Role of cell block technique as a novel diagnostic approach in odontogenic cysts and ameloblastomas of the jaw region

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Abstract

Background: Fine-needle aspiration cytology (FNAC) involves puncturing a lesion for microscopic analysis. Smears are prepared using aspirated material for cytology and for cell block (CB) preparation. FNAC is a preoperative technique and does not provide sufficient information for precise diagnosis, and the risk of false diagnosis or intermittent diagnosis always exists. To overcome the deficiencies of FNAC, the CB technique (CBT) is considered a novel innovative technique for odontogenic lesions. The present study is carried out to evaluate the efficacy of CBT in diagnosing odontogenic cystic lesions and ameloblastoma of the jaw and comparing it with aspiration cytology and histopathology.

Methods: In this prospective study, 17 patients with clinical diagnosis of odontogenic cysts and ameloblastoma are obtained. FNAC smears and CBs are prepared and compared with cytology and histopathology.

Results: A total of 17 cases of odontogenic cysts and ameloblastoma are divided into seven odontogenic keratocyst (OKC), three dentigerous cysts, three radicular cysts and four ameloblastoma. In seven cases of OKC, five cases (71.4%) are showing positive features and two cases (28.6%) are showing negative features. Three dentigerous and three radicular cysts are showing three positive CB features (66.7%) and three negative features (33.3%). Among four cases of ameloblastoma, two cases (50%) are showing positive CB features and two cases (50%) are showing negative features.

Conclusion: CB is a novel diagnostic technique in oral pathology for odontogenic cysts, tumors, metastatic tumors and bony lesions. Compared to FNAC, it gives accurate diagnostic architecture and apparent histopathological features. This technique can be used alternative to FNAC.

Keywords: Aspiration cytology, cell block technique, odontogenic cyst, parakeratin

INTRODUCTION

Exfoliative cytology is the microscopic examination of shed or desquamated cells from the epithelial surface usually the mucous membrane. It also includes the study of those cells that have been collected by scraping the tissue surface or collected from the body fluids such as sputum and saliva.[1] The needle aspiration is a clinical maneuver that involves puncturing a lesion for microscopic analysis. This analysis can be performed after smearing the aspirated fluid directly onto a glass slide or using the cell block technique (CBT).[2]

Fine-needle aspiration cytology (FNAC) of the superficial...
lesion or deep anatomical site is an increasingly common procedure in the diagnosis of neoplastic and cystic lesions. Sometimes, FNAC does not yield sufficient information for precise diagnosis, and the risk of false negative or intermediate diagnoses always exists. To overcome these problems, CBT has been resorted to make the best use of the available material.[8] CB is a histological technique largely used in medical pathology for pleural fluid, peritoneal fluid, bronchial washings, fine-needle aspirations and other cytological specimens. CB prepared from the residual tissue fluids and FNA can be useful adjuncts to smears for establishing a more definitive cytopathologic diagnosis.[4] The cytomorphologic features are clearly visualized, and also the storage of CB is easier. An additional advantage of this technique is the possibility of executing immunohistochemistry (IHC) to identify the structures.[2] CBT are used as a complementary method in the diagnosis of odontogenic cystic lesions of the jaw. The aspiration of a cystic lesion can provide additional information about its content (if liquid or serous, or if absent) and aid in the presumptive clinical diagnosis at the moment of its application.[4] The present study is carried out to evaluate the efficacy of CBT in diagnosing the odontogenic cystic lesions and ameloblastoma of the jaw and comparing it with aspiration cytology and histopathology.

**Review of literature**

A study constituting of 17 cystic jaw lesions showed that in 9 cases, CB revealed a large number of erythrocytes, inflammatory cells and few epithelial cells with cholesterol clefts. Histopathological analysis associated with clinical and radiographic characteristics rendered the diagnosis of inflammatory cystic lesions odontogenic cysts; three residual cysts and six radicular cysts. In other 8 cases (47%), diagnosed with keratocystic cystic lesions odontogenic tumors (KOT) by the histopathological analysis, the CB method showed the predominant occurrence of keratin and the presence of parakeratin in some areas. The CB method proved to be very effective in distinguishing KOT from other lesions with similar clinical and radiographic aspects through parakeratin identification.[4]

