ASSESSMENT OF HPV PREVALENCE IN NORTHERN INDIA: INSIGHTS INTO RISK FACTORS, AGE TRENDS, AND IMPLICATIONS FOR WOMEN'S HEALTH

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ABSTRACT

Introduction: Human papilloma virus is a dominant factor for cervical carcinogenesis. First coitus at a younger age, having a high number of pregnancies, and marrying at an early age are identified as significant risk factors for HPV infection.

Objective: The current study was initiated to evaluate the overall prevalence of HPV in women from the northern state of India.

Materials and method: This cross-sectional study was conducted on 120 married women within the age group of 21 to 65 years, who reported specific complaints such as vaginal discharge or bleeding per vaginum. Already diagnosed with cervical cancer, received HPV vaccination, and confirmed cases of tuberculosis or any other carcinoma were excluded from the study. Cervical smears were taken by trained technician using modified AYRE'S SPATULA. Ethical approval for the study was obtained from the Institutional Ethical Committee.

Result: Prevalence of HPV among women patients, who reported specific complaints, such as vaginal discharge or bleeding per vaginum, 82.50% were tested positive, while 17.50% were tested negative. The study also contributes valuable insights into age-specific trends of HPV infection, indicating an increased risk among women aged 25 to 34 years**.**

Conclusion: The Study reveals a higher prevalence of HPV among women with normal cytology, but minor gynecological complaints and the complex relationship between HPV and biomarkers, suggests the need for further research.

Keywords: Prevalence, HPV, Biomarker, Cervical smear, Cervical cancer

Introduction:

Cervical cancer is a significant global health concern, ranking as the second most prevalent cancer among women worldwide and accounting for approximately 12% of all female cancers [1]. In India, this malignancy takes on even greater prominence, standing as the most common cancer and a leading cause of cancer-related deaths among women in the country [2,3]. Each year, an estimated 132,000 new cases of cervical cancer are reported in India, resulting in 74,000 deaths [1]. The prevalence of Human Papillomavirus (HPV) infection is particularly noteworthy in the context of cervical cancer, especially among young women under the age of 25 years. HPV, a known risk factor for cervical cancer, exhibits varying prevalence rates across different age groups. It reaches its peak, around 20%, in sexually active young women, and then declines with increasing age [4,5]. Regional disparities in the distribution of HPV types are also observed, with Asia displaying notable diversity, likely attributed to the heterogeneous nature of its population [6,7]. Recognizing the link between persistent infection with high-risk HPV types and the development of cervical cancer [8], research efforts have predominantly focused on women over the age of 30 years. This emphasis has resulted in a scarcity of data regarding age-related infection patterns and HPV prevalence among younger women in India [9]. Furthermore, studies investigating HPV prevalence and type distribution in women with a normal cervix in India often encounter limitations, such as smaller sample sizes and a predominant focus on hospital settings. A noteworthy exception is a community-based study conducted in South India by Franceschi et al. [9], which boasts the largest sample size to date. However, a gap in similar data persists, particularly from the northern part of India. Therefore, the current study was initiated to address

this gap by evaluating the overall prevalence of HPV in women from the northern state of India, contributing valuable insights to the understanding of cervical cancer dynamics in this region.

Material and Methods:

This cross-sectional study was conducted on 120 cervical samples. The samples were taken by using modified AYRE'S Spatula, at Outpatient Department (OPD) of Obstetric and Gynaecology, and processed at Central Molecular Research Laboratory, Shri Mahant Indiresh Hospital (SMIH), Patel Nagar, Dehradun (U.K). The study was planned to be completed in the period of one year. In a study [10], the prevalence of infection by high-risk HPV types was 25.4%~25%. The sample size was calculated using the formula $n=4pq/d^2$ [11], where n is the required sample size, p=prevalence of cause, q=1-q, d=Precision. Taking 80% power, 5% significance level with 0.08 precision, the calculated sample size was, $n=4*0.25*0.75/(0.08*0.08) \approx 120$

