Optimization of FNAC findings as a preoperative diagnostic aid for odontogenic cysts

ABSTRACT
Background: Fine-needle aspiration cytology (FNAC) is not a definitive preoperative diagnostic procedure done for all cases of odontogenic cysts. This is because of the inconsistent results obtained with it.
Aims: This study was done to optimize FNAC findings and help in preoperative characterization of odontogenic cysts.
Materials and Methods: Cystic fluid was collected and centrifuged from 50 odontogenic cysts that were planned for excision. Three smears were prepared from the cell sediment obtained after centrifugation and stained. The stained sections were examined for presence and type of epithelial cells, to formulate a preoperative diagnosis.
Results: Epithelial cells were detected in 46% cases in smear 1, 48% cases in smear 2, and 52% cases in smear 3. When all three smears from one case were studied, 86% cases showed epithelial cells for evaluation.
Conclusion: Cystic aspirate should be centrifuged and the entire cell sediment should be examined by making multiple smears for evaluation of cystic epithelial lining cells.

Key words: Cell sediment; fine-needle aspiration cytology (FNAC); odontogenic cysts; smear

Introduction
Aspiration cytology dates back to 1950.[1] It is easy, noninvasive, economic, and safe.[1] Thus, it is frequently used for diagnosis of lymph nodes and salivary gland pathologies.[2] Studies have shown it to be reliable in differentiation of benign and malignant lesions.[3,4] However, up to the present date, it has not been accepted as a definite diagnostic technology in odontogenic cystic lesions, as it may not give consistent results due to relative absence of tissue architectural pattern in smears, inadequate cellular material obtained by fine needles, paucity of specific lesional cells in aspirates of cystic lesions, and the insufficient experience of the pathologist.[1] The present study was planned to overcome the problem of paucity of specific lining cells and inadequacy of tissue material, by preparing a cell suspension prior to making smears from the fluid aspirate of clinically suspected odontogenic cysts. The present study was based on the hypothesis that if the cystic aspirate is centrifuged before preparation of smears, cell dispersion is decreased.[5]

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Materials and Methods

Approval was obtained from the Institutional Ethics Committee prior to commencement of the research study. The study group comprised 50 clinically suspected odontogenic cysts that were planned for surgical excision. Written informed consent was obtained from patients willing to participate in the study. Suspected odontogenic cysts were aspirated using a 22-gauge needle by fine-needle aspiration (FNA) technique. The cystic fluid was immediately taken to the laboratory and transferred to a clean, dry centrifuge tube. The tube was then placed for centrifugation at 1500 rpm for 15 min. Following centrifugation, the cells appeared at the base of the centrifuge tube as cell sediment. The supernatant was then poured off and the resulting cell sediment was carefully removed using a micropipette. A single small drop was placed toward one end of a clean, labelled glass slide. The material was rapidly spread using another glass slide. Three smears were prepared using the above technique and fixed immediately in ethyl alcohol. The smears were then stained with Papanicolaou (PAP) stain to examine for the presence of epithelial cells, keratinized squames, keratin and parakeratin, and cholesterol crystals. The stained sections were examined under light microscope and cytological diagnosis on the basis of smears was done. After surgical treatment, the tissue was processed and sectioned using paraffin-embedded technique. Histopathologic diagnosis obtained from gold standard was compared with cytologic diagnosis and findings.

Results

The present study comprised 50 subjects, of whom 32 were males and 18 were females. The participants in the study were 5-65 years in age, with the mean age of 32.24 years. Out of these, cases diagnosed as odontogenic keratocysts (OKCs) were in the age range of 21-56 years (mean = 36.43 years); and cases of nonkeratinized odontogenic cysts were aged 5-65 years (mean = 31.56 years). The frequency of various types of cysts was as follows: 7 OKCs/keratocystic odontogenic tumors (KCOTs), 5 dentigerous cysts, 27 radicular cysts, 1 residual cyst, 4 infected odontogenic cysts, and 6 infected cyst walls.

When individual smears from each case were examined for the presence of epithelial cells, 46% cases were found to be positive in smear 1, 48% in smear 2, and 52% in smear 3 [See Table 1]. When all smears from one case were examined before commenting on the presence of epithelial cells, 86% cases were found to be positive [See Table 1]; 23.3% showed the presence of epithelial cells in all three smears, whereas 23.3% and 53.5% cases showed two and one positive smear, respectively [See Table 2]. Photomicrographs showing the epithelial lining cells from a case of keratinized [Figure 1] and non keratinized cyst [Figure 2] in the prepared smear have been included. Smear from OKC/KCOT shows numerous epithelial cells with pyknotic nuclei and smear from non keratinized cyst shows epithelial cells from a non-keratinized lining.

