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Evaluation of Liquid Based Cytology for Tongue Squamous Cell Carcinoma: Comparison with Conventional Cytology

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Abstract

Oral exfoliative cytology is now used by general practitioners in Japan to screen for oral cancer. With conventional cytology, however, the number of cells that can be sampled is small. Moreover, cell deformation and piling of cells when preparing specimens has been reported. The purpose of this study was to compare conventional and liquid based cytology (LBC), which has been employed with increasing frequency in recent years. We believe that identifying potential pitfalls in oral exfoliative cytology will help improve diagnostic accuracy. A total of 153 patients with tongue squamous cell carcinoma who were diagnosed and treated initially at our hospital between January 2000 and December 2010 were included. Of these, 124 underwent conventional cytology, while the remaining 29 underwent LBC. Histopathological and clinical findings were used as criteria. Conventional cytology yielded a positive rate of 54.8% and LBC 79.3%, while values of 28.2% and 13.8% were obtained for a suspected positive rate, respectively. Liquid based cytology yielded a significantly higher percentage of accurate diagnoses and fewer suspected positives ($p<0.05$) in cases clinically classified as endophytic and those classified as ulcerative in terms of clinical growth pattern. No significant difference was observed between conventional cytology and LBC in cases of an infiltrative growth pattern, however.

The present results suggest that LBC is superior to conventional cytology in achieving an accurate diagnosis based on oral exfoliative cytology. The present findings also suggest that exophytic type, and especially leukoderma type clinical growth patterns constitute pitfall cases in oral exfoliative cytology.

Key words: Oral exfoliative cytology — Tongue squamous cell carcinoma — Clinical growth style — Clinical growth pattern — Infiltrative growth pattern

Introduction

The oral cavity can be easily visualized and permits direct access for minimally invasive cell harvesting, thereby allowing the early diagnosis of malignant tumors and facilitating early intervention[11,14,22]. Several cases have been reported, however, where the diagnosis of precancerous lesions or early-stage lesions was difficult[11,14,22]. Recently, fluorescent optical light sources[8] and magnifying endoscopes (narrow band imaging)[18] have been utilized in screening for malignant tumors in the oral region. Oral scrape cytology remains the most commonly used method of screening for oral cancer, however[16]. Conventionally, exfoliative cytodiagnosis is used to screen for malignant tumors in multiple regions, including the genital area. It was first performed in the oral cavity in 1951 by Montgomery and Von Haam[9], after which it was soon adopted in Japan by Watanabe *et al*[21]. Specimens for exfoliative cytodiagnosis are prepared by smooth brushing of the mucosa using a curette to collect cells, followed by rapid spreading of the cells on a glass slide and immediate fixation in 95% ethanol to prevent deformation. The specimens are then treated with Papanicolaou stain for diagnostic classification[12]. Exfoliative cytodiagnosis using Papanicolaou staining and classification has advantages in that nuclear abnormalities can be detected clearly, allowing the specimens to be differentiated as benign or malignant. In addition, invaginated cell clusters can be easily detected.

In conventional exfoliative cytology, the cells are usually collected by means of a sharp curette, brush, and swab, or needle puncture and aspiration, followed by direct coating onto a slide for analysis. Some studies have reported cell adherence to the collection tool on smearing, and deformation or piling of cells when preparing specimens, however. Accordingly, since 2010, liquid-based cytology (LBC), originally used in the gynecological field, has grown in popularity in the study of oral lesions, and there have been many reports on its usefulness in diagnosis[2,3,15,17,19,20]. To our knowledge, however, no studies to date have compared clinical and other histopathological findings in patients diagnosed by using different techniques. The purpose of this retrospective study was to analyze the results of oral exfoliative cytology in patients at our department with squamous cell carcinoma of the tongue to investigate the association of those findings with clinical growth style, pattern, and tumor infiltrative growth pattern. We believe that the findings should prove useful in identifying potential pitfalls in oral exfoliative cytology, which should help improve diagnostic accuracy.

