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Oncogenic HPV Types 16 and 18: Prevalence and Risk Factors in the Sexually Inactive and Sexually Active Women Populations of Bihar, India

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Abstract

Background- Non sexual transmission of HPV is not defined and is the subject of debate. Keeping this in view, the present study has been designed to determine the prevalence of HPV types 16 and 18 and risk factors in the sexually inactive with intact hymen on clinical examination compared to sexually active healthy women population of Bihar using non-invasive urine based self-sampling. Methods- The study was conducted on 3335 healthy women. They were divided into two groups. The first one consisted of 1680 sexually active (married) women and the second one consisted of 1655 sexually inactive women. Urine samples were used for HPV DNA testing using highly sensitive Real-Time PCR technology with TaqMan chemistry which allows higher specificity and sensitivity. Results- The sensitivity and specificity of the urine as compared to cervical scrape were done in a subset of cases and found to be 94.09% (95% CI = 91.18% to 96.26%) and 91.75% (95% CI= 88.61% to 94.25%) respectively, while the PPV was 99.04% (95% CI = 98.67% to 99.30%) and the NPV was 63.29% (53.45% to 72.13%) respectively. Urine samples showed extremely good concordance for HPV DNA detection. Out of 3335 healthy women, 17.30% (577/3335) HPV type 16 was detected in both sexually active and sexually inactive populations, whereas 19.23% (323/1680) in sexually active women and 15.03% (254/1655) in sexually inactive women, however HPV type 18 was not detected in the study population. Inverse correlations between age and HPV prevalence were observed that decreased with increasing age both in sexually active and inactive healthy women. However, sexually inactive showing a correlation, $R2= 0.409$, $p< 0.001$ and sexually active showing a correlation $R2= 0.286$ with a statistical significance $p< 0.001$. In the present investigation, the association of poor genital hygiene and the prevalence of HPV infection were also observed and were found to be statistically significant ($p < 0.0001$). Furthermore, sexually active women having a male uncircumcised partner showed a strong association (Odds ratio 14.325,95% CI,7.0293 to 29.1945) $P< 0.0001$ with HPV infection as compared to other characteristics. Conclusion- Noninvasive urine-based sampling is a reliable test for HPV DNA detection using real time PCR. The prevalence of HPV infection in both sexually active and sexually inactive women suggests that poor genital hygiene practices and women with uncircumcised male partners are more prone to getting HPV infection and contribute as a risk factor in women.

Keywords: HPV, Real Time PCR, Circumcised, Hygiene, Sexually active women, Sexually inactive women.

INTRODUCTION

High risk HPV infection is responsible for developing cervical cancer in women, for which Zur Hausen received the Nobel Prize in 2008. Globally, oncogenic high-risk types 16, & 18 are frequently found in cervical cancers [1,2] and more than 80% of Indian women with cervical cancer carry HR-HPV strains 16 and 18. In India, the prevalence of HPV type16 is more common than HPV type18 and is present in 90% of cervical cancer cases of both squamous cell carcinoma and adenocarcinoma. The incidence of HPV 18 is very low, and was found to be around 3% to 5% [3-11]. Studies suggest that spontaneous clearance of HPV infection itself in 90% of females [12] and persistent infections occur in 5% to 10% of women over 35 years old and are linked to an increased risk of intraepithelial lesions. The likelihood of reactivating latent HPV and resulting in symptomatic or subclinical lesions raises various clinical problems. Furthermore, puberty is a phase during which disease can arise due to the physiological changes that occur at this point, which may predispose individuals to infection and, finally, epithelial lesion.

Among the several biological samples used to detect HPV, urine-based HPV DNA detection has been proven a non-invasive procedure, and an acceptable method that can be implemented for large-scale population screening. Urine based non-invasive method could offer more convenience to unmarried, and

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adolescents in whom insertion of speculum for cervical scrape is so painful. The presence of HPV DNA in urine samples can be elucidated by the fact that exfoliated cells containing HPV DNA or free virions shed continuously from the cervical epithelium, and even from the urethral and vulvar epithelium. These tissues are sensitive to HPV infection, hence, the chances of getting HPV in urine is more than cervical samples [13]. Urine based approach may help in sampling of large cohorts to estimate the prevalence of HPV infection in asymptomatic women.

