

# Determination and evaluation of HR-HPV genotype in different communities of Bihar, India

Akhtar Parwez<sup>1</sup>,  
Sunit Singh<sup>2\*</sup> ,  
Rahul Kumar<sup>3</sup>,  
Roushan Kumari<sup>1</sup>,  
Vikas Kumar<sup>1</sup>,  
Vidyut Prakash<sup>4</sup>,  
Mohammad Ali<sup>5</sup>

<sup>1</sup>Department of Biotechnology, Magadh University Bodh-Gaya, Bihar, India, <sup>2</sup>Department of Zoology, B.D. College, Patna, Patliputra University, Patna, Bihar, India, <sup>3</sup>Department of Pharmacy Practice, National Institute of Pharmaceutical Education and Research, Hajipur, India, <sup>4</sup>Department of Microbiology, Indira Gandhi Institute of Medical Sciences Patna, India, <sup>5</sup>Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India

#### Address for correspondence:

Dr. Sunit Singh, Department of Zoology,  
B.D. College, Patna, Bihar, India.  
E-mail: sunitsingh195@gmail.com

WEBSITE: ijhs.org.sa

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## ABSTRACT

**Objective:** Human papillomavirus (HPV)-associated uteri cervix carcinoma continues to be the 2<sup>nd</sup> highest cause of death among women in India. This study aims to identify the mode of HPV transmission in different communities such as Hindu, Muslim, Christian and Banjaran women of Bihar, India. Different patterns of life and cultural variations exist among Muslims, Hindus, Christians, and Banjarans. For example, Muslim wash their genital parts after urination and maintain genital hygiene, whereas Banjaran tribes, Christians, and Hindu communities do not maintain hygiene. Thus, the present study was undertaken to evaluate high-risk HPV (HR-HPV) infection among healthy women. We access to genuine reason for the cause of HPV transmission in women.

**Methods:** Ethical clearance was obtained from MCS and RC Patna, India. A total 154 urine samples have been used for the detection of HR-HPV through a real-time PCR technique. The DNA extraction was done from collected non-invasive urine samples. The estimation and purification of DNA purity was performed by QuantiFluor<sup>®</sup> dsDNA system and detected HPV-16 and HPV-18.

**Results:** Overall, the prevalence of HR-HPV infection was detected to be 12.34% (19/154) whereas HPV-16 was found to be 9.9% (14/154) and HPV-18 was found to be 3.25% (5/154) in women. The lowest (2%; 1/50) prevalence of HR-HPV was observed in the Muslim community, while higher (25%, 16%, and 14.71%) prevalence was found in the Banjaran, Christian, and Hindu communities, respectively.

**Conclusion:** Our study indicates that personal hygiene possibly reduces HPV infection in women and the evidence suggests that male circumcision has a protective role of HPV infection in Muslim community. Therefore, personal hygiene and circumcision may reduce the risk of HPV acquisition and transmission as well as cervical cancer development in women.

**Keywords:** Cervical cancer, community, human papillomavirus, urine sample

## Introduction

Cervical cancer is the third most diagnosed cancer among women in India.<sup>[1]</sup> About 88% of cervix carcinoma deaths and 84% of new cases occurred in low- and middle-income countries.<sup>[2,3]</sup> Population-based cytological screening program has been effectively reduce the incidence and mortality rates of cervical cancer in high-income countries.<sup>[4,5]</sup> HPV-16 and HPV-18 are related to cervix cancer in about 70% of cases<sup>[6]</sup> whereas approximately 99.7% of all uterine cervix cancers have been associated with HPV.<sup>[7]</sup> HPV is a sexually transmitted virus which plays a major role for the progression of cervical cancer in women. HPV is transmitted through sexual contact, which is recognized as a form of disease of cervix cancer in women. HPV infection can be found in the both women and men.

HPV has been classified into two risk factors; the high-risk HPV (HR-HPV) genotype and the low-risk HPV (LR-HPV) genotype. The HR-HPV genotype is mainly associated with cervical cancer, and the LR-HPV genotype is related to etiological genital warts. HR-HPV genotype associated are HPV-16, HPV-18, HPV-35, HPV-31, HPV-33, HPV-45, HPV-39, HPV-51, HPV-52, HPV-56, HPV-59, HPV-66, and HPV-69. There are two most prevalent HPV genotypes: HPV-16 and HPV-18, which are mainly responsible for the development of cervix cancer. HR-HPV is associated with cervix cancer cases and other anogenital carcinoma including about 88% of anal cancers and  $\geq 70\%$  of vaginal, 30–50% of penile cancer cases,  $\leq 30\%$  of vulvar cancer cases, and a small percentage of laryngeal carcinoma.<sup>[7]</sup> Furthermore, small cases of oral cavity carcinoma and  $\geq 25\%$  of oropharyngeal cancerous epithelial