Materials and Methods

1. Patients

This study was carried out with the approval of the Institutional Ethics Review Board of Tokyo Dental College (Approval number: 710). All the patients' personal information was anonymized. Oral exfoliative cytology specimens were obtained from 1,073 patients attending this hospital between January 2000 and December 2010. Of these, 153 who underwent initial treatment for suspected malignant lesions and who had a definitive diagnosis of tongue squamous cell carcinoma based on perioperative resected specimens were included in the analysis (Table 1). Of these, 153 underwent conventional cytology, while the remaining 29 underwent LBC.

2. Methods

For conventional cytology, cell collection was carried out using a dental curette (Fig. 1-a), followed by smearing onto slides to prepare the specimens. For LBC, cell collection was carried out using an EndoCervex-Brush (Rovers Medical Devices B.V, Netherlands) (Fig. 1-b), after which the specimens were prepared using SurePath® (BD, USA) (Fig. 1-c). Papanicolaou staining of the collected cells was carried out by the conventional method, and diagnosis was performed by a cell tester and a physician specializing in cytological diagnosis who was recognized by the

Table 1 Clinicohistopathological characteristics of patients

The method of cytology	Conventional cytology	LBC	
Case No.	124	29	153
Gender			
Male	85	14	99
Female	39	15	54
Age			
10s	0	1	1
20s	4	0	4
30s	10	3	13
40s	12	3	15
50s	35	3	38
60s	39	11	50
70s	19	6	25
80s	4	2	6
90s	1	0	1
Papanicolaou classification			
class I	3	0	3
class II	18	2	20
class III	35	4	39
class IV	26	14	40
class V	42	9	51
Clinical growth style			
superficial type	20	6	26
exophytic type	42	6	48
endophytic type	62	17	79
Clinical growth pattern			
leukoderma type	37	8	45
papillary type	4	1	5
granulomatous type	12	2	14
erosion type	10	2	12
ulcerative type	53	15	68
tumor induration type	8	1	9
Tumor infiltrative growth pattern (INF)			
no infiltration	37	10	47
INFa	17	5	22
INFb	34	6	40
INFc	36	8	44

Japan Society of Clinical Cytology. At least one cyto-screener provided the diagnosis for each specimen, after which two or more oral pathologists provided the definitive diagnosis.

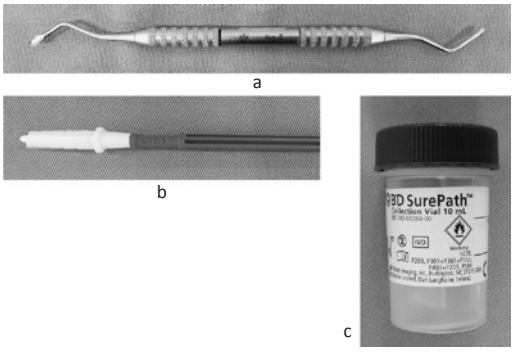

Fig. 1
a. Dental curette
b. EndoCervex-Brush (Rovers Medical Devices B.V, Netherlands)
c. SurePath® (BD, USA)

The Papanicolaou classification[12] was defined as class I or II in negative cases, class III in suspected positive cases, and class IV or V in positive cases. The sum of the class IV and V rates was defined as the"positive rate", while the class III rate was defined as the"suspected positive rate". Tumor growth style was categorized into three subtypes: superficial, exophytic, or endophytic, according to the general rules for clinical and pathological studies on oral cancer[4]. These were further subclassified into tumor growth patterns according to the Washizu classification system as leukoderma type, papillary type, or granulomatous type for exophytic growths; and erosive type, ulcerative type, or tumor induration type for endophytic growths[5]. To determine the mode of invasion, the infiltrative growth pattern (INF) was classified into 4 types. These comprised no infiltration; INFa, a solid and extensive tumor growth pattern distinct from the surrounding interstitium (expansion type); INFb, an invasive and proliferative state intermediate between INFa and INFc (intermediate type); and INFc, an unclear border between the tumor and the surrounding interstitium (invasive type)[4,5]. All diagnoses were made at the time of specimen collection.

