Contribution of the cyto-histopathological diagnosis and ultrastructural parameters to the evaluation of maxillary cysts – a 10-year multidisciplinary approach

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Abstract

Diagnostic and treatment plans in cystic jawbone tumors are often difficult to address. The etiopathogenic links involved in cell–matrix differentiation disorders are complex. Quantification of the inflammatory process in the evolution of cystic odontogenic lesions highlights a particular reactivity of the host, especially age-dependent and the endodontic–periodontal space interrelation, drawing attention to the difficulties of etiopathogenic, evolution, prognostic and treatment of these lesions. Difficulties in histopathological (HP) diagnosis are reported by the lack of morphofunctional integration of dental tissues, both topographically and evolutionarily, especially when odontogenic epithelial remains in the cystic wall, reactive bone condition, appearance and condition of the reactive epithelium are overlooked. In this study, we developed an interdisciplinary approach for the dynamics of tissue morphology found in the walls of maxillary cysts. Failure to recognize the tissues that form the cystic lesion leads to misinterpretations of pathology and to the wrong classification in the group of maxillary cysts. We analyzed by different techniques 564 biopsy fragments from maxillary cystic lesions, most of which are clinically classified as inflammatory or odontogenic ones. From our experience, we reevaluated the lesions with cystic changes and completed the diagnosis in 10–12% of cases. The most common maxillary cystic lesion encountered by us was the root cyst, an inflammatory dental cyst, which has been over diagnosed clinically, radiologically and histopathologically. Recognition and selection of embryonic remnants from odontogenesis is crucial for the HP diagnosis of maxillary cysts, allowing the clinician to monitor treatment or to develop evolutionary-prognostic perspectives of odontogenic cystic lesions.

Keywords: cyto-histopathological diagnosis, cysts tumors, interdisciplinary approach.

Introduction

The increased incidence of cysts in the maxillary and mandibular bones is clarified by the existence of ectomesenchymal and ectodermal derivatives from odontogenesis in the osseous connective tissue mass [1, 2]. The presence of epithelial remnants from the enamel organ, from the fragmentation of dental lamina, from Hertwig’s epithelial root sheath (epithelial rests of Malassez) and from the surface epithelium of maxillary embryonic processes, represents the starting point for forming odontogenic and nonodontogenic cysts [1, 2].

Nonodontogenic epithelium is found only in the maxillary bone and represents the rest of the epithelium that covered the embryonic processes from which maxillary bones are developed. These tissue remnants are found along the suture lines of embryonic maxillary processes [3].

Another source of the nonodontogenic epithelium in the maxillary bone is represented by the vestiges of the nasopalatine duct of oronasal communication in fetal life. Embryonic remnants can be found in the incisive papilla zone. These are pseudo-cysts, because they are not lined by the epithelium [4, 5].

From odontogenic cysts, more than a half are radicular cysts developed from Malassez rests activated in the periodontal ligament due to inflammation after the destruction of the dental pulp tissue [2, 6].

This study has focused on the use of all possibilities of morphological and clinical diagnosis that find etiopathogenic links involved in the development and maintenance of maxillary cyst lesions, correlating cytological aspects with those histopathological (HP), histochemical, immunohistochemical (IHC) and electron microscopic in maxillary cysts with diverse clinical and radiological aspects. This type of cyst is not difficult to diagnose.
clinically and radiologically. These cysts consist of superior tissular differentiation, in which the inductive ecto-mesenchymal aspect determines the HP subtype and the preoperative and postoperative evolution of the lesions as well. The current study finds and observe the main morphological markers used for appreciating postoperative recurrence risk of the maxillary cysts.

Materials and Methods

We studied 564 patients who underwent biopsy, presenting maxillary and mandibular cysts, clinically, radiologically and histopathologically examined between 2009 and 2019. The parameters which were examined (age, gender, initial symptomatology, anatomical localization, degree of affection of the neighboring anatomical structures, radiological dimension, postoperative evolution) were referred to the HP type of cyst. Following techniques were applied:

Histopathology

The samples were placed in hermetically sealed, sterile containers, in a 4% formalin solution, after which they were stored in the refrigerator at a constant temperature of 4°C. The samples were processed according to the paraffin inclusion technique. We performed the sections of the paraffin block, the deparaffinization, rehydration (or antigenic demasking in the case of immunohistochemistry technique) and the staining of smears.

The deparaffinization protocol involved carrying out three successive benzene baths, five minutes each, followed by two successive 100% ethanol baths, one 95% ethanol bath and one 70% ethanol bath, two minutes each.

