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Prevalence and distribution of HPV 16 and 18 with its epidemiological profile among cervical cancer patients: A prospective study from regional cancer centre of Gujarat, Western India

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ABSTRACT

Objective: Cervical cancer (CC) is the leading cause of morbidity and mortality due to cancer among women in Indian. This study was conducted to determine the prevalence and distribution of HPV16/ 18 and epidemiological profile of CC patients from, Gujarat Western India.

Materials and Methods: CC biopsy specimens of 400 patients were analyzed for HPV16 and 18 by type-specific PCR.

Results: In our study 74% of CC cases were positive for HPV, 67% had HPV16, 14.8% had HPV18 and 7.7% had both HPV16/18. The median age of the women with CC was 50 years and peak incidence (31.8%) was between 40 to 50 years. Majorities (68.5%) were postmenopausal. The median age of marriage was 20years. Squamos cell carcinoma (SCC) was found in 91% and adenocarcinoma (ADC) in 7.3% of the patients. As per study 7.8%, 16.3%, 58% and 2.5% of CC patients presented in FIGO stage I, II, III, and IV respectively. HPV16 was exhibited in, 68.8% and 66.4%, HPV18 in 14.9% and 15.9% of pre and postmenopausal patients respectively. HPV16 and HPV 18 positivity was 68.7% and 51.7% and 13.2% and 31% in SCC and in ADC respectively. HPV16 infection was higher in stage I and HPV18 in stage IV malignancy.

Conclusion: There were two peaks of HPV16, first between 21 to 30 and second between 51 to 60 years among CC patients. HPV 16 is highly prevalent among all groups. In patients with adenocarcinoma, Stage IV malignancy & who had marriage before 18years, incidence of HPV18 was relatively high.

1. Introduction

CC is the third common cancer among the women in the world.[1] It is the second leading cause of cancer deaths in women in India.[2] Mortality due to CC is higher among woman in India as most of the cases presents in late stages. Current estimates indicate that every year 122, 844 new cases are diagnosed with CC and 67,477 die from the disease.[2]

The main risk factor for development of CC is persistent infection by a high-risk human papilloma virus (HPV) which is one of the common, sexually transmitted infection.[3] The lifetime risk of HPV infection for sexually active men and women is more than 50%. In most women, immune systems eliminate HPV infection spontaneously. In a very small proportion of women, the infection will persist and can initiate precancerous changes in cells. The most common HPV types contributing to CC are HPV 16 & 18. They are also found to be responsible for almost half of vaginal, vulvar, and penile cancers.[4]

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Epidemiology of HPV infection and pattern of HPV DNA genotype distribution are not properly documented in the whole India, which affects the planning and implementation of CC prevention programs**.** There is a wide variation in the prevalence and genotypes distribution of HPV infection in India due to varied socio-economic and geo-climatic condition.[5] Looking to the reality that there are region specific variations in the prevalence of HPV infection, the present study was carried out to analyzed and evaluate the prevalence and distribution of HPV 16 and 18 in cases of CC in Gujarat, Western India.

2. Materials and Methods

This is a prospective research project carried out from 2014 to 2018 at G.C. & R.I, a Regional Cancer Centre in Gujarat. Total of 481 CC patients were enrolled for the study of prevalence of HPV 16 and 18. This study was accepted by Institutional Review Board.

2.1. Inclusion criteria

Patient in whom cervical biopsy was done at our institute for histopathological diagnosis and who did not have any prior anticancer treatment. Those patients of CC whose biopsy was done outside and had histopathology report with slides for review were not included in our study. Informed and written consent was obtained from all the patients and their relatives before enrolling them. Thus, 481 CC patients were enrolled. Part of the cervical biopsy tissue was sent from Gynecologic oncology OPD in PBS containing vial maintaining the cold chain to our cancer biology laboratory for HPV detection. As soon as the tissue samples were obtained by the laboratory, they were washed with phosphate buffer saline (pH 7.4) and immediately stored at -80°C until analysis.

