High prevalence of oncogenic HPV-16 in cervical smears of asymptomatic women of eastern Uttar Pradesh, India: A population-based study

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In developing countries like India, occurrence of *Human papillomavirus* (HPV) in cervical cancer as well as in the asymptomatic population was observed to be very high. Studies on HPV prevalence have been conducted in different parts of the country but no data were available from the eastern region of Uttar Pradesh (UP). The present study aimed to determine the status of HPV prevalence and its association with different socio-demographic factors in this population. Prevalence of HPV was investigated in a total of 2424 cervical scrape samples of asymptomatic women. Primer sets from L1 consensus region of viral genome were used to detect the presence of HPV, and the positive samples were genotyped by sequencing. Univariate binary logistic regression analysis was used to evaluate association of socio-demographic factors with HPV. 9.9% of the clinically asymptomatic women were found to be infected with HPV comprising 26 different genotypes. Among HPV-positive women, 80.8% showed single infection, while 15.4% harboured multiple infections. HPV-16 (63.7%) was the most prevalent, followed by HPV-31 (6.7%), HPV-6 (5.4%), HPV-81 (4.6%) and HPV-33 (4.2%). Significant association of HPV with non-vegetarian diet ($P<0.05$) and rural residential areas ($P<0.01$) were observed. High prevalence of HPV-16 in asymptomatic women of this population, a frequency comparable to invasive cervical cancers, highlights an urgent need for a therapeutic HPV vaccine covering HPV-16 and other high-risk types to provide protection against the disease.

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1. Introduction

Cervical cancer, the second most common gynaecological malignancy worldwide, has been reported to occur in abundance in different populations. According to World Health Organization (WHO 2010), in India approximately 1,34,420 women are diagnosed with the disease every year, and of them 72,825 die. The major causative factor of the disease is understood to be *Human papillomavirus* (HPV), a double-stranded DNA virus. More than 100 HPV types are known to occur that are categorized into three broad categories depending upon their oncogenic potential: high-risk types including HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -73 and -82; intermediate types including HPV-26, -53, -66 and low-risk types including HPV-6, -11, -40, -42, -43, -44, -54, -61, -70, -72, -81 and -CP6108 (Munoz *et al.* 2003). Infection with high-risk HPV types is the critical aetiological factor in the development of cervical cancer. Certain cervical intraepithelial neoplasias (CINs) with persistent HPV infection progress to invasive cervical cancer although a fraction of them regress during the course of time – 60% in case of CIN-1, 40% of CIN-2 and 33% of CIN-3 (Ostor 1993). Approximately 40% of all CINs persist and only about 1% of CIN-1, 5% of CIN-2

Keywords. Asymptomatic; cervix cancer; HPV; HPV genotypes; socio-demographic factors

Abbreviations used: CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, *Human papillomavirus*; OR, odds ratio

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and 15% of CIN-3 advanced to invasive cancers (Ostor 1993). Since only a certain fraction of HPV-infected CINs progress to invasive cancer after a long latent period and the incidence of tumours are less frequent than the HPV infection, additional events also play crucial role in making HPV infection persistent, leading to oncogenicity. These cofactors may be genetic, immunological as well as socio-demographic, e.g., lower age of conception, high parity, use of oral contraceptives, diet, smoking, etc. It was also evidenced that women co-infected with multiple HPV-type infections comprising of one or more high-risk types were prone to persistent HPV infection and hence advancement of the disease. Although biological significance of multiple HPV-type infection is not known, it seems that they act synergistically accelerating the process of disease progression (Trottier *et al.* 2006).

HPV-infected asymptomatic women remain at risk of developing the disease, and hence its screening is indispensable. Many studies have been performed in different populations of India to determine the prevalence of HPV and their types but no study has yet been reported on the eastern UP population. Therefore, this study was designed to determine the prevalence of HPV and its genotypes in asymptomatic women of Varanasi and adjoining areas and also to study the different risk factors involved in the persistence of the virus and progression of the disease.