The rehydration was performed by rinsing the samples with distilled water in two successive baths. Hematoxylin–Eosin (HE) classical staining protocol was used for the HP examination. Staining, regardless of the chosen technique, was followed by dehydration in three ethanol baths in increasing concentrations and by clarification in three benzene baths.

The cytological smears

The cytological smears were obtained after a fine-needle aspiration (FNA) (n=120), after minimally local anesthesia. If the bone wall was thick, a foramen was made in advance with the milling machine. The smears were examined using Papanicolaou (Pap) staining and APT (polychrome tannin blue)–Dragan original method. The smears were graded based on cellularity (grade I, II, III), the dimension of the cell group, cellular arrangement, nuclear structure alteration, the presence of nucleoli.

Immunohistochemistry

The specimens were fixed in 10% neutral buffered formalin for 48 hours and embedded in paraffin. The paraffin-embedded tissues were cut into 5 μm sections. All the samples were stained using the HE classical protocol. We performed an enzyme pretreatment (Bond Enzyme Pretreatment Kit, Leica Biosystems, Newcastle upon Tyne, UK) for 10 minutes. Endogenous peroxidase blocking was done with 3% hydrogen peroxide for five minutes. This step was followed by incubation for 15 minutes with cluster of differentiation (CD) 20, cytokeratin (CK) (monoclonal mouse anti-human CK, clone AE1/AE3) and CD68 (Novocastra, Newcastle upon Tyne, UK, rabbit polyclonal antibodies, ready to use) as primary antibodies. We utilized a set of CD antigen-specific monoclonal antibodies to detect different cell types within the tissues. These included anti-CD20 (B-cells), anti-CD68 (macrophages) and Ki67 proliferative index.

The Bond Polymer Refine Detection System (Leica Biosystems, Newcastle upon Tyne, UK) was used for visualization. 3,3’-Diaminobenzidine (DAB) tetrahydrochloride was applied as chromogen, and we used Hematoxylin for counterstaining. The entire IHC procedure was developed with Leica Bond-Max (Leica Biosystems, Newcastle upon Tyne, UK) autostainer.

Image acquisition and analysis were performed using Nikon Eclipse E600 microscope and Lucia G software for microscopic image analysis.

Transmission electron microscopy

For the examination using transmission electron microscopy (TEM), the Lehner technique (1996) allowed the reinclusion of paraffin blocks (n=15). Post-fixation was made in 1% osmium tetroxide in 0.1 M phosphate buffer solution at pH 7.3 for one hour. Dehydration was made in ethanol at different concentrations, then the sections were fixed in Epon and the blocks were cut at 0.5 μm thick. The contrasting sections were made with lead citrate and uranyl acetate.

Results

Based on the modified World Health Organization (WHO) Classification, in this study, the main type was represented by inflammatory cysts, 77.1% of the cases. In the inflammatory cyst group, radicular cysts were represented by 67% of the total cases, and residual cysts – 12%. The dentigerous cysts and the keratocysts were each identified in the same percentage of 9%. The periodontal lateral, nasopalatine, paradental, eruption cysts and other cysts were found in a value less than 3%.

We have observed three postoperative recurrence cases of radicular cysts, with clinical symptoms of infection, and nine cases of keratocyst recurrence, six without any symptoms, diagnosed after radiological exam and three cases with pain and clinical manifestations.

We present, in Table 1, morphological aspects of maxillary cyst lesions found in case studies in histology.

In most of the HP sections, lining epithelium is either incomplete or alternates as layering and cell differentiation, having aspects of reactive hyperplasia or degenerative. We noticed marked edema and aspects of exocytosis to cysts with abundant inflammatory reactions. To certain cysts, lining epithelium presents an exuberant growth, which might simulate a squamous odontogenic tumor. In some specimens histologically examined, we did not find any epithelial lining of the cyst. We found only a fibrosis wall, thickened by extensive hyalinization and abundant nonspecific chronic inflammatory infiltrate, or granuloma reaction of foreign body and supplicative micro-hotspots.

