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PREVALENCE OF HIGH-RISK HUMAN PAPILLOMAVIRUS (HR-HPV) GENOTYPES IN CLINICAL SAMPLES OF SEXUALLY ACTIVE WOMEN: A HOSPITAL BASED STUDY

Amod Kumar Yadav[1], Dr. Rohit Kumar[2], Tanuj Gupta[3], Dr. Paramjeet Singh Gill[4], Reenu Kumari[5]

1. **Amod Kumar Yadav,** Demonstrator, Department of Microbiology, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana. Kumaramod1231@gmail.com
2. **Dr. Rohit Kumar,** Assistant Professor, Department of Microbiology, Index Medical College, Malwanchal University, Indore, MP.
3. **Tanuj Gupta,** Research Scientist, VDRL, Department of Microbiology, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences, Rohtak.
4. **Dr. Paramjeet Singh Gill,** Professor, Department of Microbiology, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences, Rohtak.
5. **Reenu Kumari,** Assistant Professor, Department of Anatomy, N.C. Medical College and Hospital, Israna, Panipat.

Corresponding Author:

Amod Kumar Yadav, Demonstrator, Department of Microbiology, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana. Kumaramod1231@gmail.com

ABSTRACT:

Background: Human papillomavirus (HPV) is the most common sexually transmitted infection in the world and is classified as a carcinogenic infectious agent by the International Agency for Research on Cancer. However, only some HPV strains are oncogenic. The oncogenicity of the virus is primarily dependent on the continuous expression and activity of the viral proteins E6 and E7. HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82 are considered high-risk genotypes, Of the high-risk HPV types, HPV16 is the most frequently detected at the population level, and it is by far the predominant type causing invasive cervical cancer worldwide (~60%), followed by HPV18 (~15%) [23]. HPV serotypes 16 and 18 together cause up to 70% of invasive cervical cancer worldwide. Cervical cancer ranks as the 2nd leading cause of female cancer in India. This is the most frequently occurring type of cancer in women aged 15 to 44 years after breast cancer. **Objective:** To study the qualitative detection of Human papillomavirus (HPV) DNA in clinical samples and genotyping of 14 high-risk (HR-HPV). **Material and Method:** The present study has been done on the women attending Obstetrics and Gynaecology OPD in the regional tertiary care hospital with symptomatic gynaecological problems due to the presence of Human Papillomavirus infections. Hence in the present study, we have used Real-Time PCR (BIO-RAD, CFX96 Real-Time System) for HPV DNA testing by using "TRUPCR® HPV High Risk Genotyping Plus Kit" as per manufacturer's instructions. **Results:** The prevalence of HPV is 23.24% in the present study. In which HPV16 accounts for 62.79% of the total prevalence followed by HPV58 (9.3%), HPV31, HPV33, and HPV39 each

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having prevalence of 6.97%. Next in descending order were HPV35, HPV51, and HPV68 having a prevalence of 4.65%, 4.65% and 2.32% respectively. **Conclusion:** It has been observed that there is a high prevalence of HPV16 followed by HPV58, 31, 33, 35 and 39. Our study further recommends studying the prevalence and genotyping variations in the locality before providing a vaccine in the vaccination schedule as an effective prophylactic means.

Keywords: HPV Genotype, Cervical Cancer

INTRODUCTION:

Human papillomaviruses (HPVs) are non-enveloped icosahedral, circular, double-stranded deoxyribonucleic acid (dsDNA) viruses of approximately 50-55 nm in diameter, have icosahedral capsids composed of 72 capsomeres. It contains a double-stranded circular DNA genome of 7900-8000 base pairs [1].

Human papillomavirus (HPV) is the most common sexually transmitted infection in the world [2]. It is classified as a carcinogenic infectious agent by the International Agency for Research on Cancer [3]. Both sexually active men and women will be infected at least once without developing any symptoms or cancerous diseases in their lifetime [2]. However, only some HPV strains are oncogenic [4].

The oncogenicity of the virus is primarily dependent on the continuous expression and activity of the viral proteins E6 and E7, which are tumour-associated antigens that act in concert to alter interrelated cellular processes and promote tumour development through the interaction of cellular proteins [5, 6]

The genome encodes 6 early proteins (E1, E2, E4, E5, E6 and E7) that are responsible for virus replication, and 2 late proteins (L1 and L2) which are the major and minor viral capsid proteins respectively [7]. About two hundred HPV genotypes have been identified based on the sequence of their L1 genes [8, 9]. They can be categorized into cutaneous or mucosal types based on their tissue tropism [10]. The E6 and E7 proteins are the major oncoproteins that are involved in the transformation and immortalisation of host cells. The E6 and E7 proteins bind and inactivate the two major tumour suppressor proteins; p53 and pRb respectively [11]. The sequences of HPV E6 and E7 regions have been used in the classification of some HPV variants [12–14].

The different types of HPV vary in tissue distribution and oncogenic potential associated with anatomically and histologically distinct diseases. A different HPV type causes a wide range of infections, including common warts, genital warts, recurrent respiratory papillomatosis, low-grade and high-grade squamous intraepithelial lesions (SILs) and cancers, including cervical, anal, vaginal, vulva, penile, head and neck cancer [15,16]

The virus is primarily transmitted through direct skin-to-skin contact, typically during sexual activity, including vaginal, anal and oral sex. It can also spread through non-sexual contact, such as from mother to child during childbirth [7].

