Keratinizing odontogenic cysts with a spectrum of verrucoid morphology: investigation of a potential role of human papillomavirus

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Objective. The role of human papillomavirus (HPV) in keratinizing odontogenic cysts (OC) has only rarely been studied. We describe the clinicopathologic findings in a series of OCs that had unusual keratinization patterns and were investigated for a possible HPV etiology.

Study Design. Tissue samples from 29 patients with keratinizing OCs were studied for light microscopic features suggestive of HPV infection and by an HPV DNA polymerase chain reaction assay.

Results. The mean age at presentation was 31.1 years; 79.3% of the OCs occurred in the mandible and 46.4% were associated with an impacted tooth. The phenotypic characteristics koilocytes, hypergranulosis, and a verrucous pattern of the cyst-lining epithelium were observed in 69%, 62.1%, and 17.2% of cases, respectively. These histomorphologic features did not, however, correlate with HPV infection.

Conclusions. HPV does not appear to play a role in keratinizing OCs and is not responsible for the wart-like histomorphologic features that may be seen in these lesions. (Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122:625-630)

The orthokeratinized odontogenic cyst (OOC) is recognized as an entity distinct from the keratocystic odontogenic tumor (KCOT).[1] Since its initial description in 1981,[2] more than 100 cases have been reported, and several reviews have been documented. Radiologically, OOCs present with no features that distinguish them from any other developmental or inflammatory odontogenic cyst.[3] It is, however, the histologic features of the OOC that set this particular cyst apart from the other cysts of the jaw. The stratified squamous epithelial lining of the OOC is typically thin and uniform, often with a prominent stratum granulosum.[1] The orthokeratinized surface layers may be relatively thick, with an onion skin–like appearance.[4] The basal cells show little tendency to palisade or polarize.[1,4] Several authors have placed emphasis on the basal cell features rather than on the pattern of keratinization when differentiating between KCOT and OOC.[5,6] Mature fibrous connective tissue constitutes the OOC wall, which, unless secondarily infected, is devoid of inflammation.[2,4] The histologic picture of the OOC bears a striking resemblance to extragnathic orthokeratinizing cysts, such as epidermoid cysts and middle ear cholesteatomas.[7,8] OOCs also share histologic similarities with flat viral warts (verruca plana), which are characterized by hyperkeratosis, slight or no papillomatosis, hypergranulosis, and vacuolated keratinocytes with perinuclear clearing.[9]

Over the last 2 decades, keratinizing cysts from various sites have been rigorously studied for verrucous or wart virus–associated changes, with some studies suggesting a role for human papillomavirus (HPV) in their development.[8,10,11] In addition, there are a few reports in the literature on keratinizing odontogenic cysts (OCs) with a verrucous pattern.[12-14] Two of the reported cases were studied for a possible association with HPV.[12,14] The purpose of this article is to describe the clinicopathologic findings in a series of keratinizing OCs, which were specifically analyzed for the presence of a verrucous pattern of the lining epithelium. We also sought to investigate the presence of HPV DNA within these cysts.

MATERIALS AND METHODS

Study sample

Twenty-nine cases, coded as orthokeratinized jaw cysts, were retrieved from the archives of the Department of Oral Pathology at the University of the Witwatersrand (Johannesburg, South Africa), during the period

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Statement of Clinical Relevance

Verrucoid cystic epithelium may be encountered in odontogenic cysts (OCs). Our data suggest that although some keratinizing OCs may histologically mimic the architecture of verruca, this histomorphologic appearance is not associated with human papillomavirus in OCs.

1975-2011. Patient age and sex, the anatomic location of the cyst, the presence or absence of an associated impacted tooth, the provisional diagnosis of the lesion based on the clinical and radiographic findings, and the treatment that was undertaken were determined, whenever possible, from surgical histopathology reports. Ethics approval for this study was granted by the Human Research Ethics Committee of the University of the Witwatersrand (Protocol number: M120956).

