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Human papillomavirus infection among human immunodeficiency virus-infected women in Maharashtra, India

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ABSTRACT

Introduction: Frequency and distribution of HPV types in HIV-infected women with and without cervical neoplasia and their determinants have not been widely studied in India. We report and discuss HPV prevalence and type distribution in HIV-infected women.

Methods: HPV genotyping was done using cervical samples from 1109 HIV-infected women in a cross-sectional study.

Results: Any HPV was detected in 44.8% and high-risk ones in 41.0% women. Frequency of single and multiple high-risk infections were 26.7% and 14.3%, respectively. Frequencies of high-risk HPV infections in women with and without cervical neoplasia were 73.5% and 37.6%, respectively. HPV16 was the most common genotype, present in 11.5%, and 58.5% of women with cervical intraepithelial neoplasia (CIN) 2 and 3. Other most common high-risk HPV types in CIN 2–3 lesions were HPV 31 (22.6%); 56 (13.2%); 18 and 68a (11.3%) and 33, 35 and 51 (9.4%); and 70 (7.5%). Women under 30 or over 44 years, no abortions, and women with diagnosis of HIV infection within the last 5 years were at high risk of multiple oncogenic HPV infection.

Conclusion: We observed a very high frequency of high-risk HPV and multiple infections in HIV-infected women.

1. Introduction

Persistent infection with one of the high-risk types of human papillomavirus (HPV) infection is the necessary cause for the development of cervical intra-epithelial neoplasia (CIN) and cervical cancer [1–4]. More than 110 types of HPVs are known and about half of them infect the genital tract. HPVs that are associated with cancers are termed as high-risk HPVs and those that cause benign lesions are termed as low-risk HPVs. Of the 110 HPV genotypes described so far, IARC (WHO) has classified 13 types as 'carcinogenic' and they are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Seven genotypes are 'possibly carcinogenic' and they are HPV 26, 53, 66, 67, 70, 73, and 82. Seventeen genotypes are 'non-carcinogenic/unknown carcinogenicity' and they are HPV 6, 11, 40, 42, 54, 55, 61, 62, 64, 69, 71, 72, 81, 83, 84, CP6108, and IS39 [5].

It is well known that women infected with human immunodeficiency virus (HIV) are at increased risk of cervical cancer due to a high-risk of HPV infection and persistence among them as a consequence of immunosuppression. HIV-infected women have a high prevalence of a broad range of HPV genotypes, multiple concurrent infections and persistent infections which progress at a faster pace to neoplasia as compared to HIV negative women [6–10]. The high frequency of persistent HPV infection is responsible for the high HPV prevalence and a high risk for cervical intraepithelial neoplasia (CIN) among HIV-infected women [11–17].

In spite of having the third largest burden of HIV-infected individuals and one-fourth of the global burden of cervical cancer, very few studies have addressed HPV prevalence, genotype distribution and cervical cancer prevention in HIV-infected women in India. We conducted a cross sectional study to evaluate HPV prevalence, HPV type distribution and test performance of multiple screening tests in detecting CIN2/3 lesions in HIV-infected women in

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Maharashtra, India. Our findings of performance of multiple screening tests (visual inspection using 5% acetic acid, visual inspection using Lugol's iodine, cytology and HPV testing) in detecting high-grade CIN and their treatment using cold coagulation have been previously reported [18]. We report and discuss HPV prevalence and type distribution in HIV-infected women in this communication.

2. Methods

The study was approved by the scientific and ethical review committees of the International Agency for Research on Cancer (IARC) of the WHO, Lyon, France, and the ethics committees of Hirabai Cowasji Jehangir Medical Research Institute (HCJMRI) and Prayas Health Group, Pune, India. Screening was initiated on 9 September 2010 and completed on 3 November 2011.

2.1. Study procedures

Our detailed study procedures have been described earlier [18]. The study was conducted in a designated study clinic in Pune, India, where consecutive, serologically confirmed HIV infected women regardless of their CD4 cell counts or antiretroviral treatment (ART) but satisfying the eligibility criteria were recruited after informed consent. HIV-infected women in the age group of 21 and 60, having an intact uterus, who were not pregnant and had not received any prior treatment for CIN or cervical cancer were eligible for the study. They were explained about the study objectives and procedures and their written informed consent was obtained. They were interviewed for socio-demographic, sexual, reproductive, medical, and HIV infection related characteristics using a structured questionnaire by a female social worker.