We considered that the formation of the developmental radicular cyst is an evolutive succession of apical granuloma. The transition of granuloma to the cyst is gradual and is based on growing proportions of epithelium and gradual development of a clearly defined lumen.
Table 1 – Morphological aspects of the cysts (n=564)

| No. | Morphological aspect | Type of lesion | Patient age (average) [years] | Identification | Role in local recurrence |
|-----|----------------------|----------------|-------------------------------|----------------|--------------------------|
| 1   | Proliferative lining epithelium | Inflamed odontogenic cysts (inflammatory and developmental type) | 28 | Frequent | +/- Usually surgically removed |
| 2   | Active remnants of odontogenic epithelium | Inflamed odontogenic cysts (inflammatory and developmental type), inflamed neoplasms | | Frequent | +/- |
| 3   | Active remnants of odontogenic epithelium | Developmental cysts | 10–20 | Frequent | + |
| 4   | Inflamed polymorph infiltrate | Inflamed radicular cyst, inflamed dentigerous cyst, histiocytoid lesions | 31 | Rarely | No |
| 5   | Giant cells foreign body type | Developmental odontogenic cysts | 42 | Frequent | No |
| 6   | Giant cells foreign body type | Inflamed odontogenic cysts | 35 | Rarely | No |
| 7   | Cholesterol crystals | Developmental odontogenic cysts | 45 | Frequent | No |
| 8   | Mucous cells | Developmental cysts | 39 | Frequent | No |
| 9   | Ciliated cells | Developmental cysts | 37 | Rarely | No |
| 10  | Dystrophic calcification | Odontogenic cysts | 47 | Frequent | No |
| 11  | Rushton intraepithelial hyaline bodies | Developmental odontogenic cysts | 35 | Rarely | No |
| 12  | Connective hyaline bodies’ inflammatory reaction | Follicular cysts | 38 | Relatively often | +/- (in connection with odontogenic epithelial polypotic hotspots) |
| 13  | Fibro-myxomatous degeneration of the wall | All inflamed cysts | 45 | Relatively often | No |
| 14  | Plasma cells with Russell bodies | All inflamed cysts | 45 | Frequent | + |
| 15  | Polycystic invaginations | Lateral periodontal cysts | 45 | Frequent | + |
| 16  | Thickening of the epithelium | Lateral periodontal cysts | 45 | Inconstant | + |
| 17  | Deletion of the epithelium crests | Odontogenic keratocyst | 38 | Frequent | + |

The epithelium that is platted from the support connective tissue is observed at the odontogenous keratocysts. This finding is not a feature of other types of maxillary cysts. The three cases of odontogenic recurrent keratocysts showed not constant this separation of the lining epithelium from the subjacent connective tissue (Figure 1), this observation may explain the recurrence of these cysts (Figure 2, A–C).

The myxomatous modification on the cyst wall indicates dysfunctional local tissue, which may indicate a recurrence possibility (Figure 3). The presence of metaplasia mucous cells in ameloblastoma and their comparatively regular occurrence in odontogenic cysts shows the odontogenic epithelium’s capacity for differentiation (Figure 4).

The most important predictor of postoperative recurrence risk is active odontogenic epithelial rests in the cyst wall (Figure 5). In two cases, we discovered cyst lumen-lining epithelium with ameloblastoma aspect and basaloid type, which raises the possibility of recurrence or neoplasia transformation (ameloblastoma, the carcinoma with squamous cells, the central mucoepidermoid carcinoma) (Figure 6). Carefully and using objects of increasing the image, we identified in the cyst wall active or inactive small epithelial odontogenic islands with cells in small groups (2–4–6–8 cells), with small central nuclei, sometimes arranged in short cords or in rosettes in a semi-ordered fibrous connective tissue, of ligamental type.

Figure 1 – The wall of an odontogenic keratocyst, with detachment on extended areas of the odontogenic epithelium from the connective tissue (HE staining, ×100).
In the inflammatory process, the endothelial cells activated by local proinflammatory factors (monocytes, macrophages), cytokines, initiates reactive angiogenesis identified in the inflammatory stage of different cystic subtypes in our study (Figures 7 and 8). The infarction of vascular wall is a source of intracystic hemorrhage. The recent cystic lesions present in the wall inflammatory infiltrate predominantly with polymorphonuclear leukocytes in areas with active hyperemia. In the depth of the wall, inflammatory infiltrate is predominantly mononuclear, characterized by an abundance of plasma cells. The cystic wall presents various grades of fibrosis and hyalinization with bone tissue in peripheral areas.

The cytological aspects were used to determine the cellularity of cystic lesions, to determine the subtype of
the lesion and the activity score of odontogenic epithelia, which indicated the lesion’s evolutive prognostic possibilities. Cellular discohesivity by acantholysis, ulceration of the cyst walls, intracystic hemorrhage, the suppuration, inflammatory necrosis, the microcalcifications, the presence of foamy macrophages of resorption indicates a high level of cellular activation, implying that the lesion is unstable (Figures 9 and 10).