More than 200 human papillomavirus (HPV) genotypes (‘types’) are known; these are categorized into phylogenetic genera (designated Alpha, Beta, Gamma, Mu and Nu). The Alpha

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genus contains the types that cause particularly important human diseases. If these HPV types persist, they can cause one of the most common cancers in women that is cervical cancer as well as rarer cancers of non-keratinized mucosa and skin of the lower genital tract (vagina, vulva and penis), the anus and the oropharynx [17].

HPV genotypes recognized to date are classified into two major groups, high-risk and low-risk genotypes in terms of their malignancy-causing potential [18, 19]. HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82 are considered high-risk genotypes, whereas low-risk genotypes include HPV-6,11, 34, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89 [21]. HPV types 6 and 11 are known to be responsible for 90% of genital warts [20, 22].

Whereas high-risk HPV types, HPV16 is the most frequently detected at the population level, and it is by far the predominant type causing invasive cervical cancer worldwide (~60%), followed by HPV18 (~15%) [23]. HPV serotypes 16 and 18 together cause up to 70% of invasive cervical cancer worldwide. Along with cervical cancer, HPV types 16 and 18 are responsible for 40–50% of invasive vulvar cancer and 70% of vaginal cancer [19].

Cervical cancer ranks as the 2nd leading cause of female cancer in India. This is the most frequently occurring type of cancer in women aged 15 to 44 years after breast cancer. Every year in India 122,844 women are diagnosed with cervical cancer due to HPV and more than 67,477 die of the disease. Besides, HPV causes 88% of anal cancers, 43% of vulvar cancers and 70% of vaginal cancer, 50% of penile cancers, 70% of oropharyngeal cancers, representing nearly 4.8% of all cancers worldwide [23].

AIM AND OBJECTIVES:

AIM

The present study has been planned to screen the sexually active women attending OPD of Obstetrics and Gynaecology with symptomatic gynaecological problems for the detection of Human Papillomavirus and to understand the circulating genotypes of HPV in the regional tertiary care hospital.

OBJECTIVES

1. To study the qualitative detection of Human papillomavirus (HPV) DNA in clinical samples.
2. To study the detection and genotyping of 14 high-risk (HR-HPV).

MATERIAL AND METHODS

“WHO Guidelines for Screening and Treatment of Cervical Pre-cancer Lesions for Cervical Cancer Prevention” recommends the use of DNA-based HPV testing as a first choice screening method because these are more efficient than cytology-based HPV tests, especially for low and middle-income countries; as they are less prone to quality problems.

Detection of HPV was done by using the HPV testing kit “TRUPCR® HPV High-Risk Genotyping Plus Kit” as per the manufacturer’s instructions in Real-Time PCR (BIO-RAD,

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CFX96 Real-Time System). The study was carried out in the Department of Microbiology, Pt. B.D. Sharma, PGIMS, Rohtak (Haryana). A total of 185 samples were recruited for the study. The participating women were made aware of the study in their local language. Informed & written consents were collected from the participating women.

INCLUSION CRITERIA

a) Age group 15-65 years
b) Sexually active
c) All women subjects who were married, non-pregnant and had not gone hysterectomy.
d) Symptomatic infections like vaginal discharge, itching in the genital area, intermittent bleeding, contact bleeding, post-coitus bleeding, Dyspareunia.

EXCLUSION CRITERIA

a) Pregnancy, widow, sexually naive
b) Unwilling to participate
c) Vaccinated
d) Presence of cervical cancer
e) Women underwent hysterectomy

SAMPLE COLLECTION

The subjects were women attending Obstetrics and Gynaecology OPD due to complaints of symptoms like itching in the genital area, vaginal discharge, intermittent bleeding, post-coitus bleeding, contact bleeding, dyspareunia, genital warts etc. Cervical scrapings of these patients were collected with the help of a brush after taking all aseptic measures. The brush was immediately cut off and inserted into the liquid Storage medium "SurePath Preservative Solution" for transport and storage. All cervical specimens require genomic DNA extraction and are then amplified using Real Time Amplification and detected by using fluorescent reporter dye probes specific for high risk and low risk HPV genotypes.

SAMPLE STORAGE

The samples should be shipped at 2 to 8°C and should be stored at 4°C. To prevent significant degradation of samples should be processed within 72 hours of collection, although ideally samples should be processed within 24-36 hours. The extracted viral DNA can be store at -20°C for future use.

MATERIAL AND DEVICES REQUIRED

Adjustable pipettes with sterile filter or positive displacement tips, Disposable powder-free gloves, Sterile bidistilled water, Sterile 1.5 ml and 2 ml microcentrifuge tubes, 50 ml conical tubes, Vortex mixer, TRUPCR Termi-DNA-Tor or equivalent, in order to remove DNA from working surfaces, Real Time PCR, Laminar airflow cabinet, PCR vials (0.2 ml, thin-walled), Personal protection equipment (lab coat, gloves, goggles) DNA isolation.