lesions are related to HR-HPV infection.<sup>[8,9]</sup> LR-HPV strains are HPV-11, HPV-6, HPV-42, HPV-40, HPV-43, HPV-44. HPV-6, and HPV-11 are chiefly attributable to infection by anogenital epithelial warts. LR-HPV genotypic strains lead to recognized penile, vulvar, anal, vaginal carcinoma, as well as neck-and-head cancers. Several well-established known risk factors, such as multiple sex partners, multiple pregnancies, poor genital hygiene, early age of sexual intercourse, high parity, and early age of marriage, are related to the progression of uterine cervix carcinoma.<sup>[10-12]</sup> Personal hygiene and sexual behavior (multiple sex partners, early age of intercourse, early age of marriage, etc.) play an important role in the pathogenesis of cervical cancer.

We undertook evaluation of the prevalence of HR-HPV DNA with using non-invasive method in healthy women of Bihar, India. This study aims to determine the actual mode of transmission of HPV infection in Hindu, Muslim, Christian, and Banjaran communities.

## Materials and Methods

### Exclusion and inclusion criteria

All women who met the inclusion criteria were invited to participate. Some inclusion and exclusion criteria of this study are described below:

#### Inclusion criteria

Women –

- (i) Who were – Hindus, Muslims, Christians, and Banjaran communities,
- (ii) Who were – normal/disease free,
- (iii) Who have – not HPV vaccinated,
- (iv) Who have – not undergone liquid-based cytology (LBC), pap smear assay, etc., within the past 5 years,
- (v) Who were – age range between 17 and 52 years,
- (vi) Who have – never undergone surgery or hysterectomy,
- (vii) Who were – not mensural cycle period during sampling,
- (viii) Who were – willing to participate in the study and ready to fill out consent forms and questionnaires,
- (ix) Bihar region only.

#### Exclusion criteria

Women –

- (i) Who were – age  $\leq 17$  and  $\geq 52$  years,
- (ii) Who were – other than Muslims, Christians, and Banjaran communities,
- (iii) Who were – pregnant,
- (iv) Who were – HPV vaccinated,
- (v) Who were – with any uterine cervical abnormalities and diagnosed with uterine cervix cancer cases,
- (vi) Who were – previous surgical procedure on the uteri cervix or hysterectomy,
- (vii) Who were – mensural cycle period during sampling,

- (viii) Who were – screened for a pap smear, LBC, etc.
- (ix) Those who were not willing to fill out consent forms and questionnaires in the study.

### Study population and sample size

A community-based study was carried out in different communities which have different socio-sexual life patterns and personal hygiene practices in women of Bihar, India. We have focused on different religion of healthy women. A total number 154 urine samples were collected from different communities and divided into four groups as mentioned below:

- Group I: Hindu women,
- Group II: Muslim women,
- Group III: Christian women, and
- Group IV: Banjaran women

### Biological specimen collection

We have collected 60 mL urine samples in a sterile collection container from participants and age range between 17 and 52 years of the women. Further, we have proceeded with DNA extraction.

### DNA isolation

First of all, we have transfer 20 mL urine samples from urine container to a conical tube. Centrifuged at 4000 RPM at 4°C temp. for 15 min and kept the pallet and discarded supernatant. The first step was repeated 3 times, and a 60 mL urine specimen was collected to ensure an adequate amount of DNA was obtained. Added 200  $\mu$ L sterile phosphate buffer solution in pallet of microcentrifuge tube and vortex for 1 min for dissolving and mixing the pallet. Again, centrifuged the pallet tissue at 4000 RPM for 10 min. The previous step has been repeated once again. Finally, find the tissue pallet from the urine samples and further proceeded for DNA isolation, we have used silica membrane “MyLab Life solutions” manufacturer kit for DNA isolation. A 200  $\mu$ L sample was taken in a 2.0 mL microcentrifuge tube and 20  $\mu$ L lysis enhancer buffer solution was added to the specimen and vortexed for 10 s then 20  $\mu$ L RNAout solution was added to the specimen and mixed well by vortex for 10 s then incubated at room temp. for 2 min. A 200  $\mu$ L lysis buffer 2 was added and thoroughly mixed by vortex to obtain a homogenous solution. To enhance protein digestion, incubated at 55°C temp. for 10 min on a dry bath machine. Added 200  $\mu$ L ethanol (98–100%) was added to the lysate. Mixed well by vortex for 5 s to yield a homologous solution and proceeded for DNA binding process.