The inclusion criteria for this study encompassed married women within the age group of 21 to 65 years, who reported specific complaints such as vaginal discharge or bleeding per vaginum. On the other hand, certain criteria were established for exclusion from the study, including women below 21 years or above 65 years of age. Additionally, individuals who had already been diagnosed with cervical cancer, those who had received HPV vaccination, and those with confirmed cases of tuberculosis or any other carcinoma were excluded from participation in the study. These criteria were implemented to ensure that the study population was appropriately focused on the target demographic and specific health conditions of interest.

Informed consent were obtained from all the participants before procedures. Cervical smear was taken by trained technician using modified AYRE'S SPATULA. Cervical smears collected from patient attending gynecological clinics were sent for HPV DNA Detection in Central Molecular Laboratory of SMI Hospital, Dehradun, using Bench Top PCR, (Lab System 9600).

The Biochemical Tumor markers and Biochemical Parameters were evaluated in the study group at Central Biochemistry Laboratory of SMI Hospital, using VITROS 5600 fully automated system. Ethical approval for the study was obtained from the Institutional Ethical Committee.

Statistical analysis

The results are presented in frequencies, percentages and pie chart. The Chi- square test was used to compare the difference between categorical variables. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Results:

The present study was conducted in the Department of Biochemistry in coordination with Department of Obstetrics and Gynaecology, Shri Mahant Indiresh Hospital, Dehradun, Uttarakhand, with the objective to evaluate the prevalence of HPV in women from the northern state of India. A total of 120 women patients with vaginal discharge or bleeding were included in the study.

Figure 1: Distribution of Prevalence of HPV among women patients
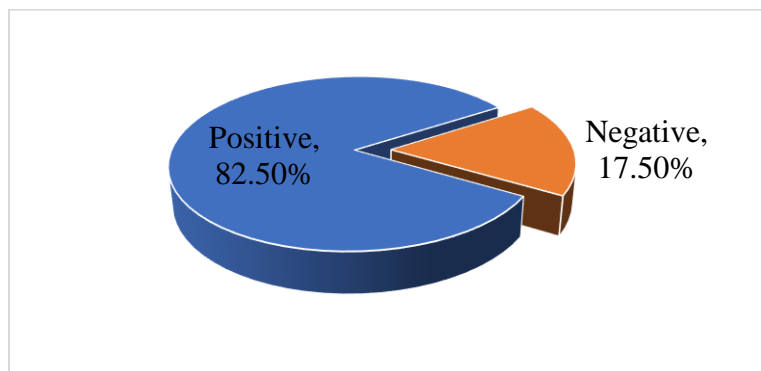
The presented data in figure 1 illustrates the prevalence of HPV among women patients attending a tertiary care hospital. The results indicate that out of the total number of women, who reported specific complaints such as vaginal discharge or bleeding per vaginum, 82.50% were tested positive for HPV, while 17.50% were tested negative.

Table 1: Association of positivity of HPV with age groups among women patients

Age in years	HPV		Total n (%)	P value
	Positive n (%)	Negative n (%)		
<30	14 (70.0%)	6 (30.0%)	20 (16.7%)	
31-40	36 (87.8%)	5 (12.2%)	41 (34.2%)	0.25
41-50	30 (88.2%)	4 (11.8%)	34 (28.3%)	
51-60	13 (81.2%)	3 (18.8%)	16 (13.3%)	
>60	6 (66.7%)	3 (33.3%)	9 (7.5%)	

Table 1, examines the association between the presence of HPV and different age groups among women patients, who reported specific complaints such as vaginal discharge or bleeding per vaginum, totaling 120 individuals. The data reveals distinct patterns in HPV positivity across various age categories. Among women aged less than 30 years, 16.7% were tested positive for HPV, with 70.0% of these cases falling within this age group. In the 31-40 years age group, 34.2% were tested positive for HPV, with 87.8% of these cases occurring within this age range. Similarly, in the 41-50 years age group, 28.3% were tested positive, with 88.2% of cases observed in this bracket. For women aged 51-60 years, 13.3% were tested positive, with 81.2% of positive cases falling in this age category. Among those aged over 60 years, 7.5% were tested positive, with 66.7% of these cases belonging to this age group. The findings suggest a potential association between age and HPV positivity, with varying rates across age groups.