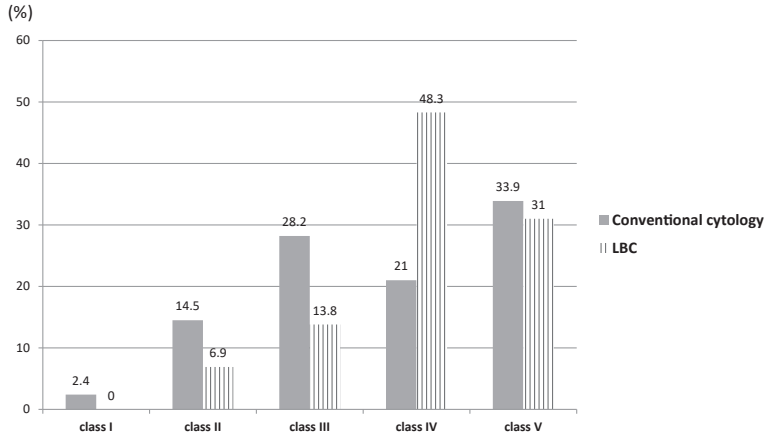

Fig. 2 Papanicolaou classification: percentage of patients

3. Statistical analyses

GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for the statistical analysis. The Chi-square test was used to compare variables. A p-value of <0.05 was considered statistically significant.

Results

1. Papanicolaou classifications

With conventional cytology, Papanicolaou class V (positive) was observed in 42 cases, class IV (positive) in 26 cases, class III (suspected positive) in 35 cases, class II (false negative) in 18 cases, and class I (false negative) in 3 cases. A positive rate was observed in 54.8% of cases, and a suspected positive rate in 28.2%. With LBC, Papanicolaou class V (positive) was observed in 9 cases, class IV (positive) in 14 cases, class III (questionable positive) in 4 cases, and class II (false negative) in 2 cases. In no case was the specimen unsuitable for testing. A positive rate was observed in 79.3% of cases, and suspected positive rate in 13.8% (Fig. 2).

2. Tumor infiltrative growth pattern

For the INF with conventional cytology, no infiltration was observed in 37 cases, INFa in 17 cases, INFb in 34 cases, and INFc in 36 cases. The positive rate was no infiltration, 48.6%; INFa, 35.3%; INFb, 55.9%; and INFc, 69.4%. The suspected positive rate was no infiltration, 29.7%; INFa, 47.0%; INFb, 32.4%; and INFc, 13.9%. For the INF with LBC, no infiltration was observed in 10 cases; INFa in 5 cases; INFb in 6 cases; and INFc in 8 cases. The positive rate was no infiltration, 60.0%; INFa, 80.0%; INFb, 83.3%; and INFc, 100.0%. The suspected positive rate was no infiltration, 30.0%; INFa, 20.0%; INFb, 0.0%; and INFc, 0.0%. No significant difference was observed between conventional cytology and LBC in terms of INF (Fig. 3).

3. Clinical growth style

The clinical growth style with conventional cytology was identified as superficial type in 20 cases, exophytic in 42 cases, and endophytic in 62 cases. The positive rate for each type was as follows: superficial type, 60.0%; exophytic type, 50.0%; and endophytic type, 56.5%. The suspected positive rate was as follows: superficial type, 25.0%; exophytic type, 19.0%; and endophytic type, 35.5%. The clinical growth style with LBC was identified as superficial type in 6 cases, exophytic type in 6 cases, and endophytic type in 17 cases. The

