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Human papilloma virus status in cervical cancer predictive and prognostic significance for chemo radiation treatment

[1*]Madisetty Adi Lakshmi, [1]M Vishnu Priya ,[2]Chinta Sanjeeva kumari

[1]Assistant Professor, Department of Radiotherapy, MNJ Institute of Oncology and Regional Cancer Center, Lakadikapool, Hyderabad, India

[2]Professor,Department of Radiotherapy, MNJ Institute of Oncology and Regional Cancer Center, Lakadikapool, Hyderabad, India

ABSTRACT

Aim: To find out the prevalence of HPV infection and genotypes in invasive cervical cancer patients.To know the predictive and prognostic significance of HPV infection to chemo radiation. **Materials and Methods:** This study was conducted in the department of radiation oncology in Patients attending to O.P.D during the period of one year are included in this study. A total of 145 women age of 30-70 years who were histopathologically proven for cervical cancer. All the patients were given radiotherapy. **Results:** For HPV detection exfoliated cells were taken by direct scrapings with cervix brush from the cervical tumour before treatment and preserved in preservCyt bottles. The DNP was extracted and was subjected to PCR by using established primers. HPV DNA was detected in 143 (98.6%) of the patients. Median age of the patients was 50 years. Most of the cases were in stage II disease (65.5%). Around 28 different HPV genotypes were found.HPV 16 is the most common genotype (77.6%).34.2% of patients were having multiple genotypes.HPV 33, 35, 52, and 58 were found as multiple genotypes and were not found in singles. Patients were followed up after chemo radiation. Mean follow up was 9.6 months and median follow up was 12 months. Only 82 patients remained for follow up at 12 months and the remaining patients did not turn up for follow up. At the end of 12 months, 57 (69.7%) patients were disease free, 11 (13.4 %) were with residual disease, and 14 (17.7%) were having recurrences. 20 patients were tested for HPV DNA post treatment; HPV DNA was cleared in 20% of patients, and was prevalent in 80% of patients in the study.20% of patients were having persistent HPV 16 genotype. All the patients with multiple genotypes prior treatment were found with single genotype post treatment.20% of patients were found to have different genotypes post treatment when compared to pretreatment.**Conclusion:** HPV 16 did not show any significance in disease response. 80% of patients had persistent HPV DNA post treatment.

Key words: Invasive cervical cancer, Chemoradiation, Human papilloma virus.

Introduction

National Cancer Institute refers cancer as to a class of disease in which a cell or a group of cells divide and replicate uncontrollably, intrude into adjacent cells and tissues (invasion) and ultimately spread to other parts of the body other than the location at which they arose (metastasis). Cervical cancer refers to proliferation of abnormal cells most commonly malignant cells that develop in the tissues of the uterine cervix. Worldwide, Cervical cancer is the fourth most common cancer in women, and the seventh overall, with an estimated 528,000 new cases in 2012. Around 85% of the global burden occurs in the less developed regions, where it accounts for almost 12% of all female cancers. High-risk regions, with estimated ASRs over 30 per 100.000, include Eastern Arica (42.7).Melanesia (33.3), Southern (31.5) and Western Asia (4.4). Cervical

**Correspondence*

Dr.M.Adi Lakshmi

Assistant Professor, Department of Radiotherapy, MNJ Institute of Oncology and Regional Cancer Center, Hyderabad ,India

