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PRE[illegible] PAPILLOMA VIRUS IN CERVICAL LESIONS AND ASYMPTOMATIC HEALTHY SUBJECTS OF CENTRAL INDIA

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ABSTRACT

Background: Detection of human papilloma virus (HPV) infection and its genotype distribution are much-needed parameters to judge the risk of cervical cancer among females. However, due to less availability of data on HPV burden in Central part of India makes cervical cancer screening difficult.

Methods: A total of 270 participants had been tested for abnormalities in cytology (Pap smear) and real-time PCR was performed for HPV DNA detection.

Result: The most common cytological finding among HPV positive patients was found to be of negative for intraepithelial lesion or malignancy NILM (93.7%), followed by atypical squamous cells of undetermined significance (ASCUS) (5.2%), low-grade intraepithelial lesion (LSIL) (0.7%) and high-grade intraepithelial lesion (0.35%) cases. HPV DNA was detected in (8.1%) patients. Highest positivity was seen in the age group of 30-39 years followed by age group of 20-29 years. The most common HPV type in our study was HPV 16 (45.4%) followed by HPV 31 infection (27.2%). Other genotypes of HPV found were HPV18 (13.6%), HPV 51 (2; 9%), HPV 58 (2; 9%), and HPV 39 or 68∗ (9%).

Conclusion: It is evident from our study that HPV DNA infection was observed with normal cervical cytology. For early detection both Pap smear and HPV DNA genotype detection should be the choice for preventing cervical cancer in women.

Keywords: Human Papilloma Virus; Genotype; Cytological Abnormalities; High Risk; Central India

Abbreviations

HPV: Human Papilloma Virus; HR: High Risk; ASCUS: Atypical Squamous Cells of Undetermined Significance; NILM: Negative for Intraepithelial Lesion or Malignancy; LSIL: Low-grade Intraepithelial Lesion; HSIL: High Grade Intraepithelial Lesion; WHO: World Health Organisation; LBC: Liquid Based Cytology; IAPCOI: Indian Academy of Paediatrics Committee on Immunization

Introduction

Cancer of the cervix is the third most common malignancy affecting women globally. It occurs most commonly in women above 30 years of age. Cancer of the cervix commonly presents with abnormal vaginal bleeding in advanced stages but in early stages it may go unnoticed as there are no obvious symptoms to raise an alarm. Every year approximately 1.2 million women develop cancer of the cervix [1]. India accounts for about 15.2% of the total mortality due to cervical cancer in the world [2].

The incidence of Cancer of cervix has decreased in the urban population due to better screening methods easily available to the women like pap smear, colposcopy and molecular testing for Human Papilloma virus. However, the incidence is still high in the rural population [3]. The progression from mild dysplasia to frank malignancy takes a few years, so it is easy to prevent the progression by timely screening and detection [4]. Infection with Oncogenic Human Papilloma virus (HPV) is the most common risk factor for developing Cancer in cervix [5]. Other surrogate risk factors include multiparity, socioeconomic status, age at menarche and menopause, multiple sexual partners, poor genital hygiene and lack of knowledge and awareness. The global prevalence of Cancer cervix is estimated to be around 9-13% [6]. The molecular, clinical and epidemiological data available point towards the causative role of HPV in precancerous and cancerous lesions of the cervix. Out of the known 80 HPV genotypes, approximately 22 genotypes infect the female genital tract out of which the types 16 and 18 are considered to be the most prevalent types responsible for approximately 70% of invasive cervical cancers. In India, the infection of HPV type 16 is found to be the very high, followed by HPV type 18 [9]. The occurrence and frequency of HPV type in cervical lesion depends on the geographical and racial variation [10].

Human papilloma viruses are obligate intranuclear residents, which transform normal cells into neoplastic cells by their oncogenic potential. HPVs are small double-stranded DNA tumour viruses with a genome size of approximately 8000 base pairs [bp] belonging to family Papillomaviridae [8]. The infection by HPV may be latent, replicative or may lead to neoplastic transformation. If the infection is detected in the latent and replicative phases, the infection can be cleared by appropriate measures.

In 1974, Professor Harald Zur Hausen first attempted to find HPV DNA in tissues from cervical cancer and genital wart by hybridizing tumour DNA with cRNA obtained from purified plantar wart HPV DNA [7]. Thus, it was established that infection of specific types of HPV was essential for the development of cervical cancer.

HPV induced cytological changes in pap smear are koilocytic changes in the squamous cells and thickening of the inner and outer layer of the cytoplasm. Apart from pap smear and colposcopy, newly developed molecular assays have made it possible to detect HPV infection in the latent phase with high degree of accuracy.

The present study aims to study the prevalence of HPV infection in females of Central India and its correlation with pap smear findings. 10

Material and Methods

Study design, settings, and participants

The cross-sectional study was conducted from January 01, 2021 to August 31, 2022 in Sampurna Sodani Diagnostic Clinic, Indore, India. Women above 20 years of age presenting with symptoms like abnormal vaginal bleeding/discharge, pain during coitus, and lower abdominal pain were included in the study. PAP smear was done on these patients. Cervical washings were taken in DNA Liquid Based Cytology (LBC) cervical sample transport medium according to the manufacturer's protocol (Easy prep, YD Diagnostics Corp., Seoul, Korea) along with the PAP smear for testing HPV DNA detection through PCR.