SAMPLE PROCESSING: The TRUPCR HPV High-Risk Genotyping Plus Kit was used for HPV Genotyping. It is a Real-Time amplification test kit for the qualitative detection and genotyping of 14 High-Risk HPV DNA in clinical samples. DNA is extracted from samples and is then amplified using Real-Time Amplification and detected by using fluorescent reporter dye

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probes specific for high-risk HPV 14 Genotypes(16/18/31/33/35/39/45/51/52/ 56/58/59/66 and 68). In Real-Time PCR, the fluorescent signal is generated from the presence of an oligonucleotide probe specific to the target DNA sequence. The probe contains a fluorescent dye molecule on its 5' end and a quencher molecule on its 3' end. The probe hybridizes with one of the chains of the amplified fragment. During the synthesis of a complementary chain, Taq DNA polymerase which possesses 5' - 3' exonuclease activity cleaves the probe. As a result, the fluorescent dye and quencher dye are separated, and the total fluorescence of reaction volume increases in direct proportion to the number of amplicon copies synthesized during PCR. The fluorescent signal is measured in each cycle of reaction, and the threshold cycle value is determined from the obtained curve. The threshold cycle is proportional to the initial number of DNA copies in a sample and its value allows qualitative comparisons of analyzed and control samples.

RESULT ANALYSIS

Data was collected, compiled and entered in the MS Excel sheet and further statistical analysis was done using the latest version of Statistical Package for Social Sciences (SSPS).

DISPOSAL OF WASTE

All the biomedical waste generated during this study in the laboratory will be discarded after proper disinfection or sterilization as per Bio-Medical Waste Management and Handling Rules 2016 guidelines, and Bio-Medical Waste Management (Amendment) Rules, 2019 guidelines.

In the present study, a total of 185 samples were analyzed by Real-Time PCR, out of which 43 samples were positive for HPV. The HPV prevalence was found to be 23.24% as shown in the figure and chart.

TABLE 1. Prevalence of HPV in the total Samples

Total no of Samples	HPV Positive Samples	% of HPV Positive Samples
185	43	23.24%

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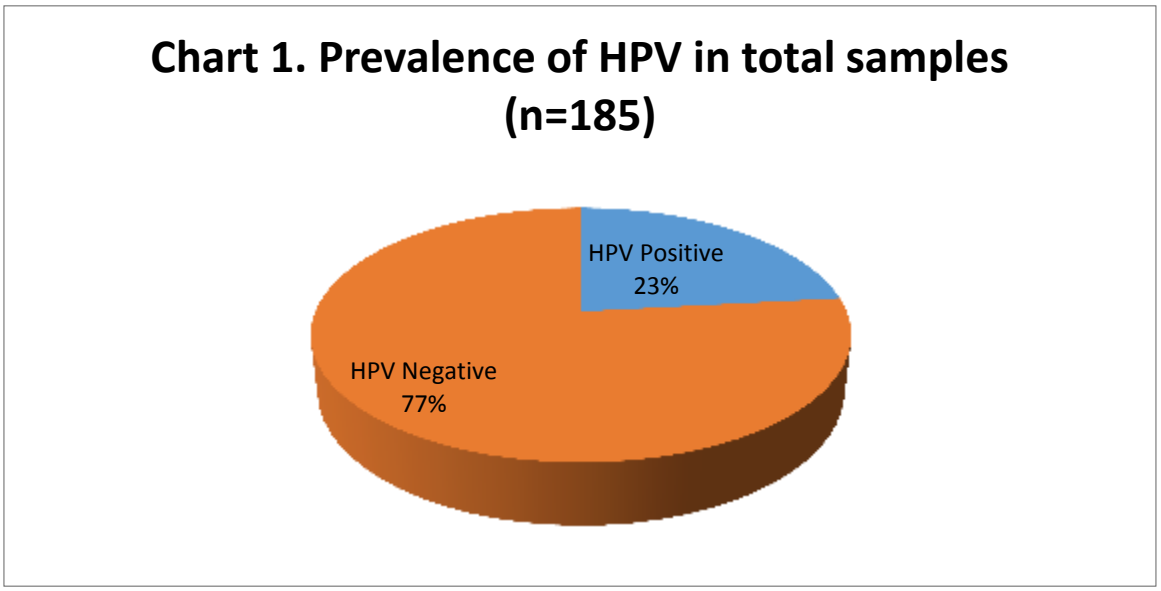

Among the total 43 HPV-positive samples, genotyping was performed by using TRUPCR kit. It was observed that 27/43 samples were positive for HPV16 which accounts for 62.79% of total prevalence (n-43). Furthermore, it is 14.59% of the total sample (n=185). 2nd most prevalent genotype was HPV58 (9.30%) followed by HPV31, HPV33, HPV39 each having prevalence of 6.97% (n-43). Next in descending order were HPV35, HPV51, and HPV68 having a prevalence of 4.65%, 4.65% and 2.32% (n-43) respectively. Interestingly, HPV18 which is the 2nd most prevalent high-risk HPV genotype in other available literature was not reported in the present study. Similarly high-risk HPV45, HPV52, HPV59, and HPV66 were also not reported in the present study (Table2, Chart2).

