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Human papillomavirus DNA in urine samples of women with or without cervical cancer and their male partners compared with simultaneously collected cervical/penile smear or biopsy specimens

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Abstract

Infection of specific types of high-risk human papillomaviruses (HPVs) causes cervical cancer in women. Conventional test for genital HPV infection requires collection of scraped cervical cells or biopsy specimens, which involves invasive procedures. Utility of non-invasive urine sampling for detection of HPV in women and their male sexual partners is controversial. The validation of this urine-based HPV DNA test is of immense value not only in screening large population and children but also for HPV vaccine monitoring in adolescents. We examined the frequency of high risk HPV types 16 and 18 in simultaneously collected urine samples and cervical scrapes or biopsy specimens from women with cervical cancer and their single lifetime male sexual partners in order to validate the utility of urine sampling as a reliable non-invasive method for detection of genital HPV infection. Thirty women with invasive cervical cancer and their husbands along with 30 age-matched normal healthy women including their husbands were recruited for the study. Cervical biopsies/scrapes from women subjects and penile scrapes from their husbands and urine samples from all of them were collected before taking biopsy or scrapes. HPV-L1 consensus primer as well as high-risk HPV (HPV 16 and 18) type-specific oligo-primers were used for PCR detection of HPV DNA. The total frequency of HPV in women with cervical cancer was found to be 83% (25/30) while it was only 67% (20/30) in their male partners but there was virtually no difference in results between urine and scrape or tissue biopsy either in women or their male partners. Although healthy women and their husbands showed similar frequency of HPV infection both in urine and scrape samples, there was a significant difference ($p = 0.05$) in the prevalence of high risk HPV type 16 in women with cervical cancer (70%) and their male partners (30%). Similar was the trend between control women and their male partners. The results also showed a very high prevalence of HPV type 16 among Indian women with cervical cancer while its frequency was significantly low in their single lifetime male partners. The case by case matching of HPV positivity and negativity between urine and cervical/penile scrapes or biopsies obtained from women and their male partners demonstrated that the non-invasive urine sampling can be reliably used for screening genital HPV infection in both men and women.

Keywords: Human papillomavirus; Cervical cancer; Women male partner; Urine; Cervical scrapes; PCR

1. Introduction

Cervical cancer is the most common cancer in Indian women with an annual incidence of about 120,000 new cases, constituting almost 16% of world's annual incidence (WHO, 1986). Several epidemiological risk factors are associated with the development of this cancer including multiple sexual partners, sexual intercourse at an early age, number of pregnancies, poor genital hygiene, smoking, oral contraceptives etc. Specific types of high risk-human papillomaviruses (HR-HPVs) have been strongly implicated as principal causal agents in the development of cervical cancer (Das et al., 1992a, 2000; Vizcano et al., 2000; Zur

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Hausen, 1979; Munoz et al., 2003). Of more than 100 HPV types thus far described, about 30 types are associated with anogenital cancers and HPV types 16 and 18 are considered to be the major high-risk HPV (HR-HPV) types. In India, as high as 98% cervical cancer cases show presence of HPV as compared to 5–20% in normal healthy controls (Das et al., 2000). HPV 16 is the most prevalent type (90%) while a very low frequency (3%) of HPV type 18 is found in India (Das et al., 1992a, 2000). Recent reports also indicate a low prevalence of other high-risk HPV types in India (Franceschi et al., 2003, 2005; Sowjanya et al., 2005).

As with all other sexually transmitted diseases, the approach to control and treat HPV-associated diseases should include assessment of both the index case as well as his or her sexual partner(s). Therefore, understanding manifestations, diagnosis, and treatment of HPV-related diseases of male genital tract is an important part of caring women with genital HPV infection. Thus, screening of male sexual partners for HPV infection has been suggested for women presenting with cervical squamous intraepithelial lesions (Palefsky and Barrasso, 1996). Despite strong evidence implicating specific HR-HPV types in cervical carcinogenesis and an apparent role of male partners in causation of this disease, genital HPV infection in men has not been thoroughly investigated. In particular, the male reservoir for HPV types detected in cervical neoplasia is poorly understood. Studies using PCR-based HPV detection among male partners of women with cervical neoplasia are few and none from India where cervical cancer and infection of HPV are highest in the world and it forms a major public health problem. The present study has therefore been designed to investigate the prevalence of high risk HPV type 16 and HPV type 18 in women with cervical cancer and their male sexual partners in comparison to normal healthy women and their husbands.

