RESEARCH COMMUNICATION

Human Papillomavirus Screening in North Indian Women

Saumya Pandey[1], Malvika Mishra[1], Chandrawati[1,2]*

Abstract

Objectives: Human papillomavirus (HPV) is the major etiological agent of cervical cancer, a leading cause of morbidity and mortality in women worldwide. Screening strategies for reducing the burden of HPV-mediated carcinogenesis are emerging as an effective means for cervical cancer control and prevention in developing countries. Our study, therefore, aimed to identify HPV infection status in North Indian women during random population screening. Methodology: Cervical/vaginal exfoliated cells and/or Pap smear specimens were collected from 890 women of North Indian ethnicity residing in Lucknow and adjoining areas, during random population screening from June 2009-March 2012. HPV viral loads in clinical specimens were determined by the Hybrid Capture (hc)-2 HPV DNA assay, and subsequently, positive/negative/borderline HPV status was calculated. Results: The HPV incidence in the present study was 11.7%. 751 out of a total of 890 women (84.4%) participating in our HPV screening program were HPV negative (HPV -), 104 (11.7%) tested positive (HPV +) while 35 (3.9%) showed borderline (HPV *) infection status. Furthermore, in the HPV + subjects (N=104), 18 (17.3%) showed strong positivity. We observed that HPV positivity tends to increase with age in North Indian women; the higher the viral load with increasing age, higher is the susceptibility to HPV-mediated cervical cancer. Conclusions: HPV viral load/genotyping may help in identifying women at risk of developing cervical cancer. However, cost-effective HPV screening protocols with a wider population coverage are warranted so as to reduce the burden of cervical cancer in women worldwide in the vaccine-era.

Keywords: Cervical cancer - human papillomavirus - north India - screening - viral load

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Introduction

Cervical cancer is a leading cause of morbidity and mortality in women worldwide (Vizcaino et al., 2000; Walboomers et al., 1999). The high-risk Human Papillomavirus (HPV) types 16 and 18 are the major etiological agents of cervical cancer (Bosch et al., 2002; Zur, 2002); despite being a preventable disease, cancer of the uterine cervix claims the lives of almost half a million women worldwide each year (Stamenkovic, 2000) and about a fifth of the global cervical cancer cases are still in India (Ferlay et al., 2004). There are approximately 130,000 new cases of cervical cancer in India per year and the age-standardized incidence rate is 30.7 per 100,000 (Dabash et al., 2005). The link between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold zur Hausen, a German virologist; although HPV is considered as a major causative agent of cervical cancer, yet the viral infection alone is not sufficient for cancer progression and/or malignancy (Ganguly and Parihar, 2009).

HPV is a double-stranded DNA virus that is non-enveloped and has an icosahedral capsid; the virus replicates as an extrachromosomal DNA inside the nucleus of the host cell (Longworth and Laimins, 2004). At present, about 118 different types of HPV have been characterized (Jo and Kim, 2008); depending on the risk of malignancy, HPVs are further grouped as high risk or low risk types. The etiopathogenesis of cervical cancer is indeed complex, and the progression to cancer generally takes place over a period of 10 to 30 years. HPV 16 and 18 are considered the most prevalent high risk types for carcinogenesis while HPV types 6 and 11 are the most prevalent low risk types with benign and genital warts (Motoyama et al., 2004; Li et al., 2005). Further, there are thirteen more high-risk HPV types viz. 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, and three probable high risk types viz. 26, 53, 66 (Zur, 1991). Out of these, HPV 16 and 18 are estimated to account for about 70% of all cervical cancers and altogether HPV 16, 18, 45, 31, 33, 35, 52, 58 are responsible for about 90% of all cervical cancers worldwide (Bosch, 2003). In addition to the most prevalent low-risk HPV types HPV 6 and 11, other types are 40, 42, 43, 44, 54, 61, 70, 72 and 81 (Villers et al., 2004).

Cervical cancer is a major public health problem in India. Screening strategies for reducing the burden of HPV-mediated carcinogenesis are emerging as an effective means for cervical cancer control and prevention in developing countries. Organizing screening programs in developing nations is indeed a big challenge. The key to reducing cervical cancer morbidity and mortality is

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early detection coupled with timely treatment of cervical precancerous lesions. Cervical cytology referred to as the Pap smear is perhaps the most well known screening method; however, newer screening techniques such as visual inspection methods and high-risk HPV DNA testing have also demonstrated potential for early detection and/or management of patients with atypical cytologic findings (Miller, 1992; Solomon, 2001; Bovicelli, 2009). Our study, therefore, aimed to identify HPV infection status in North Indian women during random population screening.

Materials and Methods

Selection of study subjects

HPV screening was conducted in a random population during May 2009 to March 2012. A total of 890 study subjects participated in our screening program at Krishna Medical Center, Lucknow. Females of North Indian ethnicity residing in Lucknow and adjoining areas in state of Uttar Pradesh were selected for HPV screening; a personal interview was conducted wherein the participants were informed/educated about HPV and HPV-mediated cervical cancer; this was followed by group discussion so as to increase awareness about HPV-related malignancies. Written informed consent was taken from screening participants.

