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Human papillomaviruses in intraepithelial neoplasia and squamous cell carcinoma of the conjunctiva: a study from Mozambique

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Abstract

The infection with human papillomavirus (HPV) has been described as a risk factor for squamous cell carcinoma of the conjunctiva (SCCC), although the evidence is conflicting. To assess the relation between HPV infection and intraepithelial neoplasia or SCCC, we evaluated archived material from biopsies of the conjunctiva performed at the Maputo Central Hospital (Mozambique) in patients with suspected eye cancer. The quality of DNA was assessed by PCR using β -globin-specific primers. A total of 22 consecutive biopsies (intraepithelial neoplasia, SCCC, and benign conditions) positive for β -globin were further tested for HPV infection by PCR using the general primers GP5 + /GP6 + and CPI/CPII. In addition, PCR with type-specific primers HPV 16 and HPV 18 was performed. Nineteen biopsies corresponded to intraepithelial neoplasia (two low-grade and nine high-grade) or SCCC ($n = 8$), from which 11 (57.9%) tested positive for HPV infection; nine were positive for CPI/CPII, including one case also positive for GP5 + /GP6 + and HPV 18, and the remaining two tested positive only for HPV 16. HPV DNA was not detected in any of the three biopsies of benign conditions. These results suggest a stronger association between infection with cutaneous HPV and SCCC than for mucosal HPV. However, further research is required to clarify the relation between HPV and SCCC as well as to understand the potential of the HPV vaccine currently available for cervical cancer to prevent SCCC.

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Conflicts of interest

There are no conflicts of interest.

Keywords

human papillomavirus; Mozambique; squamous cell carcinoma of the conjunctiva

Introduction

Squamous cell carcinoma is the most frequent cancer in the ocular conjunctiva (Kiire and Dhillon, 2006). In Mozambique, data on the frequency of squamous cell carcinoma of the conjunctiva (SCCC) are scarce. In 1956–1960, the incidence of cancer of the eye in Maputo city was estimated at 1.5 and 2.7/100 000 inhabitants, for men and women, respectively (Prates and Torres, 1965). More recent data from the Department of Pathology of the Maputo Central Hospital show a nine-fold and 18-fold increase in the number of cancers of the conjunctiva diagnosed between 1991–1993 and 2006–2008, in men and women, respectively (Lorenzoni, 2010).

Although the exposure to ultraviolet light is considered to play a major role in the etiopathogenesis of conjunctival intraepithelial neoplasia and SCCC, infection with HIV/AIDS was shown to increase the risk of SCCC by almost 10-fold (Guech-Ongey *et al.*, 2008; Ateenyi-Agaba *et al.*, 2010). This may explain the increasing incidence of SCCC reported in Sub-Saharan Africa (Wabinga *et al.*, 2000), along with the increasing importance of the HIV/AIDS epidemic, whereas the frequency of other cancers also considered to be associated with HIV infection was not found to follow the pattern of HIV prevalence in five African populations (Chaabna *et al.*, 2012). Infection with the human papillomavirus (HPV) has also been described as a risk factor for SCCC, although the available evidence is conflicting (Tornesello *et al.*, 2006; De Koning *et al.*, 2008; Ateenyi-Agaba *et al.*, 2010), and SCCC is not always considered among the most important HPV-related cancers (De Souza *et al.*, 2012). Furthermore, to our knowledge, only a few studies have addressed the role of both mucosal and cutaneous HPV types (Tornesello *et al.*, 2006; De Koning *et al.*, 2008; Ateenyi-Agaba *et al.*, 2010).

We assessed the presence of HPV DNA, including mucosal and cutaneous types, in a consecutive series of conjunctival biopsies performed in patients with suspected eye cancer.

Materials and methods

We used archived material from the 68 biopsies of the conjunctiva analyzed in the Department of Pathology of the Maputo Central Hospital between 1992 and 1997. Every biopsy had been fixed in 10% formalin and embedded in paraffin. A total of 30 cases (two low-grade intraepithelial neoplasia, 10 high-grade intraepithelial neoplasia, one invasive squamous cell carcinoma, and 17 benign conditions) were excluded because the sample available was insufficient for further analysis. The age of these patients ranged from 6 to 61 years (median: 26 years), and 36.7% were male. The remaining 38 biopsies encompassed cases of low-grade intraepithelial neoplasia ($n = 4$), high-grade intraepithelial neoplasia ($n = 16$), invasive squamous cell carcinomas ($n = 13$), and clinically and histologically benign conjunctival conditions in patients with no other cancer diagnosis ($n = 5$, namely three

pingueculae, one melanosis, and one conjunctivitis). The age of the patients ranged from 12 to 68 years (median: 35 years) and 39.5% were male.

