cellular proliferation markers in ameloblastomas (AMs) and unicystic ameloblastomas (UAMs).

Findings: Immunohistochemical and western blotting results determine that for both variants, AM and UAM, the Label index (Li) showed a major value for MCM6 protein, followed by MCM5, MCM4 and lastly by Ki67 expression (p value <0.05). The immunoexpression of Ki67 and MCM5 was exclusively nuclear in basal tumoral cells of both variants. On the other hand, MCM4 and 6 were located in the nucleus and cytoplasm of basal and columnar epithelial cells and those that resemble the stellar reticulum. There were no significant differences in the results between the AM and UAM.

Conclusions: Results suggest that MCM5 protein could be a good proliferation marker, with greater sensitivity in comparison with Ki67. Moreover, MCM markers could be used to predict AM and UAM cell proliferation. Further studies with the inclusion of others odontogenic tumors are necessary to confirm the real potential of MCM proteins, more specifically MCM5.

ASSOCIATION OF MAPK/ERK PATHWAY ACTIVATION WITH KRAS MUTATIONS IN ADENOMATOID ODONTOGENIC TUMORS.

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Objectives: Adenomatoid odontogenic tumors (AOT) are benign tumors derived from odontogenic epithelium, and they account for 2-7% of all odontogenic tumors. Intraosseous AOTs are thought to be associated with unerupted permanent teeth, although their pathogenesis is still unclear.KRAS mutation, which is involved in the pathogenesis of some malignant tumors as driver mutation, was recently detected in AOT suggesting its association with tumorigenesis.The aim of this study was to assess the frequency of KRAS mutation and his association with the presence of the MAPK / ERK signaling pathway proteins.

Finding: Paraffin-embedded tissue samples from 9 AOT patients (3-47 years old, mean 24.7 years) were obtained for this study. Genomic DNA was extracted from each sample, and in one case, genetic mutations in 50 cancer-associated genes were examined by next-generation sequencing. A KRAS G12D missense mutation was detected in the DNA sequence of the tumor cells, but it was not detected in that of the stroma tissue. Based on this result, hotspot mutations in the RAS family were analyzed by PCR-rSSO using the remaining 8 cases. KRAS G12V and KRAS G12R mutations were detected in 2 and 4 cases, respectively. Subsequently, in the paraffin blocks, immunohistochemistry was performed to visualize the presence of the proteins involved in the MAPK / ERK signaling pathway. All the cases were EGFR, KRAS,CRAF, BRAF positive, one case was ERK negative, and one case was MEK and ERK negative, all the other remaining cases were MEK and ERK positive.

Conclusions: In conclusion, KRAS mutation was frequently detected in AOT, suggesting its association with tumorigenesis of AOT. However, since EGFR was positive, how the mutation affects the tumor development is still unclear.

CLINICOPATHOLOGICAL SIGNIFICANCE OF EXPRESSION OF MIR-26A, MIR-107, MIR-125B AND MIR-203 IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS.

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Objectives: MicroRNAs play an important role in the development and progression of head and neck squamous cell carcinomas (HNSCC). In the current study, we compared the expression levels of microRNAs in primary HNSCC with and without cervical lymph node metastasis and determined their clinicopathological significance. The expression levels of miR-26a, miR-107, miR-125b and miR-203 in primary HNSCC with cervical lymph node metastasis (n=16), and their matched lymph node metastasis, and primary tumors without metastasis (n=16) were determined by quantitative RT-PCR. Furthermore, we evaluated the association of those microRNAs with clinicopathological features and survival of patients with HNSCC.

Findings: miR-26a ($p<0.05$) and miR-125b ($p<0.01$) expression levels were significantly higher in primary HNSCC with lymph node metastasis than in tumors without metastasis, while that the levels of miR-203 ($p<0.01$) were significantly lower in the metastatic tumors. Compared with matched metastatic lymph node tissues, miR-125b ($p<0.01$) exhibited a significantly lower expression and miR-203 ($p<0.01$) demonstrated higher expression in the primary tumors. The expression of the microRNAs was associated with various HNSCC clinicopathological risk features, including miR-26a high expression and N stage (p=0.04), poor histological differentiation of tumors (p=0.005) and recurrence (p=0.007), miR-125b high expression and N stage (p=0.0005) and death (p=0.02), and low levels of miR-203 and N stage (p=0.04). Importantly, high expression of miR-26a was significantly associated with shortened disease-free survival (disease relapse) and high miR-125b levels was an independent risk factor for poor disease-specific survival patients with HNSCC.

Conclusion: These findings suggest that miR-26a and miR-125b may be associated with progression and metastasis of HNSCC.

INFLUENCE OF RADIATION DOSE IN COLLAGEN IV AND MMP20 IMMUNOEXPRESSION AND THE TOOTH IMMEDIATE ADHESIVE PROPERTIES.

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Objectives: Radiation-related caries is an important collateral effect in patients with head and neck cancer subjected to radiotherapy, with rapidly progressive, asymptomatic and ample lesions, associated to direct and indirect effects of radiation. The present study has the aim of determine the alterations of the immunoexpression of collagen IV and MMP20 in the amelodentin junction and its relationships with odontoblasts according to the radiation dose (0, 20, 40, and 70Gy) and its influence on the

immediate adhesive properties (μTBS and μSBS) of enamel and dentin. The immunoexpression was performed with immunofluorescence confocal microscopic analysis.
Forty caries-free extracted third molars were divided into eight groups according to the factors: radiation dose (0, 20, 40, and 70Gy) for the experiments. Data from immunofluorescence was analyzed descriptively and adhesive properties were analyzed using two-way ANOVA and Tukeys test.