Clinical specimen collection

Cervical/vaginal exfoliate cells and/or Pap smear specimens were collected after detailed gynecological examination; cell scrapes/tissues were collected from suspicious lesions in sample collection tubes containing STM medium and stored at 4°C prior to HPV DNA testing. HPV genotyping/infection status: HPV genotyping was carried out using the Digene Hybrid Capture (hc) 2 HPV DNA test (Oncquest/Gentech, India). The hc 2 HPV DNA test is a semi-quantitative test (5000 viral DNA copies/ml cut-off) that uses RNA probes specific for full-length HPV genomes of 13 viral types responsible for the pathogenesis of high-grade cervical cancer HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68. A cut-off ratio of 0 to 0.80 is negative for high risk HPV; a cut-off ratio of 0.81-1.20 is considered borderline, and a cut-off ratio greater than 1.2 is positive for high risk HPV. Furthermore, a cut-off ratio at 1.0 corresponds to a viral DNA load of 5000 copies/ml or 1 picogram/ml at a threshold of finding a clinical disease or prognosis of a precancer. The clinical sensitivity to detect high grade squamous intraepithelial lesion (HSIL) is greater than 99%.

Data analysis

The descriptive statistics for the continuous variables were given as means with standard deviations while those for categorical data were given as frequency distributions.

Results

In the present study with a total of 890 participants in the age group of 20-70 years (average age of 35.5 ± 8.0 years), HPV incidence was observed to be 11.7%. Thirteen high risk HPV types, viz. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 were detected during the HPV screening program at our study center in Lucknow. Out of 890 study subjects, 751 (84.4%) participants were HPV negative (HPV-) [35.5 ± 8.0 years], 104 (11.7%) tested positive (HPV+) [mean age 35.9 ± 8.3 years], while 35 (3.9%) showed borderline (HPV*) [mean age 34.8 ± 7.4 years] infection status (Figure 1).

Furthermore, in the HPV+ subjects (N=104) [average viral load/cut-off ratio of 151.3], 18 (17.3%) showed strong positivity (HPV+++), as indicated by the HPV cut-off ratio (Figures 2 and 3). The mean age in stratified HPV+++ women was 39 ± 11.5 years, with an average viral load/cut-off ratio of 826.3 (Table 1).

A positive HPV infection status suggested current high risk HPV infection and was strongly predictive of cervical squamous intraepithelial lesion (SIL) and cervical cancer severity. A negative HPV status suggested the absence of high risk oncogenic HPV strains. Higher the viral load in terms of test cut-off value, higher is the susceptibility to develop cervical cancer. The viral load of HPV infection may vary with the age of the patient; we, therefore, correlated the viral load with age in the HPV positive group comprising of 104 women. However, as

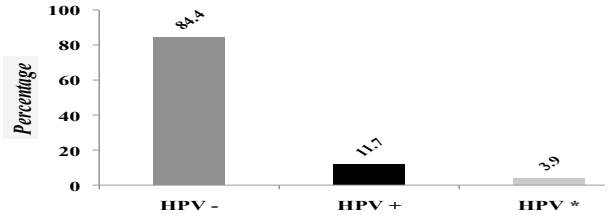

Figure 1. HPV infection status in North Indian women (N = 890 study subjects). 751 women (84.4%) were HPV negative (HPV -), 104 (11.7%) tested HPV positive (HPV +), and 35 (3.9%) showed borderline (HPV *) HPV infection status.

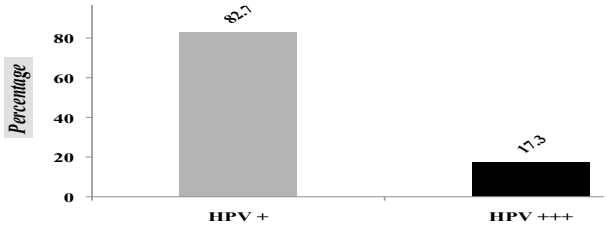

Figure 2. HPV Positivity in North Indian Women (N = 104 Study Subjects). 86 Women (82.7%) Tested HPV Positive (HPV +) While 18 (17.3%) Showed Strong Positivity (HPV

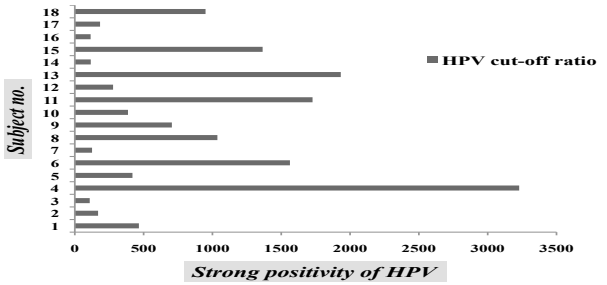

Figure 3. Schematic Depiction of Strong HPV Positivity in Study Subjects (N = 18), as Indicated by the HPV Cut-Off Ratio.

Table 1. Strong HPV Positivity in North Indian Women. Out of a Total of 890 Women Participating in our HPV Screening Program, 18 Showed Strong Positivity (HPV +++).