Histopathologic study

For light microscopy, 5-μm sections were cut from the formalin-fixed paraffin-embedded (FFPE) tissue blocks and stained with hematoxylin and eosin. The cyst linings were reviewed for the type of keratinization present (orthokeratin or parakeratin) and for the characteristics of the basal cells. For inclusion in this study, either pattern of keratinization was accepted, provided that the cyst linings did not exhibit a polarized layer of hyperchromatic basal cells with a palisaded or picket-fence appearance that is characteristic of the KCOT. The cyst linings were examined for the presence or absence of the following features: hyperkeratosis (orthokeratosis, parakeratosis, and/or verruciform hyperkeratosis), hypergranulosis, and vacuolated keratinocytes with perinuclear clearing. *Verruciform hyperkeratosis* was defined as hyperplastic squamous epithelium with a papillomatous surface or cyst lining, exhibiting hyperkeratotic pointed projections and involving at least one-third of the cyst lining examined. Descriptive statistics were used and data were analyzed in Statistica version 7.1 (StatSoft, Inc., Tulsa, OK). A significance level of $P = .05$ was used.

Polymerase chain reaction

Two to four sections, each 10 μm in thickness, were prepared from each FFPE sample. These sections were deparaffinized using 1 mL xylene and subsequently were treated with 1 mL ethanol. After centrifugation, DNA was extracted from the samples using the DNA Micro QIAamp kit (Qiagen, Valencia, CA), according to the manufacturer's protocols. HPV amplification with GP5+/6+ primers for the L1 conserved region (GP5+: 5′-TTT GTT ACT GTG GTA GAT ACT AC-3′; GP6+: 5′-GAA AAA TAA ACT GTA AAT CAT ATT C-3′) was made in a reaction mix containing 5 μL of template DNA, 200 μM dNTPs (deoxyribonucleotide triphosphates; Roche Diagnostics, Mannheim, Germany), 0.2 μM of each primer, 1.0 U Taq DNA polymerase (Roche Diagnostics), 10× reaction buffer (with $MgCl_2$, 15 mM) in a total volume of 50 μL. The thermal conditions of amplification were as follows: initial denaturation at 95°C for 4 minutes and subsequently 40 cycles at 95°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. An additional extension at 72°C for 5 minutes was performed. Polymerase chain reaction (PCR) analysis was carried out using the 9700 Gene Amp PCR System (Life Technologies, Grand Island, New York, NY).

The extraction procedure was assessed by PCR amplification of the ß-globin gene. The quantity and quality of DNA extracted was determined using the Nanodrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA). This yielded an average DNA concentration of 69.9 ± 83.6 ng/μL (−6.8 to 359.8 ng/μL). Of the 29 odontogenic cyst samples, 19 samples yielded a DNA concentration higher than 20 ng/μL.

Real-time (RT) amplification of the ß-globin gene was performed using a Corbett Research Rotorgene 6000 (Qiagen) RT-PCR machine, using the SensiMix SYBR No-Rox kit (Bioline, Taunton, MA). A final volume of 20 μL reaction mix was made using 0.2 mM of each primer, 10 μL 2× SensiMix SYBR No-Rox Master Mix (with magnesium chloride, 50 mM) and 2 μL of template DNA. The thermal cycling profile of this assay consisted of an initial denaturation step at 95°C for 10 minutes, followed by 50 cycles of 95°C for 10 seconds, 55°C for 10 seconds, and 72°C for 15 seconds. After amplification, melt curve analysis was carried out at 95°C with a ramp rate of 1°C per 5 seconds. The average melting temperature (T_m) of the β-globin amplicon is 85.5 ± 1.0°C. Randomized RT-PCR products were run on a gel to confirm the presence of the ß-globin (PC04/GH20) (PC04: 5′-CAA CTT CAT CCA CGT TCA CC-3′; GH20: 5′-GAA GAG CCA AGG ACA GGT AC-3′) PCR fragment.

Amplified PCR products were examined by agarose gel electrophoresis. Samples were electrophoresed for 1 hour at 100 V by using a 3% agarose gel (Bioline) in TAE buffer (Invitrogen, Grand Island, NY) and stained with ethidium bromide (Merck, Kenilworth, NJ). The gel was visualized under ultraviolet light. A 50 base pair (bp) DNA ladder (Thermo Scientific) was used to determine the molecular size of the PCR products. Positive HPV samples and the β-globin internal control appeared as a visible band with a molecular size of approximately 150 bp and 268 bp, respectively. Two FFPE tissue blocks of cervical carcinomas that had previously tested positive with HPV PCR were used for the positive control. A blank wax block served as a negative control from the initial DNA extraction to the endpoint of the PCR assay. A second negative control included a no-template control, in which nuclease-free water was substituted as a template.

RESULTS

Keratinizing OCs in this study showed a male predominance, with 20 cases occurring in males and 9 in females, yielding a 2.2:1 male:female ratio. The overall mean age at presentation was 31.1 ± 13.1 years