Conventional test for genital HPV infection requires collection of scraped cervical cells or biopsy specimens, which involves invasive procedures in a clinic. Such invasive methods are not feasible for a large-scale population screening. Thus there is a need for a simple, reliable and a non-invasive method for screening general population. Urine sampling has been utilized for detection of various genital infections (Hillman et al., 1993a; Tamin et al., 2002) including HPV infection (Brinkman et al., 2002, 2004; Das et al., 1992b; Gopalkrishna et al., 1992; Hillman et al., 1993a,b; Stanczuk et al., 2003). However, utility of urine sampling for detection of HPV infection is controversial (Powell et al., 2003; Fife et al., 2003; Lazcano-Ponce et al., 2001a,b). We have therefore compared the frequency of HPV infection between cervical and penile scrapes or tumor biopsies and urine samples of both partners to validate the utility of urine sampling as a reliable non-invasive method for detection of HPV infection.

2. Materials and methods

The study group comprised 30 women with histologically confirmed invasive cervical cancer and their husbands and the control group consisted of 30 age-matched (±5 years) women with normal or inflammatory or negative cervical cytology and their husbands. The women having multiple male sexual partners were excluded from the study. Informed consent was taken from all enrolled subjects and Institutional Ethical Committee approved using part of human materials obtained for routine diagnostic purposes for research investigation. All male partners were interviewed regarding their sexual behavior by male interviewers using a structured questionnaire.

Cervical biopsies from women with cervical cancer and scraped cervical cells from normal healthy women were collected. Cervical cells were collected by scraping the ectocervix or the surface of cervical portio with a wooden Ayre's spatula. The spatula along with cervical materials were transferred to collection bottles containing phosphate buffered saline (PBS) and stored at −70 °C deep freezer till further processing. The penile cell samples were collected by swabbing the intrameatal and distal urethra, the external surface of the glans and the coronal sulcus of the penis with wet thin cotton-tipped swabs. A smear on glass slide was also prepared for Pap test and remaining cells were eluted in PBS and stored at −70 °C. Urine samples were collected from both women and their male partners before taking biopsy or cervical scrape or penile swab.

DNA extraction was done by using both organic and non-organic methods (Gopalkrishna et al., 1992). The PCR amplification of HPV-DNA sequences was carried out first by L1 consensus primers and then by HPV types 16 and 18-specific primers using the method of Saiki et al. with some modifications (Saiki et al., 1988). The primers used were: (HPV16 upstream 5′-AAG GCC AAC TAA ATG TCA C-3′, HPV16 downstream 5′-CTG CTT TTA TAC TAA CCG G-3′; HPV18 upstream 5′-ACC TTA ATG AAA AAC CAC GA-3′, HPV18 downstream 5′-CGT CGT TTA GAG TCG TTC CTG-3′). All the samples were initially screened by the L1 consensus primers (L1 upstream 5′-GCM CAG GGW CAT AAY AAT GG-3′, L1 downstream 5′-CGT CCM AAR GGA WAC TGA TC-3′, where, M = A or C, R = A or G, W = A or T, Y = C or T) with beta globin gene (5′-GAA GAG CCA AGG ACA GGT AC-3′ and 5′-CAA CTT CAT CCA CGT TAC ACC-3′) as an internal control. Statistical significance was calculated using χ^2 as well as κ statistics where $k = 1$, $p < 0.001$.

3. Results

Comparison of various epidemiological characteristics of cervical cancer cases and their male partners along with controls is presented in Table 1. The mean age of women with cancer was 41.7 years, while 42.1 years was for the control group. The mean age of husbands of women in the study group was 46.4 years, and that of the control group was 46.9 years (see Table 1). Substantial difference was also observed in sexual behavior of male partners of cancer cases when compared to that of controls. They reported significantly a higher