Women were examined in a modified lithotomy position. A trained nurse performed a speculum examination, exposed the cervix and collected cervical samples for HPV DNA test by Hybrid Capture 2, cytology and HPV genotyping. The cervical cells for HPV genotyping were collected in PreservCyte medium and stored in $-80\,^{\circ}\mathrm{C}$ before transportation to the testing laboratory. After the collection of cervical cells, freshly prepared 5% acetic acid was applied on the cervix with the help of a cotton swab to perform visual inspection with acetic acid (VIA) and the nurse recorded her findings independently. A trained doctor performed colposcopy in all enrolled women and when the doctor applied Lugol's iodine as part of the colposcopic assessment, the findings of naked eye visual inspection using Lugol's iodine (VILI) were recorded by the nurse. The criteria described in IARC manuals were used for reporting findings of visual screening tests [19] and that of colposcopy [20]. Pap smears were processed and reported by cytotechnicians and pathologists using 2001 Bethesda classification at the Nargis Dutt Memorial Cancer Hospital (NDMCH) in Barshi; HPV testing by HC2 was also performed and reported by NDMCH [18].

Women having colposcopically detected ectocervical abnormalities involving three-fourths or less of the transformation zone (TZ) suggestive of CIN underwent directed multiple cervical punch biopsies and had ablative treatment with cold coagulation in the same session; those with colposcopic abnormalities characteristic of CIN involving the entire TZ or with lesions extending into the canal or with high-grade positive Pap smear but with no colposcopic abnormalities or with unsatisfactory colposcopy underwent excision of the TZ with loop electrosurgical excision procedure (LEEP) [18]. Biopsies and excised tissue specimens were processed and reported by consensus by two experienced pathologists according to the CIN system. The final diagnosis of CIN and invasive cancer was based on histopathology. Women diagnosed with invasive cervical cancer were referred for appropriate anti-cancer treatment.

2.2. HPV genotyping

DNA extraction and HPV genotyping was carried out at the Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum, India, where a dedicated and quality assured laboratory for HPV genotyping has been established in collaboration with the IARC. For the first consecutive 600 participants an aliquot from the specimen transport medium (STM) which was used for HC2 test was used for DNA extraction for HPV genotyping whereas for the remaining consecutive participants from 601 to 1153, we collected cervical samples in PreservCyte medium.

HPV genotyping was done by HPV type-specific E7 PCR bead-based multiplex genotyping (TS-MPG). The multiplex HPV type-specific E7 PCR utilizes HPV type-specific primers targeting the E7 region for the detection of 19 high-risk (HR)/probable HR-HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a, 68b, 70, 73, 82), and two low-risk (LR)-HPV types (HPV 6 and 11), with detection limits ranging from 10 to 1000 copies of the viral genome. The amplicon size varies between 210 and 258 bp. Two primers for amplification of the β-globin gene were also included to provide a positive control for the quality of the template DNA. Following PCR amplification, 10 μl of each reaction mixture was analyzed by MPG using the Luminex technology (Luminex Corporation, Austin, TX) as described previously [21–23]. Briefly, the PCR products were generated, denatured, and hybridized to the bead-coupled probes in 96-well plates, which allowed the PCR products from 96 samples to be processed in parallel. After transferring the products into wash plates with filter bottoms, the unhybridized DNA was removed. Subsequently, the biotinylated PCR products were stained with a streptavidin-R–phycoerythrin conjugate. After further washing steps, the beads were analyzed in the Luminex reader, which contains two lasers to identify the bead set by the internal bead color, and to quantify the reporter fluorescence on the bead. The results are expressed as the median fluorescence intensity (MFI) of at least 100 beads per bead set. For each probe, the MFI values obtained when no PCR product was added to the hybridization mixture were considered the background values. The cut-off point was computed by adding 5 MFI to $1.1\times$ the median background value.

2.3. Data management and statistical analysis

Data were entered using Access 2000 software and statistical analysis was carried out using STATA software, version 12.0 (StataCorp, College Station, Texas, USA). The distribution of HPV infection and genotypes was presented as proportion. Comparison of proportions between women with and without cervical neoplasia was done using the test of equality of proportions using large-sample statistics. Multinomial logistic regression was used to assess the effect of socio-demographic, sexual, reproductive, medical, and HIV infection related characteristics on the single and multiple high-risk HPV infections. Adjustment in the multivariate model was carried out using only characteristics significant at 5% level in the univariate regression analysis. For the regression analysis of a particular endpoint, individuals with the other endpoint and those with no high-risk but with probable high-risk types were excluded, whereas those with only low-risk types were included in the no high-risk category.

3. Results

We enrolled 1153 HIV-infected women and their characteristics have been described in detail earlier [18]. The mean age of the participants was 34.9 (SD 6.67, range 21–62) and only 13% did not