3. Exclusion criteria

Patients with positive viral markers like HIV, HBSAG and HCV. Malignancy not confirmed on cervical biopsy tissue and poor DNA quality. As per above mentioned criteria, 81 patients were excluded. Finally, 400 cases of CC were analyzed. DNA was isolated using commercially available DNA mini kit (Qiagen, USA) following manufacturer's instructions. Type specific PCR was carried out using HPV 16 and 18 specific primers.[6]

The details such as age, menopausal status, number of children & their age were obtained by asking a detailed questionnaire to all the patients and their relatives. The Clinical and pathological details of the patients were collected from their file.

Statastical analysis is calculated by Chi-square test in Medcalc Software, $p \leq 0.05$ was regarded as statistically significant.

4. Results

In our study, total 296/400(74.0%) cases of CC were positive for HPV. HPV 16 was positive in 268(67%), HPV 18 was positive in 59 (14.8%) and both were found in 31 (7.7%) cases (Table 1).

The age range of the CC women was from 21 to 80 years with median age of 50 years. Majority, 233/400(58.3%) were below the age of 50 years and only167/400(41.8%) were above the age of 50 years (Table 2). However on further analysis of CC in various age group, we found that maximum number (59.5%) of patients were between 40 to 60 years of the age. Peak incidence was between 40 to 50 years (31.8%). Only 26.5% were below 40years and 13.5% were above 60 years of age (Figure-1). In the age group of less than 50 years, HPV 16 and 18 infections was exhibited in 67.3% and 15.8% respectively. Age group of more than 50years had HPV16 and18 infection of 66.5% and 13.7% respectively (Table 3). Detailed analysis of HPV infection in various age group showed that HPV 16 infection had two peaks (Figure- 2). One peak was between 21 to 30 years (83.3%) and other between 50 to 60 years (70%). HPV 16 infection was higher in younger age group while HPV 18 increased with age.

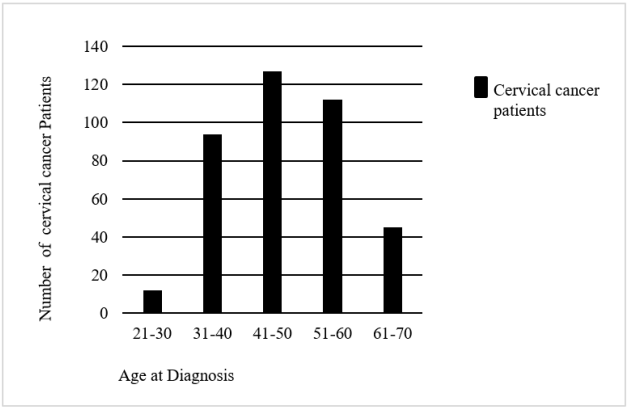

Figure 1: Age wise distribution of CC* patients from Gujarat, Western India Patients were divided into different age group as per the age at the time of diagnosis. Peak incidence of CC was between 40 to 50 years of age group. Source: Gujarat Cancer & Research Institute, India

Majority of the women, 274(68.8%) were postmenopausal as compare to only 167(41.8%) women, who were premenopausal (Table 2). HPV 16 positivity in pre and post-menopausal patients was 68.8% and 66.4% respectively. HPV 18 positivity in pre and post-menopausal patients was 14.9% and 15.8% respectively (Table 3).