TABLE 2. Frequency of HPV genotypic distribution in positive samples

Sr. No.	HPV Genotype	No of Patients	Percentage of HPV genotype in total positive samples (n=43)	Percentage of HPV genotype in total tested samples (n=185)
1.	HPV 16	27	62.79	14.59
2.	HPV 18	00	00	00
3.	HPV 31	03	06.97	1.62
4.	HPV 33	03	06.97	1.62
5.	HPV 35	02	04.65	1.08
6.	HPV 39	03	06.97	1.62
7.	HPV 45	00	00	00
8.	HPV 51	02	04.65	1.08
9.	HPV 52	00	00	00
10.	HPV 56	00	00	00
11.	HPV 58	04	9.30	2.16
12.	HPV 59	00	00	00

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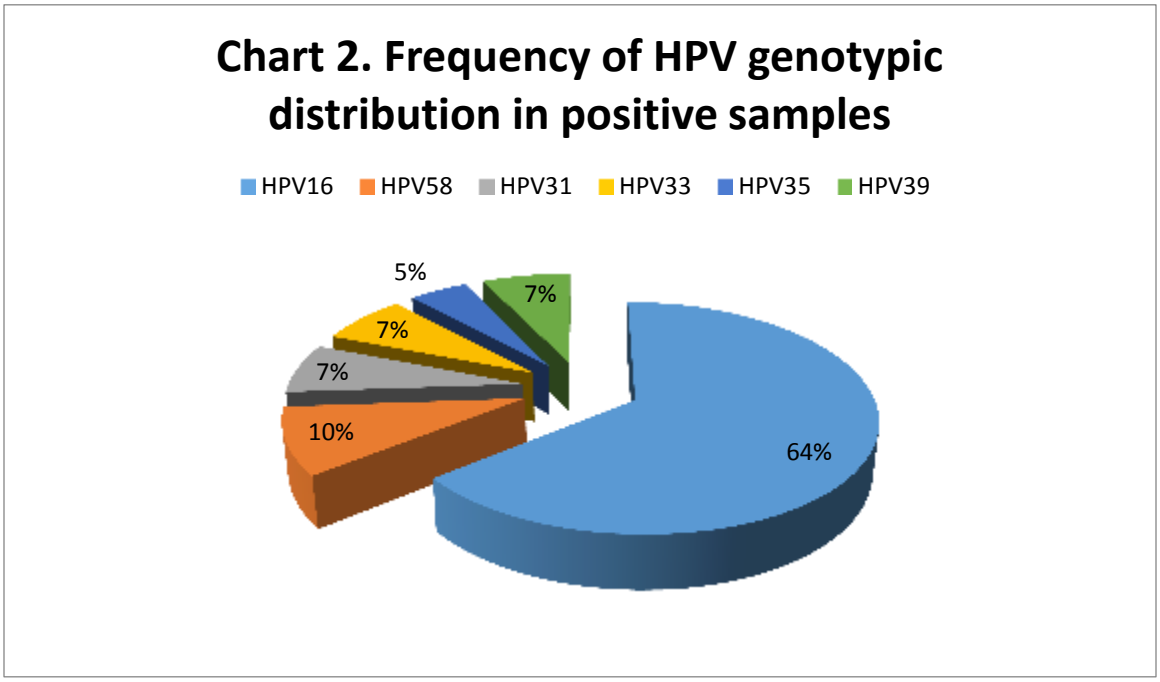
13.	**HPV 66**	**00**	**00**	**00**
14.	**HPV 68**	**01**	**2.32**	**0.54**



DISCUSSION

Cervical cancer is the most frequent malignancy in women, world accounting for 17% of all cancer deaths among women aged between 30 and 69 years. Although, the incidence of cervical cancer is steadily declining in the developed world; it is most common in developing countries [24]. Despite being curable and preventable at an early stage, cervical cancer still causes more than 67,477 deaths annually in India due to the lack of organized screening programs and intervention approaches [25].

Although India is a diverse country with extensive ethnicity and socio-cultural diversity, the incidence of HPV infection may vary significantly in different regions, it is required to study the prevalence of HPV and its genotypes in every part of the country. The available literature shows the prevalence of HPV in women in different parts of India ranges from 9 to 94%. [26]

In India, annually the incidence of cervical cancer is quite high [25] therefore, early screening of HPV infections and cervical cancer can be a better solution to this question. The major obstacles in India and other developing countries to low screening prevalence are either educational barriers or behavioural barriers [27]. Persistent high-risk HPV infection increases the risk of cervical intraepithelial neoplasia or invasive cervical cancers. The distribution of HPV varies geographically however, HPV 16 was found to be the commonest type followed by HPV 18 [28]. However, in the present study, HPV 18 was not reported at all.

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In the present study, The HPV prevalence was found to be 23.24%. Kulkarni et al in 2023 studied the genotypic diversity of Human Papillomavirus types in Central India. The overall prevalence of high-risk HPV was present in populations at 7.3% in individuals and 37% in combinations [29]. Senapati R et al in 2017 studied HPV genotype distribution in Odisha, Eastern India. A study of 607 women revealed an overall prevalence of 60.33% [30].