Human papillomavirus (HPV) infection is transmitted through sexual activity. It is presumed that sexually active women at least once in their lifetime get infected with HPV. Transmission of HPV in women during coital practice is well accepted. [14-16] Generally, harboring of HPV infections is associated with sexual activity. However, HPV infections are also observed in sexually inactive women. [17,18]. The prevalence of HPV infection in sexually inactive women raises a big question about the transmission of the virus. It means genital hygiene is also contributing the risk factor for HPV infection in sexually inactive women.

In this context, the present study has been designed to determine prevalence of HPV types 16 and 18 in women population of Bihar region (India) using a non-invasive urine-based method. To date, there has been no data on the prevalence and risk factors for HPV infection in women population of Bihar, where incidence rate of cervical cancer cases is high. Thus, the aim of the present study was to investigate the prevalence and risk factors for HPV infection in sexually inactive who had an intact hymen on clinical examination compared to sexually active women using a non-invasive urine-based approach.

MATERIALS AND METHODS

Study Population

The study was conducted on 3335 healthy women. They were divided into two groups. The first one (Group 1) consisted of 1680 sexually active (married) women in which 325 with age 19-23, 450 with age 24-28, 410 with age 29-33, 280 with age 34-38 and 215 with age >39 years respectively were kept who reported sexual activity with one partner. The second group (Group 2) consisted of 1655 sexually inactive women in which 512 with age 14-18, 655 with age 19-23, 328 with age 24-28 and 160 with age 29-33 years respectively and reported for nonintercourse sex and who had an intact hymen upon physical examination performed by an experienced gynecologist. In the present investigation to assess the risk factors, study populations were interviewed by the expert person using a questionnaire regarding awareness of genital hygiene during menstrual period, washing genital area after every pee and post coital washing habits. The study was carried out between the periods of August 2022 to December 2023. All Participants were native of Bihar (India).

Sample Size

For this prospective study, we recruited 3335 subjects of healthy controls from different districts of Bihar under the roof of health camp with the help of Primary Health Centre (PHC) workers, door to door visits and students of local colleges. The sample size (N= 3335) has been calculated using the standard formula $N=z^2 [P(1-P)/(D^2)]$ where, N is required sample size, Z is the confidence level, P is the prevalence and D is the margin of error. The prevalence of HPV infection in cervical cancer and healthy controls was taken as 85% and 11%, respectively. The D value taken as 10% and Z value as 95% for all the cases.

Sample collection and processing of biological samples

Thirty to forty ml of self-collected urine was taken in a sterile 50 ml falcon tube containing 10 ml of a 0.5M EDTA preservatives and stored at 4°C for ~2hours before processing. Urine sample was centrifuged at 3,000rpm for 10 min. Samples were kept in freezing box at 4°C and transported to Mahavir Cancer Sansthan and Research Center, Patna, India for isolation of DNA. The resulting pellet was washed twice with sterile 1x phosphate buffer saline (PBS pH 7.4). For the detection of HPV, pellet was suspended in 1ml of 1x PBS (pH 7.4) and stored at -20°C.

DNA Extraction from Urine Samples

Genomic DNA was extracted using MyLab Discovery solutions kit (Pune, Maharashtra, India) which is based on silica membrane technology, and the whole process involves the steps of sample lysis, DNA binding to silica columns, and washing elution.

Quantification of DNA: Used the QuantiFluor® dsDNA protocol for quantification of DNA. The isolated DNA specimens ere quantified using fluorometer. In the present study 50ng to 85ng concentration of DNA was used for PCR reaction.

Amplification of specific E6/E7 region of HPV types 16 and 18 using qRT-PCR

qRT-PCR reactions were performed for DNA extracted from urine samples using MyLab Diagnosis Kit (Pune, Maharashtra, India) based on TaqMan technology which allows higher specificity and sensitivity. Endogenous gene was used as a reference control. Positive and negative control were also used to meet the control conditions of the valid test run. The threshold cycle data were determined using the default threshold settings. Same concentration of DNA was used for the amplification of specific conserved target sequence of E6/E7 region of HPV 16 as well as 18 to maintain consistency and same efficiency. The reaction conditions were 10 min at 95°C, followed by 45 cycles of 95°C for 15s and 60°C for 1 min. All experiments were run in triplicate, along with a negative and a positive control. The fold change of DNA expression for each sample in relation to normal control was calculated based on the threshold cycle (CT) value using the following formula: Relative Quantification (RQ) = $2^{-\Delta\Delta CT}$.