The appearance of cholesterol crystals in dentigerous cysts indicates an aggressive evolutionary potential. In fact, cholesterol crystals are an indicator of periapical disease, their accumulation being consecutive to an inefficient endodontic therapy.

In our study, we had selected, since the collecting phase, different types of smears – sampling error. To reduce misinterpretation and screening errors, and we also used intraoperative imprint cytology. False negative results may appear in the case of hypocellular smears due to a poor technique FNA or intraoperative imprint cytology or in the case of intense hemorrhagic lesions. Table 2 summarizes the results of cytodiagnosis.

The diagnostic value of TEM was confirmed by a positive diagnosis of HP subtype of cystic lesions. Thus, by the morphological methods of cellular structure identification, TEM, due to its superior resolution, allowed us to localize to an ultrastructural level the following substrates (Table 3).

We frequently found in the cytoplasm of fibroblasts intracellular tropocollagen, some of them included in electron clear vacuoles with or without keeping the periodicity of the characteristic. These ones represent temporal sequences of intracellular collagen degradation (Figures 11 and 12).

Odontogenic cells are inactive (Figures 13 and 14), without aspects of division, corresponding immunohistochemically to a Ki67 index of 0% in the walls of radicular and dentigerous odontogenic cysts, suggestive for the phase of morphological stabilization of the cyst that became symptomatic. The absence of nervous filets or their lysis in the thickness of the cystic wall suggests a painless, latent increasing of the cyst, even in the presence of activated inflammatory cells.
Table 2 – Results of cytodiagnosis

| Cyto-diagnosis guide | Histodiagnosis                                | Cellularity | Dimension groups epithelial cells | Cellular arrangement | No. of isolated epithelial cells | No. of cases |
|----------------------|-----------------------------------------------|-------------|-----------------------------------|----------------------|---------------------------------|--------------|
|                      |                                               | grade I     | grade II                          | grade III            | small  | big  | mixed | simple | complex | grade I | grade II | grade III |            |
| Benign smear          | Inactive odontogenic cyst                     | 1           | 0                                 | 0                    | 1      | 0    | 0     | 1      | 0       | 0       | 1           | 0           | 18          |
| Benign smear          | Odontogenic cyst with marked inflammation     | 0           | 0                                 | 1                    | 1      | 1    | 0     | 1      | 0       | 0       | 1           | 0           | 27          |
| Benign smear          | Active odontogenic cyst with ameloblastic potential | 0         | 1                                 | 1                    | 1      | 1    | 0     | 1      | 1       | 1       | 1           | 0           | 30          |
| Benign smear          | Inactive odontogenic cyst in remission        | 1           | 0                                 | 0                    | 1      | 1    | 0     | 1      | 0       | 0       | 1           | 0           | 18          |
| Benign smear          | Nonodontogenic cyst                           | 0           | 1                                 | 0                    | 0      | 0    | 0     | 0      | 1       | 0       | 1           | 0           | 6           |
| Benign smear          | Pseudocysts                                   | 0           | 1                                 | 0                    | 0      | 0    | 0     | 0      | 1       | 0       | 0           | 0           | 3           |
| Benign smear          | Mesenchymal benign tumors                     | 1           | 1                                 | 0                    | 0      | 0    | 0     | 1      | 0       | 0       | 0           | 0           | 15          |
| Malignant smear       | Neoplasms and other maxillary bone lesions    | 1           | 1                                 | 0                    | 0      | 1    | 1     | 0      | 1       | 1       | 0           | 1           | 6           |

Table 3 – Aspects of cells commonly found in TEM in the walls of maxillary odontogenic cysts

| Morphological substrate | Fibroblast | Benign odontogenic epithelial cell | Macrophage |
|-------------------------|------------|-----------------------------------|------------|
| Nucleus                 | Non-crenellated with numerous nuclear pores  | Indented   | Non-crenellated or irregular consequence of morphofunctional differentiation – cell invaginations | Scintillation |
| RER cisternae           | Numerous parallel                            | Numerous   | Depending on the morphofunctional differentiation |
| Golgi sacs              | Dilated numerous                             | Dilated    | Numerous |
| Microtubules            | Non-oriented                                  | Non-oriented | Numerous depending on the morphofunctional differentiation |
| Intracellular fibrillar collagen | Present “Intracellular collagen profiles” | Present | Present endocytic material |
| Cellular invaginations  | Present                                        | Absent     | Inconstant |
| Solitary cilium toward matrix | Sometimes present                           | Absent     | Inconstant |
| Intercellular contacts  | Gap Desmosomes Zonula adherens and zonula occludens | Gap Desmosomes Zonula adherens and zonula occludens | Present with other inclusions (lipids, hemosiderinic pigment) |
| Inclusions of glycogen  | Present Poorly represented in the cells of the active proliferative group | Present Poorly represented in the cells of the active proliferative group | Present with other inclusions (lipids, hemosiderinic pigment) |
| Basal lamina            | Film stress                                    | Fragmented | Absent |
| Lysosomes               | Numerous Inconstant diffusely scattered       | Numerous autophagosomes |