2. Materials and methods

2.1 *Study group*

After obtaining institutional ethical clearance, cervical scrape samples were collected from women visiting the Post-Partum Out Patient Department (PP-OPD) of Sir Sunderlal Hospital, Varanasi between October 2005 and December 2010 for family planning, medical termination of pregnancy or for routine gynaecological examination. Several healthcare camps were also organized in the nearby villages to collect the samples from women living in rural areas. All the women were physically examined and cervical scrape samples were collected and stored for HPV testing. The women selected were asymptomatic with no previous history of HPV infection or any cervical neoplasia and were clinically normal. Unmarried women, women in their second or third trimester of pregnancy or with past history of hysterectomy were not included in the study. Since HPV infection is a sexually transmitted disease, there were less chances of infection in unmarried women and, in the Indian scenario, it is not possible to get the correct information regarding premarital sexuality. Consent was taken from each of the participants included in the study. A questionnaire form consisting of questions related to different socio-demographic factors including age of women, marital status, parity, age of conception, diet, socio-economic status, place of residence, history of any disease or HPV infection, pre and post-menopause status and pregnancy were filled and recorded for each participant.

2.2 *Sample collection and DNA extraction*

After pelvic examination, exfoliated cells from the ectocervix region were collected from each woman using a wooden Ayers' spatula. For sample collection, Ayers' spatula was inserted in the cervix using its long arm and rotated in clockwise direction. A total of 2480 women aged 17 to 80 years were examined and samples were collected in 5 mL

Table 1. Oligonucleotide sequences used as primers for detection of HPV and its different types

S. No.	Primer sequence (5′-3′)	Ann Temp	Region	Product size (bp)
MY09/11	FP- GCM CAG GGW CAT AAY AAT GG RP- CAA CTT CAT CCA CGT TAC ACC	50°C	L1	450
GP 5+/6+	FP- AAT GCC TGT GTT CAT TGC TG RP- TTC AAG GTC AGC CCC TAC AC	38°C	L1	150
HPV 16	FP- AAG GCC AAC TAA ATG TCA C RP- CTG CTT TTA TAC TAA CCG G	50°C	LCR	217
HPV 18	FP- TGA GGT ACC ATT CGA TAT TT RP- TAG CAA AAA GCT GCT TCA CGC	51°C	L1	118
HPV 31	FP- TAA GCT CGG CAT TGG AAA TAC CCT RP- CCT TCC TCC TAT GTT GTG GAA TCG	55°C	E6	350
HPV 33	FP- AAC GCC ATG AGA GGA CAC AAG RP- ACA CAT AAA CGA ACT GTG GTG	58°C	E7	211
HPV 35	FP- CCCGAGGCAACTGACCTATA RP- GGGGCACACTATTCCAAATG	57°C	E7	230
β-globin	FP- GAA GAG CCA AGG ACA GGT AC RP- CAA CTT CAT CCA CGT TAC ACC	55°C		268

pre-chilled PBS, immediately kept on ice, transported to the lab and stored at −70°C until further use. DNA was extracted from each cervical scrape sample according to the protocol previously described by Gravitt *et al.* (1998). Briefly, samples were vortexed properly to dislodge the cells from the spatula, centrifuged at 3000 rpm, and the pellet was resuspended in 600 μL of lysis buffer (0.3% SDS, 1xTE) and incubated with 80 μg of proteinase K at 55°C for 16 h. Then extractions were performed using phenol:chloroform:isoamyl alcohol and chloroform:isoamyl alcohol, and the DNA was precipitated with 1/10 volume of 3 M sodium acetate and 0.7 volume of isopropanol, dried and dissolved in TE (Tris-EDTA, pH 8.0) buffer. Samples were electrophoresed on 1% agarose gel to check the quality of the DNA. Quantification of the samples was carried out using Nanodrop spectrophotometer (Nanodrop, ND-1000).