Statistical Analysis

Data were analyzed using Graph pad prism statistics software program version 5 and IBM SPSS statistics software program version 26. The data were exhibited as the mean $\pm$ SD (standard deviation). Mann-Whitney test as well as student's t-test was employed to compare the significant mean difference of E6/E7 gene expression correlation between two groups. Kruskal-Wallis test was done to check the significant mean difference between different sampling groups. The correlation analysis to assess the relationship between HPV infection with age and genital hygiene in women population expression and clinical features was performed by Pearson's correlation coefficient. Logistic regression analysis was performed to estimate the unadjusted odds ratio with 95% confidence interval (CI). Binary logistic regression was used to estimate odds ratios and corresponding 95% confidence intervals for the association between various factors and HPV infection. p value<0.05 was considered statistically significant. The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of HPV detection in urine samples were also calculated using standard statistical formula.

Ethics Approval & Consent to Participate

Informed consent was obtained from all subjects and parents/legal guardian(s) of all the minor participants. Ethics approval and consent to participate obtained by Mahavir Cancer Sansthan Ethics Committee with IEC No. MCS/Admin/2018-19/1223 dated 23/08/2018. The study was carried out in accordance with the guidelines and principles of the Helsinki Declaration.

RESULTS

Prevalence of HPV infection in urine of sexually active and sexually inactive healthy women

HPV detection was done using urine, a non-invasive method and to validate the sensitivity and specificity of the HPV detection, samples were compared with paired cervical scrapes in subset of cases. Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were also calculated. The sensitivity and specificity were found to be 94.09% (95% CI = 91.18% to 96.26%) and 91.75% (95% CI= 88.61% to 94.25%) respectively, while the PPV was 99.04% (95% CI = 98.67% to 99.30%) and NPV was 63.29% (53.45% to 72.13%) respectively. A very high concordance for HPV DNA detection was observed. The analysis of HPV infection and its genotyping was conducted by qRT-PCR using E6/E7 HPV primers designed by Mylab life solutions company (Pune, Maharashtra, India). Patients were considered to be HPV positive at ct value ≤ 30.

A total of 3335 urine samples were used for the analysis of HPV type 16/18 from sexually active (married) and sexually inactive (unmarried, no sexual intercourse) women. Amplification of endogenous control gene confirmed the presence of DNA in urine samples. Overall HPV type 16 prevalence was 17.30% (577/3335) in both sexual and nonsexual population. HPV type 18 was not detected in the study population. Out of which sexually active was 1680 and HPV 16 positive was 323 (19.23%) and sexually inactive was 1655 and number of HPV 16 positive cases was 254 (15.03%) as shown in Fig 1.

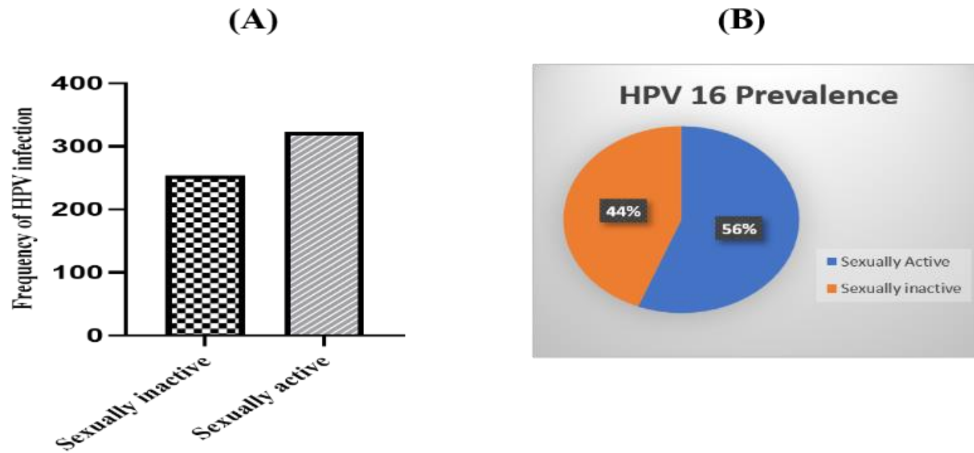

Figure 1: (A & B) Bar diagram and Pie-chart representing the frequency and prevalence of HPV infection in urine of sexually active and sexually inactive healthy women

