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Detection of Human Papilloma Virus DNA and its Genotypes in Cancer Cervix by using Polymerase Chain Reaction and its Correlation with Histopathology

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Abstract

Introduction: carcinoma cervix is the fifth most common female cancer worldwide as well as in India. Human Papilloma Virus (HPV) is the cause of this cancer in western world whereas there are other causes which are more common in causing this cancer in Indian women. In order to see the association of HPV with Indian cancer cervix patients we conducted a pilot study to see HPV and its common genotype in cancer cervix patients. *Material and Methods:* We took 52 cancer cervix cases that presented to this hospital and tested the biopsy tissue for HPV and its genotype along with the histopathological examination. *Results:* 44 patients of squamous cell carcinoma showed presence of HPV with strains 16 and 18 in 81% cases either alone or together. On history it was seen that most of these patients did not use barrier contraceptive which may protect from viral infection. *Conclusion:* HPV 16 and 18 are commonly association with squamous cell carcinoma cervix along with other causative factors common in India. More studies are required to establish the prophylactic use of HPV vaccination in Indian girls which may help in preventing cancer cervix in some cases.

Keywords: HPV; Cervix; Causes; Vaccination.

Introduction

Cancer cervix is the fifth most common cancer in all and it ranks second in women worldwide and is a major cause of death among Indian women. 80% of cervical cancer occurs in the developing countries including India. Among middle aged Indian women about 1, 32,000 new cases and 74,000 deaths occur due of to cervical cancer every year [1].

Human Pappilloma virus(HPV) is one of the most common sexually transmitted viral disease associated with development of cervical intra epithelial neoplasia and invasive cervical cancer [2]. Estimates suggest that more than 80% of sexually active women acquire genital HPV by age of 50 years [3]. Based on their association with cervical cancer and their precursor lesions, HPV can be grouped in low and high risk. Low risk HPV types include types 6,11,42, 43 and 44 where as types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70 are high risk types [4]. At any given time 6.6% of Indian women harbour cervical HPV infection and serotypes 16 and 18 account for nearly 76.7% [1]. Worldwide HPV 16 and 18 contribute over 70% of all cervical cancer cases, the most prevalent being HPV-16 in at least 50-60% and 18 in 10-12% cases.

Considering the magnitude of burden of cervical cancer in India there are very few Indian studies sharing prevalence on type distribution of HPV. So we started a pilot study to see association of HPV strains 16 and 18 with patients suffering from cancer cervix which may show the prevalence and distribution of high risk oncogenic types in local population.

Material and Methods

52 patients from January 2012 to December 2013 who presented in gynaecological and cancer outpatient department with bleeding per vaginum

were included in the study. A detailed history including age, religion residential area, literacy, contraceptive use, number of pregnancies, age at first intercourse and number of sexual partners was taken. After clinical examination and staging of the patient a punch biopsy from the growth was taken for histopathological confirmation and testing for high risk oncogenic variety HPV-DNA by PCR was taken. The tissue was sent in a sterile container containing 5ml of buffer provided with the Biotypop Kit for HPV-DNA detection. A piece of biopsy tissue was sent in formalin container for histopathological examination. The piece in the buffer was transported to the lab in ice packs as per guidelines for preservation of tissue. In the lab buffer was warmed to 55 degree centigrade for extraction and then amplification of DNA as per kit manufacturer instructions [5] and then the analysis of the amplified DNA products was done by gel electrophoresis.

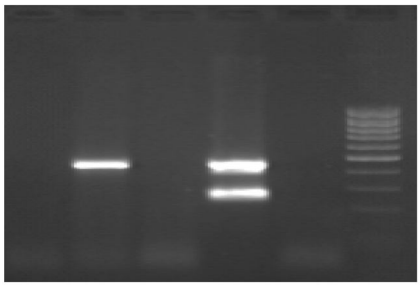
Table 1: Distribution of presence of Human Pappiloma Virus

Hpv dna pcr	Dysplasia	Squamous carcinoma	Adeno carcinoma	Total
Positive	0	44	0	44(84.6%)
Negative	2	4	2	8(15.3%)
Total	2	48	2	52

Table 2: Distribution of high risk HPV genotype

High risk HPV DNA genotypes	Positivity	Percentage
HPV-16	26	50
HPV-18	8	15.38
HPV-33	1	1.92
HPV-62	1	1.92
HPV-16,18	8	15.38
Negative	8	15.38
Total	52	100

Fig. 1:



Results

42 patients were between 36 years to 65 years of age and only 3 patients were less than 35 years. 75% of patients were illiterate from rural area with a lower socio economic background. It was seen that 61.3% females never used any contraceptive methods during intercourse and few had more than one sex partners due to remarriage. Regional and religious correlation could not be done due to small sample size. Histologically 92.3% cases had invasive squamous cell carcinoma, 3.8% had adenocarcinoma and 1.92% each had mild dysplasia and severe dysplasia.

84.6% patients showed positive high risk HPV-DNA and only 8 patients showed negative test for HPV (Table 1). All 44 patients with positive test had invasive squamous cell carcinoma with a p value of 0.0001. On analysing different serotypes, HPV-16 was most commonly seen in 50% , HPV-18 15.38% and Both 16 and 18 were seen in 15.38% (Table 2).

Discussion

Cervical cancer is one of the most common malignancies and is the major cause of cancer mortality among Indian women. Human papilloma virus (HPV) is one of the most common causes of sexually transmitted disease in both men and women worldwide and is thought to be the most common sexually transmitted viral disease in the United States. As a major public health problem, more than 80% of the cervical cancer cases occur in the developing countries [6]. WHO estimates that the contribution of cervical cancer to adult female death is 35% [7]. India's cervical cancer age-standardized incidence rate (30.7 per 100,000) and age-standardized mortality rate (17.4 per 100,000) are the highest in South-Central Asia [8].