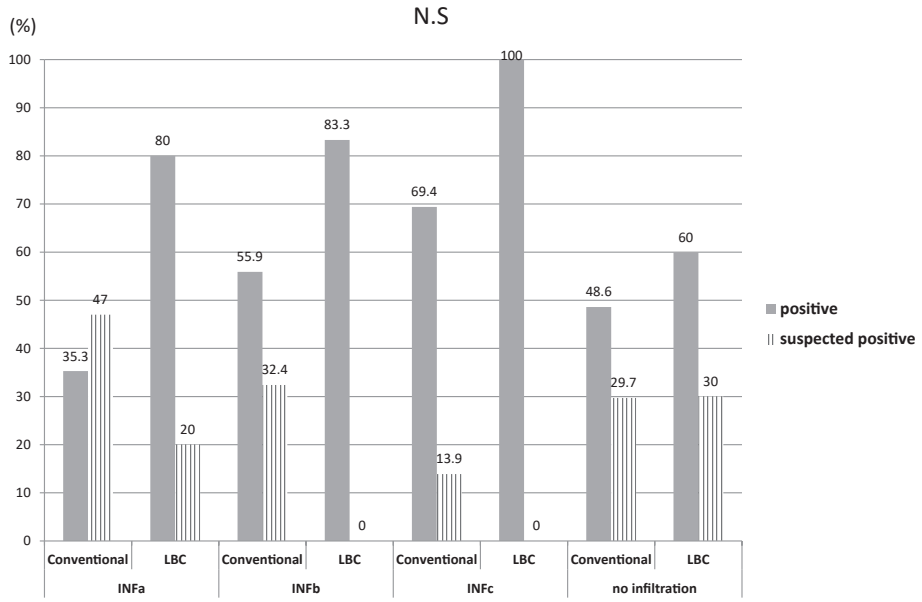

Fig. 3 Positive and suspected positive percentages with conventional cytology and LBC for INF
No significant was observed between conventional cytology and LBC (Chi-square test).
INF: Infiltrative growth pattern

positive rate for each type was as follows: superficial type, 50.0%; exophytic type, 66.7%; and endophytic type, 94.1%. The suspected positive rate was as follows: superficial type, 33.3%; exophytic type, 16.7%; and endophytic type, 5.9%. For clinical growth style, the positive rate was significantly higher with LBC than with conventional cytology, and the suspected positive rate was low ($p<0.05$), especially in endophytic-type cases (Fig. 4).

4. Clinical growth pattern

The clinical growth pattern with conventional cytology was identified as leukoderma type in 37 cases, papillary type in 4 cases, granulomatous type in 12 cases, erosive type in 10 cases, ulcerative type in 53 cases, and tumor induration type in 8 cases. The positive rate was as follows: leukoderma type, 37.8%; papillary type, 50.0%; granulomatous type, 50.0%; erosive type, 60.0%; ulcerative type, 60.4%; and tumor induration type, 55.6%. The suspected positive rate was as follows: leukoderma type, 27.0%; papillary type, 50.0%; granulomatous type, 8.3%; erosive type, 40.0%; ulcerative type, 30.2%; and tumor induration type, 33.3%. The clinical growth pattern with LBC was as follows: leukoderma type in 8 cases, papillary type in 1 case, granulomatous type in 2 cases, erosive type in 2 cases, and ulcerative type in 15 cases. The positive rate was as follows: leukoderma type, 37.5%; papillary type, 100.0%; granulomatous type, 100.0%; erosive type, 100.0%; and ulcerative type, 93.3%. The suspected positive rate was as follows: leukoderma type, 37.5%; and ulcerative type, 6.7%. The positive rate was significantly higher with LBC than with conventional cytology, and the suspected positive rate was low ($p<0.05$) (Fig. 5), especially in ulcerative type.

Discussion

Many studies have reported positive oral exfoliative cytology rates of 70.4 to 92.3%[22], rates similar to those seen here. Oral exfolia-