Table 2. Frequency of high- and low-risk HPV types detected in 240 HPV-infected women

HPV type	No. of women with single HPV infection	No. of women with multiple HPV infections	Total no. of HPV-infected women (%)
High-risk type	**166 (69.2%)**		
16	133	20	153 (63.7)
18	6	3	9 (3.7)
31	4	12	16 (6.7)
33	6	4	10 (4.2)
35	2	5	7 (2.9)
39	1	0	1 (0.4)
45	2	2	4 (1.7)
51	1	3	4 (1.7)
56	3	1	4 (1.7)
58	3	1	4 (1.7)
59	2	2	4 (1.7)
67	1	2	3 (1.2)
68	1	0	1 (0.4)
72	1	1	2 (0.8)
73	0	2	2 (0.8)
Low-risk type	**26 (10.8%)**		
6	7	6	13 (5.4)
11	3	2	5 (2.1)
41	0	1	1 (0.4)
42	3	3	6 (2.5)
54	1	0	1 (0.4)
61	1	0	1 (0.4)
70	1	0	1 (0.4)
81	7	4	11 (4.6)
86	1	2	3 (1.2)
90	2	1	3 (1.2)
Intermediate-risk type			
66	2	2	4 (1.7)
Multiple infections	**37 (15.4%)**		
Not genotyped	10 (4.2%)		
Total positive			**240 (9.9)**

2.3 *HPV detection by PCR*

PCR was first performed for human β-globin gene, which serves as an internal control to check the integrity and adequacy of DNA. The primer set used for the amplification of β-globin gene was PC04 and GH20 (Vossler *et al.* 1995), which generate a 268 bp amplicon. Samples negative for β-globin gene were excluded from further analysis. PCR for HPV detection was carried out using MY09/11 (Baay *et al.* 1996) and GP5+/6+ (Evans *et al.* 2005) primers of L1 consensus region. 25 μL of reaction containing 50 ng DNA samples, 10 pmol of each forward and reverse primers, 4 μL of dNTP mix (containing 200 μM each of dATP, dTTP, dCTP and dGTP), and 0.3 U of Taq polymerase (Bangalore Genie) was set up in 1X PCR buffer (10 mM Tris Cl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) provided along with the enzyme. Positive and negative controls were kept in each reaction along with the samples. Positive controls used were known HPV-positive DNA, and the reaction without DNA serves as negative control. DNA was amplified using thermocycler (Applied Biosystems) and the PCR products were checked on 2% agarose gel having ethidium bromide under UV transilluminator. A 450 bp amplicon was detected

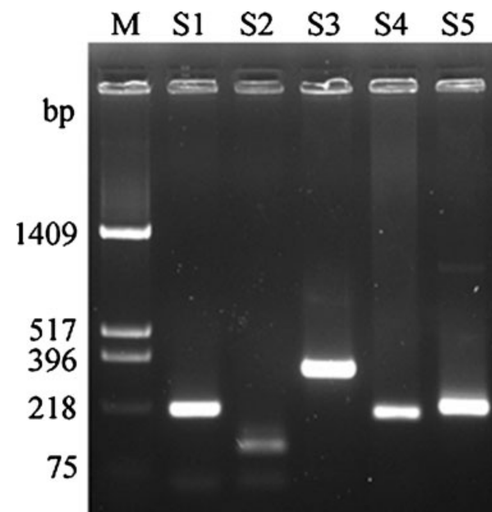


Figure 1. Agarose gel showing amplification of different HPV genotypes in cervical scrape samples using type-specific primers. M: pUC12-HinfI marker, S1: HPV-16 (217 bp), S2: HPV-18 (118 bp), S3: HPV-31 (350 bp), S4: HPV-33 (211 bp), S5: HPV-35 (230 bp).

with MY09/MY11 and 150 bp with GP5+/6+ primer sets, respectively. Primer sequences used are given in table 1.

