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LETTER TO THE EDITOR

# Prevalence and Type Diversity of Human Papillomaviruses in Penile Cancers in Hungary

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To the editor

Penile cancers are one of the rare forms of oncological diseases as in developed countries their prevalence is less than 1 %. Epidemiological studies suggested the role of oncogenic HPV-types as a causative agent of penile tumors [1, 2]. Around 40 % of patients with penile cancer had also been affected by HPV with type 16 being the most prevalent [3]. Currently available literature data explain HPV-induced tumors with the integration of virus into the epithelial cells' genome, and its genetic manipulation of the host DNA. Another interesting fact is that HPV infection is much more frequently associated with certain types of penile cancers, than other malignant manifestations [3]. Clinical course and prognosis of patients with penile cancer is unequivocally determined by the lymphatic node status. Five year survival rate of pathologically negative lymph nodes (pN0) is 85–100 %, whereas the involvement of pathologically verified metastatic lymph nodes in the inguinal region dramatically reduces this rate [4]. A retrospective study including 145 male patients describes: tumor thickness and lymphatic or vascular invasion as prognostic factors for lymph node involvement. Interestingly, no statistical correlations can be indicated in lymphatic involvement in connection with T status and the grade of cancer [5].

The aims of the present study were i) to identify and estimate the prevalence of high-risk HPV (hrHPV) genotypes in both primary penile tumors and metastatic lymph nodes, ii) to analyse the potential correlation between the hrHPV positivity and the severity and progression of the cancer. Tissue samples were taken from both the primary tumor and the regional lymph nodes, in over the course of operations of penile cancers in the Department of Urology, University of Pécs, Hungary, between 2002 and 2012. Samples were forwarded to histopathological processing where tissues were fixed in formalin and embedded into paraffin for histological processing. For retrospective molecular studies, 10 μm sections of the paraffin blocks were deparaffinated. Subsequently, DNA was extracted for the purpose of HPV-identification. Cells were disintegrated using TissueLyser (Qiagen), and subcellular structures were digested enzymatically using Proteinase-K. DNA was purified from tissues using QIAmp DNA FFPE Tissue Kit (Qiagen), according to the manufacturer's recommendations. HPV DNA was detected by virus-specific TaqMan PCR (DIAGON Ltd., Hungay). In case of HPV positive samples Linear Array HPV Genotyping Test (Roche) was further used for genotyping.

A total of 35 patients were involved in the current clinical study. High-risk HPV was identified from primary tumors in 17 cases (48.5 %), regional (inguinal) lymph nodes were positive in 3 cases. The average age of hrHPV positive males was 55 years (range: 44–87 years) while HPV negative patients were slightly older, averaging 66 years of age (range: 50–82 years). Genotyping using high-sensitivity molecular assays was available for 14 cases out of the 17 hrHPV-positive patients. HPV 16 was identified in 11 of 14 samples (78.5 %), HPV 59 and 82 were detected in two separate cases, while

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simultaneous detection of HPV 51 and 82 occurred also in one patient. For the 3 patients who all had positive results from both the primary tumor and lymph node, HPV 16 was identified. Clinical and pathological investigations (Table 1) showed that in 47 % of the hrHPV cases pTa-pT1, while in 53 %, pT2-pT4 stages were apparent. Histopathological processing of tumors from the HPV-negative patients (18 individuals) revealed pTa-pT1 and pT2-pT4 pathological stages also

**Table 1** Virological tests and clinical manifestations

| No. of patients | Primary tumor | | Lymphoglands | | TNM | Surgery | Age (year) |
|---|---|---|---|---|---|---|---|
| | HPV positivity | HPV type(s) | HPV positivity | HPV type(s) | | | |
| **1** | **pos** | **16** | **neg** | **-** | **pT1pN0GII** | **excision** | **61** |
| 2 | neg | - | neg | - | pT1pN0MxGI | partial amputation | 81 |
| **3** | **pos** | **na.*** | **neg** | **-** | **pT3pN0MxGII-III** | **amputation** | **53** |
| **4** | **pos** | **16** | **neg** | **-** | **pT1pN0M0GI** | **excision** | **87** |
| 5 | neg | - | neg | - | pT2N2MxGI | partial amputation | 82 |
| 6 | neg | - | neg | - | pT1pN0GI | excision | 56 |
| **7** | **pos** | **16** | **pos** | **16** | **pT4pN2MxGIII** | **emasculinisation** | **44** |
| 8 | neg | - | neg | - | pT1pN0MxGI | excision | 54 |
| **9** | **pos** | **na.** | **neg** | **-** | **pT3pN2MxGII-III** | **amputation** | **53** |
| 10 | neg | - | neg | - | pT1pN2MxGI-II | excision | 79 |
| 11 | neg | - | neg | - | pT1pN0GIII-IV | amputation | 63 |
| 12 | neg | - | neg | - | pT1pN3MxGIV | excision | 56 |
| 13 | neg | - | neg | - | pT2pN2MxGII-III | amputation | 50 |
| **14** | **pos** | **16** | **pos** | **16** | **pT4pN2GIII-IV** | **emasculinisation** | **52** |
| **15** | **pos** | **16** | **pos** | **16** | **pT3pN1MxGIII-IV** | **emasculinisation** | **52** |
| 16 | neg | - | neg | - | pT2pN0MxGII-III | partial amputation | 78 |
| **17** | **pos** | **59** | **neg** | **-** | **pT1bpN0MxGIII-IV** | **excision** | **60** |
| 18 | neg | - | neg | - | pT1apN0MxGI-II | partial amputation | 74 |
| 19 | neg | - | neg | - | pT1pN0MxGI | excision | 73 |
| **20** | **pos** | **51, 82** | **neg** | **-** | **pT1CISpN0MxGIII** | **excision** | **61** |
| **21** | **pos** | **na.*** | **neg** | **-** | **pT1bpN3MxGIII-IV** | **amputation** | **53** |
| 22 | neg | - | neg | - | pT1pN0MxGII | excision | 72 |
| **23** | **pos** | **16** | **neg** | **-** | **pT1pN0MxGII** | **excision** | **59** |
| **24** | **pos** | **16** | **neg** | **-** | **pT2pN0MxGII** | **amputation** | **44** |
| **25** | **pos** | **16** | **neg** | **-** | **pT2pN0MxGII-III** | **amputation** | **60** |
| **26** | **pos** | **82** | **neg** | **-** | **pTapN0MxGI** | **excision** | **59** |
| **27** | **pos** | **16** | **neg** | **-** | **pT2pN0MxGI-II** | **amputation** | **57** |
| **28** | **pos** | **16** | **neg** | **-** | **pT1pN0MxGII** | **excision** | **46** |
| 29 | neg | - | neg | - | pT3pN3MxGI | amputation | 78 |
| 30 | neg | - | neg | - | pT2pN3M1GIII-IV | amputation | 59 |
| 31 | neg | - | neg | - | pT3pN2MxGIII-IV | amputation | 61 |
| **32** | **pos** | **16** | **neg** | **-** | **pT2pN0MxGIII** | **amputation** | **48** |
| 33 | neg | - | neg | - | pT3pN2MxGIV | amputation | 71 |
| 34 | neg | - | neg | - | pT3pN2MxGII | amputation | 56 |
| 35 | neg | - | neg | - | pT2pN0GII | excision | 50 |

