HUMAN PAPILLOMAVIRUS INFECTION AND P16 INK4A HYPERMETHYLATION IN A CASE OF ESOPHAGEAL PAPILLOMATOSIS

Larissa A. Afonso, Luciana F. Pesca, Fernanda N. Carestiato, Silvia M. B. Cavalcanti*

Laboratório de Diagnóstico Virológico, Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Rua Ernani de Melo n°101 sala 321 São Domingos CEP: 24210-130 Niterói, RJ, Brasil

ABSTRACT

Esophageal squamous cell papilloma (ESCP) is a prevalent lesion worldwide, with increasing occurrence rates. The possible role of Human Papillomavirus (HPV) in the etiology of such lesions has aroused the interest on elucidating its natural history. In this report we studied an esophageal papilloma from a 27 year old female patient. HPV DNA was detected by generic PCR using MY09/11 primers and RFLP revealed the presence of HPV70, usually associated to benign genital lesions. Hypermethylation of p16 INK4A gene was also investigated, due to its relation to malignant transformation and a hypermethylation pattern was detected in the host gene. Except for recurrent dysphasia, risk factors were not recorded: cigarette smoking, alcohol abuse, esophageal reflux or infected sexual partner. Since ESCP may have malignant potential, HPV detection and typing are useful tools in terms of follow-up. Besides that epigenetic events may represent additional risk for malignant transformation and thus can be a complementary biomarker.

Keywords: HPV, PCR, Esophageal papilloma, RFLP

INTRODUCTION

Squamous papillomas are uncommon benign lesions of esophagus, of unknown etiology and controversial malignant potential. Esophageal squamous cell papillomas (ESCP) have been found to be associated with different conditions, including gastro-esophageal reflux, esophagitis, hiatal hernia, inflammation and trauma; also by the fact that most of the ESCP are located in lower level of the esophagus (Mosca et al. 2001; Bohn et al. 2008). Talamini et al (2000) found that in the Italian population, ESPs are located mostly in the middle third and secondarily, in the lower third; this study also reported a very low prevalence of cases associated with HPV (human papillomavirus) and implied that alcohol intake and cigarette smoking were risks factors.

HPV has been implicated in the genesis of malignant neoplasms in squamous epithelial-lined tissues, including the nasal cavity, pharynx and anogenital tract; however, previous studies have not consistently identified HPV in ESCP (Bohn et al. 2008). Several studies have reported the presence of HPV DNA in benign ESCP (Mosca et al. 2001, Bohn et al. 2008; Afonso et al. 2010), however, HPV was not consistently identified (Poljack et al. 1995; Talamini et al. 2000). The role of HPV in these benign lesions still remains contradictory and studies report a wide variation on prevalence rates ranging from 0% to 100%. Diverse genotypes have also been described. Such differences could be the result of the interaction of the host, the HPV and other etiological factors, and/or differences in the methodology applied to detect it (Syrjänen & Syrjänen 2000; Iftner & Villa 2003; Hubbard 2003). As suggested by Odze et al (1993), the detection of HPV in ESCP may have prognostic value, since specific genotypes can result in the genesis of premalignant and malignant lesions in squamous epithelial-lined tissues, including nasal cavity, pharynx and the anogenital tract. Once ESCP studies are scarce, no definite conclusions on the role of HPV in the etiology were obtained till now. Syrjänen (2002) made an extensive report on all related cases and was the first to suggest the association of HPV with both benign and malignant squamous cell lesions of the esophagus. There are 32 reports on the subject available in the literature, and only a few studies are from Brazil (Afonso et al. 2010; Damin et al. 2006; Herbster et al. 2012), although our country represents a high risk area for ESCC (esophageal squamous cell carcinoma) development.

