducible reporting system for classifying salivary FNA cytology specimens in 2018 (5). Since then, several studies have addressed the usefulness of this system (6-12), and it has been used worldwide in daily diagnostic practice of salivary gland cytological examination. The second edition of MSRSGC was published in 2023 (13). The philosophy of this system is risk-stratification based on the assumed risk of malignancy (ROM) and recommendation of therapeutic

management for each category (13). MSRSGC is classified into seven diagnostic categories according to the cytomorphological findings: I, non-diagnostic; II, non-neoplastic; III, atypia of undetermined significance (AUS); IVa, benign neoplasm; IVb, salivary gland neoplasm of uncertain malignant potential (SUMP); V, suspicious of malignancy; and VI, malignant (13).

Various types of neoplastic and non-neoplastic salivary gland lesions can present as cystic lesions (14). For example, benign non-neoplastic salivary gland lesions, including LEC, exhibit cystic features, and benign salivary gland tumours sometimes exhibit cystic changes. Warthin's tumour, the second most common salivary gland tumour, frequently exhibits cystic changes. Moreover, malignant salivary gland tumours, including low-grade mucoepidermoid carcinomas, can also demonstrate the presence of cystic components (14,15). Cytological diagnosis of cystic salivary gland lesions is considered challenging due to the fact that cytological specimens contain only watery or mucinous cystic contents with few or no cellular components (14,15). Although diagnostic algorithms for cytological examination of cystic lesions of the salivary gland have been proposed (15), only one article has addressed the application of MSRSGC in cytodiagnosis of cystic salivary gland lesions (14). Moreover, only a few articles have reported the cytological features of LECs of the salivary glands (16), and detailed cytological features of case series have not been reported yet. In the present manuscript, we reviewed the cytological features of a case series of LEC of the salivary gland and evaluated the application of the second edition of MSRSGC for the first time.

Materials and methods

Patient selection. Consecutive patients who were diagnosed with LEC of the salivary gland by postoperative pathological examination at Osaka Medical and Pharmaceutical University Hospital (Osaka, Japan) and also underwent preoperative FNA (from 1 January 2010 to 30 June 2023) formed our study population.

This retrospective, single-institution study was conducted in accordance with the tenets of Declaration of Helsinki and the study protocol was approved by the Institutional Review Board of Osaka Medical and Pharmaceutical University Hospital (Approval #2023-073). All data were anonymised. The Institutional Review Board waived the requirement for informed consent due to the retrospective study design, as medical records and archived samples were used with no risk to the participants. Moreover, the present study did not include minors. Information regarding this study, such as the inclusion criteria and opportunity to opt out, was provided through the institutional website (https://www.ompu.ac.jp/u-deps/path/img/file19.pdf).

Cytological analysis. FNA specimens were stained using Papanicolaou and/or Giemsa stains. Cytological characteristics of pre-operative FNA specimens of salivary gland lesions, such as background features (presence of proteinaceous contents and crystalloids) along with presence and/or types of inflammatory and epithelial cells, were evaluated. Moreover, the second edition of MSRSGC was used to classify these FNA specimens into the following seven categories: I, non-diagnostic; II, non-neoplastic; III, AUS; IVa, benign neoplasm; IVb, SUMP; V, suspicious of malignancy; and VI, malignant (summarised definitions and diagnostic criteria for cystic lesions are shown in Table I) (13,14). At least two researchers independently re-evaluated the cytological features of all the specimens after the routine cytological diagnosis.

Histopathological analysis. Surgically resected salivary gland specimens were fixed in 10% buffered formalin, dehydrated, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. At least two researchers independently evaluated the histopathological features of all the specimens. The diagnostic criteria for LEC were: presence of well-circumscribed unilocular cysts surrounded by dense lymphoid tissues composed of small lymphocytes and lymphoid follicles with germinal centres (1,2). Histopathological diagnostic criteria of LEC are as follows: Cysts are usually lined by squamous epithelium, and a variable combination of ciliated, columnar, and mucous epithelia has also been noted (1,2). Histopathological features, such as type of epithelium and cystic fluid content, were re-evaluated and compared with the cytological features of the FNA specimens after the routine histopathological diagnosis.