2.4 HPV genotyping

HPV-positive PCR amplicons were excised from agarose gel and purified using Gel extraction kit (Fermentas, USA) according to the manufacturer's protocol. The purified products were subjected to automated DNA sequencer (Applied Biosystems 3130 four capillary Genetic Analyser; ABI, USA) using Big Dye terminator v1.1 cycle sequencing kit (ABI) according to the manufacturer's protocol. GP6+ primer was used for sequencing as it detects a 34 to 50 bp hyper-variable region upstream to GP5+ primer site. It can be used as a signature sequence for most of the HPV types except for some variants (Lee *et al.* 2009). Sequence alignment was done by ClustalW and NCBI BLAST algorithm. Samples showing more than 95% identities with the GenBank database were considered as matched genotypes. Samples showing multiple genotypes were differentiated by the presence of overlapping peaks. Mixed infections were verified using type-specific primers of HPV-16, -18, -31 (Vinayagamoorthy *et al.* 2003), -33 and -35 (Karlsen *et al.* 1996). Clones for HPV-31, -33 and -35 were used as positive controls during the PCR, while known HPV-positive DNA samples were used as positive control for HPV-16 and -18.

2.5 Statistical analysis

Data were statistically analysed using SPSS statistical software (version 16.0). Univariate binary logistic regression analysis was performed to assess the strength of association of HPV and different demographic factors. Odds ratio at 95% confidence interval was calculated. The tests were considered significant if *P*-value is ≤0.05. Stepwise backward logistic regression analysis was performed to evaluate the most significantly associated factors.

3. Results

A total of 2480 women participants were screened for HPV infection; however, samples of 56 women were excluded due to negative results for β-globin. From each woman complete questionnaire form along with the consent was collected. All samples were first analysed for HPV positivity by PCR using L1 consensus primer sets, and then the amplified regions were sequenced to detect the genotypes. Samples with multiple infections were further confirmed by PCR using type-specific primers. Table 2 gives a summarized picture of different HPV types detected in the analysed population and a representative picture of PCR-amplified products is shown in figure 1. In total, 240 (9.9%) women were found to acquire HPV infection although genotyping for 10 samples was not done due to inadequate DNA amount. Twenty-six different HPV genotypes were detected including 15 high-risk, 10 low-risk and 1 intermediate-risk types. 166 (69.2%) women were infected with single high-risk HPV-type, while single low-risk and intermediate-risk types were 26 (10.8%) and 2 (0.8%), respectively. Multiple infections comprising two or three different HPV types were observed in 37 (15.4%) women. Infection with two different types of HPV was more common than with three or more types of infection (table 3).

HPV-16 was observed to be the most prevalent high-risk types (63.7%) comprising both single and multiple infections followed by HPV-31 (6.7%), HPV-33 (4.2%) and HPV-18 (3.7%) in the high-risk group. In the low-risk group HPV-6 (5.4%) and HPV-81 (4.6%) were the most frequent types, followed by HPV-42 (2.5%) and HPV-11 (2.1%).

Table 4 gives a closer look of the data showing presence of HPV in relation to different socio-demographic parameters. The different factors taken into consideration were age

Table 3. Distribution of multiple HPV genotypes present in HPV-infected women

Multiple HPV types	No. of infected women	% of infected women
HPV 16/18	1	0.4
HPV 16/31	12	5.0
HPV 16/33	1	0.4
HPV 16/42	1	0.4
HPV 16/90	1	0.4
HPV 16/11	1	0.4
HPV 33/35	2	0.8
HPV 51/35	1	0.4
HPV 59/45	1	0.4
HPV 59/73	1	0.4
HPV 18/56	1	0.4
HPV 35/51	1	0.4
HPV 58/86	1	0.4
HPV 42/73	1	0.4
HPV 81/66	1	0.4
HPV 6/66	1	0.4
HPV 6/67	1	0.4
HPV 6/81	2	0.8
HPV 16/18/72	1	0.4
HPV 16/45/86	1	0.4
HPV 16/6/67	1	0.4
HPV 35/6/42	1	0.4
HPV 33/35/11	1	0.4
HPV 81/51/41	1	0.4