A total of 6 cases of infected cyst wall were included in the present study. All 6 cases showed the presence of nonkeratinized epithelial cells in the aspirated fluid.

Discussion

For cystic lesions of the oral and maxillofacial region, fine-needle aspiration cytology (FNAC) is emerging as an alternative technique for open biopsy for preoperative diagnosis, but its diagnostic value is not yet established.\(^1\) In addition, the procedure is not a part of routine protocol for every suspected cystic lesion. The probable reasons could be the following: the difficulty to perform FNAC from intraosseous lesions as compared to soft tissue lesions, due to the thick bony wall; or obtaining material that is quantitatively sufficient but poor in cellularity; or false negative results due to small amounts of lesional cells.\(^1\) The commonly used methods for evaluation of cystic aspirate are protein estimation and direct smears.

The present study showed an improvement in epithelial cell detection for preoperative diagnosis of odontogenic cystic lesions, by centrifugation of the cystic aspirate. Cytocentrifugation has been used for urine samples and other body fluids for a long time, and now the methodology has extended to FNA samples from salivary gland, thyroid

### Table 1: Frequency of presence of epithelial cells in each smear

| Smear | Epithelial cells present | Frequency | Percentage (%) |
|-------|--------------------------|-----------|----------------|
| Smear 1 | Yes | 23 | 46 |
| No | 27 | 54 |
| Smear 2 | Yes | 24 | 48 |
| No | 26 | 52 |
| Smear 3 | Yes | 26 | 52 |
| No | 24 | 48 |

### Table 2: Frequency of presence of epithelial cells in different smears

| No. of smears in which epithelial cells were present in each case | Frequency | Percentage (%) |
|---------------------------------------------------------------|-----------|----------------|
| One smear | 23 cases | 53.5 |
| Two smears | 10 cases | 23.3 |
| Three smears | 10 cases | 23.3 |
tissue, and breast tissue. Sirkin et al. in their paper on cytospin technique for FNAC specimens of breast wrote that unsatisfactory aspirates due to unskilled direct smear technique could be eliminated by cytospin technique.[7]

The present study highlighted the possibility of detection of epithelial cells in cystic aspirate of odontogenic cysts increasing severalfold when multiple smears are examined for their presence, as compared to the evaluation of a single smear.

August et al.[4] in a similar study on fine-needle aspiration biopsy (FNAB) of intraosseous jaw lesions found 2 samples to be inadequate for evaluation due to poor cellularity out of the total 32 samples.

Additionally, they were not able to correctly diagnose 3 out of 7 samples of odontogenic cyst due to the inadequacy of epithelial cells for evaluation.[4] Some other studies using aspirates from intraoral lesions by Dereci et al., Baykul et al., Gillani et al., Khan et al., Vargas et al., and Singh et al. have also found some inadequate smears by the direct smearing technique.[2,3,8-11] The present study strongly advocates that multiple or all smears made from the cell sediment after centrifugation of cystic aspirate must be examined to overcome the inability to provide preoperative diagnosis due to inadequacy of cells.

According to Dereci et al., the quantity of the keratinized cells is important in the diagnosis of OKC when evaluating the aspirates.[2] The amount of parakeratinized cell clusters required to safely diagnose a lesion as OKC is not yet known or documented.[2] All centrifuged aspirates from OKCs (also known as KCOTs (keratinizing cystic odontogenic tumors) in the present study showed abundant epithelial cells for diagnosis.

In the present study, a greater number of epithelial cells was observed in smears prepared from keratinized cyst lesions than in those from nonkeratinized cysts. These findings were similar to the finding of Oenning et al.[12] Oenning et al. explained in their paper that epithelial cells were significantly higher in OKCs due to the high epithelial proliferation rate of these lesions. The authors also wrote that the junction between the thin epithelial lining and the connective tissue wall is very smooth and thus can be a source of stimulation for the desquamation process.[12]

