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Prevalence of anal HPV infection in solid-organ transplant patients prior to immunosuppression

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Abstract Patients that undergo organ transplantation have a high risk of developing various malignancies, depending on the duration and magnitude of immunosuppressive therapy. Among others, a 10-fold increased relative risk has been reported for the development of anal cancer. There is a strong association between persistent infection with high-risk mucosal types of human papillomavirus (HPV) and anogenital neoplasia. In this study we analysed the prevalence of anal HPV infection in organ transplant patients before starting immunosuppressive therapy. In a university transplant unit, patients ($n=60$, 40 male, 20 female) that were undergoing solid-organ transplantation (kidney, liver) for the first time were routinely screened for anal HPV infection. Anal swabs were obtained within 24 h after transplantation and analysed for the presence of mucosal-type HPV DNA by liquid DNA/RNA hybridization [hybrid capture (HC) 2 test]. Overall, some type of HPV DNA was detected in 14/60 (23.3%) patients; 9/60 (15%) were positive for high-risk HPV and 8/60 (13.4%) were positive for low-risk HPV, and 3/60 (5%) were positive for both types. Prevalence of HPV infection tended to be higher in patients that were receiving liver transplants than in those receiving kidney transplants (29.4% vs. 20.9%), but the difference did not reach statistical significance. In our series of organ transplant patients the prevalence of previous HPV infection (23.3%) before immunosuppressive therapy was started was higher than that found in previous epidemiological studies or in a control group. In particular, there was a high rate (15%) of infection with oncogenic HPV types. These findings have important implications on screening and surveillance policies in this patient group at risk of developing neoplasias, including anal cancer.

Keywords Human papillomavirus · Anal neoplasia · Organ transplantation

Introduction

There is an increased cumulative incidence of malignancy in organ transplant recipients, which has been correlated with duration and extent of immunosuppressive therapy. The cumulative risk of the development of a solid-organ neoplasm after transplantation is 5%–6% [1, 2, 3] and can reach 82% for single tumour entities in regions of high risk for skin cancer (e.g. Australia, 20 years follow-up) [4]. The standardized incidence ratio (SIR) for anogenital malignancies in renal allograft recipients has been reported to vary

between 3.3 and 55.8, depending on tumour entity [5, 6]. The incidence of anal cancer in those patients was increased 30-fold to 100-fold when compared to that in the general population [4]. The incidence of anal cancer has been reported to be between 0.5/100,000 per year [7] to 0.7/100,000 per year [8]. At our department, the incidence of anal cancer in liver transplant recipients is 86.2/100,000 per year.

Human papillomaviruses (HPVs) infect squamous epithelia of skin or mucous membranes. Infection occurs asymptomatically by minor trauma that exposes basal cells. More than 100 HPV types have been identified up to now. Low-risk HPV types generally induce benign papillomas or warts. High-risk HPV types, mostly HPV 16, are causally related to the development of anogenital neoplasia, in particular, cervical cancer. In addition, there is a strong association between high-risk type HPV infections and the development of anal cancers [9].

Epidemiological studies on anal HPV infection have been performed only in risk groups (HIV-positive patients, men who have sex with men (MSM)). The prevalence of anal HPV infection in a healthy population is unknown. The high incidence of anal cancer in transplant patients and the strong association with high-risk type HPV infection prompted us to evaluate the prevalence of anal HPV infection in these patients before they underwent transplantation. Patients that require immunosuppressive therapy are at increased risk of acquiring HPV infection and developing malignant anogenital lesions. Therefore, a high incidence of anal HPV infection could have implications for HPV screening and surveillance policies in transplant patients.

Patients and methods

In all patients who received a kidney or liver transplant for the first time between April 2003 and August 2003 anal swabs for HPV-DNA detection were routinely obtained. All patients gave their informed consent. Within 24 h after transplantation anal epithelial cells from the anal verge to the dentate line were collected on a cotton swab and a Dacron swab in STM (Digene Diagnostics, Gaithersburg, Md, USA). Sample preparation and the hybrid capture 2 assay (Digene Diagnostics) that used the low-risk and the high-risk probe set were performed according to the manufacturer's instructions as described previously [10].

Results

Forty patients (66.7%) were male and 20 (33.3%) were female. Median age was 52.5 years (range 19–67 years). Patients had neither history nor clinical evidence of anogenital HPV infection. Results are shown in Table 1. In general, HPV infection was detected in 14 patients (23.3%). The prevalence was 20.9% (9/43) in patients that received kidney transplants and 29.4% (5/17) in liver transplant patients. High-risk HPV was detected in 6/43 (13.9%) kidney transplant patients and in 3/17 (17.6%) patients that received liver transplants. In total, 9/60 patients (15%) were high-risk HPV-DNA positive.

Discussion

HPV infection is an extremely common sexually transmitted disease. In approximately 1% of sexually active adults visible genital warts are present, and at least 15% have subclinical infection as detected by colposcopy or anoscopy or by HPV-DNA assays [11]. An additional 60% of sexually active adults have antibodies to genital papillomaviruses, which suggests prior infection [11, 12].