No immunohistochemical analysis was performed in the present study.

Results

Patient characteristics. Table II summarises the clinicopathological features of the study cohort. The cohort included 13 patients with LEC of the salivary gland. The median age of the patients was 50 years (range: 25-76 years). The study population comprised seven males and six females. The lesions were located in the parotid gland in all the patients (six and seven patients on the right and left sides, respectively). All the patients tested negative for HIV infection. Patient 1 also had a pleomorphic adenoma of the parotid gland on the same side; however, no continuity was observed between the two lesions.

Cytological features. Table II summarizes the cytological features of the 13 patients with LEC of the salivary gland. Proteinaceous background was noted in all the patients (Fig. 1A). Small-sized lymphocytes were observed in all patients (Fig. 1A). Neutrophils were observed in five patients. Foamy cells were observed in eight patients (Fig. 1A) and giant cells in six patients (Fig. 1B). Amylase crystalloids, characterised by needle-shaped or rectangular crystalline structures stained orange with Papanicolaou staining, were observed in two patients (Fig. 1C).

Epithelial cells were absent in eight patients (62.5%). Non-keratinising squamous cells without nuclear atypia were observed in three patients (Fig. 1D). Only few epithelial cells with relatively rich cytoplasm and round-to-oval nuclei were observed in Patient 4 (Fig. 1E). Epithelial cell clusters with slightly enlarged nuclei and relatively rich cytoplasm were present in neutrophilic and lymphocytic background along with amylase crystalloids in Patient 2 (Fig. 1F).

Categorization of LECs according to the second edition of MSRSGC. Eight, three, one, and one patients were categorised as I, II, III, and IVa, respectively, according to the second

Table I. Diagnostic categories of the second edition of Milan System for Reporting Salivary Gland Cytopathology and its definition and criteria (13,14).

Category	Definition	Diagnostic criteria for cystic lesions
I. Non-diagnostic	Insufficient cellular material for a cytological diagnosis	Non-mucinous cystic fluid only
II. Non-neoplastic	Benign entities, such as chronic sialadenitis and infection	Benign appearing acinar or ductal epithelial components, abundant inflammatory cells, and/or inflammatory cells with amylase crystalloids
III. Atypia of undetermined significance	Limited atypia and indefinite for neoplasm	Abundant mucin with or without rare epithelial cells, or rare atypical cells
IVA. Benign neoplasm	Benign neoplasms diagnosed based on established cytological criteria	Warthin tumor or cystic pleomorphic adenoma
IVB. Salivary gland neoplasm of uncertain malignant Potential	Diagnostic of neoplasm, however, a diagnosis of a specific entity cannot be made	Epithelial cells such as oncocytic neoplasms with a cystic background
V. Suspicious of malignancy	Showing features that are highly suggestive of, but not unequivocal for malignancy	Atypical cells in a mucinous background; suspicious for low-grade mucoepidermoid carcinoma
VI. Malignant	Diagnostic of malignancy	Malignant cells in a cystic background

edition of MSRSGC (Table II). All eight patients without epithelial cells were categorised as MSRSGC I, and three with non-keratinising squamous cells without atypia were categorised as MSRSGC II. Presence of few epithelial cells without nuclear atypia was categorised as MSRSGC III (Patient 4). Epithelial cell clusters with slightly enlarged nuclei and relatively rich cytoplasm was categorised as MSRSGC IVa (Patient 2) because Warthin tumour was suspected. None of the patient was cytodiagnosed with LEC.

Histopathological features. The lesions were characterized by well-circumscribed unilocular cysts surrounded by fibrous tissue around the salivary gland in all patients (Fig. 2A). Dense lymphoid aggregates with lymphoid follicles accompanied by reactive germinal centres were observed in the cyst wall (Fig. 2A). The types of epithelia covering the cyst wall were non-keratinising squamous in 12 patients, keratinising squamous in one, ciliated in three, and mucous in one (Table II) (Fig. 2B-E). Epithelial cells showed no nuclear atypia and mitotic figures were not observed (Fig. 2B-E).

