Original Article

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Incidence and Association of HPV16 and 18 with Various Risk Factors in Cervical Cancer Patients in Population of Haryana Region, India

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: Cervical cancer is the most leading malignancy in women with the highest mortality rates in most of the countries and Human Papilloma Virus (HPV) 16 and 18 confer a greater risk of having cervical cancer than other genotypes.

Aim: To assess the incidence of high risk type HPV 16 and 18 infections in cervical cancer patients and to study their association with various risk factors in females of Haryana, India.

Materials and Methods: In present study, total 110 cervical cancer samples were collected from PGIMS, Rohtak, Haryana, India. These samples were screened to confirm the presence of HPV infection by using degenerate primers for L1 open reading frame of HPV genome. Samples found positive for HPV infection were further studied by type-specific PCR for HPV 16 and 18. Statistical analysis were performed by using Medicalc software version 18.9. Odd ratio (95% confidence interval) was calculated to study association of sociodemographic and histopathological grades with HPV type specific infections.

Results: Out of 110 samples, 107 (97.27%) were recorded positive for HPV DNA. A total of 90 (84.11%) and 78 (72.89%) patients were found infected with HPV 16 and 18 respectively. Risk factors like elder age, age at marriage, post menopause and poor genital hygiene were found to be significantly associated ($p \leq 0.05$) with HPV 18 infection and rural background showed significant association with HPV 16 infection in cervical cancer ($p \leq 0.05$).

Conclusion: Results suggest that HPV 16 and 18 infections are highly prevalent in the cervical cancer patients of Haryana. These results will be useful in establishing the future guidelines for reducing risk of cervical cancer with the help of screening programs and by providing proper vaccines targeting HPV16 and 18.

Keywords: Awareness, Human papillomavirus 16 and 18, Screening, Sociodemographic and histopathological factors

INTRODUCTION

Cervical cancer is the second leading malignancy in women worldwide as approximately 510,000 new cases of cervical cancer is diagnosed annually with approximately 288,000 deaths worldwide [1]. More than 1,22,844 women in India are diagnosed with cervical cancer and more than 67,477 die of the disease every year [2]. It is expected that in India, cervical cancer occurs in around 1 in 53 women contrast with 1 in 100 women in more developed regions of the world [3]. Age, multiple pregnancies, number of abortions, use of oral contraceptives play an important role in development of cervical cancer. The relationship of Human Papillomavirus (HPV) and cervical cancer is well studied and the association of HPV in cervical cancer is as high as association of smoking in lung cancer [4].

HPV is very common worldwide and primarily transmitted through sexual contact in both males and females [5]. Approximately, 90% of HPV infections are asymptomatic and cleared by immune system. Epidemiological data and molecular observations have revealed that a persistent infection with high-risk HPV is the most crucial risk factor for cervical, anogenital and other organ sites cancer [6]. There exist more than 100 types of HPVs out of which more than 40 HPV types are found in the genital tract that is passed through sexual contact. Ten HPV types i.e., 16, 18, 31, 33, 35, 45, 51, 52, 58 and 59 have been adequately evaluated as high-risk types or oncogenic types [7]. Previous studies have strongly supported HPV infection as the prime risk factor in cervical cancer [8-12]. Being important risk factor, HPV needs to be studied in every part of the country as the incidence of HPV and its subtypes are different in different geographical regions of the country. Association of HPV with various risk factors like age, age at marriage, parity, residential background, menstrual status and menstrual hygiene also needs to be studied in cervical cancer for designing future screening and vaccination programs.

After considering all facts, the present study was done for the first time to account the prevalence of HPV infection in Haryana, India and aims to determine the incidence of high risk HPV types 16 and 18 in cervical cancer patients by using highly sensitive Polymerase Chain Reaction (PCR) technique. An effort was also made to find out association of HPV infection with socio-demographic factors and with histopathological factors in cervical cancers.

