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Original Research Article

Prevalence and Association of High Risk Human Papillomavirus (HPV) Type 16 and Type 18 with Various Risk Factors in Cervical Cancer Patients in North Western Rajasthan

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Abstract

Introduction: Cervical cancer is one of the commonest cancers of female and the leading cause of morbidity and mortality. It is second most common cancer in India. In 2020, Globocan estimated 123,907 incident cases and 77,348 deaths, with an age-standardized incidence rate of 18 per 100,000 women and a cumulative risk of 2.01%. The major risk factor is persistent infection of Human papilloma virus (HPV) infection, HPV 16 and 18 are found with the highest frequencies and account for approximately two thirds of all cervical carcinomas worldwide, with HPV-16 occurring most frequently.

Aim: To find out the prevalence and association of high risk type HPV 16 and 18 with various risk factors in cervical cancer patients.

Materials and Methods: In present study, total 200 cervical carcinoma patients above the age of 25 years were included. Cervical biopsy samples were collected from Acharya Tulsi Regional Institute of Cancer Research, Bikaner. Genomic DNA were isolated from commercially available DNA isolation kit as per manufactures instruction. For detection and amplification of HPV type 16 and type 18 Real time PCR technique was used. Amplified product was identified by fluorescent signal generated from the presence of an oligonucleotide probe specific for target DNA sequence.

Results: Mean age of Cervical cancer Patients was 50.28 ± 10.01 Years. Out of 200 samples, 179 (89.5%) were recorded positive for HPV DNA. A total of 152 (76%) and 43 (21.5%) patients were found infected with HPV 16 and 18 respectively. There was statistically significant association between elder age, menstrual status and parity with HPV 16 and HPV18.

Conclusion: In cervical cancer patients HPV 16 and 18 infections are highly prevalent and various risk factors like elder age, parity were significantly associated with HPV. These results will be useful for better management of disease in term of screening and early detection and prevention.

Keywords: Cervical carcinoma, Human Papilloma Virus, Risk factors, Real time PCR

Introduction

Globally, non-communicable diseases are accountable for the highest mortality in which cancer ranks as the major cause of mortality and the important obstacle in increasing the life expectancy of humans in the 21st century. [1] Cervical cancer is one of the commonest cancers of female anogenital tract and the leading

cause of morbidity and mortality. [2] In India it is the second most common cancer among women. In 2020, Globocan estimated 123,907 incident cases and 77,348 deaths, with an age-standardized incidence rate of 18 per 100,000 women and a cumulative risk of 2.01%. [3] The 5-year relative survival rate of approximately 46% (range 34–60%) is much lower than that of other Asian countries. [4] This is due to the fact that cervical cancer is diagnosed at advanced stage in more than 80% cases, resulting in high mortality. [5]

Various epidemiological studies have identified a number of risk factors that contribute to the development of Cervical Intraepithelial Neoplasia (CIN) and cervical cancer. Human Papilloma virus (HPV) is associated with almost all cervical cancer worldwide. [6] The association of HPV and cervical cancer was first suggested by zur Hausen in 1976. It is now believed that 94-100% of cervical cancers - as well as tumours of the penis, anus, vagina, and vulva - are associated with sexually transmitted genital infection by the human papilloma virus (HPV). [2] The other risk factors include sexual promiscuity and multiplicity of sexual partners, exposure to sexual intercourse at an early age, number of pregnancies , cigarette, smoking, use of oral contraceptives, dietary and other factor.