### Binding and washing of DNA

Approx. 640  $\mu$ L of prepared lysate was placed in a spin membrane containing column and centrifuged at 10,000  $\times$  g RPM for 1 min at room temp. The collection tube was discarded and placed in a spin column into a clean collection tube supplied by the manufacturer. The proceed for washing

of DNA. Added 480  $\mu\text{L}$  wash buffer 1 into a spin column at the center. Centrifuged spin column at  $13,000 \times g$  for 3 min at room temp. Discarded the solution from the collection tube. Given an empty spin by centrifuging at  $13,000 \times g$  for 1 min at room temp. and discarding the collection tube. Placed the spin column in a sterile 1.5 mL microcentrifuged tube and proceeded to Elution buffer of DNA.

### Elution buffer of DNA

Added 50  $\mu\text{L}$  elution buffer to spin column, incubated for 1 min at room temp., centrifuged the spin column at  $15,000 \times g$  for 1 min at room temp. The microcentrifuge tube contains purified genome DNA and discarded spin column from the microcentrifuge tube, stored the eluted DNA at  $-20^\circ\text{C}$  until used for further processing.

### Quantification of DNA

We have used the QuantiFluor<sup>®</sup> dsDNA protocol for the quantification and purification of DNA. It comprises a fluorescent DNA-binding dye that allows sensitive quantitation of a small amount of double-stranded DNA in purified samples. The test is highly selective for dsDNA over other nucleic acids and has a linear range of 0.05–200 ng of dsDNA input. The dye-based system provides concentrated QuantiFluor<sup>®</sup> dsDNA dye, dilution buffer, and DNA standard. Reagent was stored at  $2-10^\circ\text{C}$  temp. and protected from sunlight. QuantiFluor<sup>®</sup> dsDNA system contains a 20X TE buffer solution (pH 7.5), dsDNA dye, and a DNA standard reagent.

We prepared a 1X TE buffer solution to added 19 mL nuclease-free water, mixed well, and protected from sunlight. Prepared a working solution in 1X TE buffer to dilute the QuantiFluor<sup>®</sup> dsDNA dye 1:400. Prepared blank solution to added 200  $\mu\text{L}$  of QuantiFluor<sup>®</sup> dsDNA dye to an empty 0.5 mL PCR tube and protected from sunlight. A 200  $\mu\text{L}$  of the prepared working solution was dispensed into each 0.5 mL PCR tube, then 1  $\mu\text{L}$  of the sample was added to the prepared sample incubated at room temperature for 5 min. The prepared sample should be protected from light.

First, calibrate the Quantus<sup>™</sup> Fluorometer for standard solution and save it, then calibrate the fluorometer for blank solution. A fluorometer was used to measure the DNA isolated specimen. The number displayed represented the concentration of DNA. In our study, the concentration of DNA purity of urine samples was ranged from 10 ng/mL to 85 ng/mL.

### Prepared master mixed for RT-PCR

The Pathodetect<sup>™</sup> HPV real-time PCR protocol is designed for *in vitro* detection of HR-HPV DNA from isolated DNA specimen. The MyLab Lifesolutions kit is based on TaqMan fluorogenic probe chemistry, which uses the 5' end nuclease activity of Taq DNA polymerase to detect specific PCR products as they accumulate during RT-PCR cycles. Following

reagent provided by MyLab Lifesolutions manufacturer for the master mix:

- PCR mix
- Nuclease-free water
- HPV-16 detection mix
- HPV-18 mix
- IC detection mix.

Kept all the reagents on icepack during the experiment setup (as per manufacturer's instruction). Take and thaw all the components thoroughly before using, them mixed gently, spin down the content for 5 s, and then test it immediately. Test controls: HPV-16 and HPV-18 positive control. Used nuclease-free water as HPV-negative control. Determined the number of reactions (N) perform for each experiment. It is necessary to make an excess reaction cocktail to allow for the control reaction and pipetting error. Prepared two master mix in a 2.0 mL microcentrifuge tube for RT-PCR. One is for HPV-16 and another is for HPV-18. Prepared 17.0  $\mu\text{L}$  each volume of master mix for genotype HPV-16 and HPV-18 in PCR tube to add specimen number of reactions to be set up for each reaction reagent mixed well by pipetting up and down gently but did not require vortex. Centrifuged for a few seconds to collect contents at the bottom of the tube and placed the tube on an icepack. Set up reaction strip tubes or plates and dispense 17  $\mu\text{L}$  of the above cocktail into each well as per the plate set up. Before moving the plate to the DNA handling area, pipette 8  $\mu\text{L}$  of sample DNA/positive control/negative control into respective wells as per set up to make 25  $\mu\text{L}$  of total volume. Cap the wells or sealed the plate with an optical sealer then centrifuged the plate for a few seconds. Make sure that bubbles are eliminated from the bottom of the reaction tubes. Now setup a plate experiment for RT-PCR and run the RT-PCR machine. Each specimen was put on the wells of RT-PCR and assigned a target of genotype HPV-16, HPV-18, and internal control each other, respectively.