Email: mnjadi1962@yahoo.com

cancer remains the most common cancer in women in Eastern and Middle Africa. There were an estimated 266,000 deaths from cervical cancer worldwide in 2012, according for 7.5 of all female cancer deaths. Almost nine out of ten (87%) cervical cancer deaths occur in the less developed regions. Mortality varies 18-fold between the different regions of the world, with ranging from less than 2 per 100,000 in Western Asia, Western Europe and Australia/New Zealand to more than 20 per 100,000 in Melanesia (20.6), Middle (22.2) and Eastern Africa (GLOBOCAN 2012). India had a national program for cancer since 1975, when the emphasis was on equipping premier cancer institutions, which, by 1984-85, shifted to primary prevention and early detection of cancer cases and, by 1990-91, to the district cancer centres. Strengthening of existing regional cancer centres, development of oncology wings in medical college hospitals, the district cancer control program, and the decentralized NGO scheme were the priorities of the program[1].So far, NCCP has supported 85 oncology wings in medical colleges including 27 tertiary cancer centres across the country. Keeping in view the preventable common risk factors of cancer and other non-communicable diseases (NCDs), the ministry has formulated a National Program for prevention and control of cancers, diabetes, cardiovascular diseases and stroke (NPCDCS) after integrating the NCCP with other NCDs. The major components of this program have been to strengthen 100 districts in 21 states for cancer care services, strengthen 65 centres as tertiary cancer care centres (TCCCs) throughout the country. An analysis of population-based survey indicates that coverage of cervical cancer screening in developing countries is 5% compared to 63% in developed countries[2]. Most of the cases (85%) are presented in advanced and late stages, and more than half (63%-89%) have regional disease at the time of presentation [3].Cervical cancer diagnosis and treatment in the advanced stages makes it a costly exercise, with a poor prognosis resulting in poor compliance. Despite its high incidence large scale population based studies on the HPV prevalence and the genotype distribution are very few from this region. The inconsistency in the overall reported prevalence and genotype distribution in various Indian studies warrant more studies to understand the distribution of HPV in different parts of the country. The reported incidence of multiple HPV infection in patients with cervical cancer varies greatly and ranges between 0% and 36% have been published[4].A recent study suggested an average of 3.3 different HPV types in each patient with cervical dysplasia[5]. Patients with multiple HPV types may have a higher risk of persistent infection compared to those with single HPV[6]. Persistent HPV infection in turn, is necessary for the development of cervical cancers[7]. Although the standard treatment of locally advanced cervical cancer includes radiotherapy, little is known about the impact of HPV on the response to radiotherapy and on the patients clinical outcome. While concurrent chemo radiotherapy is the main treatment modality for locally advanced cervical cancer, treatment failure in the central pelvis occurs in approximately 20-25% of patients in terms of radiotherapy outcome, several past studies showed that HPV persistence is associated with high rates of local recurrence and poor overall survival in patients with cervical cancer. Since there is limited data available regarding the status of HPV genotypes in cervical cancer and its role in predicting and prognosticating the disease after treatment in Andhra Pradesh. I envisage undertaking the study to estimate the prevalence of high risk group HPV genotypes in histologically proven cases of cervical cancer (carcinoma cervix) cases and its predictive and prognostic significance to the treatment among the women attending MNJ Cancer Institute, Hyderabad for treatment. The purpose of the study was to determine the association between HPV Genotypes and the invasive cervical cancer and its role in likelihood of response to radiotherapy and survival.

Materials and methods

This study was conducted in the department of radiation oncology, Mehdi NawabJung Institute of oncology & Regional cancer centre at Hyderabad.It was a prospective observational cohort study. Patients attending to O.P.D in department of radiotherapy in this hospital during the period from October 2013 to March 2014 are included in this study. A total of 145 patients who were histopathologically proven for cervical cancer were included in the study after taking consent from the patient. All the patients were given radiotherapy.

Inclusion criteria were age of 30-70 years, women willing to give consent, women willing to take treatment in this hospital, no history of prior treatment for CIN or Cervical Cancer, women with intact uterus, women with no history of debilitating disease or mental illness and women with FIGO stage I – stage III.

Exclusion criteria were women with pregnancy, bleeding disorders contraindicating blood sample collection, women with stage IV disease, women who

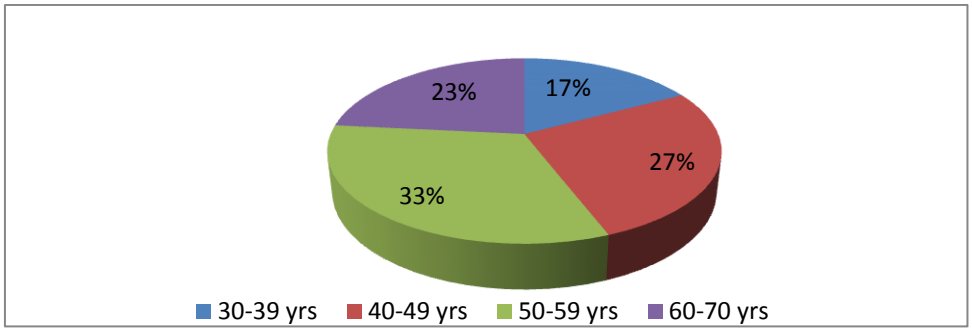
had taken radiation to pelvis earlier.Informed consent is taken from the women with histopathological proven cervical cancers who are willing to take part in this study. Pre-treatment evaluation was complete history was taken, general and physical examination, local examination includes Abdomen, pelvic, rectal examination, systemic examination, hematological investigations like complete blood picture, renal function tests, liver function tests, screening of HIV/HBs Ag, biopsy from the primary tumor (edge of gross tumor or 4 quadrants), X-ray chest, ultrasound abdomen and pelvis.

