(e) ISSN Online: 2321-9599
(p) ISSN Print: 2348-6805

ORIGINAL ARTICLE

HPV ASSOCIATION WITH CARCINOMA CERVIX, CLINICAL IMPLICATIONS AND PROGNOSTIC VALUE IN CARCINOMA CERVIX

Ratika Gupta[1], Pardeep Garg[2], Lajyadevi Goyal[3], Neerja Jindal[4], Sapna Marcus[5], Raja psbanipal[6], HP Yadav[7]

[1]Senior Resident, Department of Radiotherapy, Medanta, Gurgaon, Haryana, India,
[2]Assistant professor, Department of Radiotherapy, GGS Medical College Hospital, Faridkot, Punjab, India,
[3]Professor and Unit Incharge, Department of Gynecology, GGS Medical College Hospital, Faridkot, Punjab, India,
[4]Professor and Head, Department of Biochemistry, GGS Medical College Hospital, Faridkot, Punjab, India,
[5]Assistant Professor, Department of Radiotherapy, GGS Medical College Hospital, Faridkot, Punjab, India,
[6]Associate professor, Department of Radiotherapy, GGS Medical College Hospital, Faridkot, punjab, India,
[7]Professor and Head, Department of Radiotherapy, Institute of Liver and Biliary Sciences, Vasantkunj, Delhi, India,

ABSTRACT:
Aim and Objective- Cervix cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in females worldwide. Some 99 per cent of cervical cancer cases are linked to genital infection with human papillomaviruses (HPVs). The clinicopathological parameters and response to radiotherapy varies with the genotypes of HPV. This study was aimed at association of HPV with cervical carcinoma and to evaluate the correlation of different HPV genotypes with parameters such as histological type, age at onset, clinical stage and response to radiotherapy in diagnosed cases of carcinoma cervix.HPV DNA was detected by Real time PCR technique in the cervical smears of 50 patients of FIGO stage- IIA to IIIB**. Materials and Method** -Association was then estimated between HPV genotypes and various clinicopathological parameters. External beam radiation with concurrent chemotherapy followed by intracavitary brachytherapy was delivered to all patients. Response to radiotherapy was then assessed according to different HPV genotypes. Chi Square test of significance was used for all the calculations.A total of 50 patients were analyzed. **Results-** HPV DNA sequences were detected in 84% of the specimens. Among HPV positive samples, HPV 16 and HPV 18 were found in 67% and 21 % of the samples respectively.Poorer response to radiotherapy was observed in the patients negative for HPV genotype or positive for HPV-18 genotype. No correlation was seen between HPV genotype and other clinicopathological parameters like age, stage, histopathological type. **Conclusion-** These data suggest that the HPV type present in cervical carcinoma is related to clinical behavior and response to radiotherapy.
Key Words: Ca cervix, HPV, response

Corresponding author: Dr. Ratika Gupta, Senior Resident, Department of Radiotherapy, Medanta, Gurgaon, Haryana, India

This article may be cited as: Gupta R, Garg P, Goyal L, Jindal N, Marcus S, Psbanipal R, Yadav HP. HPV association with carcinoma cervix, clinical implications and prognostic value in carcinoma cervix. J Adv Med Dent Scie Res 2017;5(2):24-28.

Access this article online	
Quick Response Code	Website: www.jamdsr.com
	DOI: 10.21276/jamdsr.2017.5.2.7

INTRODUCTION:

Several studies have demonstrated world over that 99 per cent of cervical tumors are having the presence of human papillomaviruses (HPV).[1]More than 111 genotypes of HPV have been described, but only about 30 of them are associated with ano-genital cancer. HPV 16/18 is estimated to account for more than 80 per cent of invasive cervical cancer.[2] Altogether HPV 16, 18, 45, 31,33, 35, 52, 58 are responsible for about 90 per cent of all cervical cancers worldwide.[3] HPV has been correlated with treatment response in some studies with varying results. Increased mortality is seen in HPV negative patient as compared to HPV positive patients. [4]HPV 18, multiple HPV types have a more aggressive phenotype, higher recurrence rate and a worse prognosis than those with HPV 16.[5,6] This study was conducted to find out the association of HPV infection in patients with carcinoma cervix and the variation in response to treatment with different genotypes of HPV.

