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Prevalence and age-wise distribution of Human Papillomavirus type 16/18 infections among hospital screened women of a peri-urban area in West Bengal: Impact of socio-demographic factors

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ABSTRACT

Background: We undertook the current study on cervical Human Papillomavirus (HPV) prevalence along with cytology in women visiting the Gynecology Out-patient Department of a hospital for common gynecological ailments, subsequent to our earlier population-based study on HPV prevalence from India.
Methods: We analyzed data on cervical-cytology (Pap smears) and PCR-based molecular detection of HPV infection along with socio-demographic variables (N = 696).
Results: We identified 36.84% HPV-positive women amongst whom, HPV16 and 18 together predominated (79.37%) over other HPV types (20.63%). Contrarily, only 6.4% women revealed abnormal cytological lesions, of which, 46.51% were HPV-positive and 95% of such women harbored HPV16/18, while 5% harbored other HPV types. Individuals with normal cytology portrayed 36.09% HPV infections, of which, 77.97% were HPV16/18-positive and 22.03% harbored other HPV types. Overall HPV prevalence decreased significantly (p_{trend} = 0.047) with increase in age, but HPV16/18 infections were significantly over-represented compared to the other HPV types across all age-groups. Specifically, HPV16 prevalence increased (p_{trend} < 0.01) with increase in severity of cervical lesions. HPV16 prevalence did not differ between the Hindus and Muslims but HPV18 was significantly higher among the cytologically normal Muslim women (24.14%, p = 0.02), compared to the Hindus (11.91%), specifically among those ≥ 30 years of age. There was a significant (p < 0.05) over-representation of HPV16 prevalence among women who were users of oral contraceptive-pills, irrespective of cytology.
Conclusions: Our study highlights the need for HPV16/18-based screening of cervical cancers in India considering the immense socio-cultural and genetic diversity at the population level.

1. Introduction

Infection with Human Papillomavirus (HPV) and viral persistence in the host cervical epithelial cells are established as major etiologic factors for cervical cancer (CaCx) development [1–3]. The disease development process is often preceded by a prolonged pre-cancerous phase

Abbreviations: HPV, Human Papillomavirus; PAP, Papanicolaou; PCR, polymerase chain reaction

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after initial infection with high-risk HPVs (HR-HPVs). Thus, unlike most other types of cancer, cancer of the cervix is preventable through early detection of pathogenic infections and treatment of precursor lesions [4]. Global data suggests that both the incidence and mortality due to cancer of the cervix has fallen dramatically in the developed countries [4,5] since the advent and widespread application of cytology-based and/or molecular HPV screening. However, in the absence of screening, nearly 70% of cancer of the cervix patients in India present in stages III and IV [4,6].

At present, in India, cancer of the cervix ranks as the second most prevalent cancer type among women, specifically across sexually active women of the age-group 15–44 years. Almost 469.10 million Indian women aged 15 years and older are under the potential threat of developing cancer of the cervix, while annually 122,844 women are diagnosed with cancer of the cervix and out of them 67,477 die each year from the disease [7]. Screening for cancer of the cervix and HPV infection at population level is thus a fundamental necessity as women often do not experience symptoms until the disease has progressed to advanced stages. Current estimates suggest that HPV16 and 18 are the most prevalent types among women with normal cytology. About 5.0% of such women with normal cytology harbor cervical HPV16 and/or HPV18 infection at a given time point, while, 83.2% of the invasive cervical cancers are attributed to either HPV16 and/or 18 [7]. After HPV16/18, HPV-types such as HPV31, 33, 35, 45, 52 and 58 are found to be the most commonly oncogenic across the globe, accounting for an additional 20% total incidence of cancer of the cervix worldwide [8].

India harbors an enormous population burden, portraying an immense socio-cultural and genetic diversity. This seems to be concomitant with a wide diversity in prevalence and type-distribution of HPV infection across different geographical regions within India [7,9–22], as recorded worldwide [23]. Thus, data on relative prevalence of different HPV types are extremely crucial for the development of optimal strategies for disease containment and/or management either through HPV screening or vaccination [24,25].