Correlation between cytological and histopathological features. Patients in whom cytological specimens demonstrated non-keratinising squamous cells (Patients 3, 10, and 12) showed non-keratinising squamous epithelium in the histopathological specimens (Table II). Ciliated epithelial cells were observed in the histopathological specimens of three patients (Patients 7, 11, and 13), and mucus epithelium was noted in one patient (Patient 7). However, these epithelial components were not present in the cytological specimens of these patients.

Discussion

In the present study, we reviewed the cytological features of a case series of LEC of the salivary gland and analysed the application of the second edition of MSRSGC to the features elicited for the first time. Cytodiagnosis of LEC was difficult due to the fact that eight of 13 patients (62.5%) in the present cohort did not have epithelial component and were categorised as non-diagnostic (MSRSGC I). Epithelial components were present in five patients; and three, one, and one patient were categorised as MSRSGC II, III, and IVa, respectively.

Few previous cytological studies dealt with LEC of the salivary gland as a differential diagnostic consideration or one of the lists of diagnostic categories of cystic salivary gland lesions or lesions containing cystic fluid (14,15). However, detailed reports on cytological features of LEC are limited (16). A recent case report of LEC described a cytological specimen containing scattered lymphocytes with few neutrophils and macrophages in a proteinaceous background. However, the report provided no information regarding availability of the epithelial component (16). The present study demonstrated that the cytological features of LECs are not specific due to the fact that cytological specimens contain proteinaceous fluid, lymphocytes, and/or foamy cells, with occasional giant cells, and less frequently, epithelial cell components, including non-keratinising squamous cells. Moreover, LECs of the salivary gland are usually associated with HIV infection (1); however, in the present cohort, none of the 13 patients had HIV infection.

Several studies have addressed the usefulness of the first edition of MSRSGC in cytodiagnosis of salivary gland lesions (6-12). Although it is well recognised that cytodiagnosis of cystic salivary gland lesions is challenging because of the absence or small number of epithelial cells, the utility of MSRSGC in cystic salivary gland lesions has also been reported (14). Maleki *et al* analysed the MSRSGC categorization (1st edition) of 178 cases of cystic salivary gland lesions and proposed the cytological diagnostic criteria for cystic

Table II. Clinicopathological and cytological features of lymphoepithelial cyst of the salivary gland.

				Cytological features								Histopathological features				
						Inflammatory cells							Type of epithelium			
Patient no.	Age	Sex	Location	Background	Crystalloids	Lymphocytes	Neutrophils	Foamy cells	Giant cells	Type of epithelium	MSRSGC category	Cystic content	Non-keratinizing squamous epithelium	Keratinizing squamous epithelium	Ciliated epithelium	Mucus epithelium
1	74	F	Parotid	Proteinous	-	+	+	+	+	None	I	Proteinous	+	-	-	-
2		M	Parotid	Proteinous	+	+	+	-	-	Epithelial cell clusters	IVa	Proteinous	+	-	-	-
3	49	M	Parotid	Proteinous	-	+	-	+	+	Non-keratinizing squamous cells	II	Proteinous	+	-	-	-
4	37	M	Parotid	Proteinous	-	+	-	-	-	Few epithelial cells	III	Proteinous	+	-	-	-
5	75	F	Parotid	Proteinous	-	+	-	+	+	None	I	Proteinous	+	-	-	-
6	76	F	Parotid	Proteinous	-	+	-	+	+	None	I	Proteinous	+	-	-	-
7	46	M	Parotid	Proteinous	-	+	+	-	-	None	I	Proteinous	+	-	+	+
8	74	F	Parotid	Proteinous	-	+	-	+	+	None	I	Proteinous	+	-	-	-
9	66	F	Parotid	Proteinous	-	+	-	+	-	None	I	Proteinous	+	-	-	-
10	25	M	Parotid	Proteinous	-	+	-	-	+	Non-keratinizing squamous cells	II	Proteinous	+	+	-	-
11	49	M	Parotid	Proteinous	-	+	-	+	-	None	I	Proteinous	-	-	+	-
12	50	F	Parotid	Proteinous	+	+	+	-	-	Non-keratinizing squamous cells	II	Proteinous	+	-	-	-
13	48	M	Parotid	Proteinous	-	+	+	+	-	None	I	Proteinous	+	-	+	-

F, Female; M, Male; MSRSGC, the Milan System for Reporting Salivary Gland Cytopathology.