Results

A total of 188 patients who were histopathologically proved to have cervical cancer were enrolled into the study. But only 145 patients satisfied the inclusion and exclusion criteria and were included in the study. Reasons for exclusion of the patients were treatment with surgery alone, unwillingness to take treatment in this hospital.

Demographics: Median age was 50 (range 30-70 years). Mean age is 49.26 ± 10.7. 25 patients (17.2%) are in 30-39years of age, 39 (26.9%) in 40-49 years age group, 47(32.4%) in 50-59 years and 34 (23.4%) are in 60-70 years of age. More than 50% of patients were in the age group of 40-60 years.

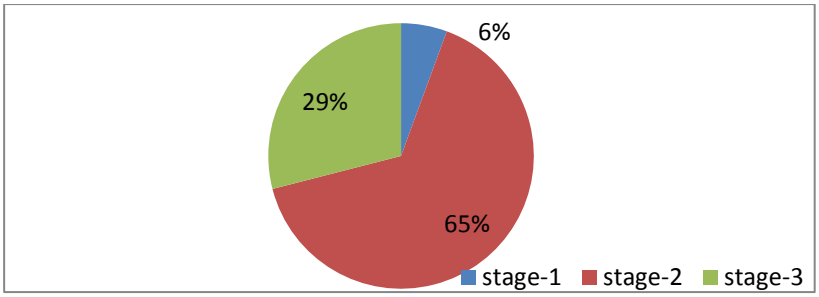
Graph 1: Distribution of cases according to age



Histology: Patients had histopathological diagnosis of squamous carcinoma in 141 patients (97.2%), adenocarcinoma in 2 patients (1.37%) and adenosquamous carcinoma in 2 patients (1.37%). **Degree of differentiation:** 132/145 (91%) patients were having well differentiated carcinoma's, 8 (5.5%) were moderately differentiated carcinoma, 3 (2.1%) were poorly differentiated carcinoma and2 were (1.1%) neuroendocrine differentiation.

Stage Wise grouping:

Graph 2: Distribution of cases according to stage



Patients were in stage I (n=8;5.5%), stage II (n=95;65.5%) and stage III (n=42;29%). More number of cases was found in stage II disease.

HPV infection prevalence: Out of 145 patients only 2 patients (1.37%) were HPV negative and 143 patients. Out of 2 HPV negative patients, one did not complete the treatment and absconded. One patient lost for follow up after completing the treatment.

Prevalence of different HPV genotypes: 28 different types of HPV DNA were found in our study. Seventeen high risk types (HPV 16, 18, 31, 33, 35, 39, 45,51, 52, 56, 58, 59, 68, 73, 82, 53, 66) and eleven low risk types (HPV 6, 42, 54, 61, 62, 64, 70, 72, 81, IS39, CP108) were found. All the low risk types were found along with high risk type and did not occur separately. Out of 143 patients, HPV 16was prevalent in 111 (77.6%) patients. HPV 18 was found in 15 (10.48%) patients. HPV 52 in 12 (8.39%) patients, HPV 35 and HPV 58 in 11 patients (7.69%), HPV 33 in 10 (6.99%) patients, HPV 62 in 7 (4.89%) patients, HPV, 31 in 6 (4.19%) patients, HPV 56,59,53,54 in 5 (3.49%) patients, HPV 51, 42, 58 in 4 (2.79%) patients, HPV 45,61,58 in 3 (2.09%) patients, HPV 73,83 in 2 (1.39%) patients, and HPV 39,66,68,6 CP6108 in 1(0.69%) patients.