Latent HPV infection and pre-cancerous changes are often asymptomatic [4] and remain undetected unless a woman undergoes screening. It is likely that women coming to a hospital with common gynecological ailments might be exposed to HPV infection as well [26] and could be at heightened risk of developing cancer of the cervix. Earlier, we identified that approximately 6.70–11.60% of women from the general populations of the eastern and north-eastern regions of India harbored HPV infections, while, the oncogenic HPV16/18 infections ranged between 3.27–7.85% [27]. However, hospital-based studies including a large cohort covering a wide geographical area, are limited in numbers [28–32]. We, therefore, undertook the current study to determine the prevalence of overall HPV infection along with that of types HPV16/18 in cervical scrapes of different cytological grades from women attending the Gynecologic Out Patients Department (GYN-OPD) of a major hospital, catering to a peri-urban population, in an around Kalyani, located 50 km s towards the North of Kolkata. Besides exploring the association between HPV infections, including HPV16/18, and the degree of severity of cervical lesions, we also aimed to identify the association of key socio-demographic factors, particularly age and religion, as epidemiological determinants of such infection among women from such a population, previously unexplored. This study by virtue of its hospital-based nature and a decent sample size with multiple associated risk-factors as parameters will probably highlight the utmost need of an organized cervical cancer screening program in India.

2. Materials and methods

2.1. Subjects and samples

Subjects (n = 696) for this study were recruited from the College of Medicine and Jawaharlal Nehru Memorial Hospital (J.N.M. Hospital), Kalyani, with the approval from the institutional ethical committee for human experimentation. Only those women, fulfilling the inclusion criteria [27], were enrolled for this study (Supplementary material) and ecto and endo cervical cell scrapes were collected for identification of cellular abnormalities and testing for the presence of HPV infections.

2.2. HPV screening by PCR- using generic primers from viral L1 region

Samples were screened for overall HPV infection by pursuing a nested PCR based approach, that first uses the consensus L1 (MY 9/11) primers [33] amplifying ~450-bp fragment from viral L1 region, followed by a second round of amplification using BSGP 5/6 primer pools [34] that amplify ~150-bp region from the same L1 region (Supplementary material). A primer pair for β-globin gene was used as internal control to ensure the integrity of the DNA samples [35].

2.3. PCR- based detection of HPV16/18 using type-specific primers

Based on the fact that HPV type 16 and 18 are the most prevalent types to be present [7], samples positive for HPV infection, were further screened for HPV16 and 18 employing HPV E6-specific primers corresponding to HPV16 and 18 [36]. The protocols and programs remained unchanged [22,27]. Samples that turned out to be positive for overall HPV infection but negative for either HPV16/18 or both were considered as HPV positive for additional types other than HPV16/18. The primer sequences used for HPV screening are given in Supplementary Table 1.

2.4. Statistical analysis

The association of epidemiologic variables with HPV infection in women across different cytological grades were determined by logistic regression analysis and interpreted in terms of age- adjusted odds ratios [OR] along with 95% confidence intervals [CIs]. Chi-square test, trend Chi-square test were performed wherever necessary, and p–values were determined to interpret the significance of the findings. For all the cases, $p \leq 0.05$ was regarded as statistically significant. All statistical analyses were performed using the R- software package, SPSS v16.0 and EXCEL–Windows 7 version.

3. Results

3.1. Prevalence of HPV overall, HPV16/ 18 and other HPV types among cervical scrape samples

Out of 696 collected samples, DNA from 12 samples was of poor quality and hence was excluded from analysis. HPV status of 684 samples was thus available of which, 36.84% (252/684) women were found to be HPV positive [Table 1]. Across HPV positive women, HPV16 and 18 together predominated (79.37%, 200/252) over types other than HPV16 and 18 (20.63%, 52/200), thereafter referred to as “other HPV types”.

Table 1
Prevalence of overall HPV infection and selected High-risk HPV types (HR-HPVs) among hospital-screened women (N = 684).

HPV Types	No. of Positive cases	Prevalence (%)
HPV Positives	252	36.84
HPV16	108	15.79
HPV18	71	10.38
HPV16/18 Both	21	3.07
HPVs other than HPV16/18	52	7.60