Sample collection and processing

Pap smear was taken for each patient according to the protocol in LBC vials (Easy prep, YD Diagnostics Corp., Seoul, Korea). Cervical washings were taken in DNA LBC cervical sample

transport medium according to the manufacturer's protocol [11] along with the PAP smear. Collected samples were stored at 4°C till further processing.

DNA extraction and HPV genotype detection

DNA extraction was done using TRUPCR Tissue DNA extraction kit (Cat. No. 3B207, 3B Black Bio Biotech India Ltd.) as per the manufacturer's instructions. TRUPCR® HPV HR Genotyping Kit Cat. (No. 3B1423/3B1424, 3B Black Bio Biotech India Ltd.) was used for detection and genotyping of Human papilloma virus DNA in clinical samples [12]. This multiplex PCR commercial kit uses fluorescent reporter dye probes specific for detection and genotyping of high-risk HPV 14 genotypes (16, 18, 31, 33, 35, 39,
45, 51, 52, 56, 58, 59, 66, and 68).

Results

Total 270 female patients were referred for PAP smear and HPV testing. The most common cytological finding among HPV positive patients was found to be of negative for intraepithelial lesion or malignancy NILM (253/270; 93.7%). Abnormal cytology was observed among 17 patients; of those 14 patients had atypical squamous cells of undetermined significance (ASCUS) (14/270; 5.2%), two cases had low-grade intraepithelial lesion (LSIL) (02/270; 0.7%) and one was high grade intraepithelial lesion (01/270; 0.35%) ḤSIL case (Table 1).

Age group (years)	NILM	Ascus	LSIL	HSIL	Total
21-29	84	2	0	0	86
30-39	111	6	1	0	118
40-49	44	3	0	0	47
50-59	9	3	1	1	14
>60	5		0		
Total	253	14	2	1	270

Table 1: Demographic profile of the patients (pap smear report).11

All 270 study participants were further tested for Human papilloma virus [HPV] DNA detection.

Among them, HPV DNA was detected in 22 (8.1%) patients. Cytology and HPV DNA PCR reports were compared in these patients. Sixteen HPV DNA PCR positive patients belong to NILM category, ASCUS was found in four patients, whereas one each case of LSIL and HSIL reported (Fig 1).

Mono infection (n=18)						**Coinfection (n=4)**			
HPV 16	HPV 18	HPV 31	HPV 51	HPV 58	HPV 39/68	16 and 31	16 and 58	16, 18 and 45	31 and 39/68
7	3	4	2	1	1	1	1	1	1

Table 2: Distribution of HPV genotype in single and coinfection.

Analysis of genotype distribution in 22 patients showed that

18 patients had single genotype infection whereas multiple genotype infection was observed in four patients (Table 2). The most common single type of HPV infection in our study was HPV 16 (n = 7; 31.8%) followed by HPV 31 infection (n = 4, 18.1%). Other genotypes of HPV found were HPV18 (n = 3; 13.6%), HPV 51 (n = 2; 9%), HPV 58 (n = 1; 4.5 %), and HPV 39 or 68* (n = 1; 4.5 %) as shown in Table 2. Four patients reported multiple genotype infection; among those HPV 16 was found in three patients along with other genotypes.

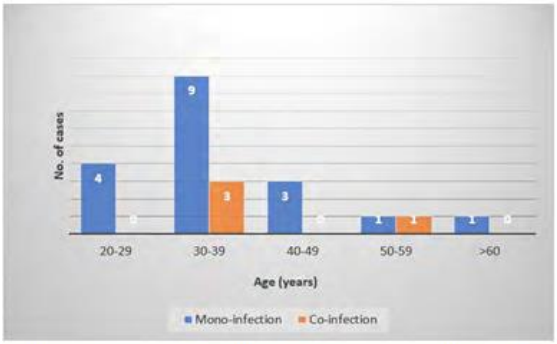

Figure 2: Age wise distribution of single and multiple HPV genotype cases.

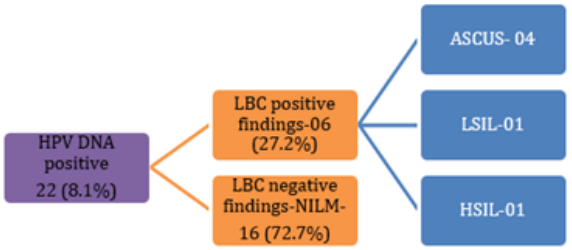

Figure 1: Comparison of HPV DNA positive samples with cytology findings. Most of HPV DNA positive patients had normal cytology or NILM category (16/22; 72.7%).