Table 1: Single Vs multiple HPV genotypes and HPV genotype distribution

HPV	Frequency	Percent
Single genotype	94	**65.73%**
Multiple genotype	49	**34.2%**
Total	143	**100.0**
HPV Type	**Frequency**	**Percent**
HPV 16	76	**53.1%**
HPV 18	7	**4.9%**
other single types	11	**7.7%**
HPV16+Other Types	35	**24.4%**
Non HPV 16 (Multiple)	14	**9.79%**
Total	**143**	**100**

Out of 143 patients 94 (65.73%) patients were infected with single type of HPV genotype and 49 (34.2%) patients were infected with multiple genotypes.

Distribution of cases according to single/multiple genotypes: HIV positive patients and HPV: 4/145 patients were HIV positive.4 (100%) of them had multiple HPV genotypes in their cervical smears. All of them did not complete the treatment.

Prevalence of HPV 16 genotypes: Among 143 patients who were positive for HPV genotypes HPV 16 were found in 111 (77.6%). HPV 16 as single genotype were found in 76 (53.1%) patients and HPV 16 along with other genotypes were found in 35 (24.4%) patients.

HPV 16 among different grades: 104/136 (76.47%) patients were found to have HPV 16 as single or in multiple genotypes.3/3 (100%) patients who were classified as poorly differentiated carcinoma were having HPV 16 as a single genotype.2/2 patients with neuroendocrine differentiation were having HPV 16, one as single genotype and as multiple genotytpe. Other genotype HPV 18 were found in 15 (10.4%) patients, as single genotype were found in 7 (4.89%), and as multiple genotype were found in 8 (5.59%) patients. Other single genotypes were found in 11 (7.69%) patients. Multiple genotypes without HPV 16 were found in 14 (9.79%).

HPV 33, 35, 52, 58 genotypes: these genotypes were found in (11-12) 8% of patients and all the genotypes were found together in multiples and were never found in single.

Table 2: shows single and multiple genotypes among stages.

Stage	Single genotype	Multiple genotypes	Total	X^2	P
stage I	7 (4.9%)	1 (0.7%)	8 (5.6%)		
Stage II	60 (41.9%)	34 (23.7%)	94 (65.7%)		
stage III	27 (18.9%)	14 (9.8%)	41 (28.6%)		
Total	**94(65.7%)**	**49(34.2%)**	**143(100%)**		

Out of 94 patients (65.7%), with single genotypes, 7(4.9%), 60 (41.9%), 27(18.9%) patients are present in stage I, stage II, and stage III disease. Out of 49 patients with multiple genotypes,1

(0.7%), 34(23.7%), 14 (9.8%) are present in stage I, stage II, stage III disease. The chi square ($\chi 2$) value for significance of correlation between single and multiple genotypes for FIGO staging was 1.83 and p value was 0.400. As the p value being ≥ 0.05, With regard to stage at presentation, there was no significant difference between single and multiple genotypes.

Table 3: shows HPV 16 vs non HPV 16 genotypes

Stage	HPV 16 genotype	Non HPV 16 genotypes	Total	X^2	P
Stage I	6(5.4%)	2 (5.8%)	8 (5.5%)		
Stage II	72(64.8%)	24 (70.5%)	94 (65.7%)		
Stage III	33 (29.7%)	8 (23.5%)	41 (28.6%)		
Total	**111(77.6%)**	**34(23.7%)**	**143(100%)**	0.49	**0.78**

Out of 111 patients with HPV 16, 6 (5.4%), 72(64.8%), 33(29.7%) patients were found in stage I, stage II, stage III disease. Out of 34 patients with non HPV genotypes 2(5.8%), 24 (70.5%), 8(23.5%) were found in stage I; stage II, stage III disease. The chi square ($\chi 2$) value for significance of correlation between HPV 16 and non HPV 16 genotypes for FIGO staging was 0.49 and p value was 0.78. As the p value being ≥ 0.05, With regard to stage at presentation, there was no significant difference between HPV 16 and non HPV 16 genotypes.

Treatment response assessment: 139/145 patients were planned for chemo radiation and 6/145 patients with FIGO IB/IIA underwent surgery followed by post op adjuvant radiotherapy. 47/145 (32.4%) patients absconded in between treatment and did not turn up for completion of treatment. Phone calls were made to trace the patients. One patient died during treatment due to sepsis. Patients were followed every 3 months.