DNA was extracted and purified according to standard techniques and the protocol used as described previously (Carrilho *et al.*, 2003). Each tissue section was digested with 10 mg/ml proteinase K in a buffered solution. For the PCR reaction, 10 µl aliquots of DNA were used. The quality of DNA was assessed by PCR using β -globin-specific primers. Detection of HPV was performed by PCR using two pairs of HPV consensus primers, namely general primers GP5 + /GP6 + , to amplify a region of 150 bp from the L1 open reading frame of a broad spectrum of mucosotropic HPV genotypes, and CPI/ CPII, to amplify a 188 bp fragment from the E1 open reading frame of a broad spectrum of both mucosal and cutaneous HPV genotypes, as described previously (Cruz *et al.*, 1996). In addition, PCR with type-specific primers for HPV 16 (E6 HPV 16) and HPV 18 was performed. To facilitate PCR-based HPV detection and typing, a colorimetric microtitre plate-based hybridization assay (EIA) was also performed systematically, as described previously (Carrilho *et al.*, 2003).

Results

Twenty-two of the 38 samples were positive for β -globin, corresponding to 19 cases of intraepithelial neoplasia or SCCC and three biopsies of benign conditions. From the former, 11 (57.9%) tested positive for HPV infection; nine were positive for CPI/CPII, including one case also positive for GP5 + /GP6 + and HPV 18, and the remaining two cases were only positive for HPV 16. HPV DNA was not detected in any of the three samples from patients with benign conjunctival conditions (Fig. 1).

Discussion

The numerous cases in which HPV was only detected with CPI/CPII primers suggest the predominance of infection with cutaneous HPV type(s), although the confirmation of these results by sequence analysis of the PCR products would allow stronger inferences on the differential contribution of cutaneous and mucosal HPV types toward the occurrence of SCCC.

These findings are limited by the small number of biopsies, especially from patients with benign conditions. However, the study was based on a consecutive series of samples with the necessary quality for a valid assessment of HPV infection, and it is the first investigation addressing this issue in Mozambique, adding to the scarce evidence on this topic. Ateenyi-Agaba *et al.* (2010) evaluated 133 cases and 285 controls, showing no relation between infection with mucosal HPV types and SCCC/ dysplasia [odds ratio (OR) = 1.0, 95% confidence interval (CI): 0.4–2.7] whereas infection with cutaneous HPV types was associated with a higher risk (single infection, OR = 2.3, 95% CI: 1.2–4.4; multiple infection, OR = 18.3, 95% CI: 6.2–54.4). De Koning *et al.* (2008) evaluated 81 cases of conjunctival intraepithelial neoplasia and carcinoma and 29 controls; the OR was 1.0 (95% CI: 0.4–2.7) for genital and 8.0 (95% CI: 1.0–169) for cutaneous HPV types. In the study by Tornesello *et al.* (2006), DNA of cutaneous HPV types was identified in 13% of the 86

cases and none of the 63 controls, whereas infection with HPV 6 or HPV 18 was detected in 2% of cases and controls. Our results from Mozambique are in agreement with the results from these studies carried out in Uganda, but further research is required to clarify the relation between HPV and SCCC as well as to understand the potential of the HPV vaccine currently available for cervical cancer to prevent SCCC.

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HPV infection in conjunctival benign conditions and in intraepithelial neoplasia and carcinomas. HG, high-grade intraepithelial neoplasia; HPV, human papillomavirus; IC, invasive carcinoma; LG, low-grade intraepithelial neoplasia. *Two pingueculae and one conjunctivitis.

Fig. 1.

HPV infection in conjunctival benign conditions and in intraepithelial neoplasia and carcinomas. HG, high-grade intraepithelial neoplasia; HPV, human papillomavirus; IC, invasive carcinoma; LG, low-grade intraepithelial neoplasia. *Two pingueculae and one conjunctivitis.