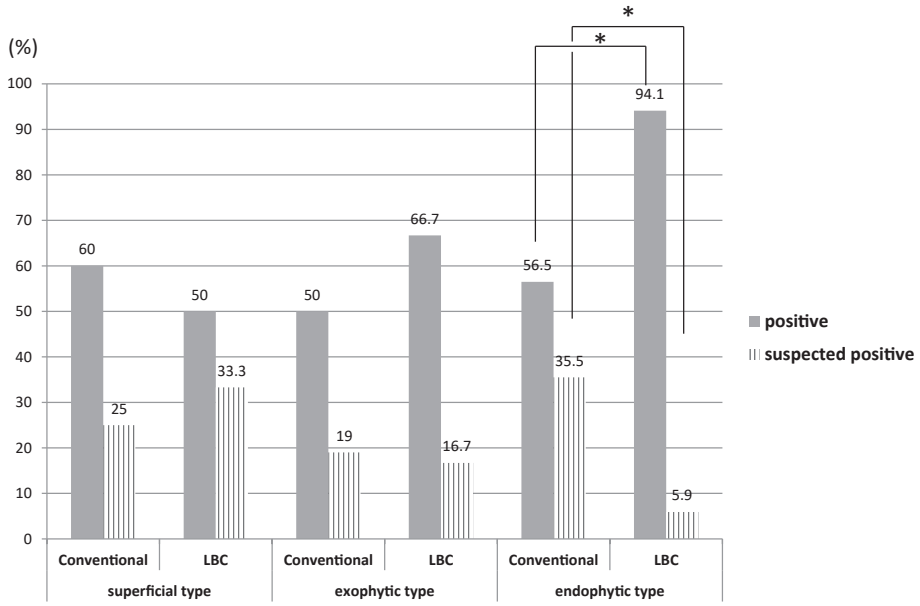

Fig. 4 Positive and suspected positive percentages for conventional cytology and LBC for clinical growth style

Positive rate was significantly higher with LBC than with conventional cytology; suspected positive rate was low, especially in endophytic cases (Chi-square test: $p<0.05$).

*$p<0.05$

tive cytology has recently moved away from conventional methods of collection in favor of LBC, which is commonly used in the field of gynecology[7,10]. Liquid based cytology offers distinct advantages over conventional cytology. For example, LBC results in a greater number of harvested cells; superior specimen quality due to less drying and smearing; improved diagnosis due to the absence of blood contaminants; and better observation of necrotic tissues, *Candida* species, and other background factors. In addition, when using the LBC method, it is easier to harvest deep-layer cells and achieve a cell monolayer to aid morphological, immunohistochemical, and molecular analyses[14,22]. Several studies have reported a superior rate of accurate diagnosis with LBC[1,6,7,10,13,14]. The inferiority of diagnosis based on conventional harvesting compared with LBC is partially explained by the above factors. Further accumulation of cases is needed, however, to determine the definitive reasons for the superiority of LBC.

There was a difference in the number of patients diagnosed based on each technique in the present study. The result showing that LBC yielded a higher positive rate and lower suspected positive rate than conventional cytology, however, suggests that LBC improves the precision of diagnosis in oral exfoliative cytology.

The superiority of LBC over conventional cytology was not seen with the INF, however. One reason for this may have been that mainly surface keratinocytes and atypical middle layer cells are collected in exfoliative cytology, and these are unsuitable for determining the mode of invasion at the tumor-host boundary.

The results for clinical growth style and clinical growth pattern revealed significant improvements in the positive and suspected positive rates with LBC compared with conventional cytology in endophytic, and particu-

