Evaluation of the cell block technique as an auxiliary method of diagnosing jawbone lesions

Abstract: This study investigated the viability of the cell block technique as an auxiliary method of diagnosing jawbone lesions. Thirty-three clinically diagnosed jawbone lesions with a cystic appearance were subjected to aspiration. The aspirated material was processed by the cell block technique, and the lesions were biopsied and treated. Cytological findings (cell block) and histopathology analyses (gold standard) were compared by the chi-square test. There were associations between cysts and cholesterol crystal clefts, between keratocystic odontogenic tumors (KOT) and epithelial cells, and between KOT and parakeratin. The occurrence of cholesterol crystal clefts in cell block slides was correlated with cystic lesions, and the parakeratin presence was a KOT indicator. The cell block technique proved to be fast, easy-to-handle, and low-cost, making it an attractive auxiliary method for the preliminary diagnosis of jawbone lesions.

Descriptors: Biopsy, Needle; Jaw Cysts; Odontogenic Tumors.

Introduction

Several disease entities, of both odontogenic and non-odontogenic origin, may affect the maxillomandibular complex. These lesions can exhibit similar clinical and imaging appearances associated with a wide range of histopathological and behavior features.

In most cases of bone lesions, a biopsy and histopathological analysis are necessary to establish the final diagnosis. Biopsy may be a complex procedure at some maxillomandibular sites, and some systemic conditions could contraindicate the procedure. The search for new, less-invasive techniques which could eventually replace biopsy and simplify the diagnostic process would benefit both professionals and patients. Aspiration is recommended in submucosal lesions or in those cases where a very thin layer of cortical bone persists. The collected material should first be visually examined, with a special focus on color and consistency. However, this material is frequently discarded without being properly referred for laboratory analysis.

The cell block technique is a histological approach, often used in medical pathology, which consists of aspirating the lesion with the use of an 18-gauge needle followed by laboratory processing. The aspirated material is centrifuged, paraffin-embedded, cut, stained, and histologically analyzed. The great advantage of cell blocks in these cases is the...
decrease in cell dispersion.²

The aim of this study was to evaluate the viability of the cell block technique as an auxiliary method for the diagnosis of maxillary bone lesions with a cystic aspect.

Methodology

This study was approved by the Human Research Ethics Committee of the authors’ institution (approval number 143/08). Sampling included patients with intrabony jaw lesions with a clinical recommendation of aspiration, and being cared for at the Stomatology Ambulatory at the University Hospital, and at the Dental Clinic of the Federal University of Santa Catarina. All participants were volunteers who were informed about the study and were asked to sign an informed consent agreement.

Clinical procedures

All the patients underwent clinical and imaging examinations. The aspirations were performed in the same session, immediately after the examination or during the biopsy procedure. An 18-gauge needle coupled to a 20-mL syringe was inserted into the lesion at its maximum fluctuation or at the “papyrus crackling” consistency point. The aspirated material was analyzed macroscopically, and the syringe was immediately packaged in an ice-cooled container and referred to the Oral Pathology Laboratory for processing. An incisional or excisional biopsy was later performed according to the clinical indication for each case. The treatment prescribed followed pre-established protocols by the respective services.

Laboratory procedures

The material was removed from the syringe, transferred to a test tube, and centrifuged at 1500 rpm for 20 min. The pellet obtained after centrifugation was transferred to absorbent paper and fixed in a 10% formaldehyde solution for 24 h. After that, the material was sequentially processed as follows:

- dehydration,
- diaphanization,
- impregnation, and
- embedment in paraffin.

Three-micrometer-thick sections were obtained and stained with haematoxylin-eosin (H&E). The cell block slides were analyzed by light microscopy (Axiostar Plus; Carl Zeiss, Oberkothen, Germany) for evaluation of the presence of epithelial cells, acute and chronic inflammatory cells, keratin, and cholesterol crystal clefts. This evaluation was performed by a calibrated examiner, first independently and later by consensus with an oral pathologist. The biopsied tissues were subjected to routine processing for the histopathological diagnosis of the lesions.

Statistical analysis

From the cell block data and histopathology analyses, a database was created with the SPSS software package (“Statistical Package for the Social Sciences” for Windows, version 16.0, SPSS Inc., Chicago, IL, USA). The chi-square test was used to check the occurrence of variable associations at a 5% significance level.

Results

The final sample consisted of 33 patients, 20 (60.6%) male and 13 (39.4%) female, with a mean age of 41 years, ranging from 9 to 73 years. The complete profile of the sample is shown in Table 1.

The data for the association of cell block features with histopathological diagnosis are shown in Table 2. There was a statistically significant association between cystic lesions and cholesterol crystal cleft occurrence, between KOT and epithelial cell occurrence, and between KOT and parakeratin occurrence.