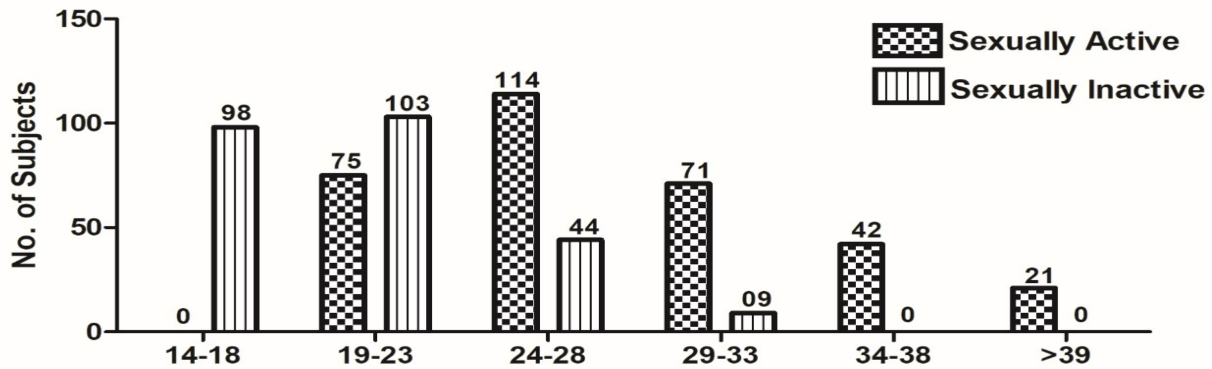

Figure 2: Bar graph represents the age wise distribution of HPV type 16 in sexually active and sexually inactive population of healthy women. $p < 0.05$ (chi-square test), considering the association between two populations

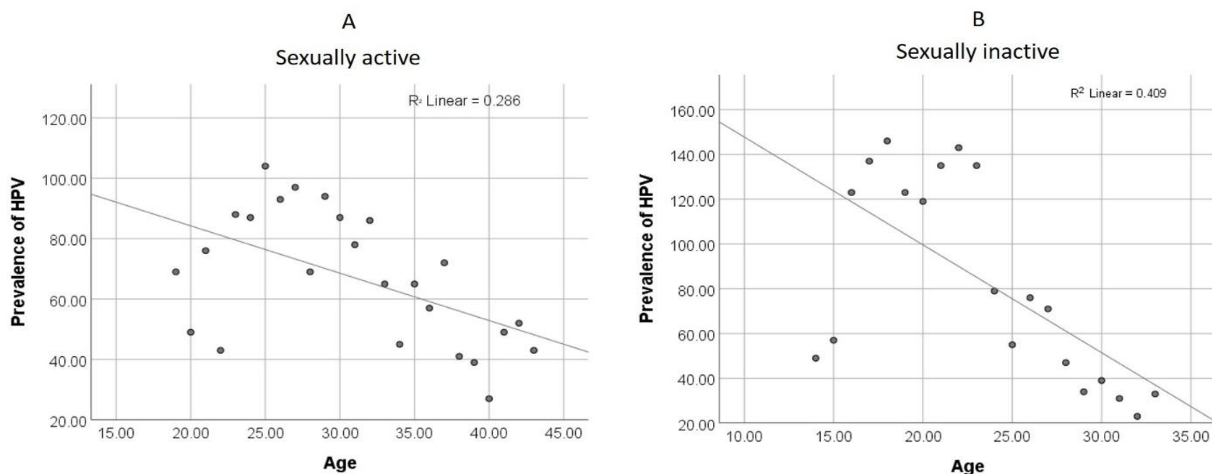

Figure 3: Correlation of prevalence of HPV and age (A) Sexually active (B) Sexually inactive using Pearson's correlation coefficient. Pearson's correlation scatter plots for the correlation between the prevalence of HPV and age in random population ($p < 0.001$)

Table 1: HPV DNA prevalence by Age in sexually active and sexually inactive women

Age (yrs)	Number of Healthy Sexually Active Women	Number of HPV 16 positive	p-value	Number of Healthy Sexually inactive Women	HPV type 16 positive	p-value
14-18	-	-	-	512	98(19.14%)	0.012
19-23	325	75 (23.08%)	0.006	655	103(15.73%)	0.000
24-28	450	114(25.33%)	0.001	328	44(13.41%)	0.004
29-33	410	71 (17.32%)	0.001	160	09(5.63%)	0.757
34-38	280	42 (15%)	0.028	-	-	-
>39	215	21 (9.77%)	0.049	-	-	-