Table 2: Association of positivity of HPV with tumor markers among women patients

Biochemical tumor markers	cut-off value	HPV		P value
		Positive n (%)	Negative n (%)	
CEA (ng/ml)	≥4.5	57 (47.5%)	10 (8.3%)	0.403
	<4.5	42 (35.0%)	11 (9.2%)	
CA125 (u/ml)	≥20	68 (56.7%)	11 (9.2%)	0.065
	<20	31 (25.8%)	10 (8.3%)	
HCG (IU/l)	≥25	37 (30.8%)	8 (6.7%)	0.950

LDH (u/l)	<25	62 (51.7%)	13 (10.8%)	
	≥220	42 (35.0%)	6 (5.0%)	0.239
	<220	57 (47.5%)	15 (12.5%)	
AFP (ng/ml)	≤4	50 (41.7%)	8 (6.7%)	0.301
	>4	49 (40.8%)	13 (10.8%)	

Table 2, investigates the relationship between the presence of HPV and various biochemical tumor markers in women patients, who reported specific complaints such as vaginal discharge or bleeding per vaginum. The table presents, the prevalence of HPV based on different cutoff values for each tumor marker, accompanied by the corresponding percentages of positive and negative cases. For CEA with a cutoff value of ≥4.5 ng/ml, 47.5% of patients tested positive for HPV, while 8.3% were negative. Conversely, for CEA levels below 4.5 ng/ml, 35.0% were HPV positive, and 9.2% were negative. For Cancer Antigen 125 (CA125), a cutoff value of ≥20 u/ml, yielded 56.7% HPV-positive cases and 9.2% HPV-negative cases. Below the cutoff value, 25.8% were HPV positive, and 8.3% were negative. HCG at a cutoff value of ≥25 IU/l showed 30.8% HPV-positive cases and 6.7% HPV-negative cases. Below the cutoff, 51.7% were HPV positive, and 10.8% were HPV negative. LDH with a cutoff value of ≥220 u/l resulted in 35.0% HPV-positive cases and 5.0% HPV-negative cases. Below the cutoff, 47.5% were HPV positive, and 12.5% were negative. AFP levels of ≤4 ng/ml showed 41.7% HPV positivity and 6.7% negativity, while levels above 4 ng/ml had 40.8% HPV positivity and 10.8% negativity.

Discussion:

India has a female population of 366.58 million aged 15 and above who are susceptible to cervical cancer. Approximately 8% of women in the general population are estimated to have cervical HPV infection at any given time, with 82.5% of invasive cervical cancers linked to HPV types 16 or 18. In India, HPV type 16 alone accounts for 70-90% of cervical cancer cases, while the occurrence of HPV type 18 ranges from 3 to 20%. While HPV is deemed a necessary factor in cervical cancer,