Table 4. Association of selected socio-demographic parameters with HPV-positive and HPV-negative women using univariate binary logistic regression analysis

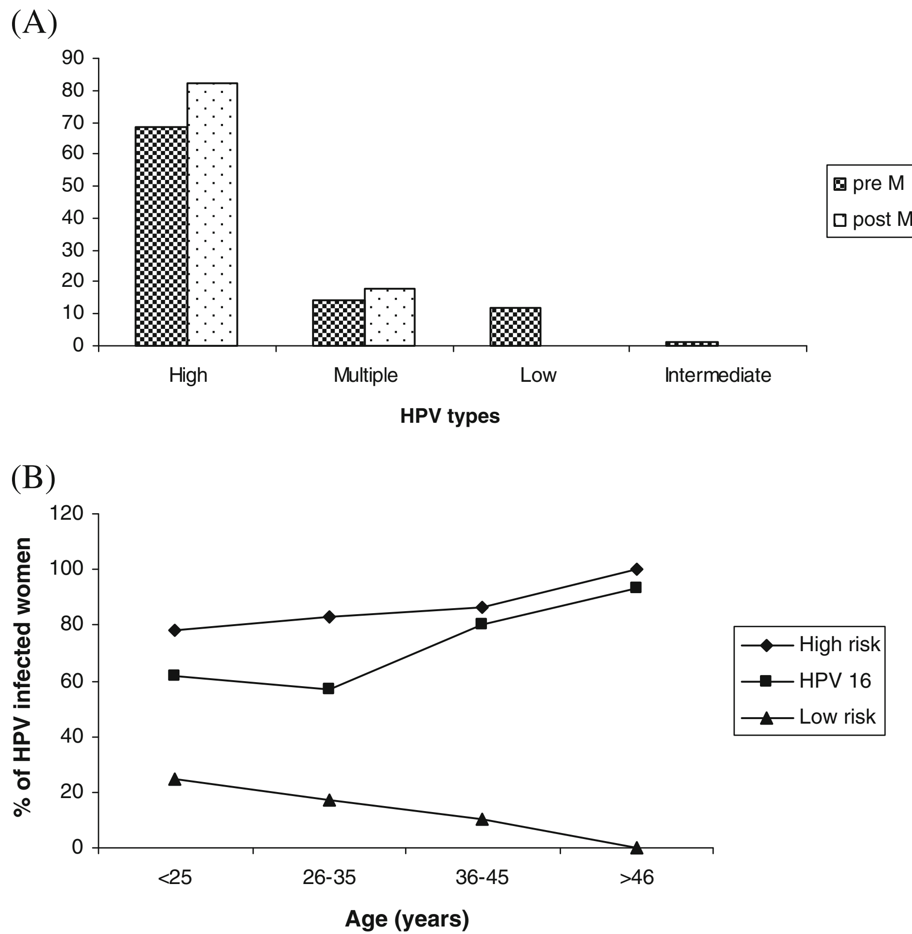
Socio-demographic factors	No. of cases[a]	HPV-positive	% infection	Odds ratio (CI 95%)	*P*-value
Age (years)					
≤25	742	68	9.2	0.68 (0.38–1.22)	0.19
26–35	1298	126	9.7	0.72 (0.41–1.25)	0.24
36–45	249	30	12.0	0.88 (0.46–1.68)	0.70
≥46	109	15	13.7	1.0 (reference)	
Parity					
0–1	435	45	10.3	1.0 (reference)	
2	794	60	7.5	0.67 (0.45–0.99)	0.05
3	580	61	10.5	0.95 (0.64 –1.41)	0.79
≥4	598	73	12.2	1.09 (0.74 –1.61)	0.66
Age at first intercourse (years)					
≤ 20	1152	127	11.0	1.12 (0.86–1.46)	0.38
>20	1115	112	10.0	1.0 (reference)	
Place of residence					
Rural	1086	129	11.8	1.42 (1.08–1.85)	0.01
Urban	1307	108	8.3	1.0 (reference)	
Diet					
Non-veg	1229	137	11.1	1.31 (0.99–1.72)	0.05
Veg	1158	102	8.8	1.0 (reference)	
Income group					
Low	1049	117	11.1	1.22 (0.93–1.59)	0.15
Middle	1342	109	8.1	1.0 (reference)	
Pregnancy					
Yes	385	46	11.9	1.22 (0.86–1.72)	0.26
No	2024	193	9.5	1.0 (reference)	
Menopause					
Pre	2280	222	9.7	0.75 (0.45–1.25)	0.27
Post	136	17	12.5	1.0 (reference)	