Table 2: HPV DNA prevalence by hygiene in sexually active women

Sexually Active	Number of cases (n=1680)	HPV 16 +ve	HPV 16 -ve	p- value
Maintain hygiene during menstrual period	454	7(2.17%)	447 (32.94%)	<0.0001**
Not Maintain hygiene during menstrual period	1226	316 (97.83%)	910 (67.06%)	
Cleansing the genital after urination by water	185	8 (2.4%)	177 (13.04 %)	<0.0001**
Not Cleansing the genital after urination by water	1495	315 (97.53%)	1180 (86.96%)	
Cleansing the genital after post coital by water	51	3 (0.9 %)	45 (3.37%)	0.021*
Not cleansing the genital after post coital by water	1632	320 (99.07%)	1312 (96.68%)	
Women with male uncircumcised partner	1310	315 (97.52%)	995 (73.32%)	<0.0001**
Women with male circumcised partner	370	8 (2.48%)	362 (26.68%)	

Table 3: HPV DNA prevalence by hygiene in sexually inactive women

Sexually Inactive	Number of cases (n=1655)	HPV 16 +ve	HPV 16 -ve	p- value
Maintain hygiene during menstrual period	364	5 (1.97%)	359 (25.63%)	<0.0001**
Not Maintain hygiene during menstrual period	1291	249 (98.03%)	1042 (74.37%)	
Cleansing the genital after urination by water	98	6 (2.36%)	92 (6.5 %)	0.009**
Not Cleansing the genital after urination by water	1557	248 (97.63%)	1309 (93.43%)	

Correlation of HPV prevalence with age group of sexually active and sexually inactive healthy women

To examine the prevalence of HPV infection in age group of sexually active (married) women, 1680 participants (Group I) were stratified into five age groups in which 325 with age group 19-23, 450 with age 24-28, 410 with age 29-33, 280 with age 34-38 and 215 with age >39. The overall prevalence of HPV infection was 19.23% (323/1680) in sexually active women and prevalence in different age groups was 23.08% (75/325), 25.33% (114/450), 17.32% (71/410), 15% (42/280), 9.77% (21/215) for 19-23, 24-28, 29-33, 34-38 and >39years, respectively. Age wise stratification was also done in the other study group of sexually inactive women who reported for non-sexual intercourse. 1655 participants (Group II) were stratified into four age groups in which 512 with age group 14-18, 655 with age 19-23, 328 with age 24-28, 160 with age 29-33. The overall prevalence of HPV infection was 15.03% (254/1655) in sexually inactive women and prevalence in different age groups was 19.14% (98/512), 15.73% (103/655), 13.41% (44/328), 5.63% (09/160) for 14-18, 19-23, 24-28, and 29-33 years, respectively. The association between age and HPV prevalence was found to be statistically significant ($p < 0.0001$) in both sexually active and sexually inactive population. (Table 1,S1 & Fig 2). Pearson's correlation scatter plots were done for observing the correlation between the prevalence of HPV infection and age in random population, which was found to be statistically significant. ($p< 0.001$). (Fig 3).

To determine whether there is a similar trend of HPV prevalence in urine of sexually active and sexually inactive women in correlation with age group, it was calculated using Pearson's correlation coefficient. Interestingly the HPV prevalence shows inverse correlation, decreased with increasing age both in sexually active and sexually inactive healthy women. However, sexually inactive showing a correlation, $R^2= 0.409$, $p< 0.001$ and sexually active showing correlation $R^2= 0.286$ with a statistical significance $p< 0.001$, which shows moderate correlation.

Geographical distribution of HPV type 16 in healthy women

Map of Bihar showing the district wise distribution of HPV type 16 in healthy women population. Oncogenic high-risk type 16 prevalent in healthy women population of Bihar indicates the high risk of cervical cancer cases in future (Fig 4).

Prevalence of HPV 16 and Genital Hygiene

In the present investigation, study population were interviewed by the expert person using a questionnaire regarding awareness of genital hygiene during menstrual period, washing genital area after every pee and post coital washing habits. To validate the sensitivity and specificity of the genital hygiene in sexually active, and sexually inactive women, we have compared genital hygiene during menstrual period in sexually active women, and sexually inactive women. Out of 1680 sexually active women, only 20 % (336) women were reported for maintaining their menstrual hygiene, 29% (487) women using water for

washing their genital area after every pee, and there is least awareness regarding post coital hygiene (Table 2). On the other hand, out of 1655 sexually inactive population only 18% (298) were reported for maintaining menstrual hygiene, and 17% (281) women using water for washing their genital area after every pee (Table 3). It was observed in the study that HPV infection was detected in those women who reported for poor genital hygiene. The association between HPV infection with poor genital hygiene and study populations was found to be statistically significant (p < 0.0001). (Table 2).