Summary of virological tests and clinical manifestations (i.e. oncology status, surgical interventions). Data of HPV positive patients are emphasized by bold

**na.*: *genotype data not available*

in the same ratio. The worst stages (pT3-pT4) were observed in those three patients who had hrHPV both in the primary tumor and regional lymph node. For all three patients, extensive removal of primary tumor (emasculinisation) was necessary. The available staging and pathological results along with the rapid cancer progression suggested the use of adjuvant chemotherapic treatment, nonetheless, due to the patients' wrong compliance, further oncologic treatment was not continued.

HPV as a risk factor is well-known; hence it can be identified in 15–71 % of primary tumors [6]. In contrast to the high prevalence of HPV-induced cervical cancer, its significantly lower prevalence in penile cancer is most probably the result of remarkable mitotic activity of virus-affected epithelial cells in the cervix [7]. Both diseases show a close relation to hrHPV 16 and 18 even though this relation is 10 times lower in the case of penile cancers [8]. Although, different hrHPV types were described previously in penile cancer cases, type 16 is considered to have a greater significance. As shown in the current study, HPV-infection could be identified in the primary tumor in nearly 50 % of patients, among them the virus was also detected in the metastatic lymph nodes in three cases. HPV 16 was present in 78.5 % of positive samples, in addition three hrHPV (types 51, 59 and 82) were also detected. HPV-mediated cancers presented at a younger age, while non-HPV associated manifestations rather obtainable at the age of 70–80. The current study showed an average age of 56 years (range: 44–86) for patients with HPV-associated primary tumors. Although Cubilla et al. reported a difference in age distribution between HPV-positive and negative cases, no other studies confirmed this observation [9]. There are only a limited number of studies about HPV-induced penile cancer available in Europe. Lont et al. found HPV DNA in 50 cases among 171 penile cancers, among them HPV 16 was identified in 38 patients, while type 18 was detected in only 3 cases [10]. Heidmann et al. found an HPV-positive rate of 55.4 %, among which 56 % and 6 % proved to be type 16 and 18, respectively [11]. Danish researchers identified HPV 16 DNA from primary tumor tissues in 65 % of 37 penile cancer cases, while low-risk HPV 6 was also found in 3 % [12]. Our current data highly correspond to the literature regarding both virus-associated disease prevalence and HPV genotypes.

Here we report local malignant progression of stages pT2-pT4 in 53 % and pTa-pT1 stages in 47 % of hrHPV-positive patients. Three patients were classified into pT3-pT4 stage, among which both the primary tumor and the adjacent lymph node block tested positive for HPV 16. Similar rates were found in the case of HPV negative patients, i.e. pTa-pT1 and pT2-pT4 pathological stages appeared in 50–50 %. Some studies claimed to discover a correlation between the HPV type of the primary tumor and the progression of cancer, while others showed no survival difference between HPV-positive and negative tumors [10, 13–15]. The current work support the latter conclusions, i.e. no any difference was found between HPV positive and HPV negative tumors in the post-operative survival and the progression of the disease.

Although the pathological role of HPV is lower in males than in females, vaccination might serve as a preventive measure for penile cancers. The Food and Drug Administration in the USA previously authorized the vaccination of male population with quadrivalent vaccines; in consequence, vaccination of Hungarian male population would be justifiable, concerning the results of our study.

#### Compliance with Ethical Standards

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**Competing Interests** None declared.

**Ethical Approval** Study was approved by the Regional Medical Scientific Ethical Board of the University of Pécs, Hungary (UP-RMSEB No#4828).

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