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Original Research Article

Prevalence of High Risk Human Papillomavirus (HPV) Type 16 and Type 18 in Carcinoma of Cervix Patients in a Tertiary Care Hospital in Western Rajasthan

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Abstract

Introduction: Cervical carcinoma is one of the commonest cancers of female anogenital tract and the leading cause of morbidity and mortality. It is the second most common cancer among women in this country. The major risk factor is persistent infection of Human papilloma virus (HPV) infection, High risk HPV 16 and 18 confer a greater risk of having cervical cancer than other genotypes.

Aim: To find out the prevalence of high risk type HPV 16 and 18 infections in carcinoma of cervix 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. On the other hand 16 (8%) patients were infected with both HPV type 16 and HPV 18.

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

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

Introduction

Cervical cancer is one of the commonest cancers of female anogenital tract and the leading cause of morbidity and mortality. [1] It is the fourth most common type of

cancer in women worldwide and serious Global Public health problem with 604,127 new cases and 341,831 deaths reported in 2020. Burden of cervical cancer is high in low and middle-income developing countries where 83% of all new cases and 85% of cervical cancer death occurs. [2] In Indian scenario, Cervical cancer is the second most common malignancy in women aged from 15 to 44 years accounts for 14% of all female cancer cases. [3] India accounts for one quarter of the worldwide burden of cervical cancer. It has a population of 483.5 million women ages 15 years and older who are at risk of developing cervical cancer. [4] The incidence of cervical cancer in India accounts for 23.3% of all newly diagnosed cases and almost 25% of all deaths caused by the disease worldwide. [5] In spite being curable cervical cancer has taken a devastating form in India therefore it is vital to understand the epidemiology of cervical cancer in India. Geographical variation in occurrence of cervical cancer is related to social and economic conditions as well as to religion and the influences of this factor on sexual practices. [6]

The cervical cancer initiates on the "transformation zone" squamocolumnar junction of cervix between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix, a region that face continuous metaplastic changes throughout women's lifecycle. [7] Histological subtypes of cervical carcinoma include Squamous Cell Carcinoma (70%), Adenocarcinoma (20%) and other unspecified like Adenosquamous carcinoma, small cell carcinoma accounts for 5%. Squamous cell carcinoma (SCC) is preceded by well-recognized epithelial alterations, the precancerous lesions which progress through a variety of grades: cervical intraepithelial neoplasia (CIN) grades I to III; or low-grade squamous intraepithelial lesions (LSIL) to high-grade SIL; or mild, moderate, or severe dysplasia leading to carcinoma-in-situ (CIS) [8] .The predominant risk factor for Cervical intraepithelial neoplasia and cervical cancer is persistent infection of sexually transmitted Human Papillomavirus (HPV). An HPV infection remains inert at its initial phase and takes 10-20 years to develop cancer. [7] Persistent HPV infection is essential but not sufficient for development of cervical cancer. HPV is a DNA virus of papillomaviridae family , its genome encoding eight genes performing early (E) and late (L) functioning in the life cycle. Proteins encoded by early genes E6 and E7 have oncogenic properties required for malignant transformation of cervical squamous epithelial cells. [9] According to their transforming capacities, they are subdivided in two categories the high risk and low risk HPV types. HPVs causing malignant transformation are labeled as high risk types and those causing benign transformation included in the low risk group. Fifteen high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) are directly associated with human cervical and other carcinomas.5 About 7.9% of women in the general population are estimated to harbour cervical HPV infection at a given time, and 82.5% of invasive cervical cancers are attributed to HPVs 16 or 18. In India, HPV type 16 alone in cervical cancer is 70-90% while occurrence of HPV type 18 varies from 3 to 20%. [10] Owing to strong association between HPV and cervical cancer, HPV DNA detection can be used for early detection and screening of this disease.

Detection and diagnosis for HPV with conventional methods was difficult until the advent of molecular methods, particularly amplification technology such as Polymerase Chain Reaction (PCR). PCR is highly sensitive and specific technique that allows detection of even low-level virus copy numbers in clinical samples. Early detection can help to

prioritize the high-risk patients and help to either prevent the disease or improve the prognosis and efficacy of treatment. Because geographical variation in type distributions may exist, knowledge about the distribution of HPV types in cervical cancers and HPV types circulating in the communities in different regions of India would be useful in devising the optimum strategy for vaccination. Thus, the present study was aimed to assess the prevalence of HPV Type 16 and Type 18 among cervical carcinoma patients.