Human Papillomavirus (HPV) is a double-stranded DNA virus transmitted via sexual skin to skin contact, and a persistent infection may result in malignant tumors. They are ubiquitous and able to adjust to their hosts. They have the ability to hide efficiently from immune reactions. [7] The HPV genome is divided into 3 regions: i. Upstream Regulatory Region (URR), ii. “Early” region - which include the genes E1, E2, E3, E4, E5, E6, E7 and E8 and iii. “Late” region - which encodes the L1 and L2 structural proteins. [8] Based on the integration properties and differential tendency to associate with benign processes or malignant hyperplasias, HPV has been classified as high risk types which are type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and low risk types 6, 11, 40, 42, 43,44, 54, 72 and 81. [9] Infection with one or more of the oncogenic HPV types may result in the integration of the viral genome into the host cellular genome resulting in the formation of cervical neoplastic cells, the proliferation of which leads to various grades of CIN, which may progress to invasive cervical cancer, Functionally high- risk HPV infection contributes to carcinogenesis and tumor progression predominantly through the actions of two viral oncogenes, E6 and E7. Both of these oncogenes interact with and inhibit the activities of critical components of cell cycle regulatory systems, in particular E6 with p53 and E7 with RB. [8] Virtually all cervical cancer cases are thought to be preceded by persistent high risk HPV genital infection out of which HPV 16 and 18 are found with the highest frequencies and account for approximately two thirds of all cervical carcinomas worldwide, with HPV-16 occurring most frequently. [9,10] In India, HPV type 16 alone in cervical cancer is 70-90 per cent while occurrence of HPV type 18 varies from 3 to 20 per cent. [8] India is a large and heterogeneous country, where, similar to cancer incidence, region-specific prevalence of HPV varies considerably. This is likely attributable to genetic and cultural diversity, as well as heterogeneity between studies.11 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, a parity, residential background, menstrual status also needs to be studied in cervical cancer for designing future screening and vaccination programs. After considering all facts, the present study was done to find out the prevalence 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 sociodemographic factors and with histopathological factors in cervical cancers.

Material & Method

Present study was descriptive study, conducted in Department of Anatomy, Sardar Patel Medical college, Bikaner the study incorporated total 200 histopathological confirmed cervical carcinoma patients above the age of 25 years. Patients having other malignancies or previous cancer treatment were excluded. Prior approval of institutional ethics committee and consent of the subjects in form of informed consent form was taken. Demographic details were entered in Proforma .After taking consent from the patient fresh biopsy tissue were collected by trained medical professional at Acharya Tulsi Regional Institute of Cancer Research, Bikaner. From biopsy tissue formalin fixed paraffin embedded blocks were made for Genomic DNA isolation by TRUPCR tissue DNA extraction kit by 3Bblackbio Biotech India as per kit protocol. Quality and competence of DNA for gene amplification, is confirmed by spectrophotometer. HPV genome was amplified by Real time PCR technique for which TRUPCR HPV 16 &18 Detection kit (3Bblackbio Biotech India) designed for an in vitro detection and amplification of HPV 16&18 DNA from extracted DNA samples was used as per kit instructions.

PCR conditions: Initial denaturation for 10 minutes at 94ºC for the first cycle followed by followed by 30 sec each of denaturation at 94ºC, annealing/extension at 60 ºC for 60 sec for 40 cycles. Reporter channel for HPV 16 was FAM, for internal control Texas Red and for HPV 18 was HEX. Amplified product was identified by fluorescent signal generated from the presence of an oligonucleotide probe specific for target DNA sequence.

Statistical analysis: The data was entered into Microsoft Excel and then analysed with the help of IBM SPSS software

Results

Total 200 cases were incorporated in study. Cases were above the age of 25 years, range between 25 to 75 years . Mean age of cases was 50.28 ± 10.01 Years. Out of total 200, 119 (59.5%) belongs to rural area and 81(40.5%) belongs to urban background. There were three categories of socioeconomic status : Low, Middle and High. Majority of cases belongs to middle income group (n=99), followed by low income group(n=66). Out of total 200 cases around 57.5% were illiterate or had primary education. Menstrual status of cases showed that majority of cases, 138(69%) were postmenopausal and 62(31%) were premenopausal. Parity of 3 or more were observed in 70% of cases. Out of total 200 cases, 179 cases were HPV positive which was 89.5% and 21 cases (10.5%) tested negative for HPV(Figure 1).