All KOT cases (n = 11) included in this study

<table>
<thead>
<tr>
<th>Table 1 - Description of the lesion sample.</th>
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<tbody>
<tr>
<td><strong>Histopathological diagnosis</strong></td>
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<tr>
<td>---------------------------------------------</td>
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<tr>
<td><strong>Cysts</strong></td>
</tr>
<tr>
<td>Inflammatory cysts</td>
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<td></td>
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<tr>
<td>Development cysts</td>
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<tr>
<td><strong>Tumor</strong></td>
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<td><strong>Total sample</strong></td>
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</table>
showed parakeratin in cell block preparations (Figure 1A). Among 22 cystic lesions, 15 (68.2%) revealed the presence of cholesterol crystal clefts (Figure 2A). These clefts were also found in one KOT case that showed an intense inflammation upon histopathological examination. Epithelial cells resulting from desquamation were found in 7 KOT cases (Figure 1B) and in a nasopalatine duct cyst.

Discussion

Biopsy may be complex at some maxillomandibular sites, such as the mandibular ramus and condylar lesions, due to the difficulty of surgical access.4,6 Systemic conditions either contraindicate biopsy or render it difficult. In these cases, aspiration could be indicated to verify the absence or presence of blood or liquid inside a mandibular lesion, to allow for an evaluation of the coloration, consistency, and amount of the material extracted.4 Fine-needle aspiration cytology (FNAC) is a valuable resource, especially to differentiate malignant from benign lesions.3,7 However, the use of this technique for jaw lesions is not unanimously accepted.2,3,7,9 In this study, the biopsies were performed with thicker-gauge needles, followed by the processing of the aspirated material by the cell block technique, with the goal of decreasing cell dispersion and providing a larger amount of material for analysis.

The occurrence of inflammatory cells was an expected finding in aspirations of inflammatory

<table>
<thead>
<tr>
<th>Cell block feature</th>
<th>Histopathological diagnosis</th>
<th>Feature occurrence</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td></td>
<td>KOT</td>
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<tr>
<td>Acute inflammation</td>
<td>Cysts</td>
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<td>3</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>KOT</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Cholesterol crystal clefts</td>
<td>Cysts*</td>
<td>15</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>KOT</td>
<td>1</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>Cysts</td>
<td>1</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>KOT*</td>
<td>7</td>
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<td>11</td>
</tr>
<tr>
<td>Parakeratin</td>
<td>Cysts</td>
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<td>22</td>
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<tr>
<td></td>
<td>KOT*</td>
<td>11</td>
<td>0</td>
<td>11</td>
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* Statistically significant.
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origin, since leukocyte transmigration toward the radicular and residual cyst epithelium is common. Nonetheless, a statistically significant association between the occurrence of inflammatory cells and the lesions in this study was not found. Other authors, upon examining maxillary aspirates through FNAC, found a large quantity of inflammatory cells in radicular cysts, in addition to fibromyxoid tissue and epithelial cells. Despite the lack of statistical relevance, probably due to the small sample in our study, in a comparison of cytological slides of radicular cysts (Figure 2B) with those of residual cysts, inflammatory cells were found to be more abundant in the former. This can be explained by the continuous contact of the lesion with bacterial components from the tooth root canal, presenting devitalized pulp.

A statistically significant association between cysts and cholesterol crystal clefts was found. Cholesterol crystal clefts have been reported in the lumen and/or inflammatory cyst capsule. Epithelial cell occurrence showed a statistically significant association with KOTs. Such findings can be explained by the high epithelial proliferation rate of these tumors, which is responsible for the growth mechanism of these lesions. The smooth junction between the thin epithelium and the connective tissue of the capsule may also be a likely source of stimulation for the desquamation process.

All KOTs (n = 11) showed parakeratin. In cytological findings by August et al., only one out of 3 KOTs analyzed showed keratin. However, these authors used a fast staining method or immediate fixation in ethanol without centrifugation and embedding, which generated an inevitable loss of material for analysis, due to the typical cell dispersion of these materials. Radhika et al. stated that, when liquid is aspirated from extensive lesions, its centrifugation can improve diagnosis due to the concentration of lesion-typical cells.

The literature shows that FNAC, with or without cell block association, is a valuable diagnostic technique for other jaw lesions, such as giant-cell lesions and ameloblastomas. However, it is important to emphasize that cytology is more complex than histology in maxillary lesions, and further studies should be developed on this matter so that a better understanding of the cytological features of these lesions can be achieved.

Conclusion

A preliminary diagnosis of cystic lesions and KOTs can be conducted by the cell block technique. The occurrence of cholesterol crystal clefts in cell block slides suggests cystic lesions. The presence of parakeratin in the cytological analysis is highly suggestive of KOT, eliminating the need for incisional biopsy in the therapeutic planning for these lesions.

Figure 2 - Cytological analysis (cell block) of cysts. (A) Cholesterol crystal clefts (arrows), a few inflammatory cells, and a large number of erythrocytes in a residual cyst (H&E 400x). (B) Inflammatory profile in a radicular cyst (H&E 400x).
References