[a] Due to some missing data, numbers may not add up to total.

of the women, diet, place of residence, income groups, parity, age at first intercourse, pre- and post-menopause status and pregnancy (Ist trimester). Univariate binary as well as backward logistic regression analysis results showed diet and place of residence as the significantly associated factors with HPV infection. Women taking non-vegetarian diet showed higher association ($P \leq 0.05$) with HPV than women taking vegetarian diet. Further, women belonging to rural population showed significant association with HPV infection ($P \leq 0.01$) in comparison to women living in urban areas.

A non-significant positive association of HPV with parity was observed, indicating an increase in risk of HPV infection with the increase in number of births, while women having two pregnancies with the lowest OR value (0.67) were found to show the least association with HPV ($P \leq 0.05$). An increase in association of HPV infection was also observed with the increase in age of the women. Although not statistically significant, pregnant women (11.9%) and women belonging to lower income group (11.1%) showed high percentage of HPV infection, consequently making them a highly susceptible group. Interestingly, post-menopausal women showed greater risk of infection (12.5%) in comparison with pre-menopausal women (9.7%). Although women in their pre-menopausal status were inversely correlated to HPV infection (OR= 0.75; 95% CI 0.45–1.25), showing they were at low-risk level yet they were more susceptible to low and intermediate HPV-type infection in comparison to post-menopausal women (figure 2A).

The frequency of HPV-16 also shows an increasing trend with age, with lower infection in women less than 25 years of age and increases with age, hence showing positive correlation (figure 2B).



Age (years)	≤ 25	26–35	36–45	≥ 46
Total HPV +ve	68	126	30	15
No. of high-risk HPV	53	105	26	15
No. of low-risk HPV	17	22	3	0
No. of HPV16 (%)	42	72	24	14

Figure 2. (**A**) Prevalence of high- and low-risk HPV types among pre- and post-menopausal women. (**B**) Frequency of occurrence of high-risk HPV types, HPV-16 and low-risk HPV types with increase in age.

Not much difference in the frequency of high and low-risk HPV-type infection in different socio-demographic factors was observed. But, with increase in age, an increase in the frequency of oncogenic (high-risk) HPV-type infection was observed, while the opposite is true for non-oncogenic (low-risk) HPV types (figure 2B).

The present study shows that the risk of HPV infection increases significantly with residence in rural areas and non-vegetarian diet, while other factors like increase in age, parity, low income group and pregnancy might have some influence although no association was observed with young age and the age at first sexual intercourse.

4. Discussion

In India, cervical cancer ranks first among all the female malignancies, and as observed, it is mainly associated with HPV infection. So HPV screening is one of the best ways to check if a woman is at the risk of developing cervical cancer. We have studied the overall HPV incidence in Varanasi and its adjoining areas along with detecting different prevalent types in this population.