In this report we studied an esophageal papilloma from a female patient with 27 years old.
The patient was submitted to surgical extraction of the papilloma that was then submitted to histopathologic diagnosis and HPV DNA detection by the Polymerase Chain Reaction (PCR). Patient reported recurrent dysphasia since the age of 6 years old. Together with intermittent reflux and abdominal pain, these signals and symptoms were indicative of endoscopic procedure that revealed an esophageal papilloma at the upper third of the esophagus, characterized as a 5mm polypoid, soft, smooth, whitish-pink tumor. Histopathological analysis revealed an acanthotic superficial epithelial hyperplasia and papillomatosis with cytopathic alterations suggesting viral commitment, including koilocytosis and parakeratosis. For HPV detection, DNA was extracted with QiAmp Viral Kit for paraffin embedded tissues (Qiagen, Germany). HPV DNA was detected by generic PCR using MY09/11 primers, used as described elsewhere (Cavalcani & Oliveira 2003). HPV was then typed by RFLP as described by Bernard et al (1994) using a six restriction enzyme panel (BamHI, DdeI, HaeIII, HinfI, PstI, Rsal). After electrophoresis on a 1.5% agarose gel, RFLP analysis showed the presence of HPV 70, usually associated to benign genital lesions (Muñoz et al. 2003). We also tested the methylation status of p16 INK4A from DNA sample as described by Herman et al (1996). Briefly, DNA was modified by sodium bisulfite 3M and hydroquinone 10mM. The amplification of p16 INK4A was achieved by MSP technique. Two primer pairs (p16U and p16M) were used to distinguish methylated from unmethylated DNA in bisulfiite-modified DNA. Our result showed a methylated pattern of p16 INK4A gene, usually associated to p16 INK4A gene silencing, compromising cell DNA repair and increasing the risk of malignant transformation.

Although presenting a low risk of malignant transformation, persistent HPV infection can lead to anogenital cancer. Besides that, it is interesting to notice that HPV70 is phylogenetically classified as a high risk HPV type because its similarities with HPV 18 (Muñoz et al. 2003). Few studies were done concerning HPV natural history in esophageal mucosa and the malignant potential of ESCP is still unclear (Gupta et al. 2012). As described by Talamini et al. (2000), ESCP did not represent a risk of development of malignancy. In agreement with them, Bohn et al. (2008) found prevailing HPV infection by low-risk types, with no association with dysplastic changes or malignant transformation. Nevertheless, Syrjänen & Syrjanen (2012) described that, similarly to cervical cancer development, there are no doubts that ESCC develops through distinct precursor lesions, namely ESCP but no prospective follow-up study has been published till now. Furthermore some authors suggested that HPV infection is frequently associated with ESCC in patients from geographic regions presenting high incidence rates of ESCC, such as parts of China, South Africa and Iran (Syrjänen & Syrjanen 2000; Ferlay et al. 2000).

HPV has been repeatedly found in esophageal carcinoma tissues. However, detection rates of HPV DNA in these tumors have varied markedly. Differences in detection methods, sample types and geographic regions of the sample origin have been suggested as potential causes of this discrepancy (Syrjänen & Syrjanen, 2012). The studied conducted by Gupta et al (2012) suggested the possibility that HPV is involved in esophageal carcinogenesis, especially the non-keratinizing type of SCC. Several risk factors have been proposed, such as cigarette smoking, alcohol abuse, smoked and hot food, HPV infection in sexual partner, history of recurrent or chronic dysphasia, suggesting a multi-factorial pathology (Muñoz 2003).

In our study, a relevant history of recurrent dysphasia was registered. Clinical records pointed to probable perinatal HPV infection, since the patient has been presenting dysphasia, a relevant risk factor, throughout her life. An incipient reflux was also recorded but, besides that, the patient was not a cigarette smoker or alcohol addict and her sexual partner did not present history of HPV infection. Nevertheless, it is important to notice that ESP etiology remains controversial and prospective cohort studies are mandatory to evaluate the impact of HPV in its pathogenesis(Syrjänen & Syrjanen, 2012).

Recently, epigenetic modification of p16INK4A by hypermethylation has been implicated in cancer development, leading to gene silencing of tumor suppressor proteins (Bohn et al. 2008). Our results pointed to a methylated host DNA. Although we cannot assure that this malignant feature would predict a bad prognosis to the patient, we suggest that clinical and endoscopic follow-up should be recommended to patients with history of recurrent lesions showing HPV infection, in order to investigate transformation events.

**REFERENCES**