The assay is based on RT-PCR for the amplification of specific conserved target sequences of the E6/E7 region of HPV-16 and HPV-18 and detection of the target specific probe.  $\beta$ -globin gene is also detected as a housekeeping gene to check for extraction efficiency and PCR inhibition. The test principle is based on TaqMan technology, which allow for higher specificity and sensitivity. This kit uses single steps RT-PCR with TaqMan fluorogenic probes chemistry that uses the 5' nuclease activity of the Taq DNA polymerase enzyme and enables the detection of a specific PCR product as its accumulation during PCR cycles. We have used specific primer and probe for HPV-16, 5'-CCGGACAGAGCCCATTACAAT-3' was forward primer sequence and 5'-ACGTGTGTGCTTTGTACGCAC-3' for reverse primer and probe sequence was 5'-TGTTGCAAGTGTGACTCTACGCTTCGGT-3'. Similarly, HPV-18 was 5'-GACTCAGAGGAAGAAAACGATGAAA-3' for forwarding primer and reverse primer sequence was 5'-GTGACGTTGTGGTTCGGCT-3' and probe sequence (5'-TGGAGTTAATCATCAACATTTACCA-3').  $\beta$ -globin

gene 5'-GACAGGTACGGCTGTCATCA-3' for forwarding primer and 5'-TAGATGGCTCTGCCCTGACT-3' for reverse primer and probe sequence was 5'-CTAGGGTTGGCCAATCTACTCCCAG-3'.

## Statistical analysis

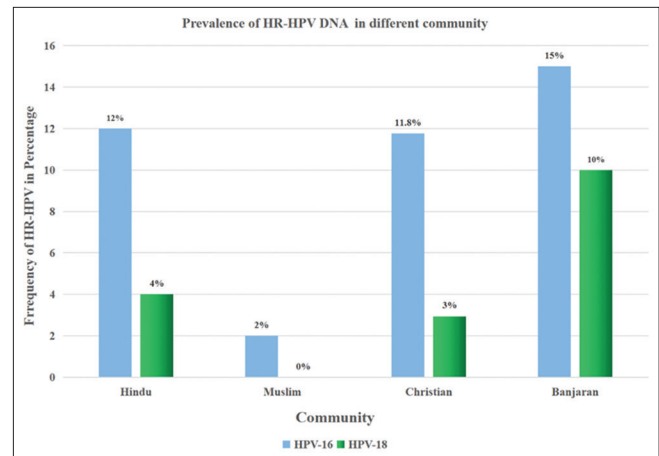
Results were analyzed by *t*-test. Statistically significant value  $P < 0.0001$  was considered. The analysis was done using the GraphPad Prism 5.0 program (GraphPad Software, Inc., San Diego, USA).

## Results

A total no. of 154 urine samples were used for the detection of HR-HPV DNA in an age range between 17 and 52 years in healthy women. Overall, the prevalence of HR-HPV infection was detected to be 12.34% (19/154) whereas HPV-16 was detected to be 9.9% (14/154) and HPV-18 was detected to be 3.25% (5/154) in the study subjects of all communities. Based on our results, the prevalence of HR-HPV infection was found to be 16% (8/50) in the Hindu community, whereas HPV-16 was detected to be 12% (6/50) and HPV-18 was detected to be 4% (2/50). HR-HPV infection was detected to be 14.71% in the Christian community, whereas HPV-16 was identified to be 11.8% (4/34) and HPV-18 was identified to be 3% (1/34). The prevalence of HR-HPV infection was found to be 25% in the Banjaran community, whereas HPV-16 was found to be 15% (3/20) and HPV-18 was found to be 10% (2/20). The prevalence of oncogenic HPV infection was detected to be 2% in the Muslim community, whereas, HPV-16 was detected 2% (1/50) and HPV-18 was not detected in our study group [Figure 1]. The lowest HR-HPV infection was observed in the Muslim community as compared to other communities, such as the Hindus, Christians, and Banjaran women (16%, 14.71%, and 25%) of this study's subjects, respectively. Such results reveal that Muslim women have fewer HPV infection, which may be because, it is possible to practice washing the genital area after urination. Circumcision is one of the most important factors in reducing the burden of HPV infection or cervical cancer in women. Our results showed that the circumcised male partners (Muslim) of the female were less HPV infected than the uncircumcised male partner.

