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Evaluation of Frequency of Human Papilloma Virus DNA in Odontogenic Cysts

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Abstract

Aim: The aim of this study was to determine the prevalence of HPV in odontogenic cysts. Materials & Methods: The DNA of 85 samples of odontogenic cysts consisted of odontogenic keratocyst (OKC), dentigerous cyst (DC), calcifying odontogenic cyst (COC) and periapical cyst were extracted. Amplification of HPV genes were performed using GP5+/GP6+ primers. The results were statistically analyzed using chi-square test. Results: Three samples (15%) of periapical cysts, 4 samples (20%) of OKCs, 5 samples (25%) of DCs and 7 samples (28%) of COCs (9.09% of type Ia, none of type Ib, 66.6% of type Ic and 50% of type II) were HPV-positive. Statistical analysis showed that the frequency of HPV DNA was significantly different only between type Ia and Ib of COC samples. Conclusion: The high prevalence of HPVs in odontogenic cysts proposed that these microorganisms may have a potential role in the pathogenesis of odontogenic lesions. Clinical significance: Recognition of etiologic and predisposing factors of odontegenic lesions could introduce new approaches in the management of these lesions. Due to the possible role of HPV in the pathogenesis of odontogenic lesions, antiviral therapy has the potential to be considered as an adjunctive treatment to reduce the recurrence rate after surgical procedure.

Keywords: HPV, DNA, Odontogenic Cysts.

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Introduction

Human Papilloma Virus (HPV) family consists of 200 DNA viruses that is present as a natural flora in healthy mucosa. It is suspected that few of these viruses have the potential to cause malignant changes (Dalgleish, 1991). HPV DNA has been detected in healthy saliva (Baumforth et al., 1999). Both mucosal and dermal HPVs can contaminate the epithelium of the upper respiratory and digestive tract and transmission of this virus through saliva or cross-infection is probable (Praetorius, 1997). Both low and high risk viruses could be found in HPV family which associated with benign epithelial proliferation or malignant changes (Melrose, 1999). HPV seems to play a role in the pathogenesis of tumors and malignancies through induction of oncoproteins expression and making the genome unstable (Münger et al., 1992), but the role of HPV in the development of pre-malignant lesions and oral squamous cell carcinoma (SCC) is still under debate (Clifford et al., 2003). E6 and E7 onco-proteins of HPV make malignant changes by inducing the genomic instability or other possible mechanisms (Paget, 1854). Several theories have been proposed about the etiology of cysts and odontogenic tumors. Factors such as trauma, tooth extraction, infection, history of root canal treatment and genetic factors seem to be effective in the etiopathogenesis of these lesions (Stoelinga, 1988). Over the past two decades, a number of studies have shown the association between HPV and the development of neoplastic epithelial lesions (Anneroth G, Johansson, 1985; Tsuchiya et al., 1991; Ostwald et al., 1994). Studies on the role of polymavirus in the development of ameloblastoma have been described the role of viruses in the pathogenesis of odontogenic cysts and tumors (Gollard, Slavkin and Snead, 1992). Further studies evaluated the presence of HPV in ameloblastoma (Migaldi et al., 2005) and odontogenic keratocyst (Hong, Ellis and

Hartman, 1991). These studies had controversial results about the presence and the role of these viruses in the development of odontogenic lesions. Evidences seem inadequate to reveal the role of HPV in the pathogenesis of odontogenic lesions and also the mechanisms underlying this association. The aim of this study was to determine the prevalence of HPV in odontogenic cysts.

Materials and Methods

Sample collection

A total of 85 odontogenic cysts were studied. These samples consisted of odontogenic keratocyst (OKC), dentigerous cyst (DC), calcifying odontogenic cyst (COC) and periapical cyst. All samples used in this study were formalin fixed parafine embedded (FFPE) from department of pathology of Mashhad dental school. All of the samples were intra-bony and there was not any peripheral lesion. The hematoxylin and eosin (H&E) stained slides were re-evaluated to confirm the diagnosis. Demographic data including age and gender were recorded for each sample.

DNA Extraction

Each FFPE sample was cut to a thickened slice. The samples were then deparaffinized by Xylen and hydrated again using reduced concentrations of ethanol and distilled water. After centrifugation, the DNA of samples was extracted using the Micro QIA Amp DNA kit (Qiagen, Stanford, CA, USA) based on the manufacturer's instructions.