MATERIALS AND METHODS

The present retrospective study was conducted on cervical tissue biopsies collected from 110 patients with cervical abnormalities (94 cases of cervical cancer and 16 cases of chronic cervicitis) of different age group from Department of Obstetrics and Gynaecology, Pandit Bhagwat Dayal Sharma Health University, Rohtak, Haryana, India, from September 2016 to August 2018. Married women were enrolled in the study after clinical examination. Relevant information regarding age, age at marriage, parity, number of abortions, menstrual hygiene, menstrual status, chief complaints, clinical diagnosis, examination findings, etc. were recorded.

Biopsy samples were histopathologically confirmed by histopathologists as cervicitis, squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma. Written informed consent was obtained from all participating patients. Processing of all samples was done in a laminar flow cabinet in Department of

Genetics, Maharishi Dayanand University, Rohtak, Haryana, India. Ethical approval for sample collection was taken from institutional human ethical committee (IHEC) with Number IHEC/2016/80-13.06.16.

Sample collection and genomic DNA isolation: Cervical biopsies were collected and stored immediately at -80°C for further process. Briefly, tissues were crushed in Liquid N_2 and resuspended in STE buffer (100 mM NaCl, 10 mM Tris and 1 mM EDTA) and incubated with 100 µg of proteinase K at 55°C for 16 hour. DNA was extracted by using phenol, chloroform and isoamyl alcohol mixture (25:24:1) and further precipitated by using ethanol [13].

DNA concentration and purity assay: The purity and concentration of genomic DNA was checked by Nanodrop spectrophotometer at OD 260/280. Integrity of genomic DNA was checked on 1% agarose gel. Quality of DNA samples was tested by GAPDH, yielding a human GAPDH product of 200 bp.

HPV detection and HPV typing: Following primers were used for the PCR amplification: For GAPDH, F 5'-AGCGAGATCCCTCCAAA-3' and R 5'-CTTGAGGCTGTTGTCATACT-3'; for GP5+/6+, F 5'-TTT GTT ACT GTG GTA GAT ACT AC-3' and R 5' GAAAAATAAA CT GTAAATCAT ATTC; for HPV 16, F 5'-ATT AGT GAG TAT AGA CAT TA-3' and R 5'-GGC TTT TGA CAG TTA ATA CA-3'; for HPV 18, F 5'-CAC TTC ACT GCA AGA CAT AGA-3'and R 5'-GTT GTG AAA TCG TCG TTTTTC A-3'. HPV genotyping was done by using degenerate primers GP+5/+6 targeting 150 bp region and type specific PCR using HPV 16 and 18 specific primers targeting 109 and 334 bp region respectively. Reactions were performed as initial denaturation at 94°C for 4 minutes followed by 35 cycles with denaturation for 45 seconds at 94°C, annealing at 53-61°C for 45 seconds and extension at 72°C for 1 minute, and a final extension at 72°C for 7 minutes. PCR products were further visualised on ethidium bromide stained 2% agarose gel.

STATISTICAL ANALYSIS

Association of various risk factors and histopathological factors with the presence of HPV 16 and HPV 18 infections was estimated by using multivariate logistic regression analysis. Descriptive values were presented as mean, median, Standard Deviation (SD), percentages and frequencies. Statistical analyses were performed by using Medicalc version 18.9. Odd ratio (95% confidence interval) was calculated to check the association of HPV type 16 and 18 infections with sociodemographic factors and histological grades. A p-value of ≤0.05 was considered statistically significant.

RESULTS

Sociodemographic and histopathological parameters for enrolled cases (110) were recorded. The association of HPV 16 and 18 infections with different parameters were studied.

Demographic and clinical features of cervical cancer cases: Sociodemographic features and histological grading of all cervical cancer cases are provided in [Table/Fig-1,2]. Mean age and age at marriage of the registered cases were 55.3 and 15.95 with an age range of 38-82 years and 14-21 years respectively. Most of the cases (84.39%) were illiterate/just literate and belonged to rural background (78.18%). Bleeding after menopause and bleeding during intercourse were the main symptoms in cervical cancer cases. Differentiation degree was unknown for 46 (41.81%) biopsies, 30 (27.27%) biopsies showed moderate differentiation, 10 (9.09%) showed well differentiation and 8 (7.27%) showed poor differentiation.

