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Detection of Human Papillomavirus DNA in Intraosseus Ameloblastoma

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Abstract: Human Papillomavirus (HPV) infection has been shown as a risk factor in oral carcinogenesis. The association between HPV and benign and malignant neoplasm of oral mucosa, especially surface epithelium-derived tumors, is well established. The role of HPV in pathogenesis of odontogenic cysts and tumors has been published in few articles. The aim of this study was detection of HPV in Iranian patients with intrabony ameloblastoma and investigation of specific risk factors associated with ameloblastoma. One hundred intrabony ameloblastoma and 50 age-sex matched samples as controls were evaluated by polymerase chain reaction for the detection and typing of HPV. Fisher exact and chi square tests were used to assess the data. HPV DNA was detected in 32% of patients and 10% of controls. HPV-6 was the most prevalent genotype (31.6%) in infected cases. It was followed by HPV-11 (12.5%), HPV-16 (12.5%) and HPV-31 (3.1%). We found a significant association between presence of HPV and location of tumor ($p = 0.02$), traumatic history ($p = 0.03$) and odontologic therapy ($p = 0.01$). These findings indicated that HPV-6 probably is one of the most important etiologic agents in causing intraosseous ameloblastoma in Iranian population.

Key words: Ameloblastoma, risk factors, PCR, human papillomavirus, Iran

Introduction

Ameloblastoma has been associated with the most complex group of pathogenic viruses. Eighty-five genotypes of HPV have been fully characterized (zur Hausen, 1999). The association between Human Papillomavirus (HPV) and development of benign and malignant oral neoplasm is well established (Bouda *et al.*, 2000; Summersgill *et al.*, 2000; Van Rensburg *et al.*, 1996; Miller *et al.*, 1996; Fornatora *et al.*, 1996; Dekmezian *et al.*, 1987). The role of HPV in oral cancer is inevitable and presumably HPV is a cofactor in 100% of patients who developed oral cancer in 4-12 years of age (Nielson *et al.*, 1996). The specific HPV types, most notably types 16, 18 and a few others, have been shown to cause the majority of cervical cancers and their high-grade precursor lesions (zur Hausen, 1999). Some types also seem to be responsible for other anogenital and a subset of head

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and neck cancers (zur Hausen, 2002). Association between HPV and odontogenic cysts and tumors has been studied in a few cases (Sand *et al.*, 2000). Particularly, types 6, 11, 16 and 18 have been detected in peripheral and intrabony ameloblastoma (Sand *et al.*, 2000; Kahn, 1991, 1989). The aim of the current study was to investigate the presence of HPV in intrabony ameloblastoma by PCR, using consensus primers and identify different genotypes, using type-specific primers.

Materials and Methods

Through 1999-2000 one hundred blocks of paraffin embedded tissue intraosseous ameloblastoma were retrieved from the archives of the Department of Oral Pathology, Dental School, Shahid Beheshti University of Medical Sciences and the Department of Pathology, Taleghani General Hospital, Tehran. The tissues with ameloblastoma were providing confirmed by pathologists. The control samples selected from dental follicles of patients, referred to our clinics for excision were of their impacted teeth. They were selected if their histopathologic examinations showed no evidence of ameloblastic changes.

All of the cases and controls were classified in four groups of age. With respect to the tumor site, 90 were located in mandible and 10 in maxilla. Thirty-five patients were smoker. We also recorded history of tooth extraction, endodontic surgery and tooth repair in cases and control groups.

Four to six 5 µm sections were cut from each paraffin embedded tissue and placed in 2.5 mL Eppendorf tubes. DNA was extracted as described previously (Fornatora *et al.*, 1996). In brief, the sections were deparaffinized in 2 mL Xylene, this was repeated for three times. Xylene was pipetted off and its residue was removed using 2 mL of 100% ethanol. It was followed by vortexing and spinning. Most of the ethanol was pipetted off and the remaining was removed by incubation at 56°C for 30 min. Two hundred microliter digestion buffer (50 mM KCL, 1.5 mM MgCl₂, 10 mM HCL, 0.45% v/v Tween 20, 10 mg mL⁻¹ proteinase K (pH = 8.3) was added and incubated overnight at 36°C. Proteinase K was inactivated by incubation at 95°C for 8 to 10 min. This was followed by spinning at 7000 rpm for 5 min. The supernatant was collected in a clean and sterile tube and stored at -20°C for PCR processing.

