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Prevalence and Genotype Distribution of Human Papilloma Virus (HPV) Among Women with Abnormal Cervical Cytology at a Tertiary Care Centre in Chennai

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ABSTRACT

Background: Cervical cancer is the second most common malignancy among Indian women, with persistent infection by high-risk human papillomavirus (HPV) particularly HPV-16 and HPV-18 being the primary causative factor. This study aims to investigate the prevalence and genotypic distribution of HPV infection, along with associated risk factors, in women presenting with abnormal cervical cytology screening.

Methods: This cross-sectional study was carried out at the Shri Sathya Sai Medical College and Research Institute's Department of Microbiology in Chengalpattu, Tamil Nadu, India. The study sample consisted of 70 females over the age range of 30 to 80. The cervical tissues were sampled using a cervical brush and Pap Smear. The DNA was extracted using the Qiagen Kit (QIAamp DNA Kits for DNA Extraction). Clinical specimens were thoroughly analysed using molecular techniques for HPV genotyping using the MehrViru HPV PCR Test kit. The statistical analysis was conducted using SPSS version 17.0.

Results: 17 of the 70 women with abnormal cervical cytology were tested positive for HR HPV, which is a prevalence rate of 24.28%. Nearly, 38.6% (27) are between the ages of 41 and 50. There was no correlation found between HPV prevalence and sociodemographic, sexual, or reproductive factors. Out of 17 HPV positive cases, 41% (7) were diagnosed with atypical squamous cells of undetermined significance (ASCUS). HPV 16 was the most prevalent kind in terms of prevalence, followed by 52, 58, 18, and 33. Types 16 and 52 were the most common persistent HPV genotypes.

Conclusions: This study underscores the importance of integrating HPV DNA testing with Pap smear screening to improve the early detection of cervical cancer. The combined approach enhances diagnostic accuracy and enables timely counselling, education, and referral for affected women, ultimately contributing to better reproductive health outcomes in India.

KEYWORDS: Cervical cancer, Human papillomavirus, Abnormal cervical cytology, HPV genotyping

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INTRODUCTION

With an incidence of 660,000 new cases and around 3,50,000 fatalities in 2022, cervical cancer ranks as the fourth most frequent malignancy in women worldwide.[1] Approximately 85% of cervical cancer cases and 87% of fatalities take place in developing nations. Cervical cancer accounts for around 18.3% of all cases in India, making it the second most frequent malignancy in women. In 2020, there were 77,348 cervical cancer-related deaths and 123,907 new cases in India.[2] Cervical cancer incidence has been found to be strongly correlated with high risk persistent human papillomavirus (HPV) infection [3,4]. Finding proof of HPV infection via cervical cytology is quite challenging, though.[5]

Koilocytes in cervical smears have been generally accepted as a cytological indicator of HPV infection since the middle of the 1970s.[6,7] It is now clear that the cytological and histological characteristics of koilocytosis or koilocytotic atypia are insufficiently sensitive as markers of HPV DNA presence due to the constantly evolving developments in molecular diagnostic methods.[8] In fact, most women who test positive for HPV DNA do not exhibit cytological or histological evidence of HPV infection.[9] A variety of squamous and glandular cell alterations are examples of epithelial cell abnormalities. Atypical squamous cells of unknown significance (ASC US), high grade squamous intraepithelial lesions (HSILs), and squamous cell cancer are examples of squamous cell abnormalities. Mild dysplasia and HPV-related alterations are included in low grade squamous intraepithelial lesions (LSIL), which frequently equate to Grade 1 cervical intraepithelial neoplasia. Additionally, HSIL and LSIL have not been shown to be accurate as the only methods of diagnosing HPV infection.[10,11]

In order to aid in detect and manage any cancers, glandular cell abnormalities can range from atypical glandular cells to more serious types like adenocarcinoma in situ and adenocarcinoma.[11] Furthermore, it may be challenging to characterise koilocytes in histological sections due to fixation artefacts or inadequate dehydration, which might produce "koilocyte like" cells with perinuclear halos.[5]

Considering the causative relationship between chronic HPV infection and cervical cancers, a number of control strategies were created to prevent cervical cancer. These include the Papanicolaou (PAP) test, HPV DNA assays, and visual inspection using acetic acid or Lugol's iodine (VIA/VILI). VIA/VILI tests are less expensive and have a reasonable level of sensitivity and specificity. Although expensive, PAP smear cytology and molecular HPV testing have been shown to have great sensitivity and specificity.[12] Many wealthy nations employ the PAP test and the HPV molecular test as their main screening tools. It has been noted that these techniques have resulted in a 50% to 70% decrease in the yearly incidence of cervical cancer in those nations.[13,14]

The study was carried out to determine the prevalence of HPV positive status in the general population with abnormal cytology, taking into account the link between HPV infection and cervical cancer.