MATERIAL AND METHODS:

The present study was conducted from 1st April 2013 to 31st October 2014 at Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab. A total of 50 inoperable patients of carcinoma cervix were enrolled from outpatient department, who received radical treatment. Inclusion criteria was (a) Newly

histopathologically diagnosed carcinoma cervix patients, (b) age of more than 25 years and (c) diagnosed patients with FIGO stage- IIA to IIIB.Each participant was enquired with a standardized questionnaire on socioeconomic status, sexual behaviour, reproductive history, contraceptive practices, hygienic conditions and history of sexually transmitted infections and cervical cytological screening. Written and informed consent was taken from each patient.Cervical cells were collected with the help of cervical brush which was placed in transport media and was send to microbiology laboratory, where HPV was detected by using Real time PCR DNA kit - Biotron HPV high risk screen (12 types). EBRT was delivered through Cobalt 60 unit or linear accelerator. Individualized treatment planning was executed for each patient. A dose of approximately 50 Gray (Gy) was delivered to pelvis by either the two field parallel opposed technique (anterior/posterior field) or the four field box technique, in 25 fractions, over 5 weeks. If indicated the para aortic field was treated with a dose of 45 Gy/ 25# / 5 weeks. During radiation all the patients received chemotherapy on concurrent basis with Inj cisplatin 35mg/m^2. Per vaginum examination, complete blood count and renal function tests were done weekly for all patients while on external beam radiation.After approximately 1 week of completion of external beam radiation all the patients received high dose rate brachytherapy with intracavitary radiation applicators. Three fractions, each at a gap of one week were delivered with a dose of 7 – 7.5 Gy in each fraction. After completion of treatment, follow up was done every month for one year to see the response of radiotherapy. Every month assessment of patient was done by per vaginum examination and per speculum examination. USG abdomen/ CECT abdomen (if indicated) were done one month after completion of treatment followed by every three months to assess the response rate.Patients were considered to have good response if there was no residual disease on completion of treatment, as seen on clinical examination and USG abdomen. If suspected lesion was present, Pap smear and CECT abdomen were done for confirmation and if disease was confirmed, it was characterized as poor response. If there was residual disease or if disease recurred within one year, it was also considered to be poor response.Chi Square test of significance was used to estimate the association between HPV genotypes and factors such as clinicopathological parameters and response to treatment ,$p<0.05$ was considered significant.

RESULTS:

The present study was designed to analyze the relationship between HPV DNA status and clinicopathological parameters in order to further elucidate the role of the HPV type in relation to clinical outcome of cervical carcinoma. Finally, to determine the clinical implications and prognostic value of the HPV genotype in cervical carcinoma, we evaluated whether various HPV genotypes in patients receiving radiotherapy for cervical cancer correlate with their survival.HPV subtypes-in the present study 42 (84%) out of 50 patients were positive for HPV infection. The distribution of cases according to HPV genotype in our study was as follows, 28(67%) patients were HPV 16 positive, 9 (21%) were positive for HPV 18, 2 (4.7%) were positive for HPV 45, while only 1 (2.4%) patient was positive for HPV 33. Out of 42 patients positive for HPV, the number of patients with multiple HPV infection was 2 (4.7%). Age-the mean age of diagnosis in our study was 49.2 years. In our study, the youngest patient was 38 years old while the oldest was 75 years old. The maximum number of patients was seen in the 40-59 years age group. Distribution of cases according to HPV status and age is shown in table 1. On comparing the data, we did not find any correlation between age and genotype of HPV infection ($p = 0.97$ i.e. >0.05)