Table 4: shows response assessment at the end of 12 months

Response at 12 months	Frequency	Percent
Locally disease free	57	**69.5%**
Residual disease	11	**13.4%**
Recurrences	14	**17.07%**
Total	**82**	**100%**

Patients are followed up every 3 months. Clinical history is taken, general examination, local examination at each follow up and investigations when necessary were done.Out of 97 patients 5 of them did not turn up for follow up post treatment and 10 of them lost for follow up later. At the end of 12 months out of 97patients,15 patients lost for follow up. Out of 82 patients 57 (69.51%) patients were disease free locally, 11 (13.4%) patients had residual disease, 10 (12.1%) patients had local recurrence.4 (4.87%) had distant metastasis.

Table 5: shows disease response in relation to HPV (Single/Multiple):

Response at 12 months	Sin/multiple genotypes		Total	X^2	P
	Single	**Multiple**			
Disease free	40 (70.1%)	17 (29.82%)	57 (69.5%)		
Residual disease	4 (36.3%)	7 (63.6%)	11 (13.4%)		
Recurrence	12 (85.4%)	2 (14.2%)	14 (17%)		
Total	**56 (68.2%)**	**26 (31.7%)**	**82 (100%)**		

Among 57 patients who were locally disease free single genotypes were found in 40 (70.1%) and rest of 17(29.82%) patients had multiple genotypes. 4/11 (36.3%) patients with residual disease had single genotypes and 7 /11 (63.6%) patients had multiple genotypes.12/14 (85.7%) of patients with recurrence disease had single genotypes and 2/14 (14.2%) patients had multiple genotypes. The chi square ($\chi 2$) value for significance of correlation between single and multiple genotypes for disease response was 7.2

and p value was 0.02. As the p value is <0.05, with regard disease response, there was significant difference between single and multiple genotypes.

Table 6: Disease response in relation to HPV

Response at 12 months	HPV 16/non HPV 16		Total	X^2	P
	HPV 16	Non HPV 16			
Disease free	44 (77.1%)	13 (22.8%)	57 (69.5%)		
Residual disease	8 (72.7%)	3 (27.8%)	11 (13.4%)		
Recurrence	11 (78.5%)	3 (21.4%)	14 (17.1%)		
Total	63 (76.8%)	19 (23.1%)	82 (100%)	0.13	0.9

44/57 (77.1%) patients who were locally disease free had HPV 16 genotype and 13/57 (22.8%) patients had non HPV 16 genotypes. 8/11 (72.7%) patients with residual disease had HPV 16 genotype and 3/11(27.27%) patients had non HPV 16 genotypes.11/14 (78.5%) patients with recurrence disease had HPV 16 and 3/14 (21.4%) patients had non HPV 16 genotypes. The chi square ($\chi 2$) value for significance of correlation between HPV 16 and non HPV 16 genotypes for disease response was 0.13 and p value was 0.9. As the p value being ≥ 0.05, With regard to disease response, there was no significant difference between HPV 16 and non HPV 16 genotypes.

Table-7: Pretreatment HPV and post-treatment HPV:

	Post- treatment HPV at 12 months				
	HPV cleared	Persistent HPV 16	Persistent HPV 18	Other single genotypes	Total
HPV 16	4	5	0	4	13
HPV 18	0	0	1	0	1
HPV 16+multiple	0	1	1	2	4
Non HPV 16 multiple genotypes	0	0	0	2	2
Total	4	6	2	8	20

Mean follow up of the patients was 9.6months and median follow up period was 12 months. After 12 months, 20 patients were screened for post treatment HPV prevalence.4/20 (20%) patients cleared HPV DNA post treatment.16/20 (80%) patients were having HPV in their cervical smears. 6/20 (25%) of them had persistent HPV 16 genotype. 6/20 (25%) patients who were having multiple genotypes pretreatment, were found to have single genotype post treatment. 6/20 (25%) patients were having different HPV genotypes in their smears post treatment which are different from pretreatment HPV genotypes.