MATERIALS AND METHODS

This study is descriptive cross-sectional. The study was carried out from February 2024 to May 2025 at the Department of Microbiology, Shri Sathya Sai Medical College and Research Institute, Chengalpattu, Tamil Nadu, India, and involved 70 patients in total.The Institutional Ethics Committee (Conscience Independent Ethics Committee) gave its approval to this study under IEC- SSSMCRI-IEC-930/2024.

Females aged 30 to 80 years with abnormal cytological findings were included in the study. Normal cytological findings and Pap that are Negative for intra-epithelial lesions or malignancy (NILM) were excluded.

All patients clinical and demographic information was entered into the case record form (proforma), which included information on their age at conception, marital and menopausal status, parous state, use of family planning methods, Personal hygiene, place of residence, socioeconomic status, etc.

Clinical samples were taken from women who were attending for cervical screening. Two samples were taken in compliance with aseptic protocols. One is for the Papanicolaou test, while the other is for the molecular test.

Ayre's spatula was used to obtain the samples following a comprehensive cervical examination. The observations were documented using the 2001 Bethesda system nomenclature for reporting cervical cytology results, and each participant had a standard Papanicolaou test.For molecular testing, the cervical brush was utilised. At the start of the examination, it was inserted into the external cervical cavity and slowly rotated to extract cells from the endocervix and ectocervix. A vial of viral transport medium containing preservation fluid was then used to hold the brush head after it had been removed. After that, the vial was brought to the lab for the molecular analysis.

Genomic DNA was extracted from the collected samples using a DNA extraction kit by Qiagen (QIAamp DNA Kits for DNA Extraction) according to the standard protocol. The amplification of the HPV genome was performed by real-time polymerase chain reaction (RT-PCR) technique using the MehrViru HPV PCR Test kit for the detection and genotyping of HPV DNA in clinical specimens. In this kit, fluorescent reporter dye probes specific for the detection of specificity 12 High-risk HPV targeted are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. 7 Probable/possible High-risk HPV targeted are 26, 53, 66, 67, 68, 73, 82 and 11 Low-risk HPV targeted are 6, 11, 40, 42, 43, 44, 54, 61, 62, 89 and 90 types. SPSS Statistics for Windows version 17.0 was used to statistically analyse the recorded data once it had been abstracted into Microsoft Excel.

RESULTS:

Demographic data

This study covered 70 patients in total. Approximately 32.9% (23) of women are in the 30–40 age range, and 38.6% of the population falling into the 41–50 age range. Of the 70 women, 58.6% were from rural areas, 11 (15.7%) had completed middle school, and 24.3% (17) had a degree. Additionally, 22.9% (16) are IT professionals and 14 (20%) are stay-at-home moms. (Table 1)

A variety of sexual and reproductive traits were observed. Of the 70 cases, 48 (68.6%) were premenopausal and 22 (31.4%) were postmenopausal in terms of menstrual status. Of them, 65 (92.9%) live with their spouse and 11 (15.7%) had their first intercourse before 18 years old. were married before turning 18. In view of parity, 40 (57.1%) were in Para 2 and above. Three of them (4.3%), have two or more sexual partners. 66(94.3%) were practicing good personal hygiene. (Table 2)

Papanicolaou smear test: Every participant got HPV molecular analysis and the Papanicolaou test. 41.2% (7) of the HPV positive cases were having ASCUS and 23.5% (4) were with bacterial vaginosis, 1 (5.9%) tested positive for candidiasis following cytological evaluation. Five smears (29.4%) revealed squamous cell carcinoma (SCC), and no smear (0%) had cervical adenocarcinoma (Table 3).