This study selected five districts (Patna, Muzaffarpur, Madhubani, Darbhanga, and Saran) of Bihar, India, and detected HPV-16 and HPV-18 strains. The distribution of HR-HPV was found to be 16.3% (7/43) in Patna district, 12.8% (5/39) was detected in Muzaffarpur district, 10.5% (4/38) was detected in Madhubani district, 8.3% (2/24) was detected in Darbhanga district, and 10% (1/10) was detected in Saran district. Patna district of Bihar was the most HR-HPV-infected districts of Bihar, India [Figure 2].

The age-wise distribution of HR-HPV was detected in the Hindu, Muslim, Christian, and Banjaran communities in this



**Figure 1:** The prevalence of HPV-16 and HPV-18 in different communities of Bihar, India. As showing figure, Banjaran community was the highest (25%) HR-HPV infected whereas Muslim community is the lowest (2%) HR-HPV transmission. HPV: Human papillomavirus, HR-HPV: High-risk human papillomavirus

study. The participants of mean were 20 of age group 17–22 years where HPV-16 was detected six out of 50 samples, HPV-18 was detected two out of 50 samples with gradually decreasing trend of HPV infection with increasing the higher age group in our study [Figure 3]. Our study observed that HPV-16 and HPV-18 were detected the highest in the youngest participants (17–22) in the Hindu, Muslim, Christian, and Banjaran women and more than 40 years age group was not detected HPV infection in our study.

On the basis of genital hygiene practices, one out of nine participants was HPV infected who did not wash the genital parts after urination in Muslim community, whereas 18 out of 101 were infected in non-Muslim community, statistically significant  $P < 0.0001$ . The frequency of genital organ washing was higher in the Muslim participants as compared to the non-Muslims.

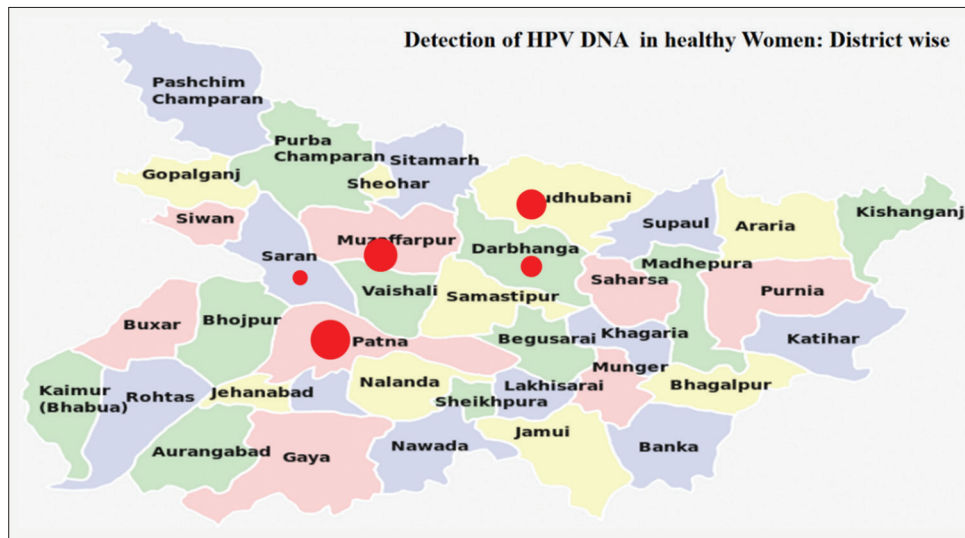
All genital hygiene variables, such as not washing genital parts during the menstrual period, not using sanitary pads during the menstrual period, not washing genital organ after sexual intercourse, and not using condoms during sexual intercourse, had a statistically significant  $P < 0.0001$  [Table 1]. All uncircumcised male partners of the female were found to be non-Muslim community where all circumcised male of female partner was found to be the Muslim community. It may probability to higher prevalence of HR-HPV infection in non-Muslim communities.