Findings: The alterations in the immunoexpresion of collagen IV and MMP20 is directly associated with the dose of radiation, showing increasing levels of MMP20 and decreasing levels of collagen IV in the most irradiated teeth.When radiation doses were applied between 40 to 70 Gy, the adhesive values were significantly lower for both strategies in the two tests performed.

Conclusion: High doses of radiation above 40 Gy affect the expression of collagenIV, MMP20 and immediate adhesive proprieties on dentin and enamel. The information obtained about the altered expression of collagen IV and MMP20, and the adhesive properties in dental irradiated tissue is crucial to understand the process of radiation-related caries and the restorative treatment of these patients.

THE PARARADICULAR RADIOLUCENCY WITH VIABLE PULP: CLINICOPATHOLOGIC FEATURES OF 21 CEMENTAL TEARS.

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Cemental tear is considered to be rare, with few case reports and no large series published.

Objective: To investigate and characterize the disease from a review of 21 new cases.

Methods: This was a retrospective review of consecutive cases collected from patient panels of the investigators.

Results: Twenty-one cases were identified during a 6 year period. All lesions presented with pain. Nineteen were vertical radiolucencies along the root of a vital or endodontically treated tooth; the remaining 2 were periapical only. Radiolucencies were: D-shaped (40%); thin regular lines (25%); thick, irregular lines (15%); J-shaped (15%). All showed focal destruction of the lamina dura, with 66.7% showing extension into the medullary bone. Maxillary incisors were most often (46.2%) affected. Histopathologic diagnoses were chronic fibrosing osteomyelitis (76.2%) or intramedullary fibrous scar (23.8%), all associated with embedded cementum fragments. Five associated teeth were also examined: all showed tears beneath remaining cementum. Four cases were successfully treated with curettage; endodontic therapy was mistakenly performed in 8 cases.

Conclusions: Cemental tears produced symptomatic, localized chronic inflammation characterized by a vertical radiolucency adjacent to a root. These lesions may not be as rare as previously thought and extraction may not be the best treatment.

A PILOT STUDY OF SELECT CELL CYCLE MARKERS IN GLANDULAR ODONTOGENIC CYSTS.

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Objectives: Glandular odontogenic cysts (GOCs) and dentigerous cysts (DCs) differ significantly in their biologic behavior. One third of GOCs have been reported to recur whereas recurrence is rare in DCs. Due to the apparent growth potential of GOCs, we evaluated and compared the presence of cell cycle markers such as cyclin D1, p53, p16, p27, Rb, and BCL-2.

Findings: Eight GOCs and a control group of three DCs were included in the pilot study. All GOCs possessed seven or more of the required features. Interestingly, we detected strong expression of Cyclin D1, a regulatory protein required for cell cycle progression, within the basilar and parabasilar layers of the cyst epithelium for GOCs and scattered positivity correlating with the level of inflammation in DCs. Expression of tumor suppressor proteins, p27 and p16, were notably different between the two cysts. For p16, the superficial layers were strongly and diffusely positive in GOCs while the basilar and parabasilar layers were essentially negative. DCs showed a patternless distribtion of p16 staining with variable intensity throughout the epithelium. The majority GOCs exhibited full thickness expression of p27 whereas DCs demonstrated scattered and weak positivity. With p53, BCl-2, and Rb, minimal appreciable difference was noted in the staining pattern and intensity between GOCs and DCs.

Conclusions: Our results revealed differential staining patterns between DCs and GOCs for the following cell cycle markers: Cyclin D1, p16, p27. Based on our staining pattern, we also hypothesize that the proliferation potential of the basal and parabasilar layers of the epithelium in particular contribute to the growth and high recurrence rate for GOCs. Our findings suggest that cell cycle disturbances exist in GOCs and may contribute to the aggressiveness of their biological behavior. Additional studies with an expanded cohort are required to confirm these initial findings and provide further insights.

MINIMALLY-INVASIVE ORAL EXFOLIATED CELLS STUDY FOR PREMALIGNANT LESIONS USING RAMAN MICROSPECTROSCOPY.

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Squamous cell carcinoma of the oral cavity ranks as the 15th most common cancer in the world and the 10th most frequent cancer in males. The present study was undertaken for the development of new methods for early oral cancer detection based on Raman microspectroscopy of exfoliated cells. Exfoliated oral cells were collected by brush biopsy from patients attending Dublin Dental Hospital Dysplasia Clinic (25) and from healthy volunteers (25). Samples of exfoliated cells from normal mucosa and from pre-malignant lesions were collected using an endocervical cytobrush and placed in ThinPrep vials. Slides were prepared using the Thinprep2000 processor with the aim of forming a monolayer of cells for analysis. Raman spectra were acquired from the nucleus and cytoplasm of each cell using an XploRA confocal Raman instrument (HORIBA JobinYvon). As source, a 532 nm laser was focused by a 100X objective onto the sample and the resultant Raman signals were acquired in the 400 to 1800 cm−1 region. Glass spectral contamination was removed using extended multiplicative signal correction Following pre-processing, spectra were subjected to principal component analysis