it is not sufficient on its own [12]. This study, unlike others in India, revealed a high prevalence of HPV infection among women with normal cytology but minor gynecological complaints [13-17]. However, the study did not investigate bacterial, fungal, or HIV infections that might increase susceptibility to HPV in symptomatic cases. The prevalence of HPV among women with specific complaints such as vaginal discharge or bleeding per vaginum was 82.5%, higher than in other studies. Senapati et al. reported a similar finding, noting a 94.28% prevalence of HPV among cervical cancer cases [18]. It is widely acknowledged that HPV is responsible for nearly 100% of cervical carcinoma cases. In comparison to other studies, the overall prevalence of HPV in cancer cases (82.3%) aligns with the findings of the present study [16,19,20]. Furthermore, in general population, Xu et al [21] reported the prevalence of HPV being 22.8%. Another study,[22] reported that the prevalence of cervical HPV infection being 25.6%. In India, the association of the infection of high-risk HPVs with the age of marriage below 18 years has been found to increase the risk of cervical cancer by 22 fold. The study carried out by Dutta S et al [2011] showed that the risk of HPV infection was higher in women aged 25 to 34 years, in married women below 20 years of age [23]. The age-specific trend of HPV infection among noncancer cases showed bimodal shaped infection peak which is similar to many other reports. HPV infection at younger age reached its peak soon after sexual initiation [24].

A meta-analysis done by Silvia de Sanjosé et al.[2007] confirms that HPV infection is most common in women younger than 25 years of age [6] . However; a second peak of HPV prevalence is seen in women aged 45 years or older in all regions. Prevalence of HR-HPV types in the 30–39 years, 40– 49 years and 50–59 years age groups were 9.8%, 10.4% and 12.2%, respectively. In the multicenter, cross-sectional study in India, these were 7.0%, 6.8% and 7.5%, respectively [25]. Using data from multiple international studies, the median oncogenic

HPV prevalence among all women was 15.1%, while the median oncogenic HPV prevalence among women age 30 and older was 9.2% [26]. The prevalence of HPV infection is highest among young women and appears to drop off with increasing age [27]. Since most HPV infections occur soon after initiation of sexual activity and are transient, women over age 30 years, who are HPV positive include, those who are persistent carriers as well as those with new infections. While most studies indicate a decrease in HPV prevalence with age, a handful of studies conducted in several different international regions have shown a peak prevalence of HPV infection in women below age 25, a decrease among women aged 35–54 and a second peak after age 55[28]. In the present study, the prevalence of HPV was higher among the patients of age 41-50 years (88.2%) followed by 31-40 years (87.8%), 51-60 years (81.2%), <30 years (70%) and 60 years (66.7%). The age distribution of cervical HPV prevalence has been observed to vary between populations [29]. Marc et al [22] found that the prevalence of cervical HPV decreased significantly with age. There was more than an 8-fold variation in the prevalence of HR-HPV between women <25 years and those ≥55 years. Moreover, Various proteins are being evaluated to see if they can be used as the potential diagnostic markers for the screening of ovarian cancer [30,31]. The most widely used serum biomarker in ovarian cancer screening is CA125[32]. An elevated level of LDH has been reported in the total serum of ovarian adenocarcinomas patients [33,34].

Similarly, Schneider et al [35] reported a significantly higher level of LDH in patients with ovarian cancers than benign ovarian tumors. While, In the present study, the level of LDH was insignificantly higher in patients with HPV positive cases than negative. In the present study, β hCG was insignificantly higher among HPV positive cases than negatives. However, no difference in the serum levels of AFP between HPV positive and HPV negative cases. Panza et al [36] showed that β hCG levels of plasma were not significantly increased in epithelial ovarian carcinoma (EOC)

and there was no correlation between EOC and β hCG levels of plasma. Aggarwal and Kehoe [37] found no significant increase in AFP level was observed in EOCs. In the present study, there was no significant difference in carcinoembryonic antigen (CEA) between HPV positive cases and negative cases. In present study, variations in HPV positivity were observed across different tumor markers, the lack of statistically significant associations, underscores the importance of further research with a larger sample size.

Conclusion:

This study reveals a higher incidence of HPV among women with normal cytology but minor gynecological complaints, suggesting the need for comprehensive investigations into other potential contributing factors, such as bacterial, fungal, or HIV infections. The study also contributes valuable insights into age-specific trends of HPV infection, indicating an increased risk among women aged 25 to 34 years. Study suggests a complex relationship between HPV and biomarkers, emphasizing the need for further research with a larger sample size.

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