Among four categories (Hygiene during menstrual period, Cleansing the genital after urination by water, Cleansing the genital post coital by water and Women with male uncircumcised partner) of sexually active women, the women with male uncircumcised partner showed strong association (Odds ratio 14.325,95% CI,7.0293 to 29.1945) P< 0.0001 with HPV infection as compared to other characteristics (Table 4).

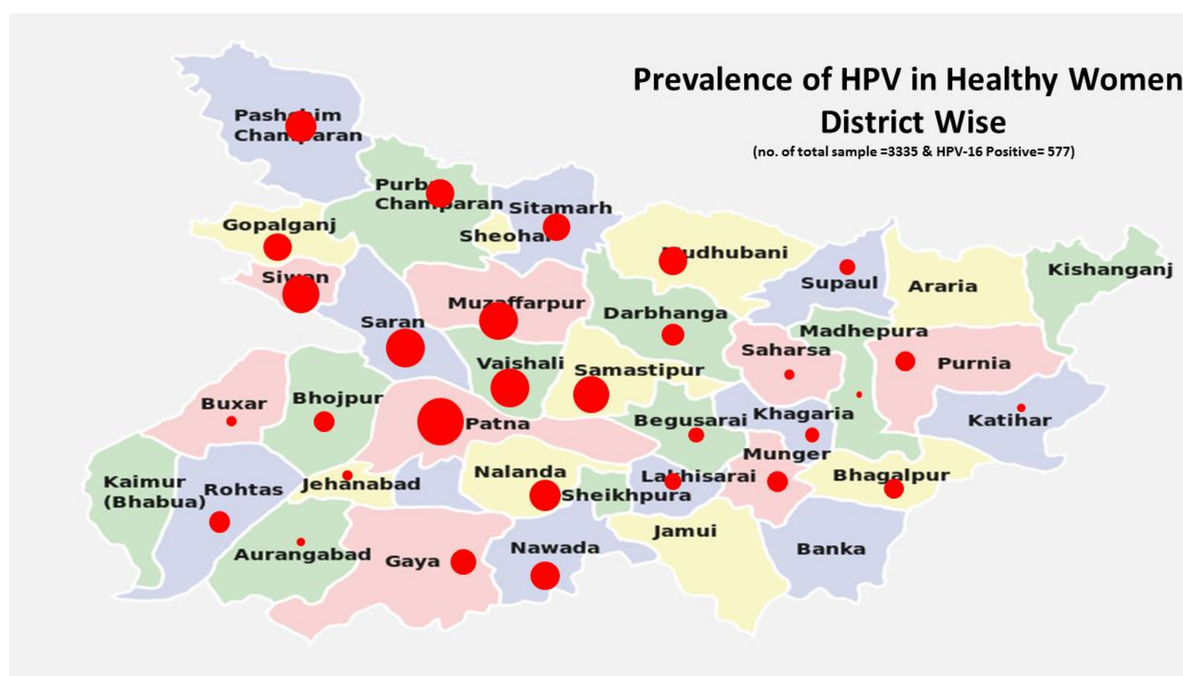

Figure 4: Map of Bihar showing the prevalence of HPV type 16 in healthy women population using urine sampling

Table 4: HPV prevalence and Odds Ratio for HPV prevalence among sexually active and inactive women using multivariate analysis

	Sexually active (n= 1680)				Sexually inactive (n=1655)			
	Total number of cases	HPV positivity	Odd Ratio (95% CI)	p-value	Total number of cases	HPV positivity	Odd Ratio (95% CI)	p-value
Characteristics								
Hygiene during menstrual period	-	-	-	-	-	-	-	-
Yes	454	7	1.4 (0.211- 1.962)	<0.0001	364	5	0.58 (0.239-1.424)	< 0.0001
No	1226	316			1291	249		
Cleansing the genital after urination by water		-	-	-	-	-	-	-
Yes	185	8	1.69 (0.825 -2.3476)	<0.0001	98	6	0.34 (0.1491 - 0.7949)	0.012
No	1495	315			1557	248		
Cleansing the genital after post coital by water		-	-	-	-	-	-	-
Yes	51	3	1.27 (0.844 -1.8852)	0.0305	NA	NA	NA	NA
No	1632	320			NA	NA	NA	NA
Women with male uncircumcised partner		-	-	-	-	-	-	-
Yes	1310	315	14.32 (7.0293 - 29.1945)	<0.0001	NA	NA	NA	NA
No	370	8						