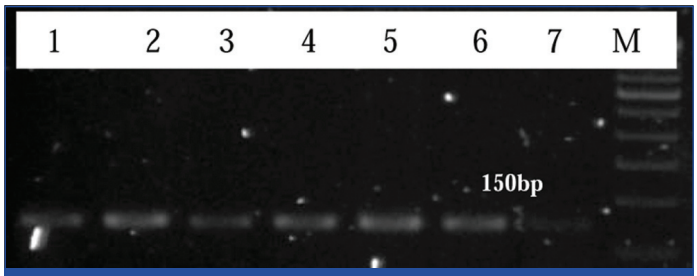
Detection of HPV by PCR in cervical cancer samples: Out of 110 cervical cancer samples, 107 (97.27%) were recorded positive for HPV DNA. Out of 107 HPV positive cervical cancer samples, 90 (84.11%) samples were found HPV 16 positive and 78 (72.89%) samples were found positive for HPV 18. These results suggest that the prevalence of HPV infection in cervical cancer is very high and HPV 16 is more prevalent along with HPV 18. [Table/Fig-3] represents 2% agarose gel showing 150 bp product for the GP05+/6+ consensus primers of HPV in cervical cancer patients. Similarly, [Table/Fig-4,5] represents 2% agarose gel showing 109bp and 334 bp product for HPV 16 and HPV 18 respectively.

Sociodemographic Factors	Total		HPV	HPV	OR (95%CI)	P	HPV	HPV	OR (95%CI)	P
	N=110	%	16+	16-			18+	18-		
Age										
≤55 years	42	38.18	33	9	0.7 (0.2-1.8)	0.49	35	7	2.9 (1.1-7.5)	0.027*
>55 years	68	61.82	57	11			43	25		
Age at time of marriage										
≤18	53	48.18	47	6	2.55 (0.9-7.2)	0.07	30	23	0.2 (0.1-0.6)	0.002*
>18	57	51.81	43	14			48	9		
Rural	86	78.18	78	8	9.7 (3.3-28.7)	0.0001*	60	26	0.8 (0.3-2.2)	0.62
Urban	24	21.81	12	12			18	6		
Number of births										
≥3	60	54.54	52	8	2.0 (0.7-5.5)	0.15	45	15	1.6 (0.7-3.5)	0.30
<3	50	45.45	38	12			33	17		
Number of abortions										
0-3	101	91.81	84	17	2.4 (0.5-10.8)	0.23	72	29	1.2 (0.3-5.3)	0.77
≤3	9	8.18	6	3			6	3		
Menopause										
Pre	43	39.09	31	12	0.4 (0.2-1.1)	0.07	28	15	0.3 (0.1-0.74)	0.009*
Post	67	60.90	56	9			58	9		
Menstrual Hygiene										
Poor	72	65.45	50	22	1.1 (0.5-2.5)	0.91	52	20	3.21 (1.4-7.3)	0.005*
Good	38	34.54	26	12			17	21		

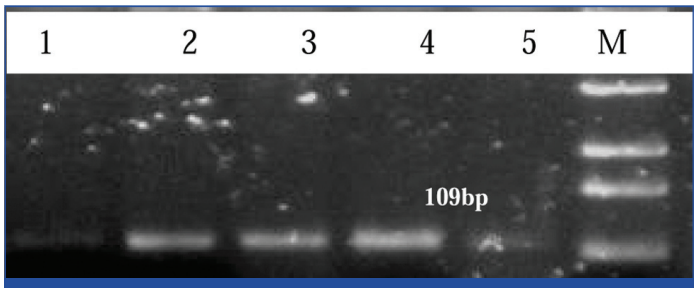
[Table/Fig-1]: Sociodemographic factors as risk factors for HPV 16 and 18 infection in the study population of cervical cancer.
OR: Odd ratio; CI: Confidence of interval; No: Number; HPV: Human papillomavirus; P: p-value. *Significant at p≤0.05