Discussion

Studies have shown that cervical cancers can occur with multiple HPV genotype infections. The incidence of multiple HPV infection is related to the method of HPV detection. In previous reports, the rate of multiple HPV infection in invasive cervical cancer ranged between 0% and 36 %. Apart from ageographical differences, the differences may be due to the type of tumour sample analysed (paraffin-embedded tumour versus cell samples of a cervical brush) and the molecular techniques used for HPV detection. Clifford et al reported the prevalence of multiple HPV infection with a frequency ranging from 11.5% in Turin, Italy, to 42.4% in Ho Chi Minh City, Vietnam [9] While Bachtiaryetal.[10] reported that multiple HR-HPV infections were seen in 46% of cervical cancer biopsies. In our study, multiple HPV infections with at least 2 HPV genotypes are present in 33.1% patients. This result is consistent with above results. Multiple HPV infection was found to be associated with persistence of HPV infection that itself leads to neoplastic transformation. Impaired immunity might play an important role in multiple HPV infection. Palefskyet. Al[11]demonstrated recently a 46% prevalence of multiple HPV infection among HIV-infected women, whose immune system was

seriously compromised, In a further study, the presence of multiple infections was strongly associated with HPV persistence in HIV infected women.100Thus, carriers of multiple HPV types might have deficient immune responses to HPV that predispose them to persistent infection. In the present study 4 of the patients were HIV-positive. All the 4 patients had multiple genotypes. But our number is less to correlate with the above study. Bao et al.[12]performed a meta-analysis of HPV genotyping in Asia and reported that there were eighteen HPV genotypes detected from cervical cancer, of which the 10 commonest HPV genotypes were HPV 16, 18, 58, 52, 33, 45, 31, 35, 39 and 51. Study by Abdul Raub in Malaysia, detected HPV 58 (10.7%), 52 (10.4%) and 33 (10.4%) were the 3 commonest HPV genotypes after HPV 16 and 18. This finding is similar to the studies conducted in Indonesia, China, Hong Kong, Taiwan, Korea and Japan13. Comparing these data with current study 28 types of HPV genotypes were found. HPV 16 (74.4%), 18 (10.3%), 33 (6.8%), 35 (7.5%), 52 (8.27%), 58 (7,5%) were found mostly. However it is slightly different when compared to US and Europe where it was reported as HPV 16, 18, 31, 45 and HPV 16, 18, 33, and 31, respectively (Smith et al.)14. While HPV 16 and 18 still remain the 2 commonest types across the world, other HPV types may differ. This suggests that the distribution of different HPV genotype differs in geographic distribution and may be due to ethnic diversity. This study is also similar to another study by Shilpi Roy15from Karnataka; HPV DNA was detected in 63 (88.73%) patients. With respect to the subtypes, out of 63 samples which showed presence of HPV, 46 samples were positive for HPV 16 (73.01%), 5 samples were HPV 18 positive (7.93%) and 2 samples were positive for both HPV 16 and 18 (3.17%). 10 samples i.e. 15.87% patients had other oncogenic HPV i.e. 31,35,52 and 58 in their study. Study by P. Basu from Kolkata in 2010, 16 determined HPV genotype distribution in 91/7% of the cases. Genotype 16 was the most common type, detected alone in 59.4% and in association with type 18 in 3.6% of cases. Genotype 18 was detected as a monotype in 13.3% cases. In total, types 16 and 18 alone or in co-infection with each other were detected in 76.3% cases. Genotype 22 was the third most common type and overall, but genotypes 16, 18, 31, 33 and 45 were the five most common types, detected in 87.1% of the total cases which is slightly different from our study. This study showed similar finding as that of study conducted by Soujanya[17] from Hyderabad which shows 87.8% of high risk HPV types in cervical cancers and among them HPV 16, 18, 33, 35, 52, 58, 45, 59 were found common. Pattern of HPV distribution remained same in this region. One explanation as to why these HPV types was a common element of multiple infections may be that these HPV types usually occurs in combination with phylogenetically closely related types (clade A9: HPV 16, 31, 35 and 58)(). It is possible that these types share 1 or more epitopes that render them particularly sensitive to loss of immune control. Recently, Liaw et al.[18] observed a reduced risk for subsequent acquisition of a new infection within the same phylogenetic clade A9 in initially cytological normal women, which implies a potential protective cross reactivity within clade A9. The immunologic situation, however, is probably different in cancer patients and cross-reactivity was possible. Future studies will focus on the role of HPV type 33, 35, 52, 58 in multiple HPV infection. In this study, type 16 was found to be far the most common, with an overall prevalence of 76.5% when taken into account both single and multiple infections, and type 16 and type 18 alone or with combination in 84.8%. This is similar to study conducted by P. Basu16 in India, who also found overall prevalence of HPV 16 as 65.5% when taken into both single and multiple infections. Excluding coinfections with other types, types 16 and 18 prevalence rate of 78.9% reported in the metaanalysis of Indian studies (Neerja et al., 2008), and 77% reported by Saranath et al in a separate Indian study of 337 cases of invasive cervical cancer[19]. 65-70% in less developed countries compared to a higher prevalence of 74-77% in more developed countries[19]. A recent study from France has reported types 16, 18 in 82% of cervical cancers, again indicating a higher attributable fraction than what is generally estimated (Pretet et al., 2008)[20].Studies from Delhi and Hyderabad also showed high prevalence of HPV 16 as 59.37% and 66.7% respectively comparatively lesser prevalence than our study. As 84.8% of patients were having HPV 16 and HPV 18, vaccination with the vaccines which are available now, (quadrivalent and nonavalent) would decrease these infections which are the main contributors for cervical cancers and as a result burden of the disease would decrease. To date, information is not available concerning the natural history of HPV infection in cervical carcinoma managed with RT[21].

