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Human papillomavirus detection and p16 methylation pattern in a case of esophageal papilloma

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Abstract

Esophageal cancer is a prevalent cancer worldwide. Some studies have reported the possible etiology of human papillomavirus (HPV) in benign and malignant papillomas of the esophagus but the conclusions are controversial. In the present study, we investigated an esophageal papilloma from a 30-year-old male patient presenting aphasia. HPV DNA was detected by generic PCR using MY09/11 primers, and restriction fragment length polymorphism revealed the presence of HPV54, usually associated with benign genital lesions. Hypermethylation of the pINK4A gene was also investigated due to its relation to malignant transformation, but no modification was detected in the host gene. Except for an incipient reflux, no risk factors such as cigarette smoking, alcohol abuse or an infected sexual partner were recorded. Since esophageal lesions may have a malignant potential, HPV detection and typing are useful tools for patient follow-up.

Key words: Human papillomavirus; Esophageal cancer; Restriction fragment length polymorphism

Esophageal cancer is the sixth most prevalent cancer worldwide, with a wide variation in incidence in different geographical regions of the world (1). An etiologic role of different microorganisms has been proposed for the pathogenesis of esophageal cancer such as fungus, bacteria, and viruses. Among the latter, are human papillomavirus (HPV), cytomegalovirus and Epstein-Barr virus. Several studies have reported the presence of HPV DNA in benign squamous cell papillomas (SCPs) of the esophagus (2-4). Esophageal squamous papillomas (ESPs) are uncommon benign lesions, usually asymptomatic and often discovered as incidental findings. The role of HPV in these benign lesions is controversial, with a wide variation in prevalence ranging from 0 to 100% having been reported in several studies (5). As suggested by Odze et al. (6), the detection of HPV in ESPs may be of a great value in terms of the follow-up of lesions associated with genotypes implicated in the genesis of premalignant and malignant lesions in squamous epithelial-lined tissues, including the nasal cavity, pharynx and anogenital tract. Because of the rarity of esophageal SCPs, the number of cases is still too small to draw definite conclusions about the role of HPV in their etiology. Syrjänen (1) published an extensive review of all cases reported and was the first to suggest the association of HPV with both benign and malignant squamous cell lesions of the esophagus. Only 30 reports on the subject are available in the literature and none from Brazil, although our country represents a high risk area for the development of esophageal squamous cell carcinoma (ESCC). Some investigators have suggested that HPV infection is frequently associated with ESCC in patients from geographic regions presenting a high incidence of ESCC, such as parts of China, South Africa and Iran (7-9).

In the present investigation, we studied an esophageal papilloma from a 30-year-old male patient with a 20-year history of hoarseness as well as swallowing difficulty. The patient was submitted to surgical extraction of the papilloma, which was then evaluated by histopathology and HPV detection by PCR. Intermittent reflux associated with progressive aphasia was the indication for endoscopy, which revealed an esophageal papilloma in the upper third of the esophagus, characterized as a polypoid, soft, smooth, whitish-pink tumor, measuring 6 mm in its largest dimension. Histopathological analysis revealed superficial acanthotic epithelial hyperplasia and papillomatosis with cytopathic alterations suggesting viral involvement, mainly

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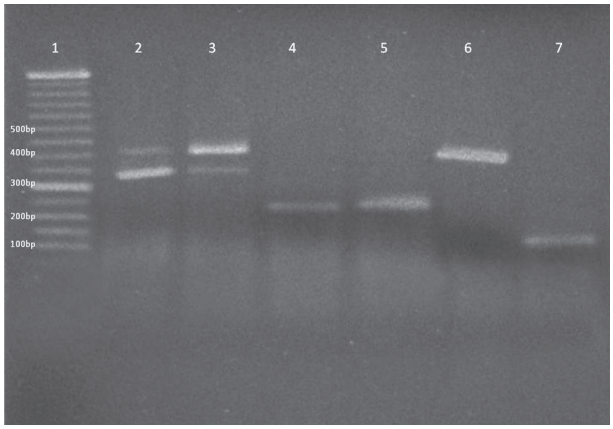

Figure 1. Restriction fragment length polymorphism (RFLP) pattern of MY09/MY11 L1 PCR products used to identify genital human papilloma virus (HPV) type. The sample was digested with restriction enzymes and applied onto the gel. According to the patterns of the bands, HPV54 was identified. *Lane 1*, 50-bp ladder (Invitrogen); *lane 2*, *Bam*HI (350 bp); *lane 3*, *Dde*I (450 bp); *lane 4*, *Hae*III (250 bp); *lane 5*, *Hin*fI (250 bp); *lane 6*, *Pst*I (450 bp); *lane* 7, *Rsa*I (150 bp). Ethidium bromide agarose gel is shown.