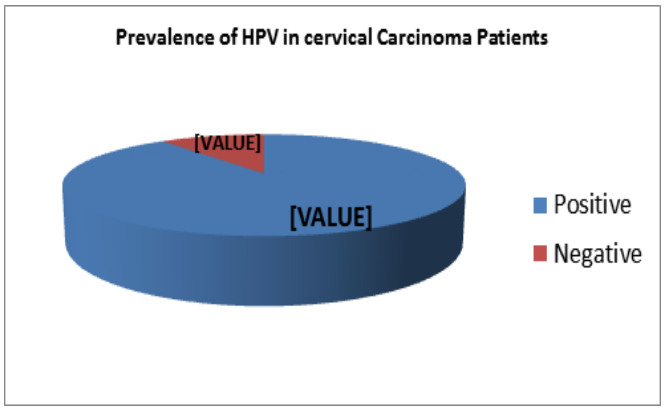

Figure 1: Prevalence of HPV in Cervical Carcinoma Patients

Overall Prevalence of HPV 16 was 76% as 152 cases tested positive, on the other hand prevalence of HPV 18 was 21.5% as 43 cases were positive for HPV 18. Total 16 cases (8%) tested positive for both HPV 16 & 18. Among 179 HPV positive patients, prevalence of HPV 16 was 76%, HPV 18 was 15% and Co infection of HPV 16 &18 found in 9% cases (Table 2).

Table 2: Type specific HPV Prevalence in HPV positive cases

Status	Number (n)	Percentage (%)
HPV 16	136	76%
HPV 18	27	15%
Both HPV 16 & HPV 18	16	9%
Total	179	100

Association of HPV with Demographic variable revealed that Statistically Significant Association was Found between age and HPV 16 (p=0.003) & also in age and HPV18 (p=0.012). Level of education and HPV16 were also significantly associated (p=0.006). Association between Socioeconomic status and HPV 16 status was also significant (p=0.011) but no such association was observed between SES and HPV 18.Parity and menstrual Status both were significantly associated with HPV 16 & HPV 18. No significant association was found between residence and HPV status (Table 3).

Table 3: Association of HPV 16 and HPV 18 with demographic variables

Characteristics	HPV 16		*P*-value	HPV 18		*P*-value
	Negative (n=48)	Positive (n=152)		Negative (n=157)	Positive (n=43)	
Age, years, (Mean ± SD)	46.58±10.08	51.45±9.74	0.003*	51.15±10.19	47.14±8.74	0.020*
Age Group			0.003*			0.012*
25 to 35 Years	7(14.6%)	10(6.6%)		14(8.9%)	3(7%)	
36 to 45 Years	22(45.8%)	35(23%)		35(22.3%)	22(51.2%)	
46 to 55 Years	9(18.8%)	57(37.5%)		56(35.7%)	10(23.3%)	
56 to 65 Years	6(12.5%)	38(25%)		38(24.2%)	6(14%)	
66 to 75 Years	4(8.3%)	12(7.9%)		14(8.9%)	2(4.7%)	
Residency			0.599			0.620
Rural	27(56.3%)	92(60.5%)		92(58.6%)	27(62.8%)	
Urban	21(43.8%)	60(39.5%)		65(41.4%)	16(37.2%)	
Education			0.006*			0.065
Illiterate	6(12.5%)	49(32.2%)		47(29.9%)	8(18.6%)	
Primary	11(22.9%)	49(32.2%)		51(32.5%)	9(20.9%)	
Secondary	19(39.6%)	34(22.4%)		35(22.3%)	18(41.9%)	
Graduation	6(12.5%)	10(6.6%)		11(7%)	5(11.6%)	
Post-Graduation	6(12.5%)	10(6.6%)		13(8.3%)	3(7%)	
Socio-Economic Status			0.011*			0.143
Lower	11(22.9%)	55(36.2%)		57(36.3%)	9(20.9%)	
Middle	22(45.8%)	77(50.7%)		75(47.8%)	24(55.8%)	
Higher	15(31.3%)	20(13.2%)		25(15.9%)	10(23.3%)	
Menstrual Cycle			<0.001*			<0.001*
Pre-menopausal	27(56.3%)	35(23%)		39(24.8%)	23(53.5%)	