In the cohort, the information regarding the age at marriage was available for 213 patients. It ranges from 9-44 years with median age of marriage being 20 years. Though median age of marriage was 20years in our study, we have taken 18 years as cut off because it's the legal age

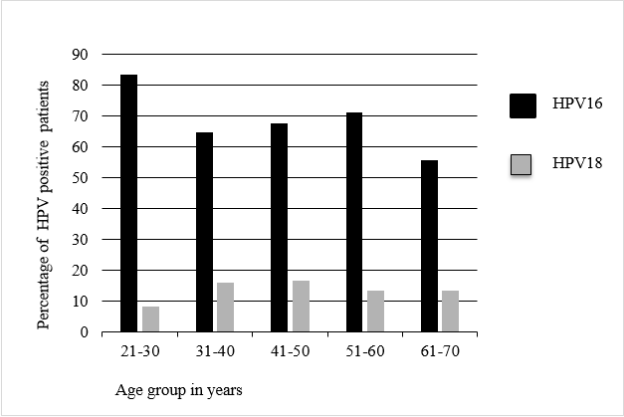

Figure 2: Age wise distribution of HPV16 & HPV18 among CC patients from Gujarat, Western India Footnote: Type specific polymerase chain reaction was carried out using specific primers. Prevalence of HPV16 was high with two peaks between 21 to 30 and 51 to 60 years of age. HPV 18 prevalence was high between 41 to 50 years of age.

of marriage in our country. Of these 213 patients, 67(31.4%) were married before the 18 years of age (child marriage) and 146 (68.5%) were married after 18 years (Table 2). HPV16 positivity in the women who had child marriage and women married after 18 years of age was 61.1% and 69.9% respectively. HPV 18 positivity among the women who had child marriage and women married after 18years of age was 19.4% and 12.3% respectively (Table 3). Thus, HPV 16 positivity was higher in women married at older age group and HPV 18 positivity was higher in women who had child marriage.

Out of 400 patients, 364(91%) had squamous cell carcinoma (SCC), and 29 (7.3%) had adenocarcinoma (ADC). Seven patients had other histopathological variant which are not included in our analysis (Table 2). In SCC patients, 68.7% had HPV 16 and13.2% had HPV 18 infection. Whereas in ADC patients 51.7% were HPV 16 and 31% were HPV 18 positive (Table 3). Notable observation here is that HPV 16 positivity is higher in SCC while HPV 18 positivity is higher in ADC. The difference was statistically significant (p value-0.018).

In this study, 31(7.8%) patients presented with FIGO stage I, 65(16.3%) with stage II, 253(58%) with stage III, and 10 (2.5%) patients with stage IV disease. HPV 16 infection rate was higher in patients with stage I disease, 71% and lower for stage IV disease, 60%. In contrast, HPV 18 infection rate was higher in stage IV patients, 30% and lower in stage I, 12.9% (Table 3).

5. Discussion

Knowledge regarding cause and pathogenesis of CC is expanding rapidly. Persistent infection with one of the

Table 1: Prevalence of HPV 16 and 18 among CC* patients (n=400)

HPV status	Number of cases	Percentage
HPV Positive	296	74
HPV 16 Positive	268	67
HPV 18Positive	59	14.8
HPV 16 & 18 Positive	31	7.7

*Cervical cancer

Table 2: Sociodemographic, clinical and pathological characteristics among CC*patients

Characteristics		No. of Cervical Cancer patients n=400 (%)
Age at diagnosis in years (n=400)	<50	233(58.3)
	>50	167(41.8)
Menopausal status (n=400)	Pre	126(31.5)
	Post	274 (68.8)
Age at marriage (n=213)	<18yrs	67(31.4)
	>18yrs	146(68.5)
Histology (n=400)	SCC**	364(91)
	ADC***	29(7.3)
Stage (n=400)	Stage I	31(7.8)
	Stage II	65(16.3)
	Stage III	232(58)
	StageIV	10(2.5)

*Cervical cancer
**Squamous cell carcinoma
***Adenocarcinoma

fifteen genotypes of high risk HPV causes almost all cases. In our study HPV prevalence is only 74% as we have studied only HPV 16 and18. HPV prevalence increased significantly from 85.9% in studies published from 1990 to 1999 to 92.9% in studies published from 2006 to 2010. Prevalence increased due to analysis of various other high risks HPV and increased sensitivity of polymerase chain reaction to detect broad range of HPV types and multiple infections.