Polymerase chain reaction (PCR) procedure

Amplification of HPV genes was performed using GP5+/GP6+ primers (Table 1). Temperature for amplification was as follows: primarily denaturation at 95 ° C for 1 minute, 55 ° C for 1 minute, 72 ° C for 5 minutes were repeated. This method is based on the movement of different single strand DNA on the polyacrylamide gel. After denaturation of double-strand DNA from PCR, single strand DNA was renatured again and through the placement of the intra-strand bases made three-dimensional structures. New three-dimensional structures move at different rates on the gel and form specific patterns of bands on the gel of electrophoresis. The β globin gene (PC04/GH20) was used as control. Also, to determine the quality of the extracted DNA, each sample was controlled by primers of albumin gene. Finally, a sample of COC was excluded due to the lack of DNA detection.

Electrophoresis

After the PCR process, DNA fragments were separated through their sizes. PCR-amplified products were electrophoresed using agarose gel. The samples were electrophoresed in 3% agarose gel and stained with ethidium bromide under a voltage of 100 mV. The gel electrophoresis was observed under ultraviolet light and the positive specimens appeared as a specific band with a molecular weight of 150 bp.

Results

Out of 85 lesions, 20 samples were periapical cysts, 20 samples were OKCs, 25 samples were COCs and 20 samples were DCs. Of 25 COCs studied histopathologically, 11 samples were type Ia, 4 samples were type Ib, 6 samples were type Ic and 4 samples were type II. Demographic data related to these lesions are illustrated in Table 2.

Of the 20 samples of periapical cysts examined in our study, 3 samples (15%) were HPV-positive. HPV also was found in 4 OKC samples (20%), 5 DC samples (25%) and 7 COC samples (28%). Regarding the COC histopathologic types, HPV DNA was detected in 9.09% of type Ia, none of type Ib, 66.6% of type Ic and 50% of type II. The chi-square test results showed that the frequency of HPV DNA was significantly different only between type Ia ($P = 0.01$) and Ib ($P = 0.03$) of COCs.

Discussion

In our study, 15% of periapical cyst and 25% of OKC samples were HPV positive. In the Alseagh et al. study, 36.8% of OKC samples were positive for HPV (Alsaegh and Zhu, 2014). However, according to Gonzales et al. study, among 83 OKC samples none of them were HPV positive (González-Moles et al., 2006). HPV DNA was detected in 15% of DCs. According to Alseagh et al. study, the HPV DNA was found in 25% of DCs (Alsaegh and Zhu, 2014). In our study, 24% of the COC samples were HPV positive. HPV DNA was detected in 18.18% of Ia type, 25% of Ib type and 16.6% of Ic type of COCs. The highest prevalence of HPV DNA (approximately 50%) was seen in Type II. The results of different studies regarding the presence of HPV and its role in odontogenic lesions were inconsistent. Some could not detect the HPV DNA (González-Moles et al., 2006; Cox, Eveson and Scully, 1991), while some others showed different genotypes of virus in odontogenic lesions by various methods (Namin et al., 2003; Kahn, 1989; Kahn, 1992; Heerden et al., 1993; Sand et al., 2000). These controversial results can be attributed to different sampling methods, samples quality and molecular methods. The behavior of maxillofacial lesions such as cystic and neoplastic changes induced by viruses may be affected by racial diversity and other epidemiologic characteristics (Dalgleish, 1991). Also different laboratory methods may be another source of controversial results. For example, in our study, GP5 + / GP6 + primers had high sensitivity and could detect low levels of microbial load and also very short sequences of DNA of the viruses in FFPE samples (Baumforth et al., 1999; Migaldi et al., 2005). With these primers there is no concern about the effect of fixation process on the viral DNA (Namin et al., 2003; Remmerbach et al., 2004). GP5 + / GP6 + primers could detect wide ranges of HPV genotypes including 6, 11, 13, 16, 18, 30, 31, 32, 33, 35, 39, 40, 43, 45, 51, 54, 55, 56, 59 and 66, while other primers had been used in previous studies only recognized few genotypes (Migaldi et al., 2005; Cox, Eveson and Scully, 1991; Namin et al., 2003; Kahn, 1992; Heerden et al., 1993). Using the high sensitive PCR primers may be a probable explanation for more HPV detection in our study compared to others (Alsaegh and Zhu, 2014; González-Moles et al., 2006; Cox, Eveson and Scully, 1991). Some studies proposed HPV as an etiologic or predisposing factor in the development of ameloblastoma and OKC (Namin et al., 2003; Kahn, 1992; Correnti et al., 2010), while some others suggested that HPV is a secondary infection caused by direct contact between the epithelium of tumor and the