Mishra R. et al (2022) in a cross-sectional study on 217 women attending tertiary care hospitals in western UP. The overall prevalence of HPV was 5.5% (12/217) [23]. Datta et al reported the prevalence to be 7% [31] while Sahasrabudhdhe et al found it to be 41.7% [32].

Mishra R et al suggested that asymptomatic women contributed 30.41% among them while 3.03% HPV positivity which was quite low as compared to Sontakke et al. who reported a prevalence as high as 44.2% [33]. However, HPV DNA testing is the most acceptable tool in the screening of cervical cancer [34].

A study of HPV prevalence in coastal Karnataka by Saxena V et al (2018) reported 18.8% overall prevalence [35]. In a study on the North Indian population, Misra M et al (2021) found the prevalence of HPV-positive cases was 20% [36]. Vora Kranti Suresh et al in 2022 reported 32.47% of HPV prevalence in urban slums of Ahmedabad [37]. Rashid A. and Sharma DC (2021) screened 380 female patients from northern India and found a 15.78% prevalence of HPV infection among the total screened patients [38].

In China, The overall HPV prevalence was 19.7% among women in northern Henan Province [39], which was similar to the results from several surveys of HPV prevalence reported in other provinces of China [42-44]. The prevalence of HPV in northern Henan Province was lower than that reported in Shandong Province (28.4%) and Fujian Province (38.3%) [45-46], but higher than Yunnan Province (12.9%) and Shanxi Province (8.92%) [40, 41]. According to previous reports, the HPV infection rate ranges from 6.7 to 44.5% in China [46].

In the present study, It was observed that the 27/43 positive samples were HPV16 which accounts for 62.79% of total prevalence (n-43). Furthermore, it is 14.59% of the total sample (n=185).

2nd most prevalent genotype was HPV58 (9.30%) followed by HPV31, HPV33, HPV39 each having prevalence of 6.97%. Next in descending order were HPV35, HPV51, and HPV68 having a prevalence of 4.65%, 4.65% and 2.32% respectively. Interestingly, HPV18 which is the 2nd most prevalent high-risk HPV genotype was nil in the present study. In other studies, it is 24.56% [30]. Likewise, Mishra R et al observed that a high prevalence of HPV 16 was not found, whereas the prevalence of HPV 18 was comparatively low 16.6% [23].

Similarly, high-risk HPV45, HPV52, HPV59, and HPV66 were also not reported in the present study. HPV 59, which was not reported in our study was found as the most predominant genotype in Ghaziabad [23] (Table 2, Chart 2).

Kulkarni et al in 2023 found the prevalence of HPV 16, 18, 31 and 45 as 29.6%, 11.1%, 12.9%, and 9.2% respectively [29]. Senapati R et al in 2017 observed the most prevalent genotype was HPV16 (87.28%) followed by HPV18 (24.56%) and HPV 51(3.46%) [30]. Other detected

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genotypes in descending order were HPV 51(3.46%), HPV 39(3.17%), HPV 66(2.8%), HPV 68(2.3%), HPV 35(1.7%), HPV 45(1.7%), HPV 44(1.1%), HPV 58 (1.1%), HPV 52(.57%), HPV 6/11(.57%), HPV 42(1.1%) and HPV 43(.57%). Prevalence of single and multiple genotypes was 76.58% and 23.41% respectively

HPV 51 was found to be the 4th most predominant genotype in the present study, while it was the 3rd most prevalent genotype in a study by Senapati et al [30]. However, it is rarely reported in other regions of the country and worldwide. Data on the prevalence and distribution of the three most prominent genotypes in cancer cases from different geographical regions of India shows a great regional variation [29, 49-53].

HPV 66, was absent in the present study population which follows the previous literature; except a study by Senapati et al reported a prevalence of about 3% for HPV66. [30]

Mishra R. et al (2022) in a cross-sectional study on 217 women attending tertiary care hospitals in western UP. The overall prevalence of HPV was 5.5% (12/217) and HPV types 59,56,51,33 and 18 were found [23]. Misra M et al (2021) reported that Infections with high-risk HPV types were found to be 41% of positive tested samples followed by 35% of moderate-risk HPV types. 41% of infections were contributed by HPV type 16 and 18 alone [36]. In the present study, HPV16 contributed to 62.79% of the infections alone and HPV18 was not seen. Other high-risk genotypes contributed nearly 37.21% which is similar to the results of Mishra M et al [36].

After analysis of several studies, it has been found that the most predominant genotypes are HPV 16 and 18 responsible for causing approximately 75% of cervical carcinoma in India [49-52,55]. In other words, we can say that they are highly mentioned and most commonly detected high-risk genotypes [54]. Interestingly, in our study HPV 18 was not reported and HPV 16 had the highest prevalence.

In contrast to our study where HPV 18 was not reported. Rashid A. and Sharma DC (2021) observed HPV type 18 ranges from 3%-20% per the WHO Report [70]. Thus we can conclude that the prevalence of HPV18 has geographical variations.

