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Original Article

Microbiology Section

# Genotype Distribution of Human Papillomavirus in Women with Cervical Cancer and Normal Cytology in Malwa Region of Punjab- A Pilot Study

SHILPA ARORA[1], AVNISHA[2], PRAGATI GROVER[3], NAVNEET KAUR[4], NEERJA JINDAL[5], RATIKA GUPTA[6]


## ABSTRACT

**Introduction:** Human Papillomavirus (HPV), a small circular double stranded DNA virus is the major aetiological agent of cervical precancerous and cancerous lesions. Genital HPV types are divided into high and low risk types, according to their oncogenic potential. The proportion of women infected with HPV varies greatly across populations as is the distribution of HPV genotypes.

**Aim:** The present pilot study was conducted to determine the most frequent genotypes of HPV associated with: a) Histologically proven cases of cervical carcinoma; b) Women with normal cervical cytology.

**Materials and Methods:** The present hospital based cross-sectional study was conducted on 132 women attending Obstetrics and Gynaecology department of a tertiary care hospital. Consecutive cervical brushings were collected from women presenting with any type of cervical lesion and were subjected to cytology, histopathological examination, HPV DNA testing and genotyping by using Sacace real time PCR.

**Results:** Of the 132 women studied between January 2014-October 2014, 50 (37.87%) had histologically proven cervical carcinoma and 82 (62.12%) had normal cytology. In proven cases of cervical carcinoma, HPV infection was found to be associated in 49/50 (98%) and all of them had high risk HPV genotypes. The most common genotypes were 16 (67.34%) and 18 (20.4%). Other genotypes present were 45 and 33. Out of 82 cytological normal women, five showed presence of HPV DNA on RT PCR. While all the five had infection of HPV genotype 18, in one there was presence of both 16 and 18 genotypes.

**Conclusion:** The detection of HPV genotypes 16, 18 along with high risk type 33 and 45 in Malwa region of Punjab confirm the importance of identifying the types of HPV that infects this community to design more effective prevention strategies and thus contribute to fight against cervical cancer.


**Keywords:** Genotyping, Molecular diagnosis, Screening

## INTRODUCTION

HPV, a double stranded DNA virus is one of the most common sexually transmitted disease agents worldwide. It has tropism for epithelial cells and causes latent or inactive infections, anogenital warts or genital tract precancerous or cancerous lesions [1]. Cancer of the cervix uteri which is the fourth most common cancer among women worldwide is totally attributed to the infection of HPV. Some studies have demonstrated the association of HPV in all the (100%) cases of cervical cancer [2]. There are over 100 different HPV genotypes which are classified into high-risk and low-risk groups according to their propensity for malignant progression of the associated lesions [1]. Among these, 15 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) are high risk and 12 (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108) are low risk types [3,4]. Molecular and epidemiological studies have solidified the association between high risk HPV types (especially HPV-16 and HPV-18) and cervical squamous cell carcinoma [1].

In addition to persistent high-risk HPV infection, other factors such as high viral loads, HPV variants, infections with multiple high-risk HPV types and genetic predisposition also contribute to the development of cervical cancer [1]. As the proportion of women infected with different HPV genotypes varies greatly across populations and there are limited studies on this subject from Malwa region of Punjab, India, the present study was undertaken to identify the most frequent HPV genotypes and multiple infections associated with cervical cancer and in women having normal cervical cytology.

## MATERIALS AND METHODS

The present hospital based cross-sectional pilot study was conducted on 132 women having any cervical complaint (bleeding per vagina, discharge per vagina, post coital bleeding, pain abdomen, low back pain) or lesion (chronic cervicitis, endocervicitis, cervical erosion, cervical laceration, cervical polyp, leukoplakia of cervix) who presented to Obstetrics and Gynaecology department of tertiary care hospital of Malwa region of Punjab during the period January 2014-October 2014. The hospital serves basically to the rural population of Punjab. The study was approved by institution with this registration no. 10850.

