Human papillomavirus 52 positive squamous cell carcinoma of the conjunctiva

Silvia Olivia Salceanu, Catalin Constantin, Ion Cijevschi, Ramona Gabriela Ursu, Mirela Grigorovici, Luminita Smaranda Iancu

Human papillomavirus (HPV) infection is strongly associated with several human cancers; the most known genotypes involved being HPV 16 and HPV 18. We report the detection of HPV 52 in a sample taken from a 47-year-old patient with squamous cell carcinoma of the conjunctiva of the left eye. The method used for the detection of HPV was real time polymerase chain reaction. The evolution was favorable after surgical removal of the tumor and the patient was explained that long-term follow-up is essential to avoid recurrence.
**Key words:** Conjunctiva, eye, human papillomavirus 52, real time polymerase chain reaction, squamous cell carcinoma

Human papillomavirus (HPV) infection is strongly associated with anogenital tumors (cervix, penis, vulva, vagina, anus), head and neck cancers (oral cavity, esophagus, larynx), and nonmelanoma skin cancers (squamous and basal cell carcinoma).

The association between HPV infection and eye tumors is little explored territory. There are two studies that detected HPV in 100% of ocular surface squamous neoplasia (OSSN),[1,2] many studies that find a HPV infection prevalence higher than 50%[3–5] and some other studies that find no HPV infection[6,7] making the role of this virus controversial and unresolved. In all positive studies, HPV 16 is the most prevalent genotype found, followed by HPV 18.[1–5] In the international literature, HPV infection appears at all ages, with a predilection to young people. Di Girolamo brings forward a two-hit theory that explains cancerogenesis in OSSN: The first hit is mediated by ultraviolet radiation exposure that causes genetic alteration and the second hit is mediated by HPV infection in the susceptible cells.[8] HPV infection of the conjunctiva is thought to be transmitted from mother to the child by natural birth or by autoinoculation with contaminated fingers.

**Case Report**

We present a unique case of squamous cell carcinoma of the conjunctiva examined and treated in June 2014. The 47-year-old patient presented at the Ophthalmology Department for mild stinging sensation and redness in the left eye for almost 1-year and a half. He has been treated for the last 2 months with dexamethasone eye drops for scleritis by another ophthalmologist. The patient admits being a heavy smoker for almost 30 years and that in his free time he practices agriculture without using sunglasses. The uncorrected visual acuity was 20/20 in both eyes. The slit-lamp microscopic examination showed a prominent reddish limbic growth between 2 and 6 o'clock which slightly covered the cornea in the periphery and which appeared not to invade the corneal stroma [Fig. 1]. All other ophthalmological findings were normal. Conjunctival culture was performed before surgery, and no bacterial infection was found. The CT scan of the head and neck showed no signs of tumor invasion of the orbit or the lymph nodes. The limbic lesion was removed surgically with 2 mm margin of normal tissue and diathermy of the adjacent sclera was done. At the end, the remaining temporal defect was restored using a supero-nasal conjunctiva graft fixed in position with interrupted and surjet 10.0 nylon sutures [Fig. 2]. The excised tumor was cut into 2 fragments: One for pathology preserved in formalin and one for HPV genotyping preserved in Cobas polymerase chain reaction (PCR) solution and refrigerated at 4°C until processing. Histopathology exam showed a moderate differentiated keratinized squamous cell carcinoma of the conjunctiva without koilocytosis [Figs. 3 and 4] and the genotyping method identified HPV 52 [Fig. 5].

The recovery of the conjunctival wound was good. Four months after surgery the slit-lamp microscopic examination showed between 3 and 5 o’clock corneal stromal neovascularization which we are monitoring closely [Fig. 6]. In case the stromal neovascularization extends in surface, we explained to the patient that enucleation is necessary.

In September, we checked for viral persistence after excision and the sample was negative for DNA/HPV. We also took a cervical sample from his wife in order to check for possible sexual transmission of the virus. The cervical cytology showed ASC-H (atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion) and the sample was HPV negative. We conclude that the HPV infection was transient, but enough to initiate the cancerous process in the conjunctiva and maybe the cervical changes of his wife.

**Human papillomavirus genotyping method**

DNA/HPV was purified using high pure PCR template preparation kit. According with the kit extraction protocol, we kept the sample biopsy in 200 µl lysis buffer with 40 µl proteinase K, at 50°C for 3 h (Biosan thermoblock). After total digestion of the sample, we followed the classical DNA extraction protocol. The purity and concentration of DNA were analyzed with NanoDrop pearl and real time PCR amplification used MX 3005P Stratagene thermocycler.

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**Figure 1:** The slit-lamp microscopic photograph of the left eye before surgery

**Figure 2:** The slit-lamp microscopic photograph 1-day after the surgery
The real time PCR method includes the detection of E6 gene/HPV for the types 16, 18, 33 and 52 and 52 b, by qualitative end point PCR method, with the Primerdesign™ genesig® kit for HPV TaqMan® principle. The amplification protocol included one cycle for enzyme activation for 10 min at 95°C and 50 cycles of 10 s denaturation at 95°C, 60 s annealing and extension at 60°C. The qualitative end point supposed the inclusion in the real time PCR plate of a positive control and of negative controls (between 1 and 3) to find out the contamination which can lead to false positive results.

**Discussion**

This study is the first of its kind done in Romania and one of the few taken in Europe and in the world. This is the first case report of squamous cell carcinoma of the conjunctiva infected with HPV 52 that is published in the international literature. Genotyping other samples from young patients with squamous cell carcinoma of the conjunctiva will show if HPV 52 is specific for this region or it was an accidental finding.
Another important fact to be underlined is the detection of E6 viral protein by our method. Reverse transcriptase PCR amplification of E6/7 mRNA is the gold standard for detection of clinically significant HPV infection in tumor samples but is considered to be time consuming and technically difficult.[9] Our method-real time PCR is sensitive, accurate, and we have detected the E6 protein, which shows transcriptional active virus in the sample. E6 is an oncoprotein which cooperates with E7 to immortalize primary human keratinocytes.

In a previous study performed on cervical samples in the same region of our country, HPV 52 was detected in 4.08% of the 514 tested women, after HPV 16 (10.5%), 53 (5.44%) and 51 (5.05%). These findings support the possibility of HPV 52 to be found in other tumors of head and neck, beside cervical cancer.[10]

Surgical treatment of the squamous cell carcinoma of the conjunctiva is the optimal treatment and HPV genotyping should be considered each time, especially if the patient is young. Doctors should check for viral persistence after excision which can lead to recurrence. Long-term follow-up is essential.

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Figure 6: The slit-lamp microscopic photograph 4 months after the surgery

Cite this article as: Salceanu SO, Constantin C, Cijevschi I, Ursu RG, Grigorovici M, Iancu LS. Human papillomavirus 52 positive squamous cell carcinoma of the conjunctiva. Indian J Ophthalmol 2015;63:166-9.

Source of Support: This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/136893. Conflict of Interest: None declared.