DISCUSSION

HPV infection has been prevalent in young women after a few years of sexual exposure [19-23]. It has been observed that most of the sexually active women once during their lifetime get infected with HPV [24]. Question for debate is: how virus enters in genital area of women despite of having single partner for both men and women throughout their lives? However, multiple partners are a prerequisite for spreading sexually transmitted diseases, but the onset of HPV infection is prevalent in women (having a single partner) after a few years of their sexual debut. Moreover, prevalence of HPV is also reported in sexually inactive women but the chances of nonsexual transmission of HPV are not well understood. The present study has been designed keeping in view the mode of transmission of HPV in women population. Therefore, the objective of the present study was to investigate the prevalence, and risk factors for HPV infection in healthy sexually active and sexually inactive women using non-invasive urine-based method in women population of Bihar (India). In India, every year, around 1,22,844 new cases of cervical cancer are reported, and Bihar itself reports more than 10,000 cases of cervical cancer. Despite the causative role of HPV in developing cervical cancer and the large number of cervical cancer cases in Bihar, to date, no work has been done on the prevalence of HPV in women population of Bihar.

In the present study, HPV types 16 and 18 testing were done through real time PCR technology using non-invasive urine-based sampling. Real-time PCR method is highly sensitive to detect HPV DNA in urine samples that allows mass screening of patients for HPV infection. Amplification detection assays, such as Real-Time PCR-based methods, are more sensitive than the Digene Hybrid Capture II (HC II) assay, as several viral molecular markers, such as cytomegalovirus DNA, hepatitis B DNA, and more recently corona virus are detected by Real-Time PCR. Several studies have been done on urine sampling for HPV detection, which is in support of my work. Scientists reported a fair level of agreement with urine samples and highly sensitive vaginal samples [25]. Researchers also reported more sensitivity in urine samples by using Roche Cobas HPV 4800 assay [26]. Studies have deciphered the use of urine sampling as HPV testing for large scale screening and it is also a non-invasive, and useful alternative [27-29]. Scientists have also confirmed the utility of urine samples for detection of HPV DNA, and observed similar features of cervical cells in urine as cervical samples collected for the investigation of abnormal cervical cytology [30]. Thus, the aforementioned studies support a fair level of agreement for the use of urine as a non-invasive method which can be reliably implemented for HPV DNA testing in cervical cancer screening programs.

In the present investigation, HPV types 16 and 18 were tested on healthy sexually active and sexually inactive women using a non-invasive urine sampling. The reason for considering only types 16 and 18 in the present study is that more than 90% of cervical cancer cases are linked with type 16 and around 3-5% cases with type 18 [31-33] in India. In our study, out of 3335 healthy women, 17.30% (577/3335) of HPV type 16 was detected in both sexual, and nonsexual populations, whereas HPV infection was detected 19.23% (323/1680) in sexually active women, and 15.03% (254/1655) in sexually inactive women. Prevalence of HPV infection in different age groups of sexually active women was found to be 23.08% (75/325), 25.33% (114/450), 17.32% (71/410), 15% (42/280), 9.77% (21/215) for 19-23, 24-28, 29-33, 34-38 and >39 years, respectively. On the other hand, prevalence of HPV infection in different age groups of sexually inactive women was found to be 19.14% (98/512), 15.73% (103/655), 13.41% (44/328), 5.63% (09/160) for 14-18, 19-23, 24-28, and 29-33 years, respectively.

The prevalence of HPV infection is well established in sexually active women [34-36] which was also observed in the present investigation. Simultaneously, the study of present results showed that HPV infection is also prevalent in sexually inactive women who have never been involved in sexual activities. In agreement with my work, HPV infection in sexually inactive women was also detected by other workers, and the reasons explained by them were autoinoculation [37], fomites [38] and other non-sexual routes [39]. The possibility of non-sexual transmission of HPV infection, and sexual transmission in single partner couple is the subject of debate and genital hygiene could be contributing as a risk-factors for HPV infection in both sexually active women having single partner, and sexually inactive women.

It was also observed in the present investigation that rate of HPV infection was high in age group <25 years in both sexually active, and sexually inactive populations while rate of infection declined in older age group. Other workers also reported a very high rate of HPV infection in sexually active women younger than 25 years of age [40-42] which supports the present study. In the present study, we observed a high rate of HPV infection in both groups of women who were menstruating at their peak. Chances of infection are very high during menstruation as the cervix opens slightly to pass blood from the uterus, which is a favorable period for travelling bacteria and viruses into the upper cervix and uterine cavity. Another thing is that on a non-period day, the pH of the vagina remains acidic, which prevents the passage of pathogens but menstruation makes the vagina less acidic and more alkaline. In this way, pathogens enter and thrive in reproductive tract. Thus, almost every bacterium, and virus poses a great threat to women when they are menstruating. In fact, period blood is like a perfect petridish which provides nutrient for sexually transmitted infections.