The role of HPV infection in the oncogenesis of cancer of the uterine cervix is well established. Riou et al. Reported that HPV is a prognostic factor for better clinical outcome when compared to non HPV patients[22]. Most previous studies examined HPV

infection only before RT. They assessed a possible correlation between a pre-treatment status of HPV infection (HPV DNA-positive/negative tumour) and prognosis in patients treated with RT[21].

Harima et al. found that HPV-negative patients had a highly significant shorter survival than the HPV-positive group. The status of p53 (58 out of 65 had a wild-type p53 gene) was only of borderline significance for clinical outcome, and HPV status remained significant in the multivariate analysis. Several studies reported a decreased rate of p53 mutations among HPV-positivetumours, and HPV-positive tumours appear to express significant amounts of functional (wild) p53, which may contribute to the cellular response to radiation therapy.21 Lindel et al.investigated HPV status and E2 gene integrity as potential prognostic parameters for clinical outcome and prediction of RT response in 40 women with locally advanced cervical cancer treated with curative RT. Their study revealed that HPV is an independent prognostic parameter for outcome and radiation response. In their study, they confirmed a trend for a better clinical outcome for women with HPV-positive cervical cancer with an intact E2 gene. By the fact that E2 protein is able to induce apoptosis independent of other viral proteins, it might be possible to use it as an antitumor agent not only in HPV-positive cancers. They concluded that exact relationship between HPV genes and response to radiation has to be further investigated to classify a special group of cancers not only in the uterine cervix[23]. Nagai et al. Reported the first study in which HPV infection status was examined before, during, and after RT. Their data demonstrated that, in HPV DNA-positive cervical carcinoma, persistence of HPV DNA in the cervix at the end of RT was highly predictive of local recurrence. In their study, sampling for HPV DNA examination was performed every 2-4 weeks during RT and every 3 months after RT. They found that cervical HPV DNA cleared in 42 patients (43.3%) and persisted in 55 patients (56.7%) at the end of RT. Moreover, multivariate statistical analysis showed that persistence of HPV DNA represented an independent and the most powerful prognostic factor24. In conclusion, the excellent clinical performance of the combination strategy with HPV genotyping and cytology is considered a powerful tool for managing the post-treatment follow-up. In our study we could not evaluate post treatment HPV prevalence in all 82 patients during follow up due to social reasons and financial constraints. Due to the constraints in the size of our study post treatment, it was not possible to test the significance of the post treatment HPV prevalence. Large scale studies will provide newer insights, to formulate better methods to meet the future challenges for cervical cancer prevention our efforts will also continue for assessing the response to the treatment. The development of HPV vaccines holds tremendous promise for developing countries Like India, where cervical cancer is the most common malignancy among middle aged women, particularly in rural areas. The availability of HPV vaccine will not only help in curbing the cervical cancer incidence and mortality, but it may also bring down the cost burden of the cervical cancer screening programmes.

Conclusion

HPV was prevalent in 98.6% of patients.More number of patients with multiple genotypes was found. HPV 16 was more prevalent among different genotypes. Single and multiple HPV genotypes play significant role in disease response.HPV 16 did not show any significance in disease response. 80% of patients had persistent HPV DNA post treatment.

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