In the 1970s an association between cervical cancer and infection with HPV was first suggested, from the observation that cervical intra-epithelial neoplasia (CIN) and cervical lesions caused by HPV could not be distinguished morphologically. Nowadays, a variety of HPV types is known. Each type seems to be associated with distinct types of lesions. These are linked to low-risk HPVs that cause cutaneous and genital warts and high-risk HPVs that are associated with anogenital cancer and its pre-malignant lesions. Of special interest in anal cancer are the types HPV-16 and HPV-18.

Many epidemiological studies on incidence of anal HPV infection have been performed in high-risk groups (MSM, receptive anal intercourse). Parallel to an increase in anogenital HPV infection, the incidence of anal cancer and its precursor lesions (anal

Table 1 Results of anal swab tests for HPV DNA

Parameter	All		Kidney		Liver	
	n (%)	High risk + Both	*n* (%)	High risk + Both	*n* (%)	High risk + Both
Patients	60 (100.0)		43 (71.7)		17 (28.3)	
HPV-positive	14 (23.3)		9 (20.9)		5 (29.4)	
Low risk	5 (8.4)		3 (7.0)		2 (11.8)	
High risk	6 (10.0)	9 (15.0)	4 (9.3)	6 (13.9)	2 (11.8)	3 (17.6)
Both	3 (5.0)		2 (4.6)		1 (5.8)	

intra-epithelial neoplasia, AIN) has increased in these patients [13]. In addition, an association exists between HPV infection, prevalence of intra-epithelial lesions and suppression of the cell-mediated immune system. Especially in HIV-positive patients, the incidence of HPV infection and AIN is high [14]. Up to 93% of MSM positive for HIV infection are found, by polymerase chain reaction (PCR), to have anal HPV DNA [14]; 73% are infected with more than one type (with the number increasing with decreasing $CD4^+$ count); 36% are diagnosed with AIN [15], and the incidence of invasive anal cancer is 70/100,000 per year [13]. Interestingly, this number approximates the incidence of anal cancer estimated for liver transplant recipients at our department (86.2/100,000 per year).

Owing to common immunological pathways (that involve suppression of T-cell activity), the risk to develop AIN due to HPV-infection in transplantation patients could be similar to that in HIV-positive patients. Only a few studies on prevalence of anal HPV infection have been carried out on patients receiving immunosuppressive therapy after organ transplantation. In a study on renal allograft recipients (8 years' median time on immunosuppressive therapy) the prevalence of HPV-16 DNA in anal swabs was 47%, compared with 12.4% of control subjects [16]. The prevalence of AIN/anal cancer in those patients was 20.3%. In another report the relative risk of anal cancer for renal allograft recipients was calculated to be ten times more than that for non-immunosuppressed individuals [5].

There are few epidemiological data on the prevalence of anal HPV infection in the normal (immunocompetent) population. Koutsky et al. [11] reported a prevalence of 15% for genital HPV infection in women [clinical or subclinical (PCR) disease]. Alterations in the cellular immune system have been reported in uraemia and cirrhosis [17, 18]. In this study on transplantation patients the prevalence of asymptomatic anal HPV infection prior to immunosuppression is considerably high. By the use of the hybrid capture 2 (HC 2) test, 23.3% of patients were shown to be positive for any mucosal HPV type and 15% were infected with high-risk HPV type(s). This non-amplifying DNA detection method is likely to underestimate the true number of infections, as would be detectable by highly sensitive amplification methods (PCR).

In addition, long-term immunosuppression has been shown to increase the number and persistence of HPV-induced lesions. Importantly, due to the increased life expectancy of transplant patients, the incidence of anal and other genital HPV-associated cancer is increasing [4]. Thus, in accord with previous reports, our findings have important implications with regard to prophylactic HPV vaccination to prevent primary infection and/or clinical surveillance of infected patients to prevent invasive disease.

General recommendations of screening programmes depend on a number of factors, including importance of the health condition (frequency, morbidity, mortality), power of the screening test (accuracy, cost, simplicity) and whether early diagnosis leads to a better outcome. Screening programmes for AIN and/or HPV have only recently been established in high-risk groups (especially for MSM and HIV-positive patients). These policies have been shown to improve life expectancy and to be cost-effective [19]. Although the HC 2 test is not as sensitive as screening by PCR for HPV DNA [20], it may preferentially detect clinically relevant lesions.

A prophylactic vaccine for HPV-16 [21] has already been tested in a placebo controlled phase III study in HPV-negative women. After a median study period of 17.4 months, vaccination prevented persistent HPV-16 infection and associated cervical neoplasia with 100% efficacy in a large group of women [22]. Multivalent vaccines with additional HPV types are currently in late-phase clinical trials. Until effective HPV vaccines are approved for general use, the introduction of organized screening programmes for transplant patients appears to be mandatory for the prevention of anogenital cancer in these high-risk groups.

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