HPV DNA was amplified with GP⁵⁺/GP⁶⁺ primers (Jacobs *et al.*, 1995), which amplify a portion of the L1 gene of most of mucosal HPV types (Table 1). Preparations were also tested by

Table 1: Polymerase chain reaction primers

Type of primers	Primers sequences	Length of PCR products (bp)
β-globin ^a	PCO3 5' ACA CAA CTG TGT TCA CTA GC PCO4 5' CAA CTT CAT CCA CGT TCA CC	110
HPVs ^b	GP5+ 5' TTT GTT ACT GTG GTA GAT ACT AC GP6+ 5' GAA AAA TAA ACT GTA AAT CAT ATT C	150
HPV 6.1 ^c	+5' TAG TGG GCC TAT GGC TCG TC 3'	280
HPV 6.2	-5' TCC ATT AGC CTC CAC GGG TG 3'	
HPV 11.1 ^c	+5' GGA ATA CAT GCG CCA TGT GG 3'	360
HPV 11.2	-5' CGA GCA GAC GTC CGT CCT CG 3'	
HPV 16.1 ^c	+5' TGC TAG TGC TTA TGC AGC AA 3'	152
HPV 16.2	-5' ATT TAC TGC AAC ATT GGT AC 3'	
HPV 18.1 ^c	+5' AAG GAT GCT GCA CCG GCT GA 3'	216
HPV 18.2	-5' CAC GCA CAC GCT TGG CAG GT 3'	
HPV 31.1 ^c	+5' ATG GTG ATG TAC ACA ACA CC 3'	514
HPV 31.2	-5' GTA GTT GCA GGA CAA CTG AC 3'	
HPV 33.1 ^c	+5' ATG ATA GAT GAT GTA ACG CC 3'	455
HPV 33.2	-5' GCA CAC TCC ATG CGT ATC AG 3'	

a-(Baay *et al.*, 1996); b-(Jacobs *et al.*, 1995); c-(Melchers *et al.*, 1994)

PCR with primers PCO3 and PCO4 (Baay *et al.*, 1996), specific to the human β -globin gene to monitor the integrity of DNA in the samples and the potential of amplification. Only samples showing successful amplification of β -globins sequences were subjected to GP⁵⁺/GP⁶⁺ PCR. All HPV positive specimens were further analyzed with type-specific primers for HPV types 6, 11, 16, 18, 31 and 33 (Melchers *et al.*, 1994) (Table 1).

Ten microliters of the DNA solution were used for PCR in 50 μ L of reaction mixture, containing 10 mM Tris-HCl (pH = 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.01% gelatin, 10 mM each dNTP (dATP, dTTP, dCTP, dGTP), 1 unit Taq polymerase and 50 pmol of each primers GP⁵⁺/GP⁶⁺. Each cycle included 1 min denaturation at 94°C, annealing step at 40°C for 2 min and extension at 72°C for 1.5 min. Forty cycles of PCR amplification were performed. The first cycle was preceded by a 5 min denaturation at 95°C and the last cycle was extended by a 4 min elongation at 72°C. Descriptive statistic was performed and were analyzed by chi-square for categorical variables.

Results

Human Papillomavirus (HPV) DNA was detected in 32 (32%) patients and 5 (10%) of the controls. HPV genotyping using type-specific-primers, detected HPV-6, 11, 16 and 31 in 10 (31.6%), 4 (12.5%), 4 (12.5%), 1 (3.1%) positive of samples, respectively. Mix infection of types 6 and 16 was revealed in 2 (6.3%) positive samples. HPV 18 and 33 were not detected. Eleven (34.4%) HPV positive specimens were not identified by the specific primers used in this study (Table 2).

HPV-6 was the predominant type, being present in 31.6% of the infected cases, either alone or together with other types. A significant association was observed between the location of tumor and the presence of HPV ($p = 0.02$). There was a strong correlation between traumatic history and HPV infection ($p = 0.03$). In addition, HPV infection showed a significant correlation with age group of 36-45 years ($p = 0.03$). We found also a significant correlation between endodontic therapy and HPV presence ($p = 0.01$). In relation to further clinicopathologic features of the analyzed patients, statistical analysis revealed no correlation between HPV infection and gender, tooth extraction, smoking, type of tumor and history of operative dentistry procedure (Table 3).