As per our study HPV 16 prevalence is higher than HPV 18, which is similar to reports from the rest of the country. In Kolkata, 59–74% and 2–13.9% of CC patients had HPV 16 & HPV 18 infection respectively.[7] As per the study conducted in South India, rate of HPV 16 and HPV18 infection was 58–69% and 5–19.4% among CC patients respectively.[8] Delhi, other parts of Central and Western India also have similar reports. World literature also showed that HPV16 is the most common type followed by HPV18. The third to eighth most frequently found HPV types were HPV31, 33, 35, 45, 52 and 58 although their relative importance slightly varied by region.[9]

CC is the commonest reason of death due to cancer among female in developing countries. Mortality because of CC is also a sign of the lack of an organized CC screening program. India contributes to 25.4% and 26.5% of the global burden of CC cases and mortality, respectively as per

Table 3: Prevalence and distribution of HPV 16 &18 among CC patients

No. of Cervical Cancer patient	HPV 16positive (%)	p value	HPV 18 positive (%)	p value
<50 (Age at diagnosis) n=233	157(67.3)	0.83	37(15.8)	0.56
>50 (Age at diagnosis) n=167	111 (66.5)		22 (13.7)	
Premenopausal n=126	86(68.8)	0.63	41(14.9)	0.82
Postmenopausal n=274	182(66.4)		37(15.8)	
<18yrs (Age at marriage) n=67	41(61.1)	0.55	13(19.4)	0.36
>18yrs (Age at marriage) n=146	102(69.9)		18(12.3)	
SCC* n=364	250(68.7)	0.095	48(13.2)	0.018
ADC** n=29	15(51.7)		9(31.0)	
Stage I n=31	22(71.0)		4(12.09)	
Stage II n=65	45(69.2)	0.90	11(16.9)	0.42
Stage III n=232	155(66.8)		30(12.9)	
Stage IV n=10	6(60)		3(30)	

Kawana et al.[10] Therefore, there is a need for population-based interventions in India to reduce the overall burden of CC globally.[11] In the present study, we aim to focus on the epidemiology of CC in Gujarat, West India and investigate HPV DNA prevalence and correlate its status with epidemiological, clinical and pathological parameters of CC.

The mean age of the CC patients was 50 years in our study. Majority of women were between fourth and sixth decade of life. Peak incidence of CC was between 40 to 50 years (31.8%), followed by 50 to 60 years (27.8%). In our study, 68.8% of patients were postmenopausal. Incidence of CC was 13.3% after the age of 60year, showing the risk of CC even in elderly women (Figure-1). This data is in accordance with many studies conducted in India. A study conducted by Kumari et al. in a tertiary center in Bihar showed median age of 51 years among the CC patients and majority were between 40 to 60 years.[12]In another study by Dutta S. et al., maximum number of CC were between the fourth and fifth decade of life and majority were postmenopausal.[13]

CC has two peak age incidence, first peak around 35 years and second around 55 years. The peak age for CC is 55–59 years in India as per many studies.[2,14] In contrast, our study showed peak incidence a decade earlier.

In perimenopausal women, periods become irregular. Most women assume this abnormal bleeding as a normal consequence before menopause, which actually may be a sign of CC and do not take any measures for it. As symptoms of CC are nonspecific, most of these women report very late. Because of lack of awareness, illiteracy and social stigma, many of the women have reluctance for routine Gynecology checkup and Pap's smear. Hence, large number of cases are diagnosed in advanced stage. This explains the higher incidence (58%) of Stage III CC in our study. Secondary prevention by Pap's smear creates some problem in postmenopausal women because of factors like, flushed cervix with the vagina and receded squamocolumnar junction inside the endocervicalcanal.[15]