Similar to the present study, the Chinese population also showed the absence of HPV18. Here, the overall HPV prevalence was 19.7% among women in northern Henan Province. The most common HR-HPV genotype was HPV16 (4.3%), followed by HPV52 (3.5%) and HPV58 (2.0%), HPV53 (1.8%) and HPV39 (1.5%) [39].

Also, HR-HPV infection accounted for all the HPV infections demonstrating the most symptomatic infections were caused by HR-HPV which were following previous literature [39]. Our study showed that the overall HPV infection rate was 23.24%, which was similar to the results from several surveys of HPV prevalence reported in other parts of India [35-36]. Similar results were seen in the Chinese population, the prevalence of HPV in northern Henan Province was lower than that reported in Shandong Province (28.4%) and Fujian Province (38.3%) [45, 46], but higher than in Yunnan Province (12.9%) and Shanxi Province (8.92%) [40, 41]. According to previous reports, the HPV infection rate ranges from 6.7 to 44.5% in China [56].

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CONCLUSION

It has been observed that there is a high prevalence of HPV16, 58, 31, 33, 35 and 39. The currently available bivalent (HPV16, HPV18), tetravalent (HPV16, 18, 6, 11) and 9-valent HPV vaccine is used to prevent infection with HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV45, HPV52, and HPV58 as there are many regional variations, especially in developing nations as described in the previous literature. Our study further recommends studying the prevalence and genotyping variations in the locality before providing a vaccine in the vaccination schedule as an effective prophylactic means.

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COMPETING INTEREST: The authors declare no competing interests. We declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

BIBLIOGRAPHY

1. Sastry A S, Bhat S. Essentials of Medical Microbiology. 3rd Edition, chapter 80, 803.
2. Kombe AJK, Li B, Zahid A, Mengist HM, Bounda G-A, Zhou Y, et al. Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation. Front Public Health (2021) 8:552028.
3. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global Burden of Cancers Attributable to Infections in 2012: A Synthetic Analysis. Lancet Global Health (2016) 4:e609–16.
4. Kilic S, Shukla PA, Marchiano EJ, Patel RH, Baredes S, Liu JK, et al. Malignant Primary Neoplasms of the Nasal Cavity and Paranasal Sinus. CurrOtorhinolaryngol Rep (2016) 4:249–58.
5. Huang, W. et al. Prevalence of Human Papilloma Virus among women in Northern Guangdong Provinceof China. Sci. Rep. 12,13353.
6. Tesfaye Elsa, KumbiBirhanu et al. Prevalence of human Papilloma Virus infection and associated factors among women attending cervical cancer screening in setting of Addis Ababa, Ethiopia. Scientific Reports (2024)14:4053.
7. Fernandes J, Carvalho M, de Fernandes T, Arau´jo J, Azevedo P, Azevedo J, et al. Prevalence of Human Papillomavirus Type 58 in Women With or Without Cervical Lesions in Northeast Brazil. Annals of Medical and Health Sciences Research. 2013; 3(4): 504–510.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE4, 2024

8. Bernard HU, Burk RD, Chen Z, van Doorslaer K, Hausen HZ, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010; 401(1): 70–79.
9. HPV Center http://www.hpvcenter.se/html/refclones.html. Accessed 29 Dec 2017. Last modified by: Davit Bzhalava 2016-05-24.
10. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zurHausen H. Classification of papillomaviruses. Virology. 2004; 324: 17–27.
11. Lee K, Magalhaes I, Clavel C, Briolat J, Birembaut P, Tommasino M, et al. Human papillomavirus 16 E6, L1, L2 and E2 gene variants in cervical lesion progression. Virus Res. 2008; 131: 106–110.
12. Villa LL, Sichero L, Rahal P, Caballero O, Ferenczy A, Rohan T, et al. Molecular variants of human papillomavirus types 16 and 18 preferentially associated with cervical neoplasia. J Gen Virol. 2000; 81: 2959–2968.
13. Xi LF, Koutsky LA, Hildesheim A, Galloway DA, Wheeler CM, Winer RL, et al. Risk for high-grade cervical intraepithelial neoplasia associated with variants of human papillomavirus types 16 and 18. Cancer Epidemiol. Biomarkers Prev. 2007; 16: 4–10
14. Zuna RE, Moore WE, Shanesmith RP, Dunn ST, Wang SS, Schiffman M., et al. Association of HPV16Thistle, P.; Parpia, R.; Pain, D.; Lee, H.; Manasa, J.; Schnipper, L.E. Prevalence and Subtype Distribution of High-Risk Human Papillomavirus Among Women Presenting for Cervical Cancer Screening at Karanda Mission Hospital. JCO Global Oncology 2020, 1276-1281.
15. E6 variants with diagnostic severity in cervical cytology samples of 354 women in a US population. Int. J. Cancer. 2009; 125: 2609–2613.
16. World Health Organization
17. Schiffman Mark, Doorbar John, Nicolas Wentzensen, Silvia de Sanjosé, Carole Fakhry, Bradley J. Monk, Margaret A. Stanley and Silvia Francesch. Carcinogenic human papillomavirus infection.Nature reviews, disease primers. Article number: 16086
18. Bihl MP, Tornillo L, Kind AB, Obermann E, Noppen C, Chaffard R, et al. Human papillomavirus (HPV) detection in cytologic specimens: similarities and differences of available methodology. ApplImmunohistochemMolMorphol. 2017;25(3):184.
19. Ozaydin-Yavuz G, Bilgili SG, Guducuoglu H, Yavuz IH, Elibuyuk-Aksac S, Karadag AS. Determinants of high-risk human papillomavirus infection in anogenital warts. PostepyDermatolAlergol. 2019;36(1):76–81.
20. Min K-J, Kwon S-H, Kim K, Kim S, Kim HJ, Seong SJ, et al. Clinical guideline for 9-valent HPV vaccine: Korean Society of Gynecologic Oncology Guideline. GynecolOncol. 2018;30(2):1–10.
21. Schmitt M, Depuydt C, Benoy I, Bogers J, Antoine J, Arbyn M, et al. Prevalence and viral load of 51 genital human papillomavirus types and three subtypes. Int J Cancer. 2013;132(10):2395–403