Prevalence of HPV genotypes: A total of 8 patients tested positive for HPV16, the prevalence of HPV16 was 47.1%. The HPV16

prevalence among the patients varied significantly. However, the prevalence of HPV52 was 23.5%, with 4 out of 17 cases testing positive, and the prevalence of HPV58 was 11.8%, with 2 cases testing positive. Two cases (11.8%) tested positive for HPV18 and one case (5.9%) tested positive for HPV33.

Table :1 Sociodemographic characteristics of study population

Demographic variable		No. of Women (n= 70)	%
Age	30-40	23	32.9
	41-50	27	38.6
	51-60	10	14.3
	61-70	8	11.4
	71 - 80	2	2.9
Place of Residence	Rural	41	58.6
	Urban	29	41.4
Educational Status	Nil	5	7.1
	Primary	10	14.3
	Middle school	11	15.7
	High school	27	38.6
	College	17	24.3
Occupation	Home maker	14	20
	Unskilled labour	6	8.6
	Skilled labour	10	14.3
	Teaching & clerical	13	18.6
	IT	16	22.9
	Others	11	15.7

Table :2 Sexual and Reproductive characteristics of study population

Characteristic	Category	No. of Women (n=70) %	HPV Positive n=17 (%)
Menstrual Status	Pre-menopausal	48 (68.6)	12 (70.6)
	Post-menopausal	22 (31.4)	5 (29.4)
Age at First Intercourse	15–18	11 (15.7)	1 (5.9)
	19–25	41 (58.6)	12 (70.6)
	≥26	18 (25.7)	4 (23.5)
Marital Status	Living with spouse	65 (92.9)	17 (100.0)
	Widow/Divorced/Separated	5 (7.1)	0 (0.0)
Age at Menarche	<12 years	11 (15.7)	1 (5.9)
	12–14 years	38 (54.3)	12 (70.6)
	≥15 years	21 (30.0)	4 (23.5)
Parity	Nulliparous	3 (4.3)	0 (0.0)
	Para 1	27 (38.6)	2 (11.8)
	Para 2 and above	40 (57.1)	15 (88.2)
Sexual Partners	Single	67 (95.7)	13 (76.5)
	≥2 partners	3 (4.3)	4 (23.5)
Use of Family Planning	Natural	31 (44.3)	7 (41.2)
	Oral contraceptive pills	6 (8.6)	1 (5.9)
	Intrauterine device (IUD)	8(11.4)	2 (11.8)
	Male condom	14 (20.0)	3 (17.6)
	Female condom	1 (1.4)	0 (0.0)
	Tubal ligation	11 (15.7)	3 (17.6)
Vaginal Discharge	Yes	33 (47.1)	8 (47.1)
	No	37 (52.9)	9 (52.9)
Personal Hygiene	Good	66 (94.3)	17 (100.0)
	Poor	4 (5.7)	0 (0.0)

Table: 3 Pap smear diagnosis and HPV infection in the study population

Pap cytology	Total n=70 (%)	HPV positive n=17 (%)	HPV negative n=53 (%)
ASCUS	19 (27.1)	7 (41.2)	12 (22.6)
AGUS	3 (4.3)	0 (0)	3 (5.7)
SCC	11 (15.7)	5 (29.4)	6 (11.3)
Adenocarcinoma	2 (2.9)	0 (0)	2 (3.8)
Candidiasis	12 (17.1)	1 (5.9)	11 (20.8)
Bacterial vaginosis	23 (32.9)	4 (23.5)	19 (35.8)

ASCUS- atypical squamous cells of undetermined significance, AGUS- atypical glandular cells of undetermined significance,

SCC- Squamous cell carcinoma, Pap smear -Papanicolaou smear

Table :4 Prevalence of top 5 common HR HPV genotypes among the study population

HPV genotype	n= 17 (%)
16	8 (47.1%)
52	4 (23.5)
58	2 (11.8)
18	2 (11.8)
33	1 (5.9)

DISCUSSION:

Cervical cancer continues to be a significant public health burden in India, ranking among the top five cancers affecting women. It accounts for 12.1% of the total cancer incidence and contributes to 9.9% of cancer-related mortality among women in the country [15]. In contrast to developed nations where the implementation of comprehensive screening programs and widespread HPV vaccination have resulted in a considerable decline in cervical cancer cases [16], developing countries still struggle with persistent incidence and mortality rates. The lack of awareness, limited accessibility to healthcare services, and inadequate implementation of screening and vaccination programs are key contributing factors.