In the present study, out of 1680 sexually active women, only 20% (336) were reported for maintaining menstrual hygiene, 29% (487) women used water for washing their genital area after every pee, and there was very little awareness regarding post coital hygiene, only 3% (51) of the sexually active population reported for post coital hygiene. However, in the sexually inactive population only 18% (298) out of 1655 were reported to be maintaining menstrual hygiene and 17% (281) of women used water for washing their genital area after every pee. Thus, awareness of genital hygiene observed in the study population was very poor and positivity of HPV infection was also observed in both groups who reported poor genital hygiene. A report from WHO suggests that genital hygiene is an important risk factor for contributing to cervical neoplasia [43]. It has been considered that poor hygiene practices among women might increase chances of HPV infection [44], which is a fair level of agreement with this present work. Scientists reported in their study that women who did not maintain menstrual hygiene showed a higher chance of developing cervical cancer [45]. Menstrual hygiene has been shown in studies to be important and to be a risk factor for developing CIN III and malignancy [46]. A study showed homemade napkins and poor hygiene during the menstrual period as risk factor in the development of cervical cancer with HPV infection [47]. A study conducted in Kerala; India confirmed the role of genital hygiene against HPV infection in the development of cervical neoplasia[47]

Thus, poor genital hygiene may greatly contribute to the entry and persistence of HPV infection in sexually active and sexually inactive women. Unlike other sexually transmitted infections, HPV can be transmitted through nonpenetrative sex, autoinoculation, and via fomites as well. Therefore, always maintaining good genital hygiene should be an essential practice for both sexually active and sexually inactive women. However, in the case of sexually active women both partners (male & female) need to maintain the genital hygiene as studies suggest that cervical cancer is prevalent in partners of uncircumcised males [48]. In an uncircumcised male, the foreskin of penis remains moist by the buildup of a whitish yellow substance known as smegma. If the smegma is not cleaned frequently by pulling the foreskin back, it provides a favorable environment for pathogens to thrive. Phimosis and balanitis are two diseases that can develop from an uncleansed smegma in an uncircumcised male.

In uncircumcised men, the moist sub preputial cavity plays an important role in the acquisition of STI, and during intercourse, HPV from infected sexual partners can be trapped by the inner mucosal surface of the foreskin.[49]. In India, state-wise study done for national HPV mapping which demonstrated that prevalence of HPV type 16 is highest in Chennai (88%), a Hindu religion dominating state where circumcision is not practiced, and lowest in Jammu and Kashmir (14.2%), a Muslim religion dominated state where circumcision is being practiced [50]. Furthermore, Muslim-dominated courtiers reported a low prevalence of HPV infection and cervical cancer burden [51-54].

In the present study, the women with a male uncircumcised partner showed a strong association (Odds ratio 14.325, 95% CI, 7.0293 to 29.1945) $p < 0.0001$ with HPV infection as compared to other characteristics, which is in favor of the aforementioned work.

CONCLUSION

Noninvasive urine-based sampling is a reliable test for HPV DNA detection using real time PCR and should be used for large scale screening purposes. In the present study, prevalence of HPV infection in healthy sexually active and sexually inactive women in the population of Bihar suggest carrying out massive screening of HPV infection in order to reduce the high burden of cervical cancer. The present study suggests that apart from multiple sexual partners, genital hygiene contributing as a major risk factor for HPV infection in both male and female. Since HPV DNA is present in the urine of infected persons and at the time of urination HPV DNA virus may attached to the object like toilet and other places where urine has been voided. If any person uses such infectious places for urination, virus may infect the healthy persons. Furthermore, women with uncircumcised male partners are more prone to get HPV infection. Uncircumcised men are more vulnerable to getting such infections as their lubricated foreskin of penis are susceptible to trapping any pathogens. Therefore, genital hygiene practice is very essential for uncircumcised men so that their sexual partner may remain free from any infection during coital practice. Similarly, females are more vulnerable to getting infected during their periods. Hence, it is highly needed to maintain genital hygiene for sexually active, and sexually inactive women.

Competing Interests

The authors declare that they have no competing interests.

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