Age wise distribution revealed abnormalities in cytology mostly in 30-39 years of age (12/22; 54.5%); of which nine females were affected by single infection and three with multiple genotypes (Fig 2). Four patients (18.1%) were in the age group of 20-29 years and three (13.6%) were in the

age group of 40-49 years.

Discussion

HPV testing in addition to PAP smear screening has become a relevant diagnostic and prognostic tool. HPV being the most common viral infection of the reproductive tract, is responsible for the majority (95%) of cervical cancer. It is the fourth most common cancer among women globally, with an estimated 604 000 new cases and 342 000 deaths in 2020 [11]. The World Health Organisation (WHO) now encourages countries to use HPV DNA tests to detect high-risk strains of HPV for cervical screening. As the treatment has a high potential for cure, the screening detects an HPV infection or pre-cancerous lesions, these can easily be treated and cancer can be avoided.

In our study, of the 270 patients, 17 (6.3%) of the participants had abnormal cytology (Table 1). Among 17 patients, 14 (82.3%),
2 (11.7%) and 1 (5.9%) were found to be ASCUS, low-grade squamous intraepithelial lesions (LSIL) and HSIL, respectively. Variations in the proportion of abnormal cytology among HPV cases were reported worldwide [12-17]. As compared to other studies from different parts of India as well as worldwide, 5-20% abnormal cytology cases were reported, which correlates well with our study [15-18].

All 270 patients were further tested for HPV DNA PCR. Among these 22 (8.1%) participants were positive for high risk (HR) HPV infection. Southeast Asian countries have a low frequency of HPV (9-16%) [17-19]. In India, frequency of HPV varies in different populations [20]. It has been reported that in Manipur, another state of eastern India, HPV prevalence is very low (7.4%), whereas studies in the peri urban areas of West Bengal reported higher prevalence (22.2%) of HPV infection [22].

Investigation shows correlation between cervical cytology along with HPV infection [22]. Studies reported the prevalence of HSIL+ squamous lesions are significantly higher in HPV DNA positive samples [22-24]. Interestingly, in our study, HPV DNA detection was higher in patients with normal cytology (72.7%; n = 16/22) belonging to negative for intraepithelial lesion or malignancy (NILM) category, 18.1% (4/22) ASCUS; 1 each (4.5%) LSIL and HSIL (Figure 1). Studies from India reported many cases of women with normal cytology but HPV infected [25,26]. HPV DNA positive and cytology negative patients might be negative for cervical cancer but continue to have an increased

risk of cervical precancer [26].

The age-specific prevalence of HPV infection showed highest percent positivity among 30-39 years of age (54.5%; n = 12/22) followed by 20-29 years of age (18.1%; n = 04/22) (Figure 2). A hospital-based study in Uttar Pradesh, India likewise reported the highest HPV prevalence (45.7%) among 31-40 years age group patients [27]. Studies by Banerjee et al, 2020 from West Bengal, India, reported highest HPV infection among females below 29 years of age (35.48%); whereas studies by Gupta et al reported highest HPV positivity in the age group of 41-50 years [12,22].

The present study revealed that the co-infection was higher (n = 03/04) in 30-39 years of age group.

In our study, the presence of single infection and multiple genotype HPV infection among gynaecological outpatients were analysed (Table 2). Monoinfection was reported among 82% [18/22] cases and 18% [04/22] were multiple infection cases. Analysis of genotypes distribution in the referred cases showed that HPV 16 was the most predominant genotype in both monoinfection (38.8%; n=07/18) and co-infection (75%; 03/04). The prevalence of multiple genotypes has varied widely [between 12%-87%] in similar studies worldwide [12,13,15,27]. Some studies find association of multiple infection with decreased immune response leading to reactivation of latent HPV genotypes, or reflective of high-risk sexual behaviours of HIV-infected women or their partners [28]. Significant association of multiple infections with increased tumour size has also been observed [29] Among ICC cases, more than 55% cases were infected with HPV 16 and 2-20% cases infected with HPV 18, reported from Northern, Eastern and Southern part of India [17,19,20]. Other parts of Central and west India also have similar reports showing HPV 16 (72-73.6%) as the most predominant genotype followed by HPV18 (5-11.9%) in cervical carcinoma cases [19-21]. In our study, HPV 31 was the second most prevalent high-risk genotype in both monoinfection (22.2 %; n=04/18) and co-infection (50%; n = 02/04), which was also reported from other parts of India [30,31].

The development of HPV vaccines holds tremendous promise for developing countries like India where cervical cancer is the most common malignancy among middle aged women. The Indian Academy of Paediatrics Committee on Immunization (IAPCOI) recommends offering HPV vaccine to all females including as young as nine years old, who can afford the vaccine.

Despite HPV vaccination, screening is considered a public health strategy to assess the impact of

HPV vaccination, risk stratification of women, and the improvement of post treatment surveillance.

Conclusion

It is evident from our study that HPV DNA infection was observed with normal cervical cytology. For early detection both Pap smear and HPV DNA genotype detection should be the choice for preventing cervical cancer in women.

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