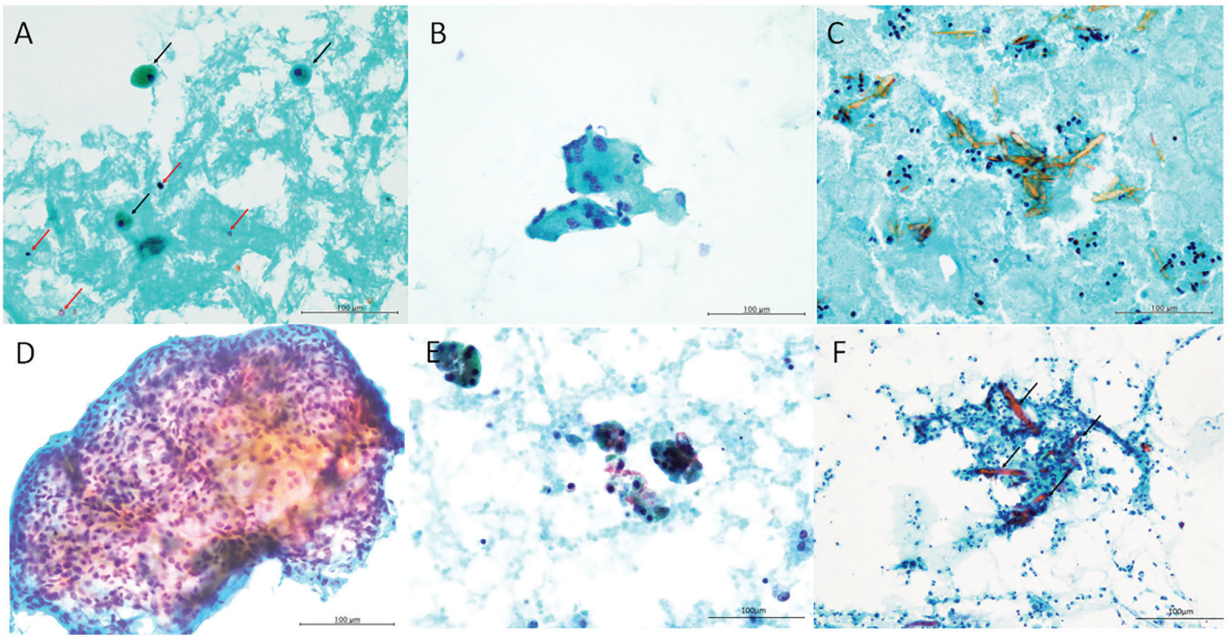

Figure 1. Cytological features of lymphoepithelial cyst of the salivary gland. (A) Proteinaceous material is observed in the background. Few small-sized lymphocytes (red arrows) and foamy cells (black arrows) are also present (Papanicolaou staining; magnification, x400). (B) Foreign body type giant cells (Papanicolaou staining; magnification, x400). (C) Amylase crystalloids (Papanicolaou staining; magnification, x400). (D) A cluster of non-keratinizing squamous epithelial cells without atypia (Papanicolaou staining; magnification, x400). (E) Few epithelial cell clusters with small nuclei and relatively rich cytoplasm are present in a proteinaceous background (Patient 4) (Papanicolaou staining; magnification, x400). (F) Epithelial cell cluster with slightly enlarged nuclei and relatively rich cytoplasm in a neutrophilic background. Amylase crystalloids are also present (black arrows) (Patient 2) (Papanicolaou staining; magnification, x400).