oral mucosa or contamination with the flora of superficial mucosa during operation (Praetorius, 1997; Heerden et al., 193). The presence of HPV may be related to neoplastic changes, ameloblastomatosis of cysts or more aggressive behaviors. Several studies suggested the role of HPV in the pathogenesis of DC and the development of ameloblastoma from the wall of DC (Kahn, 1989; Correnti et al., 2010; Gillette and Weinmann, 1958). In the Alseagh et al. study (2014), HPV DNA was detected in 25% of DCs (Neville et al., 2015). Ameloblastomatosis changes were frequently seen in DC and COC. According to the higher prevalence of HPV in ameloblastoma compared to DC and COC, HPV proposed as a probable etiologic or predisposing factor for the development of ameloblastoma from these lesions. COX et al. study (Cox, Eveson and Scully, 1991) showed that the infection of differentiated cells with HPV could induce keratosis, neoplastic or cystic changes. The histological origin of DC is still unknown, but most authors proposed that DC originates from the dental follicle. Ben et al. (1996) introduced two types of DCs with developmental or inflammatory origin, which the inflammatory type causes by the infection of their primary predecessors. HPV considered as a possible source of dental follicle infection and it can replicate within the inflammated tooth follicle. Different mechanisms have been proposed to explain the pathway through which HPV infects the odontogenic cysts and tumors. Khan et al. suggested that HPV transmitted from vaginal flora during labor and could invade the primary enamel organ (Kahn et al., 1989). To explain how the virus transmitted from the bloodstream to the infected area, Bodaghi proposed that the virus could be able to reach the target area via mononuclear cells which were considered as HPV carriers (Bodaghi et al., 2005). After infection of enamel organ, HPV stimulates the release of growth factors or prevents the secretion of inhibitory factors (Kahn, 1989).

Despite this hypothesis, still many believe that the presence of HPV in odontogenic lesions is only a secondary infection that occurs due to the contact of the lesions with the patient's mucosa (Heerden et al., 1993; McNicol and Dodd, 1990). Recurrence is a prevalent complication after surgical removal of odontogenic cysts and tumors, especially for aggressive lesions such as ameloblastoma, OKC and COC. Regarding to the possible role of HPV in the pathogenesis of odontogenic lesions, antiviral therapy has the potential to be considered as an adjunctive treatment to reduce the recurrence rate after surgical procedure. However, further studies with larger sample size and detection of different HPV genotypes are need to identify the role of HPV in the development of odontogenic cysts and tumors.

Conclusion

Regarding to our results the prevalence of HPV was 15% in periapical cysts, 25% in OKCs, 15% in DCs, 24% in COCs (18.18% in type Ia, 25% in type Ib, 16.6 % in type Ic and 50% in type II). Statistical analysis showed that there was a statistically significant difference regarding to prevalence of HPV only between Ia,Ib,Ic types of COC samples, however this difference in comparison with the other groups was not statistically significant.

IHC is another diagnostic method which despite the lower sensitivity compared to PCR, provides additional information about the location of the viruses in the tissues. For future studies it is suggested that IHC and other molecular methods be used for HPV detection.

Clinical significance

Recognition of etiologic and predisposing factors of odontegenic lesions could introduce new approaches in the management of these lesions. Due to the possible role of HPV in the pathogenesis of odontogenic lesions, antiviral therapy has the potential to be considered as an adjunctive treatment to reduce the recurrence rate after surgical procedure. However, further studies with larger sample size and detection of different HPV genotypes need to identify the role of HPV in the development of odontogenic cysts and tumors.

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Table 1: Sequence of DNA primers of HPV (GP5+/GP6+) and hemoglobin β gene (PC04/GH20)

Primer	DNA sequence
GP5+	5’- TTT GTT ACT GTG GTA GAT ACT AC–3’
GP6+	5’- GAA AAA TAA ACT GTA AAT CAT ATT C –3’
PCO4	5'-CAA CTT CAT CCA CGT TCA CC-3'
GH20	5'-GAA GAG CCA AGG ACA GGT AC-3'

Table 2: Demographic data of different odontogenic lesions of the study

Odontogenic cysts	Number	Men	Women	Mean age
Periapical cyst	20	8	12	37.4
OKC	20	5	15	40.3
COC Ia	11	4	7	54.3
COC Ib	4	3	1	48.9
COC Ic	6	4	2	67.7
COC II	4	3	1	52.4
DC	20	15	5	39.8