A study composed of 33 clinically diagnosed jawbone lesions with a cystic appearance was subjected to the aspiration. Cytological findings and histopathological analyses were done. There was association between cysts and cholesterol clefts and between KOT and parakeratin. Among KOT, 11 included in this study showed parakeratin in CB preparations. Among 22 cystic lesions, 15 revealed the presence of cholesterol crystal clefts. Epithelial cells resulting from desquamation were found in seven KOT cases and in a nasopalatine duct cyst. The CBT proved to be fast, easy to handle and low-cost making it an attractive auxiliary method for a preliminary diagnosis of jaw bone lesions.[9]

A study composing of 9 ameloblastoma cases which were subjected to prepare CB. Cytological analysis revealed that erythrocytes were present in six cases, and there epithelial cells in seven cases with a rounded-or-polygonal form. The existence of acellular amorphous eosinophilic materials was detected in five cases. All epithelial cells were observed by IHC utilizing the anti-pan-cytokeratin (AE1/AE2) antibody.[6]

Another study on CB as a forgotten tool and found out that CB is superior in diagnosing neoplasm’s as well as providing a high-diagnostic accuracy.[6]

**METHODS**

**Source of data**

FNAC samples required for the study were collected from patients of odontogenic cystic lesions and tumor from the Department of Oral and Maxillofacial Surgery and Oral Medicine, Yenepoya Dental College, Mangalore, Karnataka, India. Clinical and Radiographical findings were taken from the patient records.

**Method of collection of data**

The study group consists of 17 samples of both males and females with a history of odontogenic cystic lesions and tumor indicated for FNAC. The collected material from the lesion is visually examined for the color and consistency. Inclusion criteria are cystic lesions of the head-and-neck region. Exclusion criteria are noncystic lesions.

**Smear preparation**

Syringe containing aspirate material is smeared by holding the smearing slide against the specimen slide at a blunt angle near one end of the slide, allowing the fluid to accumulate in the angle. The smearing slide is then rapidly moved along the specimen slide, halfway or all the way depending on the amount of fluid. Smears are immediately fixed with 95% Ethanol Stained with Hematoxylin and Eosin.

**Cell blocks preparation**

Excess material in the syringe was transferred into a test tube and centrifuged at 3000 rpm for 10 min, discard the supernatant, and the sediment was mixed with ethyl alcohol (2–3 ml), mixed the content and filtered and added 10% formalin (4–5 ml), filtered again and taken sediment for a routine processing. The pellet obtained thereby was transferred into an absorbent paper, placed in the tissue cassettes and fixed in 10% formalin for 24 h. Routine
processing and embedding in paraffin wax were done. Three-micrometer-thick sections were cut and stained with hematoxylin and eosin. Stained sections were analyzed under a light microscopy. After biopsy, biopsied samples were taken for the routine processing. Three-micrometer thick sections were cut and stained with hematoxylin and eosin. Stained sections were analyzed under the light microscopy.

RESULTS

The present study consists of 17 clinically diagnosed odontogenic cystic lesions and ameloblastoma; CBs were prepared from these lesions and compared with cytology and histopathology. Among these 17 cases, there were 7 cases of odontogenic cysts, 3 cases of dentigerous cysts, 3 cases of radicular cysts and 4 cases of ameloblastoma of the jaw region were diagnosed clinically and histopathologically. Out of 17 cases, 7 were females and 10 were males with the age group of 10–60 years were considered and tabulated [Table 1].

CBs were prepared using the FNAC material and features were categorized into positive and negative of individual lesions, and CB findings are tabulated in Table 2 and compared with cytology and histopathology.

Among 7 odontogenic keratocyst (OKC), 5 cases (71.4%) were showing positive features, and 2 cases (28.6%) with negative features. Out of 3 dentigerous and 3 radicular cysts, 2 cases (66.7%) with positive CB features, and 1 case (33.3%) with negative CB feature. The ameloblastoma was showing 2 cases (50%) with positive CB features and 2 cases (50%) with negative CB features [Table 2].