Subject no.	Age in years	HPV cut-off ratio
1	*	465.68
2	32	169.10
3	45	108.43
4	31	3228.81
5	*	418.89
6	38	1563.14
7	*	125.28
8	*	1036.01
9	26	705.24
10	*	386.35
11	37	1727.36
12	60	278.06
13	60	1932.54
14	36	116.02
15	*	1364.64
16	35	114.85
17	29	183.45
18	*	949.90

the age details of 73 women were available, we stratified the HPV+ group according to age ≤ 30 (N=24) and age ≥ 30 years (N=49), and observed mean HPV viral loads of 49.4 and 197.6, respectively. Our findings suggested that HPV positivity tends to increase with age in North Indian women, and therefore, higher the viral load with increasing age, higher is the susceptibility to HPV-mediated cervical cancer.

Discussion

Cervical cancer, a major public health problem among women worldwide, is linked to persistent infection by HPV (Zur, 2002). Cervical tumors have been shown to harbor HPV sequences in as many as 99.7% of the cases analyzed, implying a need for the sustained presence of viral DNA during carcinogenesis (Dabash et al., 2005). This finding led to the assumption that HPV testing would be useful for the diagnosis and monitoring of cervical cancer. Unfortunately, the mere presence of viral DNA has been shown to have poor positive predictive value for cervical cancer because of high rates of transient infections in sexually active women (Stoler 2001; Hildesheim et al., 2004).

In the present study with 890 participants, HPV incidence was observed to be 11.7%. During the HPV screening program at our study center based in Lucknow, the HPV infection in terms of negativity, positivity and borderline status was 84.4%, 11.7% and 3.9%, respectively; the viral types detected were HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. A positive HPV status was suggestive of high risk infection and predictive of cervical cancer severity; on the contrary, a negative HPV status suggested the absence of high risk oncogenic HPV strains. We observed that HPV positivity tends to increase with age in North Indian women; higher the viral load with increasing age, higher is the susceptibility to HPV-mediated cervical cancer.

In India, the most common oncogenic types are HPV types 16 and 18 with HPV 16 being the most prevalent (80-90%); however, region specific prevalence of HPV varies in India and the most consistent variation has been reported in the prevalence of HPV 16 rather than other types and this regional variation may be due to genetic, cultural and ethnic diversity as well as heterogeneity between studies (Bharadwaj et al., 2009). Moreover, a comparative study (Das et al., 2008) has shown that the peak of HPV infection appears at a later age as compared to that of western countries; the prevalence of HPV infection/ cervical cancer in India indicates that the initiation as well as peak of HPV infection occurs at a slightly higher age (26-35 years) in women mostly in their third decade of sexual activity than that of global scenario (peak in 18-25 years). Clinical trials with two recently developed HPV prophylactic vaccines, quadrivalent Gardasil (HPV 16/18/6/11) by Merck and bivalent Cervarix (HPV 16/18) by Glaxo Smith Kline (GSK), recommended by US Food and Drug Administration (FDA), have indicated the vaccines to be highly immunogenic, well tolerated, safe as well as highly effective in preventing incident and persistent HPV infections (Villa et al., 2005: 2006; 2007; Harper et al., 2004: 2006). Moreover, in most countries, the three-stage conventional screening for cervical cancer (Pap smear, colposcopy/biopsy and treatment) repeated at regular intervals has not been sustainable, thereby warranting active research to evaluate screening alternatives (Denny et al., 2006). Studies have shown that the use of HPV DNA testing as primary screening method is significantly more sensitive than cytology-based screening, either conventional or liquid based (IARC 2005; Cuzick et al., 2006; Ronco et al., 2006; Mayrand et al., 2007). In India, a visual inspection method/VIA programme was found effective in reducing the incidence and mortality of cervical cancer (Sankaranarayanan, 2007; Bosch, 2008).

The present study had some strengths as well as limitations. The study subjects enrolled in our HPV screening program were of North Indian ethnicity, thereby reducing the possibility of heterogeneity in terms of ethnicity and geographical diversity of the HPV types. Our sample size was relatively large with a total of 890 women during a 2.8 year timeline, thereby strengthening the accuracy of our findings. Moreover, we maintained the quality of the clinical specimens, viz. cervical/vaginal exfoliate cells and Pap smears, throughout the course of the present pilot study, thereby reducing any possibility of sample contamination/degradation from the time of collection to the clinical assay. The study also had some limitations; the age of the participants was missing in a few samples during the rigorous HPV screening programme. Furthermore, parameters such as marital status, age at menarche/menopause, parity and socioeconomic status were not included in the present study. In conclusion, our pilot study strongly implicates HPV DNA testing as an effective screening strategy for cervical cancer control and prevention in North Indian women. However, cost-effective HPV screening protocols with a wider population coverage are warranted so as to reduce the burden of

cancer of the uterine cervix in women worldwide in the vaccine-era. Therefore, cervical cancer, the number one cancer affecting Indian women in the 21st century is an eradicable condition.

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