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE4, 2024

22. Mohammad Farahmand , Mohsen Moghoofei , AbolfazlDorost , SaeedehAbbasi , SeyedHamidrezaMonavari, Seyed Jalal Kiani4 and Ahmad Tavakoli. Prevalence and genotype distribution of genital human papillomavirus infection in female sex workers in the world: a systematic review and meta-analysis. BMC Public Health (2020) 20:1455
23. Mishra R, Bisht D. Distribution and Prevalence of High-risk Human Papillomavirus Infection in Women of Western Uttar Pradesh, India: A Hospital-based Study. J South Asian FederObsGynae 2022; 14 (2):91-94.
24. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. ColdSpring Harbor Laboratory Press; 1989;1:6.4-6.11.
25. Sreedevi A, Javed R, Dinesh A. Epidemiology of cervical cancer with special focus on India. Int J Womens Health. 2015;7:405–14.
26. Kadian LK, Singhal G., Sharma S, Chauhan P, Nanda S, Yadav R. Incidence and Association of HPV16 and 18 with Various Risk Factors in Cervical Cancer Patients in Population of Haryana Region, India.Journal of Clinical and Diagnostic Research. 2019 Feb, Vol-13(2): QC10-QC13.
27. Ganju SA, Gautam N, Barwal V, Walia S, Ganju S. Assessment of knowledge and attitude of medical and nursing students towards screening for cervical carcinoma and HPV vaccination in a tertiary care teaching hospital. Int J Community Med Public Health. 2017;4(11):4186-93.
28. Peedicayil A, Abraham P, Sathish N, John S, Shah K, Sridharan G, et al. Human papillomavirus genotypes associated with cervical neoplasia in India. Int J Gynaecol Cancer. 2006;16(4):1591-95
29. Kulkarni Sayali P, Paliwal Shruti and Kosta Susmit. Genotypic Diversity of Human Papilloma Virus (HPV) types and its prevalence with Cervical cancer in Central India. Cureus 2023 Feb 15(2); e35227.
30. Senapati, R., Nayak, B., Kar, S.K. *et al.* HPV Genotypes distribution in Indian women with and without cervical carcinoma: Implication for HPV vaccination program in Odisha, Eastern India. *BMC Infect Dis* **17**, 30 (2017).
31. Datta P, Bhatla N, Pandey RM, et al. Type-specific incidence and persistence of HPV infection among young women: a prospective study in North India. Asian Pac J Cancer Prev. 2012;13(3):1019–24.
32. Sahasrabuddhe VV, Bhosale RA, Joshi SN et al. Prevalence and Predictors of coloscopic histopathologically confirmed cervical intraepithelial neoplasia in HIV infected women in India. PLoSOne2010,5:8634.
33. SontakkeBR, AmbulkarPS, TalharS, etal. Molecular genetic study to detect prevalence of high-risk human papillomavirus strains (type16 and 18) in cervical lesions and asymptomatic healthy subjectsofruralCentralIndia.JCytol2019;36(1):32–37.DOI:10.4103/JOC.JOC_10_18.
34. Jin XW, Lipold L, Foucher J, et al. Cost-effectiveness of primary HPVtesting, cytology and co-testing as cervical cancer screening forwomen aboveage30years. JGenInternMed2016;31(11):1338–1344.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE4, 2024