HPV Type infection	HPV infected (107)	Cervicitis			Squamous carcinoma			Adenocarcinoma			Adenosquamous		
		N (%)	OR (95% CI)	P	N (%)	OR (95% CI)	P	N (%)	OR (95% CI)	P	N (%)	OR (95% CI)	P
HPV16+	90	6 (37.5)	0.133 (0.04-0.40)	0.0004*	81 (90)	2.0 (0.86-4.64)	0.10	1 (50)	0.22 (0.01-3.70)	0.29	2 (100)	1.13 (0.05-24.4)	0.93
HPV16-	20	10 (62.5)			9 (10)			1 (50)			0		
HPV18+	78	3 (18.7)	0.09 (0.025-0.35)	0.0005*	73 (82.2)	1.76 (0.90-3.44)	0.097	0	0.88 (0.03-1.77)	0.11	2 (100)	2.07 (0.09-44.31)	0.64
HPV18-	32	13 (81.2)			17 (18.88)			2			0		

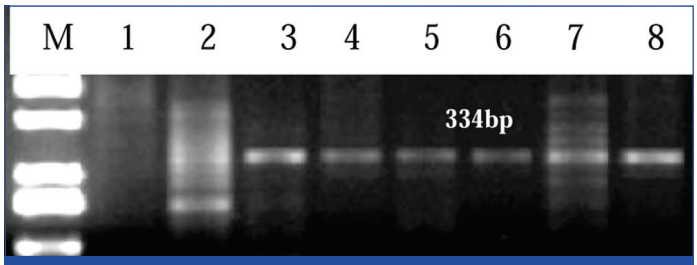
[Table/Fig-2]: Association of HPV 16 and 18 infections with histopathological factors.
OR: Odd ratio; CI: Confidence of interval; No: Number; HPV: Human papillomavirus; P: p-value. *Significant at p≤0.05



[Table/Fig-3]: Representative image of 2% agarose gel showing product of 150 bp targeting L1 gene (GP05+/6+ consensus primer) of HPV in cervical cancer patients. Lane M: Upto 1 kb DNA ladder, Lane 1 to 7 shows HPV positive samples.



[Table/Fig-4]: Representative image of 2% agarose gel showing product of 109 bp for HPV 16 in cervical cancer patients. Lane M: Upto 1 kb DNA ladder, Lane 1 to 5 shows HPV positive samples.



[Table/Fig-5]: Representative image of 2% agarose gel showing product of 334 bp for HPV 18 in cervical cancer patients. Lane M: Upto 1 kb DNA ladder, Lane 2-8 shows HPV positive samples and Lane 1 shows HPV negative.

DISCUSSION

Cervical cancer is the most frequent malignancies in women worldwide accounting for 17% of all cancer deaths among women aged between 30 and 69 years. Although, the incidence of cervical cancer is steadily declining in the developed world; it is most common in developing countries [14]. In spite of being curable and preventable at an early stage, cervical cancer still causes more than 67,477 deaths annually in India due to the lack of organized screening programs and intervention approaches [2].

Although, India is a diverse country with extensive ethnicity, and socio-cultural diversity, the incidence of HPV infection may vary significantly in different regions so, it is required to study prevalence of HPV and its genotypes in every part of the country. The available literature shows that the prevalence of HPV in women in different parts of India ranges from 9 to 94%. Incidence of HPV 16 infection along with HPV 18 in cervical carcinoma is very high as compared to other HPV type infections in India [Table/Fig-6] [9-12,15-20].

In present study, the prevalence of HPV infection (97.27%) was found very high with HPV 16 (84.11%) and HPV 18 (72.89%) as the prominent infection. The prevalence of HPV types found in this study is quite similar to a recent large-scale study reported from Odisha, India [11].

Age at marriage and menstrual hygiene showed significant association with HPV 18 infection; moreover, a significant association between HPV 16 infection and residential background (p≤0.05) was also observed in this study group.

Present study proves that women above 55 year (CI=1.12-7.51 and p=0.027) and with early age at marriage (CI=0.1-0.6 and p=0.002) were at greater risk of developing HPV infection in cervical cancer cases i.e., 68 (61.82%) and 53 (48.18%) respectively which was quite similar to other studies [21-23]. So, it is believed that if females get vaccinated with HPV in younger age before their first sexual contact then, virus exposure can be stopped. The vaccine can put a stop to almost 100% of disease caused by the four types of HPV (HPV 16, HPV 18, HPV 6 and HPV11) [1].