Human Papillomavirus (HPV) infection, particularly with high-risk genotypes such as HPV- 16 and HPV-18, plays a central role in the pathogenesis of cervical cancer. Epidemiological studies have consistently demonstrated that persistent infection with these oncogenic HPV types is a necessary cause of cervical intraepithelial neoplasia and cervical carcinoma. While cytological screening through the Papanicolaou (Pap) test remains a cornerstone of cervical cancer screening, it does not detect HPV infection unless explicitly tested for. Hence, molecular analysis, such as PCR-based detection of HPV DNA, is essential for identifying latent or asymptomatic infections that may later progress to malignancy [17].

Among women with abnormal cytology, our study identified a high HPV prevalence rate of 24.28%. This is similar to previously documented rates in similar cohorts [18,19]. This prevalence may reflect regional or population-specific risk factors. Interestingly, our demographic data indicated that 38.6% of participants were between 41 and 50 years old. This finding contrasts with many previous studies which suggest that younger women, particularly those below 30 years, are at higher risk for HPV infection due to higher sexual activity levels and cervical epithelial vulnerability [20–22]. However, our findings are supported by studies such as those by Smita et al. [24] and Bhawna et al. [23], who also reported a higher prevalence of HPV in the 41–50 year age group. Asiaf et al. [25] similarly noted a significant infection rate among women aged around 30 years.

Sexual behavior also plays a crucial role in HPV transmission. In our study, 76.5% of HPV- positive women had a single sexual partner, and 23.5% reported having two partners. This is consistent with Bhawna et al., who reported that 85% of HPV-positive women had a single partner and 14% had multiple partners [23]. Early initiation of sexual activity is a known risk factor, and our study observed that 70.6% of participants had their first sexual encounter between 18 and 25 years of age. These findings are in agreement with other Indian studies that highlight early age at marriage and sexual debut as significant contributors to HPV infection risk [23, 26].

Parity, or the number of full-term pregnancies, was another important factor evaluated in this study. We found that 88.2% of HPV-positive women were para 2 or above, indicating a significant association between high parity and HPV infection. This correlates with Bhawna et al.'s study, which reported that 77% of HPV-positive women were para 3 [23]. A potential explanation is that in multiparous women, the cervical transformation zone remains exposed on the ectocervix for longer durations, increasing susceptibility to HPV infection and other co- factors [27].

The type of contraceptive method used may also influence the risk of HPV acquisition. In our analysis, 17.6% of HPV-positive women had undergone natural family planning methods, while 17% used barrier methods such as condoms. The higher rate of HPV infection among women with natural family planning methods may be attributed to increased sexual activity due to the absence of pregnancy risk. Conversely, condom use has a protective effect against sexually transmitted infections, including HPV, by limiting viral exposure during intercourse. Previous research has associated long-term use of oral contraceptive pills (OCPs) with a heightened risk of cervical cancer among HPV-positive women [23, 28]. Moreno et al. reported that prolonged OCP usage in HPV-infected women increased the risk of cervical carcinoma by up to four times [29].

Sociodemographic factors such as education and hygiene practices were also assessed in relation to HPV positivity. In our study, more than 90% of HPV-positive women had primary education or higher, and the majority reported satisfactory hygiene. However, studies by Srivastava et al. and Chakravarty et al. suggested that rural residence and low educational attainment are associated with increased HPV prevalence [30, 31]. This discrepancy may be due to regional variations in awareness, health-seeking behavior, and access to reproductive healthcare services.

CONCLUSION

In conclusion, our study emphasizes the critical need for integrating HPV DNA testing with conventional Pap smear screening. This dual approach enhances the sensitivity and specificity of cervical cancer screening, particularly in populations at risk of under-diagnosis. Women who tested positive for HPV and/or presented with abnormal Pap results were appropriately counseled and referred for further evaluation, including colposcopy. They were educated on the natural course of HPV infection, including its frequent self-resolution and potential to cause precancerous changes. Timely diagnosis, patient education, and appropriate follow-up are essential to reduce the burden of cervical cancer and improve women's reproductive health outcomes in India.

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