Parity-In our study 47 (94%) patients were multiparous, 3 (6%) were primiparous and none of the patient was nulliparous. Sexual behaviour-the reported number of patient's having multiple sexual partners were 4 (8%), patient's husband having multiple sexual partners were 7 (14%) and male partner having warts in genital region were 2 (4%). Histological type andstage- in our study we observed that out of a total of 50 patients, the maximum number of patients 35 (70%) were clustered together in stage IIIB. The number of patients in stage II was 12(24%) and in stage III were 38(76%) (table 2).Out of 10 patients positive for HPV in stage II, 7 (70%) patients were positive for HPV 16 and 2 (20%) patients were positive for HPV 18. And in stage III out of 32 patients positive for HPV, 21 (65%) patients and 7 (21.8%) patients were positive for HPV 16 and HPV 18 respectively. So there was no difference in distribution of different genotypes of HPV according to staging ($p=0.55$).

Out of 50 patients, 48 (96%) patients were histologically diagnosed to be squamous cell carcinoma and only 2 (4%) patients were diagnosed as adenocarcinoma (table 3).

Table 1: Distribution of cases according to HPV status and age of patient (n=50)

Age group (years)	Number of patients	HPV positive patients	HPV negative patients	HPV 16 positive patients	HPV 18 positive patients	HPV 33,45 and multiple
25-39	03	03	00	02	01	00
40-59	33	28	05	18	06	04
60-79	14	11	03	08	02	01
>80	00	00	00	00	00	00
TOTAL	50	42	08	28	09	05

Table 2: Distribution of cases according to stage of carcinoma cervix (n=50)

Stage	Number of patients	HPV Positive	HPV Negative	HPV 16	HPV 18	HPV 33,45 and Multiple
II A	02	02	00	01	01	00
II B	10	08	02	06	01	01
III A	03	02	01	01	00	01
III B	35	30	05	20	07	03
Total	50	42	08	28	09	05

Table 3: Distribution of cases according to HPV status and histopathological diagnosis (n=50)

Histopathology	No.	HPV Positive	HPV Negative	HPV 16 Positive	HPV 18 Positive	HPV 33,45 and multiple
Squamous cell carcinoma	48	40	08	27	08	05
Adenocarcinoma	02	02	00	01	01	00
Total	50	42	08	28	09	05

Table 4: Distribution of patients, comparing treatment response according to the HPV status

Treatment response	No.	HPV Positive	HPV Negative	HPV 16 positive	HPV 18 Positive	HPV 33,45 and multiple
Good	31	29	02	22	03	04
Poor	19	13	06	06	06	01
Total	50	42	08	28	09	05

Relationship between HPV Status and response to radiotherapy

In our study out of 42 patients positive for HPV infection, 29 (69.1%) patients had good response to treatment, 12 (28.5%) patients had local recurrence/residual disease and 1 (2.4%) patient had distant metastasis. In the HPV negative arm, out of 8 patients, 2 (25%) had good response while 6 (75%) patients local recurrence or residual disease. On comparing the two arms we found that treatment response was better in HPV positive tumors (p = 0.019) as compared to HPV negative tumors. (table 4)

Relationship between HPV Subtypes and response to radiotherapy

Treatment response in HPV positive tumors varies with the type of HPV infection. In our study, out of 28 patients positive for HPV 16, 22 (78.6%) had good response while rest of the patients i.e. 6 (21.4%) had local recurrence or residual disease. On the other hand, out of 9 patients positive for HPV 18, only 3 (33.3%) patients had good response to treatment, while 6 (66.7%) patients had poor response (5 patients had local recurrence or residual disease and 1 patient had distant metastasis). When we compared the treatment response in HPV 16 and HPV 18 positive tumor, HPV 16 positive tumors (good response seen in 78.6% patients) were found to fare better than HPV 18 positive tumors (good response in 33.3% patients) (p=0.023). Out of two patients positive for multiple infections with HPV in our study, one (50%) patient had good response while one (50%) of them had poor response.(table 4)

DISCUSSION:

Cervical carcinoma is the most common carcinoma affecting Indian women. The incidence of cervical cancer has decreased in developed worlds in recent years but India still has the largest burden of cervical cancer. A very strong association has been demonstrated between the human papillomavirus (HPV) and cervical cancer. HPV has now been accepted as a necessary cause of cervical cancer.Previous attempts to determine the prognostic significance of the presence or absence of detectable HPV DNA and HPV genotypes in cervical cancer patients have generated conflicting results.[7-9] The maximum number of patients in our study were seen in the 40-59 years age group. (table 1). Similar results were seen in the study done by Das et al, who had also shown that most cervical cancers in India occur in 45-60 yr of age.[10]According to many of the studies mentioned above, the number of cases were evenly distributed between patients at 30 to 39 and 60 to 69 years of age.[7,8] This represents a combination of factors like, earlier onset of sexual activity (i.e. earlier acquisition of human papillomavirus [HPV] infection) and active Pap screening programs in the developed countries, which detect cancerous and precancerous lesions earlier in life as compared to developing countries like ours. The increased risk of invasive cervical cancer in women with high parity is a known fact and was also proven by Muñoz,who showed that high parity increases the risk of squamous-cell carcinoma of the cervix among HPV-positive women. A general decline in parity might therefore partly explain the reduction in cervical cancer recently seen in most developed countries.[1]In our study most of the patients were positive for squamous cell carcinoma.(table 2). An Indian study done by Shukla et al had reported similar results as ours and they found 10-15 percent cases positive for adenocarcinoma.[11]It is a universal fact that treatment outcome and survival benefit are directly related to initial stage of tumor. Almost similar results as ours were found in an Indian study by Munirajan, in which out of 43 tumors, 83%

were of stage III and 14% of stage II.[12] These results are in contrast to the results of studies done in western countries. The study done by Nakagawa had 73.9% diagnosed in early stages and only 21% cases were diagnosed in stage III.[8] The reason for different trends and late stage of presentation in developing countries as compared to developed countries was that most of the women in our country belong to low socioeconomic status and are usually ignorant towards their problems.When we studied the distribution of HPV genotype, our results matched closely with Hariharanet al, who studied HPV type distribution in a rural community in Hyderabad and found that among the HPV positive cancers, (n=41) the overall type distribution of the major HR HPV types was as follows: HPV 16 (66.7%), HPV 18 (19.4%), HPV 33 (5.6%), HPV 35 (5.6%), HPV 45 (5.6%), HPV 52 (2.8%), HPV 58 (2.8%).[13]The number of multiple genotypes of HPV detected in our study was slightly less than what was seen in a study by Lai et al. In their study multiple type of HPV were seen in 9.6% of patients.[14]In this study we did not find any correlation between age and genotype of HPV infection (p = 0.97 i.e. >0.05). Different studies have shown conflicting results in this aspect. The study done by King et al supported our results, as they did not find any correlation between genotype of HPV and age at diagnosis.[7] In another study done in 2010 by Ferdousi et al HPV 16 was found to be more commonly associated with patients of younger age group.[15]In our study two patients were positive for adenocarcinoma, and only one of these patients was positive for HPV 18, but as the sample size was less, it was difficult to comment on the correlation between the type of HPV and histopathological diagnosis.(table 3) According to a study by Das et al, in India HPV 16 was the most prevalent type both in squamous cell carcinoma as well as adenocarcinoma while global reports indicate preferential occurrence of HPV 18 in adenocarcinoma.[10]While comparing treatment response in HPV positive and HPV negative patients, our results were similar to Harima Y et al. they found that HPV-negative patients survived for significantly shorter time periods compared to the HPV-positive patients.[16]Overall in our study, when we compared the treatment response in HPV 16 and HPV 18 positive tumor, HPV 16 positive tumors were found to fare better than HPV 18 positive tumors (table 4). These results were concordant with Nakagawa et al study, in which HPV 18 had a tendency towards poorer prognosis than HPV 16.[8] The study by Stephen showed slightly different results. According to them the poorer overall survival among patients with HPV related tumors were statistically significant for patients with early stage disease, whereas there was little difference in survival by HPV type for patients with more advanced disease.[9] However in a study done by King et al there was no difference in tumor recurrence between HPV 16 positive and HPV 18 positive patients.[7]

CONCLUSION:

Current data suggests that the type of HPV infection present in cervical carcinoma may influence clinical features or biological behaviors. As a result HPV genotyping may serve as a potential biomarker of response to radiation and prognosis in cervical carcinoma patients undergoing radio- or chemoradiotherapy. So getting an HPV testing done in all the patients before starting treatment, can help predict the prognosis in carcinoma cervix patients. As in our study HPV 18 was shown to have higher chances of recurrence, aggressive treatments can be planned for such patients.Occurrence of an exclusive high prevalence of HPV16 puts this region in an advantageous position because both the new vaccines are against HPV 16 and 18. These will have maximum impact in India, as they would be able to take care of about 90 per cent of cases.

REFERENCES:

1. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiological classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.
2. ZurHausen H. Viruses in human cancers. Science 1991; 254 :1167-73.
3. Bosch FX. Epidemiology of human papillomavirus infections:new options for cervical cancer prevention. SaludPublicaMex2003;45(3):326-39.
4. RiouG, Le MG, Fane M, Jeanne D, Bourhis J, Orth G. Human papillomavirus-negative status and c-myc gene overexpression: independent prognostic indicators of distant metastasis for early-stage invasive cervical cancers. J NatlCuncerInst 1992;84:1525-6.
5. Schwartz SM, Schwartz, Janet R, Daling JR, Shera KA, et al. Human papillomavirus and prognosis of invasive cervical cancer: a population- based study. J ClinOncol 2001;19:1906–1915.
6. Bachtiary B, Obermair A, Dreier B, et al. Impact of multiple HPV infection on response to treatment and survival in patients receiving radical radiotherapy for cervical cancer. Int J Cancer. 2002;102:237–243.
7. King LA, Tase T, Twiggs LB, Okagaki T, Savage JE, Adcock LL, Prem KA, Carson LF.Prognostic Significance of the Presence of Human Papillomavirus DNA in Patients With lnvasive Carcinoma of the Cervix. Cancer 1989;63:897-900.
8. Nakagawa S, Yoshikawa H, Onda T, Kawana T, Iwamoto A, Taketani Y. Type of Human Papillomavirus Is Related to Clinical Features of Cervical Carcinoma. Cancer 1996;78:1935-41.
9. Stephen M S, Janet RD, Katherine AS, Margaret M, Barbara M, Denise A, et al. Human Papillomavirus and Prognosis of Invasive Cervical Cancer: A Population-Based Study.American Society of Clinical Oncology 2001;19(7):1906-1915.
10. Das B, Alok C. Bharti, Bharadwaj M. Human papillomavirus & cervical cancer: Looking ahead.Indian J Med Res 2009;130:210-211.
11. Shukla S, Bharti AC, Mahata S, Hussain S, Kumar R, Hedau S, Das B.C. Infection of human papillomaviruses in cancers of different human organ sites. Indian J Med Res 2009;130:222-233.
12. Munirajan AK, Kannan K, Bhuvarahamurthy V, et al. The status of human papillomavirus and tumor suppressor

genes p53 and p16 in carcinomas of uterine cervix from India. GynecolOncol 1998;69(3):205-9.
13. Hariharan I, Pillai MR. Genotypes of the human papillomavirus: Relevance to Indian field trials of the vaccine. Indian J Med Res 2009;130:247-260.
14. Lai HC, Sun CA, Yu MH, Chen HJ, Liu HS, Chu TY. Favorable clinical outcome of cervical cancers infected with human papilloma virus type 58 and related types. Int J Cancer.1999;84:553–557.
15. Ferdousi J, Nagai Y, Aoki Y. Impact of human papillomavirus genotype on response to treatment and survival in patients receiving radiotherapy for squamous cell carcinoma of the cervix. Exp Ther Med 2010;1(3):525–530
16. Harima Y, Sawada S, Nagata K, Sougawa M, Ohnishi T. Human papilloma virus (HPV) DNA associated with prognosis of cervical cancer after radiotherapy.Int J Radiat Oncol Biol Phys. 2002 Apr 1;52:1345-51.

Source of support: Nil **Conflict of interest:** None declared