Table 2 shows the demographical and clinical characteristics of several communities corresponding to oncogenic HPV infection. On the basis of the educational level of participants, HR-HPV infection was found 12.1% (7/58) in illiterate individuals, followed by 13.3% (6/45) in literate individuals, 10.8% (4/37) in secondary school participants, and 7.1% (1/14) of those with

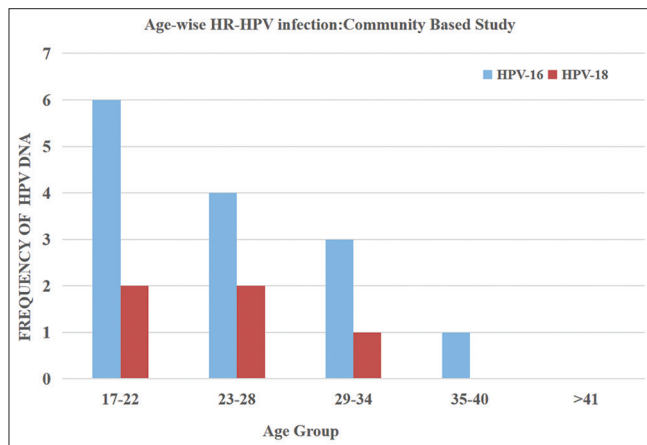
**Table 1:** Different characteristics of personal hygiene in Muslim and non-Muslim women

Variables	Muslim (n=50)	Non-Muslim (n=104)	P-value
	HPV positive (sample No.)	HPV positive (sample No.)	
Not washing after urination	1 (9)	18 (101)	<0.0001
Washing after urination	0 (41)	0 (4)	NA
Not washing during menstrual period	1 (7)	17 (89)	<0.0001
Washing during menstrual period	0 (43)	1 (11)	NA
Using sanitary pad during menstrual period	0 (38)	2 (15)	NA
Used cloth during menstrual period	1 (12)	16 (89)	<0.0001
Not washing after intercourse	1 (17)	15 (76)	<0.0001
Washing after intercourse	0 (33)	3 (28)	NA
Used condom during intercourse	0 (10)	15 (77)	NA
Not used condom during intercourse	1 (40)	3 (27)	<0.0001
Not circumcised male partner	0 (0)	18 (104)	NA
Circumcised male partner	1 (50)	0 (0)	NA

T-test was used for  $P < 0.0005$  statistically significant



**Figure 2:** Oncogenic HPV transmission across five districts (Patna, Muzaffarpur, Madhubani, Darbhanga, and Saran) of Bihar, India. As showing figure, large size in red circle, Patna is the most infected area of Bihar, India. HPV: Human papillomavirus



**Figure 3:** Higher trend of oncogenic HPV infection with young age group (17–22 years) and decreasing trends of HR-HPV infection with higher age group (>40 years). HPV: Human papillomavirus, HR-HPV: High-risk human papillomavirus

a bachelor’s degree or above. At the level of religion, HR-HPV infection was found 16% (8/50) in Hindu women, 2% (1/50) in Muslim women, 14.7% (5/34) in Christians, and 25% (5/20) in Banjaran women. About marital status of participants, HPV infection was found to be 6.25% (1/16) in single or unmarried women, 14.06% (18/128) in married women and there was no detectable HPV infection in widowed women in our study. At the parity level, HPV infection was found 6.7% (1/15) in women with single parity, 14.29% in women with two to three parities, and 11.59% in women with more than four parities. HPV infection was found in participants who had not been screened for cervical cancer in our study.

## Discussion

To date, it may be the first community-based study to evaluate the HR-HPV genotype among Bihari women

**Table 2:** Clinical and demographical characteristics in different communities of women corresponding to oncogenic HPV infection

Demographic characteristics of the HR-HPV-infected women		
(n=154)	No. of samples	% age of HPV-16/18
Education level		
Illiterate	58	7 (12.1%)
Literate	45	6 (13.3%)
Up to high school	37	4 (10.8%)
Up to graduation and above	14	1 (7.1%)
Religion		
Hindu	50	8 (16.0%)
Muslim	50	1 (2.0%)
Christian	34	5 (14.7%)
Banjaran	20	5 (25.0%)
Marital status		
Single	16	1 (6.25%)
Married	128	18 (14.06%)
Widowed	10	0 (0%)
Parity		
<01	15	1 (6.7%)
02–03	70	10 (14.29%)
>04	69	8 (11.59%)
Ever screened for cervical cancer		
Yes	0	0 (0%)
No	154	19 (154)