Sr No.	Place	Subjects enrolled	Number	Positivity	References
1	Chennai	HPV as a risk factor in cervical cancer patients	205	99.4% (HPV DNA)	Franceschi S et al., [9]
2	Tamil Nadu	Women randomly selected from general population	1179	14.2% (HPV DNA)	Clifford GM et al., [20]
3	Andhra Pradesh	HPV prevalence in cervical cancer and normal women	41	HPV DNA (87.8%) HPV 16 (66.7%) HPV 18 (19.4%)	Sowjanya AP et al., [10]
4	Chandigarh	Hospital based study of women with benign cervical cytology	472	36.8% (HPV DNA) 8.2% (HPV 16 & 18)	Aggarwal R et al., [15]
5	North-Eastern India	HPV prevalence in women of eastern India without cervical cancer	2501	9.9% (HPV DNA), 2% (HPV 16 & 18)	Datta P et al., [16]
6	Uttar Pradesh	A population based study of asymptomatic women of eastern Uttar Pradesh	2424	9.9% (HPV DNA)s	Srivastava S et al., [17]
7	Amritsar	Prevalence of HPV infection in patients with cervical lesions	100	10% (HPV DNA), 6% (HPV 18) and 2% (HPV 16)	Kaur P et al., [18]
8	Jammu and Kashmir	Hospital based study for HPV prevalence in women of Jammu Region	500	40.8% (HPV DNA), 2% (HPV 16) and 6.8% (HPV18)	Bhardwaj R et al., [19]
9	Madhya Pradesh	HPV detection in cervical cancer patients	52	84.6% (HPV DNA), 50% (HPV 16) and 15.3% (HPV18)	Bhandari V et al., [12]
10	Odisha	HPV Genotypes distribution in Indian women with and without cervical carcinoma	607	HPV DNA (93.28%) HPV 16 (87.28%), HPV18 (24.56%)	Senapati R et al., [11]

[Table/Fig-6]: Comparison of studies of HPV in cervical cancer from different regions of India [9-12,15-20].

People living in rural area are 20 times more prone to acquire HPV infection as these areas reflects poor socioeconomic condition due to lack of access to hospitals, poor genital hygiene, poor health [24]. In rural areas of Haryana, sexual notion and behavior of women are more conservative which stops women to talk about gynaecological problems. In present study, 86 (78.18%) of the cases were from rural background of Haryana with very high infection of HPV 16 (90.70%) and moderate with HPV18 (69.76%). It was observed that the women from rural areas of Haryana were at greater risk of developing HPV 16 infection than women living in urban areas of Haryana (CI=3.30-28.75, p=0.0001).

In villages, some women use old poorly stored clothes as pads and reuse them every time were considered as risk factors for infection. It was found that such women with poor menstrual hygiene (65.45%) were at greater risk of developing HPV infection (CI=1.4-7.3, p=0.004) than women with good menstrual hygiene (use sanitary pads). In these studies, no significant association was observed in post-menopausal state and HPV infection as compared to other significant study (p=0.009) [25].

The association of different histological groups of cervical cancer with HPV 16 and 18 infections was shown in [Table/Fig-2]. The risk of HPV 16 and 18 was significantly associated with cervicitis (p=0.0004 and 0.0005 respectively).

Due to high prevalence of HPV in Haryana region, it is extremely crucial to establish screening programs to make people aware about signs, symptoms, risk factors and preventive measures associated with cervical cancer.

LIMITATION

Small sample size and limited genotype (HPV 16 and 18) are major limitation for this study. Studies for larger population needs to be done to further validate these findings and provide strong evidence to make any further policies. Study of other high risk and low risk genotypes will help in knowing whether they contribute to the pathogenesis of cervical cancer.

CONCLUSION

Outcomes of the study suggest that the incidence of HPV in cervical cancer patients is high and strongly supports HPV as a causative factor in the development of cervical cancer. The infection of HPV 16 along with HPV 18 is found to be most prevalent in this study population. Thus, an effective vaccination program based on regional epidemiological profile of HPV is needed to reduce the cervical cancer burden in women of Haryana, India.

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