Cell block preparation from cell suspension aspiration sample has been recommended by many authors as it may give a better idea of tissue architecture, allows multiple sections, and is particularly useful when samples are heavily admixed with blood. However, they are relatively time-consuming and expensive compared to routine smears.[1] Various other cytocentrifugation methods including liquid-based cytology are available for liquid as well as FNA samples. According to a study by Piaton et al., urine samples can be processed by modern cytocentrifugation methods as well as by liquid-based cytology. The authors found that both methods were equally effective for cytology-based molecular studies but that liquid-based cytology methods were more expensive.[13]

In the present study, the aspirate was centrifuged but cell blocks were not made. Despite the avoidance of cell blocks for analysis, a large number of epithelial cells was observed in all OKC cases, whereas a significant number of cells was also observed in the nonkeratinizing cysts, in contrast to the studies by Oenning et al.[12] and Ramos et al.[14] In addition, keratinaceous

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Figure 1: Photomicrograph shows epithelial lining cells from a case of OKC in the prepared smear (Pap, ×400)

Figure 2: Photomicrograph shows epithelial lining cells from a case of radicular cyst in the prepared smear (Pap, ×400)
debris and parakeratin or keratin threads were found in 3 out of 7 cases, which left no doubts in categorizing the aspirate as that obtained from a keratinized cyst. Cholesterol crystals were not observed in all cases of nonkeratinized cysts in the present study. This could be explained by the fact that cholesterol crystals signify an infected cyst or inflammatory cyst, while all nonkeratinized cysts may not be infected secondarily or belong to the inflammatory cyst category. Nonpreparation of cell blocks in the present study saved time and money for preoperative analysis, without compromising much on the diagnostic accuracy.

Vargas et al.\textsuperscript{[8]} conducted a similar study to establish FNAC as an additional tool for diagnosis of OKCs. The authors prepared direct smears from the cystic aspirate, then made cell blocks with the remaining aspirate, followed by immunohistochemical (IHC) staining of pancytokeratin, CK 14, and CK 19 for all 8 samples collected. By direct smears, they found several desquamated normal keratinocytes, numerous anucleated squamas and keratinous debris, neutrophils, and macrophages. In the sections obtained from cell blocks, the authors found a number of keratin lamellae and scarce keratinocytes. On IHC staining, the authors found strong positivity for pancytokeratin (AE1/AE3) and CK19, and negative staining for CK 14. By a combination of the three techniques, the authors obtained accurate results.\textsuperscript{[9]} Our study did not use multiple techniques but only one technique with multiple smears, and our results were similar to those from the mentioned study by Vargas et al.\textsuperscript{[8]}

In the present study, we observed that all six cases that were diagnosed by histopathology as infected cyst wall due to lack of epithelial lining in the tissue section, showed the presence of nonkeratinized type of epithelial cells in the cystic fluid aspirate. This finding suggested that epithelial lining can get destroyed by long-standing inflammation or that induced due to the FNAC procedure, but as the FNAC is an initial procedure, epithelial cells can be obtained in the aspirate. Thus, the categorization was possible in this preoperative procedure. However, to be able to say whether this was a chance finding or that every infected cyst can be correctly categorized by fluid aspirate, further studies with a larger sample size are required.

Open biopsy and sampling is an alternative method for FNAC of odontogenic cysts. It may be a gold standard, but at the same time it may serve to convert an often isolated and noninflamed cyst into an open, contaminated one. Other shortcomings may include postbiopsy bleeding and inflammation, which may make histopathology of the excisional specimen difficult.\textsuperscript{[15]}

The present study utilized the FNAC technique, which is simple to perform, not contraindicated in any case, safer, minimally traumatic, and cost-effective, as compared to open incisional biopsy for categorization of lesions. With minimal training and equipment, it can yield information about the biological behavior of a lesion in the preoperative period. Even if that information is not complete and accurate, it can be a starting point for diagnosis and may make clear the next step for the surgeon.\textsuperscript{[3]} Baykul et al. mention that “FNAC materials also permit the supplementary studies such as immunohistochemistry, electron microscopy, morphometric studies for diagnosis of specific typing of lesions”,\textsuperscript{[3]}

**Conclusion**

The present study emphasized the fact that epithelial cells can go undetected if a single smear is prepared and evaluated. Centrifugation of the aspirate not only helps to reduce cell dispersion but also helps in evaluation of a larger quantity of aspirate despite preparation of fewer smears.

The authors would like to stress the importance of evaluating the entire cystic aspirate to aid in preoperative diagnosis, which will help the surgeon in better treatment planning.

Using the technique described in this study, the entire submitted aspirate should be examined in future studies.

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**Conflicts of interest**

There are no conflicts of interest.

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