After taking the informed consent, the socio-demographic profiles of the cases were recorded and scrape samples or cervical brushings were collected. The samples were sent for cytology and histopathology examination and stored at 4°C for HPV DNA testing. HPV High Risk Screen Real-TM Quant 2x (Sacace Biotechnologies Italy) was used for detection of E1-E2 region of HPV DNA using real time amplification. A β-globin gene was used as internal control. Real time amplification was done with smart cycler (Cepheid). Following manufacturer's instructions parameters were set and approximate time for amplification was two hours as shown in [Table/Fig-1].

Results were considered valid if the negative controls did not show positive fluorescence signals and the standards showed signals in all channels (Fam, Joe/HEX/CY3).

## RESULTS

On the basis of cytological and histopathological examination, the breakup of the 132 cases of the study showed 50 histologically

| Stage | Temperature | Seconds | Repeat |
|---|---|---|---|
| Stage 1 Hold | 95° | 900 | 1 |
| Stage 2 Hold | 65° | 120 | 1 |
| Stage 3 3-Temperature cycle | 95° | 20 | 5 |
| | 63° | 30 | |
| | 65° | 60 | |
| Stage 4 3-Temperature cycle | 95° | 25 | 42 |
| | 60° | 30 | |
| | 65° | 60 | |

**[Table/Fig-1]:** Various stages of amplification.

proven cases of cervical carcinoma and 82 having normal cervical cytology. Presence of HPV was detected in 49 of 50 (98%) cases of cervical carcinoma and in 5 of 82 (6.1%) cases with normal cytology. Age distribution of HPV positive cases of cervical carcinoma and that of normal cytology is given in [Table/Fig-2]. All the women included in the study were multiparous and were married at less than 20 years of age.

| Age Group (years) | Cervical Carcinoma HPV Positive (N=49) | Normal Cytology HPV Positive (N=5) |
|---|---|---|
| <35 | 00 | 04 (80%) |
| 36-59 | 37 (75.5%) | 01 (20%) |
| >60 | 12 (24.5%) | 00 |

**[Table/Fig-2]:** Distribution of Cervical carcinoma and normal cytology HPV Positive patients according to their age group.

HPV DNA study showed that in cases of cervical carcinoma the most common genotype was 16 (67.34%) followed by genotype 18 (20.4%). Other genotypes observed were 45 (4.08%) and type 33 (2.04%). On the other hand, in women with normal cytology the most common genotype was 18 which was present in all the 5 women (100%). Multiple infections with 16 and 18 genotypes were observed in 3 (6.12%) cases of cervical carcinoma and in one (20%) women with normal cytology [Table/Fig-3].

| Histological diagnosis | Type 16 | Type 18 | Type 45 | Type 33 | Type 16 and 18 |
|---|---|---|---|---|---|
| Cervical carcinoma (n=49) | 33 (67.34%) | 10 (20.4%) | 02 (4.08%) | 01 (2.04%) | 03 (6.12%) |
| Normal cytology (n=5) | 00 | 04 (80%) | 00 | 00 | 01 (20%) |
| Total (n=54) | 33 (61.11%) | 14 (25.92%) | 02 (3.7%) | 01 (1.85%) | 04 (7.4%) |

**[Table/Fig-3]:** Distribution of HPV genotypes by PCR.

## DISCUSSION

HPV is the major aetiological agent for the development of cervical precancerous and cancerous lesions [5]. While some studies have demonstrated that more than 90% of cervical cancer cases are caused by HPV, other studies observed HPV in all the cases of cervical cancer [6]. In the present study the positivity was 98% which is similar to the findings of another study from India [7]. In women of normal cervical cytology the reported HPV positivity varies greatly across populations [8]. In a systemic meta-analysis, Sanjose S et al., observed HPV prevalence as 22.1% in Africa, 20.4% in Central America and Mexico, 10.3% in North America, Europe and 8% in Asia [9]. The present study detected HPV DNA in 6.1% of these women which falls in the range reported by Clifford GM et al., [8].