based on the cultural socio-behavioral characteristics of the participants. In the present study and the close HPV distribution of global estimates, the overall prevalence of HR-HPV infection was reported to be 12.34%. In recent meta-analysis studies of asymptomatic women, the prevalence of HPV was found 11% among women who attended uterine cervix cancer screening, while it was 10% among rural and urban women.<sup>[13]</sup> Although, washing genital parts after urination are a matter of cultural variation and restricted to a particular community, it appears to be very lucrative to overcome this problem. The Muslims wash or clean genital parts after each urination, both males and females. Circumcision may also reduce the risk of sexually transmitted diseases, urinary tract infection, and penile cancer including HIV (human immunodeficiency virus).<sup>[14-16]</sup> Cervical cancer is ranked ninth in the frequency of cancer among Saudi females, with a prevalence of 3.6% in Saudi Arabia.<sup>[17]</sup> The Muslim women are less susceptible to HPV infection or cervical cancer.<sup>[18]</sup> The Muslim community is maintaining personal hygiene, such as washing the genital area after each urination. This might be one of the reasons to reduce the prevalence of HPV infection in Muslim women. The male sex partner is supposed to be the agent of the HPV infection. Male circumcision has been given credibility for providing protective shelter to the female partner by getting

less HPV infection and reducing the risk of cervical cancer. A study conducted in Iran reported that the prevalence of HPV was found to be 24% in the general population,<sup>[19]</sup> whereas global HPV prevalence in women varies from 2% to 44% across the world.<sup>[20]</sup> HR-HPV prevalence was found to be 10.3% in other study conducted on healthy Iranian women in Iran in 2018.<sup>[21]</sup> Our study found a significant (12.5%) HPV prevalence when compared to Iranian healthy women (10.3%). This study's finding of HR-HPV infection (2% in Muslim) is consistent with studies of low cervical cancer incidence in the Islamic Republic of Iran and other Muslim nations.<sup>[22]</sup> Low risk for Muslim women has also been detected in countries like India which have a large Muslim population, the country otherwise has been considered one of the high risk regions for cervical cancer.<sup>[23]</sup> Actually, a low incidence has been recognized among the Muslim women in the entire Asian region, though squamous cell cancer of the cervical has been reported as a major problem in this region.<sup>[24]</sup> Latin American and pre-dominantly the Hindu and Christian countries commonly had the highest rates of HPV infection while Middle Eastern, predominantly Buddhist and Muslim countries, tended to have the lowest rates of HPV infection.<sup>[25]</sup>

Our study shown the highest HPV infection in the youngest individuals of the participants. In some other study, it was observed that high prevalence of cervix carcinoma had been reported in younger individual.<sup>[26]</sup> A similar study was carried out in India; they did not observe any variation in the prevalence of HPV infection with age.<sup>[27]</sup>

All non-hygienic practices as not cleaning genital organ after sexual intercourse, not used condom during intercourse were showing statistically significant  $P < 0.0001$  in women [Table 1]. The religious practices of personal hygiene since childhood and male circumcision are quite associated with a religiously specific pattern in the Muslim population. Thus, regular cleaning with water of the genital parts after urination is essential to maintain good personal hygiene in both males and females. The study conducted in Jammu and Kashmir, India, reported that the prevalence of HPV infection and cervical cancer cases is the lowest in Muslim women.<sup>[28,29]</sup> In a national HPV infection mapping study, the prevalence of HPV-16 was detected the highest in Chennai (88%) whereas it was lowest in Jammu and Kashmir (14.2%) in India.<sup>[29-31]</sup> This extremely low prevalence of HPV infection in Jammu and Kashmir may be due to the circumcision of males in this dense Muslim population.<sup>[29]</sup>

The distribution of HPV infection has been found to vary depending on geographical location, and the most consistent variation has also been observed in the prevalence of HPV-16 rather than other HPV strains. An intermediate prevalence of HPV-16 was found in South America whereas a significant heterogeneity was seen across the Asian population. Not only geographical location but also cultural variations influence the

sexual behavior of women and their male partners, resulting in differential HPV acquisition.<sup>[32]</sup>

According to the Indian Cancer Registry Program (NCRP) reports, the rate of cervical cancer among Indian Muslim women is rigorously low.<sup>[14,28]</sup> Male circumcision provides protective shelter to their female sex partners, resulting in less HPV infection and a reduced burden of cervical cancer in women.<sup>[28]</sup>