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. After taking consent from the patient fresh biopsy tissue will be collected by trained medical professional at Acharya Tulsi Regional Institute of Cancer Research, Bikaner. The collected sample was stored at - 20° C until further use. Genomic DNA from cervical cancer tissue was isolated by TRUPCR tissue DNA extraction kit by 3Bblackbio Biotech India as per manufacturer's instruction. 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. 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, mean age of cases was 50.28 ± 10.01 Years. Out of total 200 cases 180 were married and 20 were single. Most of the cases belongs from rural background (119/200; 59.5%) and middle socioeconomic status (99/200: 49.5%). Out of total 200 cases around 57.5% were illiterate or had primary education. Menstrual status of cases showed that out of 200 cases, 138(69%) were postmenopausal and 62(31%) were premenopausal. Parity of 3 or more were observed in 70% of cases. Clinical features showed that III stage cancer was found in 48.55 cases followed by stage II in 37.55 cases.(Table 1).

Table 1 : Baseline Demographic Data of Cervical Carcinoma Patients

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%
Marital Status		
Unmarried	20	10%
Married	180	90%
Parity		
Nil	24	12%
1-2	36	18
More than equal to 3	140	70%
Cancer Stage		
I	8	4%
II	75	37.5%
III	97	48.5%
IV	20	10%

Out of total 200 cases, 179 cases were HPV positive which was 89.5% and 21 cases (10.5%) tested negative for HPV. 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.(Table : 2).

Table 2 : Prevalence of HPV among Cervical Carcinoma Patients

HPV Status	HPV		HPV 16		HPV 18		Both HPV 16 and 18	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)
Positive	179	89.5 %	152	76 %	43	21.5%	16	8 %
Negative	21	10.5 %	48	24 %	157	78.5%	184	92 %
Total	200	100 %	200	100 %	200	100 %	200	100 %

Discussion

Cervical cancer is an important cause of mortality and morbidity worldwide, more so in developing countries. Carcinoma of cervix is both a preventable and curable disease. The disease is preventable because the pre invasive stage can be detected by screening. It is curable because it can be cured if diagnosed at early stage before its progression to invasive carcinoma [11]. Human papilloma virus is recognized as one of the leading cause and is associated with approximately 90% of cases. It is thus extremely imperative to assess the status of HPV infection and genotype for prevention of cervical cancer in India. However due to extensive socio- cultural diversity of India the prevalence of different infectious agents vary significantly in different parts of India. Same is true for HPV also as its prevalence varies greatly across India among asymptomatic [12] Out of total 200 cases, 179 cases (89.5%) tested positive for HPV and 21 cases (10.5%) tested negative. This result was within the range of other published findings by SS Kulkarni in 2011, which represent 96.7% cases are positive for HPV [13] and study conducted by Pavani et.al represented 87.8% cases positive for HPV [14]. Similarly Das M et.al. [5] also reported 94.8% cases tested positive for HPV. In present study prevalence of HPV 16 was 76% as

152/200 cases tested positive ,HPV 18 was found in 43 cases which represents 21.5% of study population. In 16 patients (8%) co-infection with both type16 and type 18 was found. Thus, HPV16 is most prevalent type in cervical carcinoma patients. In accordance with the findings of our study Pavani et.al [14] reported prevalence of HPV 16 (66.7%), HPV18 (19.4%) and a study conducted by Kumar R [15] in Bihar in 2020 reported prevalence of HPV 16(77.08%), HPV 18 (16.67%)and Co-infection of Both HPVtype 16 & 18 (6.25%). Similarly another study from Mumbai reported HPV prevalence statistics which broadly matched the existing literature, HPV 16 and HPV 18 accounted for the bulk of the HPV infections followed by HPV 33, 31, and 45 [16]. Similar HPV type distribution has been reported from other countries (Wu et al [17] 2010, Menegazzi et.al [18](2009). However there was variation in the ranking of prevalence of HPV types. Results of our study differs slightly from that of the study in a rural area in Dindugal, Tamil Nadu district, where the major types were HPV 16 and 56, followed by HPV 31, 33, and HPV 18 [19]. HPV 16 appear to be believed the most prevalent type of HPV in cervical cancer, worldwide and in India, although with minor regional differences. The prevalence of HPV-16 over other types may be related to the complex geographical and biological interplay between virus and host immunogenetic factors. [20] Having discussed above, the results of present and previous studies, it can be observed that High risk HPV 16 & HPV 18 are associated with majority of cervical carcinoma cases, out of them HPV type 16 was more prevalent. The results also indicates that available vaccines against high risk HPV types 16 and 18 can be applied to reduce cervical cancer burden in India.

Conclusion

Outcomes of the study suggest that the incidence of HPV in cervical cancer patients is high and strongly supports HPV as a causative factor in the development of cervical cancer. The infection of HPV 16 and HPV 18 found to be most prevalent cervical carcinoma patients. The results indicate that available vaccines against high risk HPV types 16 and 18 can be applied to reduce cervical cancer burden in India. The results of present study would help planning an appropriate strategy for disease monitoring and also offers the baseline data for future research and post vaccination surveillance in the region.

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