Age is an important determinant of risk of HPV infection. In all world regions, prevalence of HPV infection is highest in women younger than 35 years of age, suggesting infection at younger age and then slow progression to cancer which is more common in women older than 35 years. In the present study, all the women of cervical cancer were above the age group of 35 years while the incidence in women with normal cytology in same age group was only 20% [Table/Fig-2]. In an another study from Punjab, Sharma P et al., had also observed that the highest proportion of HPV positive cases corresponded to patients younger than 40 years [10].

Epidemiological studies have also indicated that the risk of contracting genital HPV infection and cervical cancer is influenced by sexual activity [1]. All the women of present study were found to be multiparous and had sexual exposure at an early age which is similar to the findings of Sharma P et al., [10]. Borna NN et al., observed that almost half of HPV positive patients in their study had history of sexual exposure before the age of 18 years [1]. Another study also showed that the risk of HPV infection was higher in women who were married below the age of 20 years and in women with parity ≥4 [11]. This increased risk may be due to the fact that younger women have exposure to persistent HPV infection for a longer period of time than women having exposure at later age.

There are various HPV genotypes involved in cervical cancer. In the present study, HPV 16 was most prevalent (67.34%) high risk type followed by type 18 (20.40%) [Table/Fig-3]. In India, the reported prevalence of HPV 16 is 70-90% and HPV 18 is 3-20% (WHO, 2010). Worldwide also, 16 and 18 are the most common types followed by genotypes 45, 31, 33, 52, 58 and 35. Borna NN et al., detected HPV type 16 in 80%, 18 in 10% and 45 in 6.67% in a study from Bangladesh [1].

In the present study where we found HPV infection in women of normal cervical cytology as 6%, HPV genotype 18 was most prevalent which correlates the findings of Sharma P et al., [10]. Other genotype (16) was observed in only one woman who had multiple infections of 16 and 18. In a pooled analysis conducted on 15618 women without cytological abnormalities, genotype 16 was the most prevalent type in Sub-Saharan Africa, Europe and South America but there was heterogenicity in types reported from different areas of Asia [9]. It has also been reported that HPV types most commonly detected in women of normal cervical cytology are similar to those described in precancerous and cancer cases [9]. The variability whenever and wherever observed could be because of variation in the population characteristics such as age, sexual behaviour, geographical location and method of detecting HPV genotyping.

The present study detected co-infection with more than one types (16/18) in 3 (6.12%) cases of cervical cancer while Borna NN et al., detected it in 3.3% [1]. Another study reported multiple infections in as many as 35% [12]. This variation could be related to different methods used to detect HPV DNA.

## LIMITATION

Sample size was small as more cases could not be screened due to financial constrains. The exact prevalence of HPV types could not be identified as the present study was hospital based. Due to limited study period, it was not possible to observe the genotypes with progression of lesions.

## FUTURE RECOMMENDATION

Based on the present study, high risk genotypes (33 and 45) could also be included in the vaccine which would ultimately help in designing more effective preventive strategy to achieve the true purpose of vaccination programme in India.

## CONCLUSION

The present study recommends identification of genotypes of HPV that are associated with cervical carcinoma and in normal cervical cytology in Malwa region of Punjab. Since, along with genotype 16 and 18, infections with other high risk genotypes and multiple infections are prevalent in this region.

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**PARTICULARS OF CONTRIBUTORS:**
1. Assistant Professor, Department of Microbiology, Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India.
2. Junior Resident, Department of Microbiology, Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India.
3. Assistant Professor, Department of Microbiology, Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India.
4. Assistant Professor, Department of Microbiology, Pandit Jawahar Lal Nehru Government Medical College and Hospital, Chamba, Himachal Pradesh, India.
5. Professor and Head, Department of Microbiology, Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India.
6. Junior Resident, Department of Radiotherapy, Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**
Dr. Shilpa Arora,
St No. 1 Partap Nagar, Kotkapura, District-Faridkot, Punjab, India.
E-mail: s.arora49@yahoo.com



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