Table 1: Total number of cases and their distribution based on the lesions and sex of the individual

| Number of cases | Sex                | OKC | DC  | RC  | Ameloblastoma |
|-----------------|--------------------|-----|-----|-----|---------------|
| 17              | Females (n=7)      | 7   | 3   | 3   | 4             |
|                 | Males (n=10)       |     |     |     |               |

OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

Table 2: Comparison between cell block features and histopathology diagnosis

| Odontogenic lesions and tumor | Cell block features                        | Number of cases with positive and negative cell block features/diagnosis | Histopathology diagnosis |
|------------------------------|--------------------------------------------|------------------------------------------------------------------------|--------------------------|
| OKC (n=7)                    | Keratin flakes, epithelial cells and inflammatory cells | Five cases with positive features                                    | OKC                      |
| DC (n=3)                     | Epithelial cells, inflammatory cells        | Two cases with negative features                                      | DC                       |
| RC (n=3)                     | Inflammatory cells, cholesterol clefts and hemorrhagic area | One case with negative features                                       | RC                       |
| Ameloblastoma (n=4)          | Odontogenic tumor epithelial cells, inflammatory cells and hemorrhagic area | Two cases with positive features                                      | Ameloblastoma            |

DISCUSSION

Odontogenic cystic lesions are lined by epithelium containing liquid or semisolid material. Based on their origin, they are mainly classified into developmental and inflammatory. Radiographically, they appear as unilocular/multilocular radiolucency with or without sclerotic border depending on the type of the cyst.[4] FNAC for a cystic lesion is an alternative technique before biopsy for preoperative diagnosis, but the diagnostic value is debatable. CBT is a histological technique which allows microscopic examination of the collected fluid/semisolid material. Bahrenburg was the first person to introduce CBT alternative to FNAC for ascetic fluid. This technique is used previously in medical pathology for specimen such as urine, pleural, peritoneal, ascetic, pericardial fluid and hemorrhagic aspirates.[8] Advantage of CBT over FNAC is that histological features can be well-appreciated, multiple tissue sections can be taken and other than routine staining, IHC or special staining can be done. CB can be stored in future for tissue sections, staining and clarity is superior to FNAC smears. It can be used as a preoperative diagnostic technique due to its proper diagnostic architecture, which is similar to histopathological diagnosis. The disadvantage of CBT is that compared to FNAC, it is time-consuming.

In the present study, 17 cases of clinically and histopathologically diagnosed odontogenic cystic lesions (7 cases of OKC, 3 cases of dentigerous cyst and 3 cases of radicular cyst) and ameloblastoma (4 cases) were taken. Both FNAC and CBT were done, which were compared with histopathology. Out of 7 cases of OKC, two cases (28.6%) were showing negative CB findings, and 5 cases (71.4%) were showing positive CB findings such as keratin and epithelial cells with or without inflammatory cells [Figure 1]. Cytopathological features of 5 OKC cases were showing mixed inflammatory cells, hemorrhagic areas [Figure 2] which were compared with CB and histopathological features [Figure 3]. CB method is simple, fast and low-cost techniques where OKC can be differentiate from other lesions by the presence of
cells and hemorrhagic area [Figure 6], and 2 cases (50%) were showing negative features. Cytological features were mixed inflammatory cells and desquamated cells [Figure 7]. Odontogenic tumor epithelial cells in CBT were observed in ameloblastoma cases, which were not appreciated in cytology. Above-mentioned CB features, cytological features were compared with histopathology [Figure 8]. The presence of epithelial cells, acellular amorphous eosinophilic material and the absence of cholesterol crystal clefts or keratin were observed in ameloblastoma.\[2\] These features are similar with our study findings. In the present study, CB features of OKC is the presence of keratin, epithelial cells in dentigerous cyst, inflammatory cells in radicular cyst and odontogenic tumor epithelial cells in ameloblastoma were seen, which was not appreciated in cytology of the same cases. Hence, CBT is more accurate, clear and more precise than FNAC. This technique can be used as a preoperative diagnostic technique for jaw bone lesions.
CONCLUSION

CBT is rarely used in oral pathology for odontogenic cystic lesions, bony lesions and tumors and can be used as a novel diagnostic preoperative technique in odontogenic cystic lesions, head-and-neck metastasis for squamous cell carcinoma, where FNAC is the present option. It is superior to FNAC and as the clarity; histopathological features are well-appreciated.

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Conflicts of interest
There are no conflicts of interest.

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