Post-menopausal	21(43.8%)	117(77%)		118(75.2%)	20(46.5%)	
Parity			0.005*			0.034*
Zero	9(18.8%)	15(9.9%)		19(12.1%)	5(11.6%)	
One	2(4.2%)	6(3.9%)		8(5.1%)	0(0%)	
Two	10(20.8%)	18(11.8%)		20(12.7%)	8(18.6%)	
Three	17(35.4%)	37(24.3%)		36(22.9%)	18(41.9%)	
More than equal 4	10(20.8%)	76(50%)		74(47.1%)	12(27.9%)	

Discussion

Cervical Carcinoma have a considerable burden on our health system as well as has social and financial implications. [12] Human papillomavirus (HPV) is the world's most common sexually transmitted infection (STI) and causes significant morbidity and mortality. It is generally accepted that infection with the oncogenic HPV is a crucial step and major causative agent in the cervical carcinogenesis. Epidemiological, clinical and molecular studies have shown that infection with high-risk HPV is the most important aetiologic agent in the pathogenesis of cervical cancer. [13] High risk HPV types 16 and 18 have been attributed to nearly 70% of cervical cancer. India is a diverse country with extensive ethnicity, and socio-cultural diversity, the incidence of HPV infection may vary significantly in different regions. The available literature shows that the prevalence of HPV in women in different parts of India ranges from 9 to 94%. [14-16] Incidence of HPV 16 infection along with HPV 18 in cervical carcinoma is very high as compared to other HPV type infections in India. [17] There is therefore an urgent need to develop better understanding about this carcinogenesis at a molecular level and translate this knowledge to develop better screening, diagnostic and therapeutic tools. [18] In present study mean age of cases was 50.28 ± 10.01 years with majority of cases(n=66) belongs to age group 46-55 years, followed by 36-45 years in which 57 cases were found they constitutes 28.5% of study population (Table 1).

Table no. 1 Distribution of cases according to Demographic data

Characteristics	**Total = 200**	
Age, years, (Mean ± SD)	50.28±10.01	
Residence	Number (n)	Percentage (%)
Rural	119	59.5%
Urban	81	40.5%
Education		
Illiterate	55	27.5%
Primary	60	30.0%
Secondary	53	26.5%
Graduation	16	8%
Post-Graduation	16	8%
Socio-Economic Status		
Low	66	33%
Middle	99	49.5%
High	35	17.5%
Menstrual Cycle		
Pre-menopausal	62	31%
Post-menopausal	138	69%
Parity		

Nil	24	12%
1-2	36	18
More than equal to 3	140	70%

Similarly, a study conducted by Jain R [12] also observed mean age of cervical carcinoma patients was 50. 67 ± 10.86 year. In a multicenter study conducted by Basu P [19] mean age of Cervical Cancer patients was 51. 40 ± 11.20 years. Our results were also in accordance with Sowjanya P15, Srivastava S [20] and Kumar R [21] who reported that high mean age of carcinoma patients. High mean age of patients with cervical cancer may be because symptoms of cervix cancer have been found similar to other gynaecological ailments and its proliferation rate is very slow hence detected at later age with increased severity.

In present study, 200 histopathological confirmed cervical carcinoma patients were incorporated. All the patients had squamous cell carcinoma. HPV prevalence was 89.5% as 179 cases tested positive for HPV and 21 cases tested negative for HPV (Figure 1). HPV genotyping among HPV-positive (n=179) cases showed that HPV 16 alone was found in 136 cases 76% and HPV 18 alone was found in 27 cases which constitutes 15%. In 9 % of cases (n=16) co infection of both HPV type 16 and Type 18 was found (Table no.14). Thus HPV 16 accounts for 84.91% and HPV 18 accounts for 24.02% cases among HPV positive group. In accordance with the findings of our study Pavani et.al [15] reported prevalence of HPV 16 (66.7%), HPV18 (19.4%).

In another study from Bihar [21] on Distribution of human papilloma virus type 16 and 18 in cervical carcinoma patients it was found that all the cases(n= 96) were tested positive for HPV among them prevalence of HPV type 16 was 77.08% whereas prevalence of HPV type 18 was 16.67% and co infection was found in 6.25% cases. .