A total of 2424 women, asymptomatic for any cervical disease, were screened for HPV infection. 9.9% of the women were found to be infected with either high- or low-risk HPV types and some of them were co-infected with multiple HPV types. This is in accordance with the other populations studied in India (Bhatla *et al.* 2008; Kerkar *et al.* 2011) but different from the data available from other countries. HPV infection was reported to be 26.8% in the US (Dunne *et al.* 2007), 46% in Gabon (Si-Mohamed *et al.* 2005), 13.3% in southeast China (Ye *et al.* 2010), 15.1% in the UK (Cotton *et al.* 2007), 15.9% in Italy (Centurioni *et al.* 2005) and 21.25% in American-Indian women (Bell *et al.* 2007).

Our study demonstrates that out of 26 different HPV types detected, HPV-16 was the most prevalent, followed by HPV-6 and -81. The frequency of HPV-16 as observed in the population under study was 6.3% (figure 3), although a lower prevalence rate (4.7%) was reported in southern Asian countries (WHO 2010). Such a high frequency of HPV-16 was not reported in other regions of India (Clifford *et al.* 2005; Franceschi *et al.* 2005; Aggarwal *et al.* 2006; Kerkar *et al.* 2011) but was reported in eastern Asia (6.4%) and Eastern Europe (7.4%) (de Sanjose *et al.* 2007).

In this study we further tried to determine the association of different socio-demographic factors with HPV infection. We found that non-vegetarian diet and rural settings act as significant independent predictors for HPV infection (also reported earlier in north Indian population [Aggarwal *et al.* 2006]). Many studies reported that vegetarians have lower risk of HPV infection because of the presence of fruits and vegetables in their diet which are rich source of antioxidants like vitamin C, E, β-carotene and presence of folate (or in the form of folic acid) (Sedjo *et al.* 2002). Folate deficiency is known to cause reduced immunity (Dhur *et al.* 1991). It was found that women with high circulating concentration of folate and β-carotene were twice as less likely to develop persistent HPV infection and had greater likelihood of clearing them, and also the women with lower folate level and infected with high-risk type HPV-16 were 9 times more likely to develop CIN-2+ (Piyathilake *et al.* 2010). It was observed earlier by National Nutrition Monitoring Bureau, India, that the level of these micronutrients were less in rural population (Rao *et al.* 2010) and as observed in this study, the frequency of HPV-16 was very high in the rural population. Although we did not check the level of folate in these samples, this may be one of the contributing factors towards acquisition, persistence and progression of HPV infection.

In many studies young age (Verteramo *et al.* 2006; Datta *et al.* 2010) and age at first intercourse (Flores *et al.* 2008) were found to be associated with HPV infection but our study demonstrated no such association with these factors.

In a study (Jacobs *et al.* 2000), it was reported that high-risk HPV-type infection decreases with age while there was constant prevalence of low-risk types. On the other hand, our study indicated an increase in frequency of high-risk HPV-type infection with increase in age and a decrease in trend in the low-risk HPV type with increasing age. This may be due to changes in immune and hormonal system in older

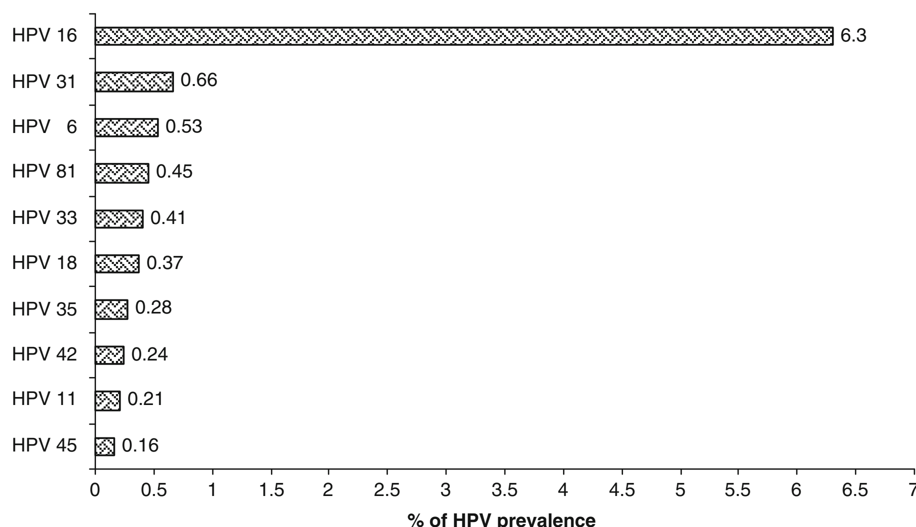


Figure 3. Ten most frequent HPV types observed in asymptomatic women of eastern UP, India.