RER: Rough endoplasmic reticulum; TEM: Transmission electron microscopy.

Figure 11 – Fibroblast indented nucleus, apparently bilobed in section, euchromatic, active and Golgi area, secretory vesicles and endocytosed fibrillar material (TEM, ×40,000).

Figure 12 – Appearance of a fibroblast surrounded by collinear “stress” fibers, interconnected with extracellular filaments via fibronexus in the conjunctival matrix of an inflammatory odontogenic cyst (TEM, ×40,000).
Discussions
The recognition and selection of embryonary remnants from odontogenesis is decisive for the HP diagnosis of maxillary cysts, allowing the clinician to monitor the treatment or to elaborate the evolutive prognosis perspectives of odontogenic and tumor cystic lesions [7–10]. The integrative results of our study identified the following parameters considered risks of recurrence: epithelial clear cell nests and islands, glycogen well represented and extended in the connective tissue; multilocular type of cyst; compartmentalization of the cavity cyst; multipolar origin of cystic lesions; mucous, apocrine, ciliated metaplasia of the epithelium (for mucoepidermoid carcinoma, ameloblastoma); epithelium parakeratinization (odontogenic keratocyst); basal buds; solid epithelial proliferations.

Some etiological factors seem to continue their cystic formative action even after the removal of the initial lesion [2, 4, 8, 11]. After complete tooth development, epithelial rests from odontogenesis remain inactive for an undetermined period. As soon as they are stimulated by factors still incompletely elucidated, these remnants of tissue proliferate and initiate the formation of epithelial lining epithelium of cysts.

Medical literature [12] brings extradata in IHC studies on tissue differentiation in the cystic wall and anticipates the evolution of cystic lesion expansion, by identifying some matrix molecules (fibronectin, tenascin, syndecan, etc.) and some growth factors (neurotrophins, endothelial origin of cystic lesions; mucous, apocrine, ciliated metaplasia of the epithelium (for mucoepidermoid carcinoma, ameloblastoma); epithelium parakeratinization (odontogenic keratocyst); basal buds; solid epithelial proliferations.

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Recent HP and cytogenetical studies cannot certainly precise which epithelial rests are more susceptible to risk of proliferation, migration and thus constitution of the cyst lining, whenever are studied either Malassez rests from which derive periapical (radicular) cysts or the reduced epithelium of enamel, which is at the origin of dentigerous cysts and cyst eruption, or rests of dental lamina (Serres rests) that are at the origin of odontogenic keratocysts, lateral periodontal, gingiva to the adult or of dental lamina to the newborn, of glandular odontogenic cyst [14].

Conclusions

Various classifications of cystic lesions of the maxillary bone point to the necessity of better knowledge of the origin and differentiation of tissue components of cysts structure. To sustain with certainty, the diagnosis of odontogenic cyst is needed clinical and radiological information convergent to the HP one. The HP aspects can be similar even to cystic lesions, which are differently classified. We can interpret that the expansion of the cystic wall depends on the imprinted tensioactive forces: the accumulation of necrotic cellular material in the lumen; proliferative activity of odontogenic epithelial rests and connective morphogenesis of the wall in which fibroblasts occupy the central place. These cells seem to have a high polarity of the migration activity, which transforms them by modulation into myofibroblasts. Knowing the morphological substrates involved in the formation and growth of cystic walls, we could speculate about the introduction of innovative therapy, complementary to surgical enucleation of inflammatory and developmental odontogenic maxillary cysts.

Institutional Review Board statement

The study was conducted according to the Guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Emergency Clinical Municipal Hospital, Timișoara, Romania (Approval No. E-5947/29.03.2019).

Informed consent statement

Written informed consent has been obtained from the patients to publish this paper.

Data availability statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to patient confidentiality.

Conflict of interests

The authors declare that they have no conflict of interests.

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