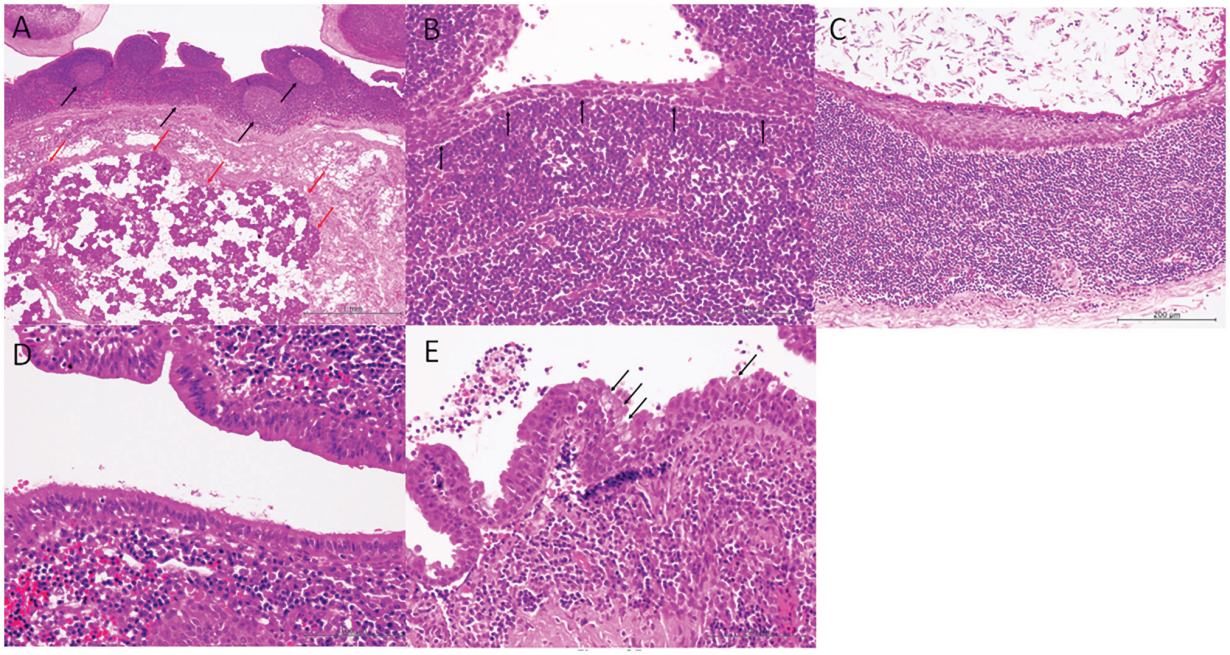

Figure 2. Histopathological features of lymphoepithelial cyst of the salivary gland. (A) The cyst is surrounded by dense lymphoid aggregates with lymphoid follicles accompanying reactive germinal centres (black arrows). Non-neoplastic salivary gland tissues are present around the cyst (red arrows) (haematoxylin and eosin; magnification, x40). (B) The cyst is lined by non-keratinizing squamous epithelium (black arrows) surrounded by dense lymphoid aggregates (haematoxylin and eosin; magnification, x400). (C) The cyst is lined by keratinizing squamous epithelium (Patient 10) (haematoxylin and eosin; ; magnification, x400). (D) The cyst is lined by ciliated epithelium (haematoxylin and eosin; magnification, x400). (E) The cyst is lined by mucinous epithelium containing mucous cells (arrows) (haematoxylin and eosin; magnification, x400).

salivary gland lesions (14). They showed that 29.2, 44.9, 19.7, 1.7, 1.7, 2.2, and 0.6% of the cases were categorised as MSRSGC I, II, III, IVa, IVb, V, and VI, respectively (14). Of these, 51 patients (28.7%) underwent surgical excision and were histopathologically diagnosed. MSRSGC II category was the most common in their series (44.9%), and all four

LEC in their series were included in this category. None of the LEC was categorized as MSRSGC I (14). LEC in their series contained prominent lymphocytes in the cytological specimens; however, no information regarding the types of epithelial cells was available (14). In the present cohort, eight of 13 patients were categorised as MSRSGC I due to the presence of proteinaceous fluid and absence of epithelial cells in the cytological specimens. Moreover, three patients of the present cohort were categorised as MSRSGC II due to the presence of non-keratinising squamous cells without atypia. One patient each was categorised as MSRSGC III and IVa, respectively, due to the presence of few epithelial cells (Patient 4) and suspicion of a Warthin tumour (Patient 2). Therefore, it can be inferred that absence of epithelial component in 62.5% of the specimens made cytodiagnosis an arduous task. However, it was not difficult to evaluate their benignity.