35. Saxena V, Pai V, RajagopalK , Abdulla Mehnaz. Molecular Detection of Human Papillomavirus (HPV) in Females and Assessment of Risk Factors for HPV Infection: A Study from Coastal Karnataka. Journal of Clinical and Diagnostic Research. 2018 Jun, Vol-12(6): DC46-DC50.
36. Misra M, et al. Human Papillomavirus Screening: Study of HPV prevalence and cervical cancer in North Indian Women Population. Ann Med Health Sci Res. 2021;11:1561-1565
37. Vora KS, Saiyed S, Joshi R, Natesan S. Prevalence of high-risk HPV among marginalized urban women in India and its implications on vaccination: A cross sectional study. Int J Gynecol Obstet. 2023;162:176-182.
38. Rashid A, Sharma D C. Molecular Characterization of High-Risk Human Papilloma Virus Infection with Abnormal Uterine Bleeding, Pelvic Inflammatory Disease and Vaginal Discharge in Women Patients. Volume 10, Issue 4, 2021, 2810 - 2819
39. Xiangpeng Wang1 , Yuan Song1 , Xiaofei Wei1 , Guanyu Wang1,2, Ruili Sun1*, Mingyong Wang1* and Lijun Zhao. Prevalence and distribution of human papillomavirus genotypes among women attending gynecology clinics in northern Henan Province of China. Virology Journal (2022) 19:6
40. Li Z, Liu F, Cheng S, Shi L, Yan Z, Yang J, Shi L, Yao Y, Ma Y. Prevalence of HPV infection among 28457 Chinese women in Yunnan Province, southwest China. Sci Rep. 2016;6:21039.
41. Yang J, Wang W, Wang Z, Wang Z, Wang Y, Wang J, Zhao W, Li D, Liu H, Hao M. Prevalence, genotype distribution and risk factors of cervical HPV infection in Yangqu, China: a population-based survey of 10086 women. Hum Vaccin Immunother. 2020;16(7):1645–52.
42. Ge Y, Zhong S, Ren M, Ge Y, Mao Y, Cao P. Prevalence of human papillomavirus infection of 65,613 women in East China. BMC Public Health. 2019;19(1):178.
43. Xu HH, Lin A, Chen YH, Dong SS, Shi WW, Yu JZ, Yan WH. Prevalence characteristics of cervical human papillomavirus (HPV) genotypes in Taizhou area, China: a cross-sectional study of 37967 women from the general population. BMJ Open. 2017;7(6):e014135
44. Mai Q, Yang X, Cheng H, Wu G, Wu Z. Prevalence and genotype distribution of human papillomavirus among women with cervical lesions in Shenzhen city. China Hum Vaccin Immunother. 2021;17(4):965–71.
45. Jiang L, Tian X, Peng D, Zhang L, Xie F, Bi C, Wang R, Wang J, Qi D. HPV prevalence and genotype distribution among women in Shandong Province, China: analysis of 94489 HPV genotyping results from Shandong's largest independent pathology laboratory. PLoS ONE. 2019;14(1):e0210311.
46. Wu C, Zhu X, Kang Y, Cao Y, Lu P, Zhou W, Zhou H, Zhang Y, Song Y. Epidemiology of human papilloma virus infection among women in Fujian China. BMC Public Health. 2017;18(1):95.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE4, 2024

47. Li K, Li Q, Song L, Wang D, Yin R. The distribution and prevalence of human papillomavirus in women in mainland China. Cancer. 2019;125(7):1030–7
48. Sauvaget C, Nene B, Jayant K, et al. Prevalence and determinants of high-risk human papillomavirus infection in middle-aged Indian women. Sex Transm Dis. 2011;38(10):902–6.
49. Sowjanya AP, Jain M, Poli UR, et al. Prevalence and distribution of high-risk human papillomavirus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh. India BMC Infect Dis. 2005;5:116.
50. Datta P, Bhatla N, Pandey RM, et al. Type-specific incidence and persistence of HPV infection among young women: a prospective study in North India. Asian Pac J Cancer Prev. 2012;13(3):1019–24.
51. Basu P, Roychowdhury S, Bafna UD, Chaudhury S, et al. Human papillomavirus genotype distribution in cervical cancer in India: Results from a multi-center study. AsianPac J Cancer Prev. 2009;10:27–34.
52. Deodhar K, Gheit T, Vaccarella S, Romao CC, et al. Prevalence of Human Papillomavirus Types in Cervical Lesions from Women in Rural Western India. J Med Virol. 2012;84:1054–60.
53. Srivastava S, Shahi UP, Dibya A, Gupta S, Roy JK. Distribution of HPV Genotypes and Involvement of risk factors in cervical lesions and invasive cervical cancer: a study in an indian population. Int J Mol Cell Med. 2014;3:2.
54. Li N, Franceschi S, Howell-jones R, et al. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication.IntJCancer2011;128(4):927–935.
55. Bhatla N, Dar L, Patro AR, et al. Human Papillomavirus-Type Distribution in Women With and Without Cervical Neoplasia in North India. Int J GynaecolPathol 2008;27(3): 426-430
56. Li K, Li Q, Song L, Wang D, Yin R. The distribution and prevalence of human papillomavirus in women in mainland China. Cancer. 2019;125(7):1030–7
57. Bruno MT, Scalia G, Cassaro N, Boemi S. Multiple HPV16 infection with two strains: a possible marker of neoplastic progression. BMC Cancer. 2020;20(1):444.
58. Yang J, Wang W, Wang Z, Wang Z, Wang Y, Wang J, Zhao W, Li D, Liu H, Hao M. Prevalence, genotype distribution and risk factors of cervical HPV infection in Yangqu, China: a population-based survey of 10086 women. Hum Vaccin Immunother. 2020;16(7):1645–52.
59. Zhang C, Cheng W, Liu Q, Guang Q, Zhang Q. Distribution of human papillomavirus infection: a population-based study of cervical samples from Jiangsu Province. Virol J. 2019;16(1):67.
60. Egawa N, Doorbar J. The low-risk papillomaviruses. Virus Res. 2017;231:119–27.