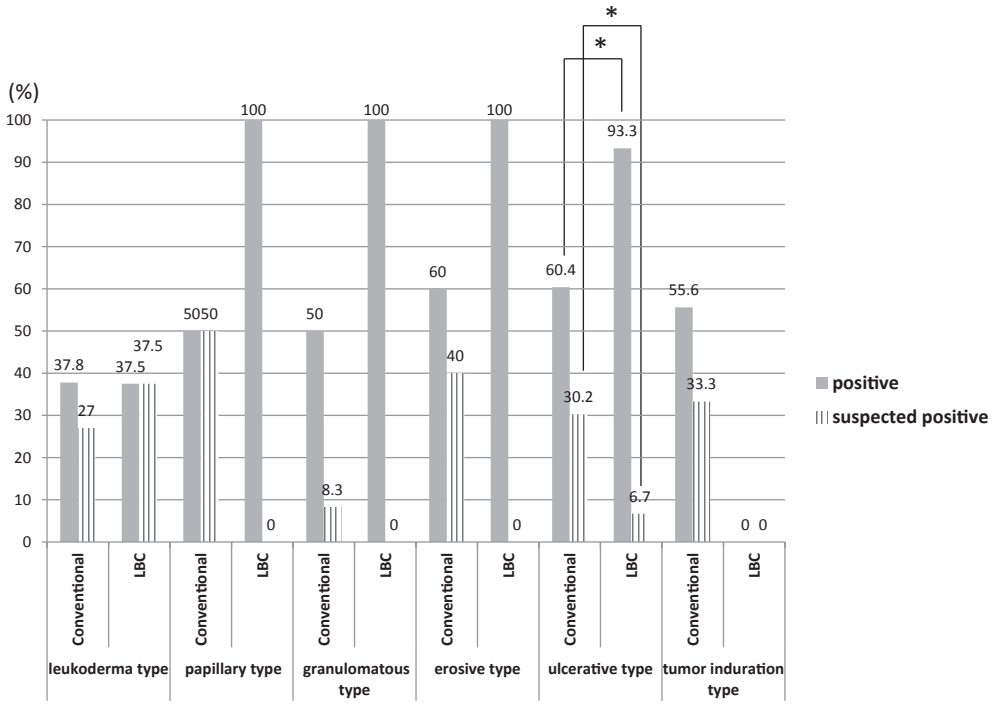

Fig. 5 Positive and suspected positive percentages for conventional cytology and LBC for clinical growth pattern

Positive rate was significantly higher with LBC than with conventional cytology; suspected positive rate was low, especially in ulcerative cases (Chi-square test: $p<0.05$).
*$p<0.05$

larly, ulcerative cases. We believe that this may have been because a susceptibility to bleeding and strong adhesion of constituents other than cells, such as blood and exudate, to the cell collection tool in the ulcerative type, which lacks keratinized cells in the surface layer, plays a greater role than in leukoderma types, which have a strong tendency to keratinization. As mentioned above, with LBC, the cells are uniformly stirred in a solution when preparing specimens. This stirring dissolves substances, such as mucus and erythrocytes, as well as collected cells in the solution, and so the final specimens have a clearer background than those prepared by conventional cytology. Therefore, the present results suggest that LBC is associated with an improvement in the positive and suspected positive rates in endophytic, and especially, ulcerative cases. This suggests that use of LBC, in particular, would improve diagnostic accuracy in such cases.

On the other hand, LBC showed no superiority over conventional cytology in exophytic, and especially, leukoderma cases, and no statistically significant difference was noted in either the rates of positive or suspected positive. One factor affecting superiority is thought to be that, in exophytic, and especially leukoderma cases, there is a strong tendency for surface layer keratinization, and when collecting cells, a large number of keratinized surface layer cells is collected, and the yield of deep layer abnormal cells, which are decisive for diagnosis, is small. We believe that no significant difference was observed in the accuracy of diagnosis between conventional cytology and LBC because of this, as the pro-

portion of deep layer abnormal cells on the specimens that are prepared is relatively low, and thus an accurate diagnosis is impossible. This result suggests that exophytic, and especially, leukoderma cases could represent a potential pitfall in oral exfoliative cytology. Therefore, tissue biopsy needs to be considered at an early stage in leukoderma cases.

Currently, oral exfoliative cytological diagnosis based on mucosal examination is widely used in general clinical practice for purposes such as screening during testing for and diagnosis of oral cancer, and early discovery of oral mucosal disorders.

The results of the present study suggest that, in future, 1) the LBC method and a special cytology brush should be used in exfoliative cytology in the region of the oral cavity; and 2) when an exfoliative lesion such as leukoplakia is noted in visual examination, tissue biopsy should be scheduled at an early stage to rule out the possibility of artefacts.

Conclusions

The results of the present study suggest that diagnostic precision would be improved with LBC compared with conventional cytology in oral exfoliative cytology. No superiority was observed for LBC, however, where the clinical growth style was exophytic or where the clinical growth pattern was leukoderma. These results suggest that these two cases represent a potential pitfall in oral exfoliative cytology, and therefore tissue biopsy should be considered at an early stage when they are encountered. Further study is needed to validate these results, however.

Acknowledgements

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