women which impair clearing of HPV infection especially of the high-risk types. In younger age group women, low and intermediate HPV types were more prevalent than in older women, which may be due to the high frequency of metaplastic changes taking place in the cervix (Burd 2003). Further, in our study multiple types of HPV infection were also observed in women of younger age group only (≤30 years). It is yet to be seen if this observation has a significance or is due to the limited number of samples.

Association of HPV infection with post-menopausal women (although not statistically significant) is being shown in our study for the first time. Earlier reports (Althoff *et al.* 2009) show persistent HPV infection in perimenopausal women mainly. As oestrogen is an important immuno-modulating hormone and stimulator of humoral immunity, an association of HPV with post-menopausal women observed here may be due to lower oestrogen level in post-menopausal women.

Further, in this study, pregnant women in their first trimester of pregnancy were also included. Risk of HPV infection was observed to be higher in them than in women who were not pregnant. Since it is known that the level of oestrogen was low in first trimester than in second and third trimesters of pregnancy (Nobbenhuis *et al.* 2002), it is likely that the low level of oestrogen is responsible for reduced immunity.

In the present study, PCR-based method for HPV detection was employed using two primer sets, GP5+/6+ and MY09/11, both of them belonging to the L1 consensus region. Since these primers can detect a large spectrum of HPV types, they were used most frequently for HPV detection, but there is also a drawback in using them. In most of the cases, integration of HPV takes place by disrupting L1, E1 or E2 regions, hence making these disrupted regions unamplified and assessing them as false HPV-negative, consequently leading to underestimation of the prevalence. So, including other regions (E6/E7/LCR) for the detection of HPV may be beneficial, but since we obtained a comparable frequency of HPV-16-positive individuals in our study and in studies reported from other labs on asymptomatic populations, as well as in cervical cancer samples (not included in this study), it is likely that our result gives a true representation of the population.

In India there is lack of screening programmes because of which large numbers of women remain undetected for HPV infection and cervical lesions in their initial stages. As this study shows that old age group women possess higher frequency of HPV infection, this emphasizes that these women should not be spared from screening.

Further, the information generated by the study regarding the distribution of different HPV types in this population is significant and can be used for proper utilization of the present prophylactic vaccines or for the generation of a new vaccine against a set of HPV types. Currently developed prophylactic vaccines are bivalent (Cervarix, GlaxoSmithKline Biologicals) and quadrivalent (Gardasil, Merck & Co.), which target only a set of HPV types 16/18 or 16/18/6/11, respectively. Although these vaccines can reduce the cervical cancer incidence, for the efficacy of the vaccines it is important to know the prevalence of different HPV types in the given population to be immunized. In UP, there was no epidemiological data of cervical cancer from National Cancer Registry Programme (NCRP) established by Indian Council for Medical Research (India), but a study including population of the districts Mahoba, Lalitpur, Agra and Banda have been shown to have highest prevalence of cervical cancer among all the female malignancies, although HPV genotypes are not known (Ganjewala 2009). Crude Incidence Rate (CIR) for cervical cancer was observed to be very high in Mahoba and Banda (28.83 and 27.63 per 100,000 persons, respectively) (Ganjewala 2009). Also the population of eastern UP covered under this study is exposed more to HPV-16, 31, 33, 6 and 81; hence, there is a need for vaccination covering these types. The study showed high frequency of HPV-16 in the given population, indicating an increase in risk of CINs in future, and hence there is an urgent need for the development of a therapeutic vaccine for this region.

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