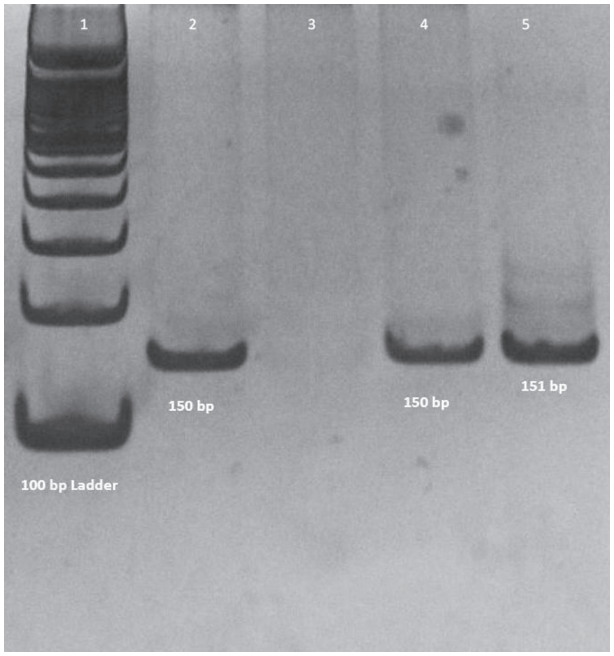

Figure 2. Methylation status of the p16 gene from a DNA sample. *Lane 1*, 100-bp ladder; *lane 2*, methylation specific PCR of bisulfite-modified DNA showing unmethylated pattern of the papilloma sample using primer p16M; *lane 3*, absence of methylation of the sample using primer p16M; positive controls (DLD-1 cell lineage) for both unmethylated (*lane 4*) and methylated (*lane 5*) pINK4A gene.

koilocytosis. For HPV evaluation, DNA was extracted with the QiAmp Viral Kit for paraffin-embedded tissues (Qiagen, Germany). HPV DNA was detected by generic PCR using MY09/11 primers, as described elsewhere (10). HPV was then typed by restriction fragment length polymorphism (RFLP) as described by Bernard et al. (11) using a panel of six restriction enzymes (*Bam*HI, *Dde*I, *Hae*III, *Hin*fI, *Pst*I, *Rsa*I). After 3% agarose gel electrophoresis, the RFLP results showed the presence of HPV54 (Figure 1), usually associated with benign genital lesions (12). We also tested the methylation status of the p16 gene from a DNA sample as described by House et al. (13). Briefly, DNA was modified with 3 M sodium bisulfite and 10 mM hydroquinone and the p16 gene was amplifed by the methylation-specific PCR technique. Two primer pairs (p16U and p16M) were used to distinguish methylated from unmethylated DNA in bisulfite-modified DNA.

Although presenting a low risk of malignant transformation, persistent HPV infection can lead to cancer. It is interesting to note that HPV54 was reported to be associated with HPV6 in a verrucous penile carcinoma (14). Nevertheless, it is still characterized as a low-risk virus (12) and DNA sequencing showed a poor relationship with the HPV16 genome (14).

Few studies have been conducted concerning the role of HPV in malignancy of the esophageal mucosa but the malignant potential of ESPs is still a matter of controversy (15). According to Talamini et al. (4), ESPs do not seem to involve a risk of development of malignancy or to be a marker of such risk. Bohn et al. (5) found a predominance of HPV infection by low-risk types and, in two cases, by high-risk types; however, no dysplastic changes, malignant transformation or recurrence were identified. Syrjänen (1) reported that, similarly to cervical cancer development, there are no doubts that ESCC develops through distinct precursor lesions, namely ESPs, but no prospective follow-up study has been published until now. In addition, the detection of HPV DNA in normal esophageal epithelium has been reported (16,17). Several risk factors have been proposed, such as cigarette smoking, alcohol abuse, smoked and hot food, and HPV infection in sexual partners, suggesting a multifactorial pathology (18).

Animal models have been developed in order to investigate such multi-factorial process. The most relevant one is related to esophageal carcinomas, common cancers associated with bovine papillomavirus 4 (BPV-4). Although BPV-4 causes benign lesions that usually regress spontaneously, the presence of carcinogenic co-factors can lead to malignant transformation. Among them, bracken fern diets are the best studied factor (19). Dietary carcinogens have already been described in humans, especially smoked and hot food (5). However, no direct relationship has been demonstrated between gastrointestinal tumors and HPV types and further studies are required to elucidate it. In the present study, except for an incipient reflux, no

risk factor was recorded: the patient was not a cigarette smoker or alcohol addict and his sexual partner had no history of HPV infection, although regularly submitting to annual cytopathological screening. Recently, epigenetic modifications of pINK4A by hypermethylation, as well as p16 protein overexpression, have been implicated in cancer development (5). Nevertheless, our results pointed to an unmethylated host DNA (Figure 2). The benign features of the studied lesion would in fact predict a good prognosis for the patient.

Hence, we suggest that clinical and endoscopic follow-up be recommended for patients with a history of recurrent lesions showing HPV infection, mainly those caused by high-risk types, which have been demonstrated by numerous epidemiologic and molecular studies to be the etiologic agents for an overwhelming majority of premalignant and malignant cervical cases (18). In conclusion, HPV should be considered to be a risk factor for esophageal carcinoma.

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