Presence of mucinous material in cytological diagnosis of cystic salivary gland lesion is very important as it indicates the possibility of mucoepidermoid carcinoma (5,14,17,18). Therefore, presence of mucinous material without epithelial cells is categorised as MSRSGC III (AUS) (5,14). Allison *et al* analysed the cytological features of cystic salivary gland lesions and classified them as mucinous, non-mucinous and lymphocytic, and non-mucinous and non-lymphocytic (18). LECs are classified into the non-mucinous and lymphocytic category, which mostly includes non-neoplastic entities. Moreover, Warthin tumour and mucoepidermoid carcinoma can be also included in this category (18).

An interesting finding in the present cohort was the presence of amylase crystalloids in two patients. Although the presence of crystalloids in salivary gland lesions is not a common finding (19), amylase-, tyrosine-rich-, and collagenous crystalloids have been reported (19-21). The most common type of crystalloid reported in salivary gland lesions is amylase crystalloid, as was seen in the present cohort (19,20). This type of crystalloid is contemplated to be composed of condensed alpha-amylase in supersaturated saliva and undergo crystallisation due to the fact that this structure is recognised as the presence of cystic lesion accompanying saliva stasis (20). Amylase crystalloids have been reported to be associated with non-neoplastic salivary gland lesions, including sialadenitis and obstructive cysts. Moreover, they are also observed in benign salivary gland neoplasms, such as pleomorphic adenoma and Warthin tumour (19,20,22). No association between amylase crystalloids and malignant neoplasms has been described (17,19). Sun *et al* analysed the incidence and types of crystalloids in cytological specimens of salivary glands (19). They reported that a total of 5.6% (37 of 663 patients) of the salivary gland cytological specimens contained crystalloids, and the most common type (75% of these cases) was amylase crystalloids, followed by the tyrosine-rich (11%) and collagenous (3%) types. The most common histology containing amylase crystalloids is oncocytic cystadenoma/oncocytic cyst, followed by sialadenitis or ductal obstructive changes (19). Only one patient with LEC in their series demonstrated the presence of amylase crystalloids (19). Only a few reports on presence of amylase crystalloids in LEC have been published (23,24). Most cases of amylase crystalloids present with sialadenitis and obstructive changes, and LECs may demonstrate this type of crystalloid in cytological specimens. Therefore, LEC must be included in differential diagnostic considerations for presence of amylase crystalloids in cytological specimens (24).

In conclusion, the present study demonstrated that 62.5% of cytological specimens of patients with LEC had no epithelial component. The remaining specimens had epithelial components, including non-keratinising squamous epithelium in a proteinaceous background with lymphocytes and/or foamy cells. Eight, three, one, and one patients were categorised as MSRSGC I, II, III, and IVa, respectively. Amylase crystalloids were present in two patients and therefore, LEC must be included in differential diagnostic considerations in the presence of this structure in cytological specimens of salivary glands. Although cytodiagnosis of LEC may be difficult, application of MSRSGC may be useful for the cytological evaluation of ROS.

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Availability of data and materials

All data generated and analysed in this study are included in this published article.

Authors' contributions

MI conceptualized and designed the study. MI, IK, MT, HO, KA, MU, CD, SO, RT, and YH performed the cytological analyses. MI and YH conducted the histopathological examinations. MI, IK, MT, HO, KA, MU, CD, SO, RT, and YH acquired and analysed the data. MI drafted the manuscript along with the tables and figures. MI and YH confirmed the authenticity of all raw data. All the authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate

This study was conducted in accordance with the tenets of Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of Osaka Medical and Pharmaceutical University (protocol no. 2023-073; Takatsuki, Japan). All data were anonymised. The Institutional Review Board waived the requirement for informed consent due to the retrospective design of the study with no risk of identity exposure for patients. This study did not include any minors.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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