A study conducted in Denmark reveals that the prevalence of HPV infection is low in circumcised men, consistent with the suggested lower risk of cervical cancer in female partners of circumcised men.<sup>[33,34]</sup> The male sex partner is supposed to be the carrier of HPV infection in women. Some other studies demonstrate that circumcised men have a lower chance having genital warts than uncircumcised men, but when they occur, warts are more often located on the distal portion of the penis in uncircumcised men. This would be described as either non-specific resistance to proximal penile warts conferred by the foreskin or heightened susceptibility to several HPV genotypes in uncircumcised men, some of which may confer subsequent immunity to genital warts.<sup>[33]</sup>

HPV infection in genital parts undoubtedly escalated by poor sexual hygiene. Deficiencies in genital hygiene are also related to certain aspects of sexual behavior, low socioeconomic status, and education. A direct role of poor hygiene conditions on the risk of HPV infection and cervical cancer has been reported.<sup>[35]</sup> Certain studies established that poor hygienic conditions were associated with a higher prevalence of HPV infection among control women but not with an increased invasive cervical cancer risk among HPV-positive women.<sup>[36]</sup>

Basically, in Bihar, India, the age of marriage is higher due to social, socioeconomic, and educational level than compared to girls. Hence, HPV vaccination (prophylaxis) at a specific age in women can reduce the burden of cervix cancer malignancies.<sup>[37]</sup> The US Food and Drug Administration recently approved two HPV vaccines: Quadrivalent Gardasil (HPV-16, HPV-18, HPV-6, and HPV-11) by Merck and bivalent Cervarix (HPV-16 and HPV-18) by Glaxo Smith Kline. The vaccines were found to be highly immunogenic, well tolerated, safe, and effective in preventing incident and persistent HPV infections.<sup>[38,39]</sup>

We used non-invasive urine samples for the detection of HR-HPV, which is the most acceptable and widely adopted non-invasive molecular diagnostic method. It is easily to collect the urine samples, low cost, and acceptable in the Indian societies. Urine samples have high sensitivity and specificity potential for use in cases of cervix carcinoma screening.<sup>[40]</sup> Cervical swab and urine specimens have similar operative characteristics to identifying HPV DNA genotype for the detection of abnormal cytological cell of cervix.<sup>[41]</sup> The rate of overall compliance in the detection of HR-HPV between cervical and urine specimens was 80% and 79%, respectively, in the PCR-based HPV genotypic assay.<sup>[42,43]</sup> According to

Parwez *et al.*, cervical swab is panic for sampling because sterile specula are injected in the vagina and rotating 2–3 times on cervix in uterus.<sup>[44]</sup> Therefore, the approach of a urine sample should facilitate HPV detection in females who do not require a gynecologist and it may be more attractive to women for cervical screening.<sup>[44]</sup>

HPV infection is exacerbated by poor genital hygiene, which leads to the development of cervical cancer or cervical dysplasia. Recently published article demonstrated that probability of genital hygiene rather than multiple sex partners stands more apt a cause of HPV infection.<sup>[44]</sup> Hence, this study among different cross-sectional communities of healthy women on the basis of sociosexual life patterns and hygiene practices is considered remarkable and need of the hour.

## Conclusion

Our study indicates that personal hygiene possibly reduces HPV infection in women and the evidence suggests that male circumcision has a protective role of HPV infection in Muslim community. Poor genital hygiene may be associated with presence of HPV infection in Hindu, Christian, and Banjaran communities. This study also observed that uncircumcised males may cause them to transmit the HPV infection in women. Therefore, personal hygiene and circumcision may reduce the risk of HPV acquisition and transmission, as well as cervical cancer development in women. Further research work is needed to establish the definitive cause behind HPV transmission in women. This study provides a strong foundation for health policymakers to implement prevention strategies for cervical cancer in low-resource setting.

## Authors Declaration Statements

### Ethical approval

This study has taken an approval from “Human Ethical committee” of Mahavir Cancer Sansthan and Research Centre (MCS&RC) Patna, India. Reference Number is MCS/Admin/2018-19/1223 Dated August 23, 2018.

### Consent of participants

Written consent form was obtained from all participants.

### Consent for publication

All authors of this study given their consent for publication.

### Competing interest

None of them declared.

### Funding statements

No funds were obtained for this study.

## Authors' Contributions

This study was conceptualized and designed by Mohammad Ali and Akhtar Parwez. Akhtar Parwez, Roushan Kumari, Rahul Kumar, and Vikas Kumar performed laboratory work. Sunit Singh verified methodology, Mohammad Ali supervised the finding of this work. Roushan Kumari, Vikas Kumar, and Vidyut Prakash did statistical analysis of this study. Akhtar Parwez took the lead in writing manuscript. All authors discussed results and contribute to prepared final manuscript.

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