Table 2: Frequency of cases and controls and the results of HPV types detection

Characteristic	Cases				Controls			
	n	%	HPV	%	n	%	HPV	%
Age/years								
16-25	34	34	13	38.3	18	36	2	11.8
26-35	35	35	6	17.2	17	34	1	5.9
36-45	21	21	11	52.4	11	22	1	9.1
46-55	10	10	2	20.0	4	8	1	25.0

Table 3: Clinicopathological feature of samples and the results of HPV detection

Samples	Age/year	Extraction						HPV+
		Yes	No	Endo	Repair	Trauma	Smoking	
Cases	16-25	25	9	10	24	7	4	13
	26-35	28	7	16	27	5	17	6
	36-45	19	2	8	20	10	11	11
	46-55	10	-	4	7	2	3	2
Controls	16-25	15	3	9	15	5	3	2
	26-35	12	5	5	12	3	9	1
	36-45	9	2	4	10	4	4	1
	46-55	3	1	1	2	1	1	1
Total		121	29	57	117	37	52	37

Discussion

The role of human papillomavirus infection in the development of benign, premalignant and malignant lesions of oral mucosa has received attention in the past several years (Van Rensburg *et al.*, 1996; Miller *et al.*, 1996; Fornatora *et al.*, 1996; Dekmezian *et al.*, 1987; Eversole *et al.*, 1987). These lesions are surface epithelium-derived. Ameloblastoma is an epithelium-derived lesion with odontogenic origin (Harada *et al.*, 1998). Some studies suggest an association between HPV and ameloblastoma (Sand *et al.*, 2000; Kahn, 1991; Kahn, 1989; Harada *et al.*, 1998; Van-Heerden *et al.*, 1993). The HPV has been proposed to be one of the initiating factors by authors. Unspecified disturbances, including pressure and nutritional disturbances, rickets, extraction, caries and tooth repair, endodontic surgery, several oral sepsis, viruses might be of important in initiating ameloblastoma (Robinson, 1973; Tsakin and Nelson, 1980; Stanley *et al.*, 1964; Ueno *et al.*, 1986; Mehlisch *et al.*, 1972; Goldberg and Friedman, 1975; Forsberg, 1954; Csiba *et al.*, 1970; Levy and Ring, 1950; Main and Dawe, 1966; Fleming, 1963). In the present study, 32% of ameloblastoma specimens were HPV positive. Kahn found HPV in three (two intrabony and one peripheral) of thirty-eight juvenile ameloblastoma (Kahn, 1989). He concluded that the HPV presence in peripheral ameloblastoma is expected and proposed that the virus is acquired in utero or at parturition and involves the invaginating primitive enamel organ. He described the HPV-6/18 in a peripheral ameloblastoma (Kahn, 1991). In other study, Sand and his co-workers investigated 12 intrabony ameloblastoma by southern blot hybridization; five cases (42%) were HPV positive (Sand *et al.*, 2000). In study carried out in Texas, America on 5 patients types 11 and 16 were spotted (Dekmezian *et al.*, 1987). Interestingly in another report articulated by other workers concerning 62 samples, types 2, 6, 11 were detected (Eversole *et al.*, 1987). Another worker detected HPV type 18 in ameloblastoma (Van-Heerden *et al.*, 1993). Present findings showed a high prevalence of HPV-6 and 11 (31.6 and 12.5%, respectively). These types of HPV were found in one peripheral ameloblastoma in Kahn's study (Kahn, 1991) and in 4 of 8 (50%) HPV positives studied by Sand *et al.* (2000) HPV-16 was detected in 4 (12.5%) cases in the present study. Kahn (1991) also found this type in one peripheral ameloblastoma it was not detected. We also found HPV-31 in one (3.1%) of HPV positive cases, it was not reported in other studies. HPV-18 that was detected (Kahn, 1991) and in 7 of 8 (87%) HPV positive cases in was not found in our investigation (Sand *et al.*, 2000). From the clinical point of view, 56 (56%) of our cases were males and forty-four (44%) were females. Kahn (1989) reported that 47.4% were males and 52.6% were females, while in Sand investigation, 9 (75%) were males and 3 (25%) were females. However, it seems that there is no significant correlation between sex and HPV presence. A significant correlation between age and HPV infection was shown in the present study. All three cases were under 20 years old and in Sand study, almost all cases were between 44 to 53 years old. These correlation derived from different studies are not consistent and therefore, further investigation is needed to clarify possible correlation between age and HPV. In present investigation for the first time, an analytical study showed a significant correlation between intrabony ameloblastoma and HPV presence. These findings emphasize the role of HPV as an important etiologic factor of ameloblastoma. However, as Kahn (1989) proposed the penetration of HPV via the primitive oral mucosa to enamel organ and then dental follicle it is possible that HPV can penetrate from the oral squamous epithelium when a trauma, tooth extraction, endodontic surgery or similar stimulation occurs. Further studies need to test this hypothesis.

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