Pilai et.al [22] conducted a study large multicentric study of region-wise distribution of High-Risk Human Papillomavirus types in squamous cell carcinomas of the cervix in India showed that 92% of invasive carcinoma of the uterine cervix (ICC) was associated with the presence of HPV DNA. HPV type-specific distribution across the regions showed that Human papillomavirus DNA sequences corresponding to type 16 or 18 were the most prevalent at 79.6% of the cases followed by HPV--45, -73, -31, -56, -52, -58, -59, -33, -68, -51, -35, -26, and -39.

The difference in type specific prevalence might be related to geographical variations. The association of high risk types HPV 16/18 might be due to their integration into the host genome which significantly increases its tumorigenicity through upregulation of E6 and E7 encoded in the early open reading frame of the virus.

In present study HPV 16 was present in 152(76%) cases and HPV 18 in 43 cases. Majority of positive cases in both HPV 16 And 18 were in age group 46-55 years. Both HPV 16 & 18 were significantly associated with age (Table 3). Shrivastava S [20] also reported that increasing age (women ≥45 years) as a significantly related factor with cervical cancer (OR= 9.4, $p<0.0001$).Similar results were also reported by Kadian L [23] that women above 55 year (CI=1.12-7.51 and p=0.027) were at greater risk of developing HPV infection in cervical cancer cases.

Though the present study reveals no significant association of HPV with residence but it showed high prevalence of HPV in Rural area which shows 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 which facilitates infection and persistence of HPV and an increasing risk of cancer development. Association between Educational status and HPV16 was statistically significant with p value = 0.006* as 64.4% positive cases had low level of education Varghese et .al [24] reported that HPV infection among the illiterates was high compared to literates. Similarly Aggarwal et al [25], observed that women who were illiterate or had less than 6 years of education had a higher rate of HPV. High incidence of HPV among illiterate or less educated people may be due to lack of knowledge and awareness about disease and its risk factors. Prevalence of HPV 16 & 18 was highest in middle socioeconomic group that is 50.7% and 55.8% respectively. Followed by low socioeconomic group. Statistically significant distribution and association (p-value-0.011*) was found between HPV 16 and Socioeconomic status of Cases. In Close agreement of our result Franceschi et al [26], Varghese et al [24] have recognized low socioeconomic status as a risk factor for cervical carcinoma as well. Majority of cases were postmenopausal in present study. There was statistically significant distribution and association was found between menstrual status and HPV 16 (p-value-<0.001**) and HPV 18 (p- value-<0.001**). Our observation closely matched the findings of Shrivastava s that women in postmenopausal group were at greater risk of developing CINs (p=0.02) and cervical cancer (p=0.001). Similar findings were also reported by Kadian L [23]. Owing to Hormonal changes in postmenopausal category insufficient adaptive immune responses contributes to HPV persistence or reactivation of latent HPV infections. Contradictory to our findings Grace N reported highly significant negative association between postmenopausal status and HPV. Distribution of HPV according to parity revealed that majority of case were multiparous having 3 or more children. Statistically significant association was observed between Parity and HPV 16 and HPV 18. Das BC [13] reported that HPV infections are at least two-times more frequent in pregnant women than in non-pregnant women showing a gradual but statistically significant ($P < 0.001$) increase in HPV infection with the increasing number of pregnancies, Mitra [27] also noted that large number of child births gave rise to high parity which increased risk factor for cervical cancer. [28]

Conclusion

In present study prevalence 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 and HPV 18 as elsewhere in India and worldwide have a high prevalence among cervical carcinoma patients. Various risk factors like age, parity, menstrual status were significantly associated with HPV. The knowledge that infection with high risk HPV is a necessary cause of cervical cancer has paved the way for a new approach to prevention of cervical cancer, both by way of new diagnostic tools as well as by vaccine development. The development of effective screening, awareness, and HPV vaccination programs in the general population are need of the hour for the better management of cervical cancer.

Recommendations:

Large population based studies of various High risk genotype in general population and cervical carcinoma patients will be helpful in better management of this disease. HPV DNA testing should be used as screening tool for early detection. Implementation of HPV vaccination will be helpful in primary prevention.

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