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Short communication

Bacteriological findings in radicular cyst and keratocystic odontogenic tumour fluids from asymptomatic patients

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ABSTRACT

Objective: In this study the potential presence of bacteria in radicular cyst (RC) and keratocystic odontogenic tumour(KCOT) fluids from clinically asymptomatic patients was investigated.

Materials and methods: Cyst fluids were collected by needle aspiration from 16 patients with asymptomatic osteolytic lesions (10 RCs and 6 KCOTs) undergoing surgery. All samples were transferred into tubes containing pre-reduced transport medium, delivered to the microbiology laboratory and processed within 1 h. The cysts, surgically enucleated, were sent for standard histopathological examination. Cyst fluid samples were cultured on selective and differential media in anaerobic (for about 2 weeks) and aerobic (for 24–48 h) conditions to detect viable microorganisms. After incubation, the colonies were counted, Gram-stained and identified by biochemical tests.

Results: Cultures were positive for the presence of bacteria in 15 (9 RCs, 6 KCOTs) out of 16 cases. RCs and KCOTs generally yielded low bacterial counts (10^2–10^4 CFU/ml) and were predominantly colonized by obligate anaerobes (64%), whereas less commonly by facultative anaerobes (36%). No significant differences in the detection frequencies of obligate and facultative anaerobes were evidenced between RCs and KCOTs. *Propionibacterium acnes* was the most common obligate anaerobe recovered both in RC and KCOT fluids. Among facultative anaerobes, *Gemella morbillorum* was more frequently isolated in KCOTs, whereas *Staphylococcus* spp. in RCs.

Conclusions: Bacteria may be present and persist within fluids of clinically asymptomatic jaw cystic lesions. The influence of bacteria and latent bacterial infection within cystic jaw lesions should be reconsidered in odontogenic cyst progression.

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[1] He passed away on 30 September 2012. He left us in a whisper. He left us here to cry... but his ideas still live with us.

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1. Introduction

Odontogenic cysts of the jaws include various pathological entities, all arising from the epithelial residues of the tooth-forming organ. These lesions have been traditionally classified into two groups: developmental or non-inflammatory cysts (''odontogenic keratocysts'', dentigerous, gingival cysts, *etc.*) and inflammatory cysts (radicular, residual, paradental cysts). Among them, radicular cysts (RCs) and ''odontogenic keratocysts'' draw special attention. RCs are the most common bone-destroying lesions and are associated with the root apex of a tooth with necrotic pulp. They comprise about 56% of all the cysts affecting the human jaws, followed by dentigerous and ''odontogenic keratocysts''.[1,2] The inflammatory dental periapical granuloma is considered to be the origin of RC formation. Humoral and cell-mediated reactions have been implicated in RC pathogenesis, whereas inflammatory cytokines in the proliferation of epithelial cell rests.[3,4] On the other hand, ''odontogenic keratocysts'' are benign uni- or multicystic intraosseous neoplastic lesions with the potential for aggressive, infiltrative behaviour and genetic factors are thought to play a major role in their aetiology.[5] They comprise about 11% of all jaw cysts and are characterized by high recurrence rate (range 3–60%) and association with nevoid basal cell carcinoma syndrome.[2] The World Health Organization in 2005, based on behaviour, histology, and genetics, reclassified ''odontogenic keratocysts'' with parakeratinized stratified squamous epithelium as keratocystic odontogenic tumours (KCOTs).[6] Through the years, there have been conflicting reports regarding the presence of microorganisms in odontogenic cysts and their role in cyst pathogenesis. Some authors suggest that bacteria are not found in RCs and do not normally penetrate into the lumen, unless as a result of a secondary infection.[7,8] Through analysis of fluids and explants media from RCs and KCOTs without any evidence of overt infection, Meghji et al. found higher level of endotoxin in RCs than in KCOTs, but no obligate or facultative anaerobic bacteria within cyst fluids.[7] In histological analysis of bacterial status in root-filled teeth with osteolytic lesions exposed to oral environment, Ricucci and Bergenholz found bacteria in the pulp canals and in the immediate region of the apical foramena, but not in the cyst walls or lamina.[8] However, other studies have shown that infected odontogenic cysts do indeed contain microorganisms and may not be sterile.[9,10] In a microbiological study of the fluids of infected jaw cysts, predominantly radicular and residual, Iatrou and co-workers[9] reported that approximately 89% of bacteria isolated from the fluid of infected cysts were obligate anaerobes, while only 10% corresponded to aerobes or facultative anaerobes. Gram positive anaerobic cocci were the most frequent bacterial group, followed by Gram negative anaerobic rods and aerobic cocci. Since no exhaustive results are available in this research-area, we investigated the potential presence of bacteria in cystic fluid from non-ruptured RCs by comparing the results with corresponding findings in non-inflammatory lesions like KCOTs, to give quantitative and qualitative information on the microorganism content within the fluids of clinically asymptomatic cystic lesions of the jaws.

2. Materials and methods

2.1. Patients

The study enrolled 16 patients with asymptomatic osteolytic lesions (6 females and 10 males, 34–82 years of age), which were clinically and radiographically provisionally diagnosed as odontogenic cysts during routine examination at the Division of Maxillofacial Surgery, Head and Neck Department, University of Torino, Turin, Italy. All patients were in general good health, without any clinical sign or symptoms of infection (pain, fever, swelling) at the time of surgery. Age, sex, type of cyst lesions, lesion maximum diameter, and lesion site of enrolled patients were recorded. Patients participating in this study gave their informed written consent.

2.2. Cyst fluids

Cyst fluids from 16 non-ruptured odontogenic cysts were aspirated (other samples contaminated with blood were not enrolled in the study). Initial local disinfection of oral mucosa with chlorexidin was performed before each surgical intervention. Surgical interventions were performed under local anaesthesia with two 1.8-ml capsules of 2% mepivacaine containing 1:100,000 adrenaline. A buccal envelope mucoperiosteal flap was raised in all cases. A further local disinfection of the exposed bone was performed by chlorexidine and new sterile surgical instruments were used hereafter, in order to avoid possible contamination. Then, the osteotomy necessary to visualize the cystic lesion was performed using a no. 8 tungsten carbide round bur mounted on a high-speed handpiece, paying attention not to damage the cystic walls. The integrity of the cystic walls was checked in each case: none of the 16 studied cysts had damaged walls. During oral surgery practice various cysts had damaged or perforated cyst walls, but they were not enrolled in the study, as they did not meet the inclusion criteria.

The cystic fluid was withdrawn by a sterile syringe through the cyst walls, paying attention not to get in contact with the bone or with eventually protruding tooth apexes. The aspirate was injected, without introducing air, in a sterile test tube with 1 ml of pre-reduced transport medium (BBL™ Port-A-Cul™, Becton Dickinson Italia S.p.a., BD, Buccinasco, Milan, Italy) to ensure specimen viability, delivered to the microbiology laboratory and processed within 1 h after collection. The quantity of aspirate was recorded: the aspirated volume ranged from 0.5 to 1.5 ml. Finally, cysts were totally enucleated and sent for histopathological examination.

2.3. Histopathological analysis

Histopathological examination of the specimens was performed by haematoxylin–eosin staining. Criteria of diagnosis of RCs were defined as the presence of multilayered nonkeratinized squamous epithelium, whereas KCOTs were diagnosed when a characteristic lining of parakeratinized stratified squamous epithelium (eventually together with thickened squamous epithelium, daughter cysts, and budding proliferation of the epithelium) was found.