The information regarding the age at marriage was available for 213(53.2%) of the CC cohort. Most of our patient did not know their age at marriage. It is calculated here from their present age and the age of their children whenever it was known exactly. The data showed that 31.4% of the women had child marriage. Though the child marriages have dropped drastically in last decade, it is not that infrequent in rural India. According to the Census data 2011, around 102.61 million women (roughly a sixth of India's female population) were married before they had turned 1.[16] Young age at first sexual intercourse (AFSI) is an important risk factor for cervical cancer. Age at first marriage (AFM), AFSI and age at first Pregnancy (AFP) are highly interrelated and has similar CC risk estimates. The increased risk of CC in women with early AFSI may be due to an increased vulnerability of the immature cervix to persistent HPV infection. Early childbearing has been linked to increased risk due to the cervical trauma experienced during early AFP or subsequently, by high-parity births.[17] The importance of HPV-vaccination programmes targeting young adolescents before first sexual intercourse can have a great effect in decreasing the incidence of cervical cancer in country like India where early AFM and early AFP is still prevalent.[18]

In this study, most patients had SCC (91%), and very few (7.2%) had ADC. The percentage of cases diagnosed as SCC, ADC and unspecified histology varied from to region. ADC ranges from 3.8% in Western/Central Asia up to 23.7% in North America. Although HPV is detected in more than 90% of SCC of the cervix, the presence of it in ADC varies, from 32% to 100%, depending on the detection method used.[19,20] Overall HPV positivity was significantly higher among SCC than in ADC. However, HPV18 positivity was exceptionally high in ADC (36.8%) compared to SCC (13.2%) as per analysis by Ni Li et al.[20] Our study also showed similar finding where HPV 16 positivity was higher in SCC while HPV 18 positivity was higher in ADC (p value 0.018). The columnar tissue, giving rise to ADC is less accessible and susceptible

to HPV infections than the squamous tissue of SCC. The establishment of ADC may require a relatively more aggressive infection like HPV 18.[21]

Very few patients in our study presented at stageI. Most of them (more than 60%) presented in advanced stage which explains the high rate of morbidity and mortality due to CC in our country compare to world stastics. HPV 16 infection is more prevalent in stage I and rate of HPV 18 increases as stage advances.

A study in Guwahati suggested that advanced stage (III-IV) of CC had increased risk of single and multiple HPV types as compared to the early stage (I-II).[22]

HPV are double-stranded DNA viruses that have a definite preference for squamous epithelium of skin and mucosa. The immune response to natural infection with HPV is slow and weak because of various reasons like absence of viremia, localized infection and lack of activation of Langhans cells. Thus, only 50% of female infected with HPV develops antibodies. It is not certain that these antibodies remain protective against re infection with the same HPV type. All these factors led to development of prophylactic vaccines.[9] Vaccine induces a strong, protective immune response which is much long lasting as compared to natural infection.[23] It is now well accepted in many of the countries and has been included in immunization program. In spite of the availability of HPV vaccines and effective methods for early detection and treatment of precursor lesions, CC still remains a major public health problem in India. Majority of the cases are detected in advanced stage due to lack of organized mass-screening program for early detection of CC.

Strength of our study is that, it is a prospective study with large number of cases from a regional cancer centre. Limitation of our study is that we have studied only HPV 16 &18. Other high risk HPV like 31, 33, 35, 45, 52 and 58 are not evaluated. Another limitation is data regarding age at marriage. It was very difficult to elicit the exact age of marriage due to illiteracy and ignorance in our patients.

In our study, majority of CC patients were postmenopausal and diagnosed at advanced stage of disease. Hence, CC screening should also be continued in postmenopausal and elderly female to detect cc early. So that preventive as well as curative treatment could be given to decrease morbidity and mortality. Our results suggest that HPV 16 and 18 infections are vastly prevalent in the CC patients of Gujarat, Western India. These results will be useful in establishing the future guidelines for reducing the risk of CC with the help of screening programs and by providing proper vaccines targeting HPV16 and 18.

6. Source of Funding

None.

7. Conflict of Interest

None.

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