Murthy et al state that the age specific incidence rates (ASIR) for cervical cancer revealed that the disease increases from age group 35 and reaches a peak between 55-64 years. WHO/ICO information centre on HPV and cervical cancer (HPV Information Centre) in their summary Report 2010 [9] report similar findings of increasing trend in the age wise incidence of cervical cancer among Indian women from third decade onward with a peak at 60-65 years age group. Similar age incidence is seen in our study group also and 73% cases are from rural area. Pandey and Bhogliwal [10] also showed that chances of developing CIN among the hospital attending women from rural areas are 3.78 times as compared to healthy rural women not attending hospital. As per Indian cancer registry highest incidence of cancer cervix is seen in Barshi (37%) which is a rural area in Maharashtra from a low socio economic status whereas it is 30.7% in Chennai, a urban areas in Tamil Nadu.The difference in incidence between the urban and rural areas i.e. provides hints towards the differences in the lifestyles of the urban and rural areas. The factors which confound in urban areas may be the presence of affluent society, mixed ethnic population and availability of abundant water supply for personal hygiene. Most of the population in the rural area are ignorant about cancer due to illiteracy and lack of proper awareness programmes. Similar illiteracy rates and poor socioeconomic class has been seen in our series also.

In this study out of 52 cases, 33(61.36%) patients did not use any form of contraception. Tubal ligation was the most use methods of contraception with16 (31.81%) patients. There are 31 cases of invasive malignancies out of the total 52 which were mainly non contraceptive users lacking the protective barrier from HPV. Mohanty et al [11] showed that in tubal ligation users the incidence of cervical cancer was 80% whereas in oral contraceptives users it was only 10%.No malignancy was detected in barrier contraception and intra uterine contraceptive users. Use of oral contraception for long term was associated with higher rates of cervical cancer as per the Oxford family planning association but it is not the oral contraception rather it is sexual behaviour that might increase the risk of development of cervical cancer. Hammouda et al [12] conclude that oral contraceptive use was unrelated to the development of cervical cancer.

As with the existing knowledge that high risk HPV is sexually transmitted and barrier contraceptives may offer partial protection against sexually transmitted high risk HPV and subsequent development of cervical cancer. Condom will protect only the covered areas of the external genitalia however the uncovered areas remain exposed to the risk of HPV viral transmission. Cavalcanti et al [13] found that oral contraception was not significant as a risk factor.

The relative type prevalence seems to fluctuate worldwide. Despite the high incidence of cervical cancer reported from India, large scale population based on the HPV prevalence and genotype distribution are very few. Sowjanya et al [4] studied the genotype distribution of high risk HPV types among the rural women who were attending regional cancer centre in Hyderabad. Among the 41 high risks HPV positive cervical cancer the overall type distribution of the major high risk 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%), HPV 59 (2.8%) and HPV 73(2.8%).

The prevalence of high risk HPV in cervicovaginal samples in women attending a cervical cancer screening pilot study conducted in southern state of Andhra Pradesh. The most frequently detected HPV types in the Medchal community are HPV 52 and 16.This is slightly different from study by Franceschi et al [14] in Dindigul district, Tamil Nadu where the prevalence of HPV of any type was 16.9%, but it varied between 14.0 among cytologically normal women and 73.9 % among those with cytological abnormalities. The corresponding proportion age standardised to the world population were 17.7% overall, 15.2% among those without and 64.9% among those with cytological abnormalities. In total, 250 women had single type and 70 had multiple type infection. High risk HPV types were subsequently more frequent (12.5% of all women)than low risk types(6.0%).Most commonly found in either single or multiple type infections were HPV 16(3.8%),HPV 42(2.2%),HPV 56(1.5%),HPV 31(1.2%),HPV 33(1.2%) and HPV 18(1.0%).High risk types were found in 70.7% of women with cytological abnormalities and in 17 out of 20 (85.0%)women with moderate or severe dyskaryosis.

Gilham et al (15) found an overall prevalence of HPV in two communities near Trivandrum, in Southern India. The overall prevalence of HR-HPV in this south Indian population was 3.9%. HPV 16 was the commonest type, accounting for 47% of the high risk HPV infections, either alone or with another type. The next most common type was HPV33, accounting for 10%. A cross-sectional biopsy study from 100 patients from South India and 30 patients from East India showed 60 % HPV16 infection,14% HPV 18 infection as the most frequent HPV types. This constitutes only 70 % of the total cases, 16 other

types were identified –HPV 26, 31, 33, 35, 42, 45, 51, 52, 53, 56, 61, 62, 64, 81 and 82.

A hospital based study in New Delhi, North India, found that, in 106 invasive carcinoma cases found that HPV 16 type was the commonest type seen in 73.6% of the cases ,followed by HPV 18(14.2%) and HPV 45(11.3%).(16)In our study HPV 16 was found to be most prevalent type in invasive carcinoma(50%), followed by HPV 18(15.3%), HPV 33 (1.9%), HPV 62(1.9%).Infection with the mixed types was seen in 15.3%. These findings are similar to all the other Indian studies as described earlier. In a hospital based study at Peru, Santos et al [17] found HPV in 93.65% in patients of cervical carcinoma. HPV 16 was seen in 83.92% patients, HPV 18 in 15.3% HPV 31 in 8.6%, HPV 35 in 0.6%, HPV 45 in 1.2%, HPV 52 in 6.2%. Infection by multiple types was seen in 11.1%.

A study conducted by Odida et al [18] in Uganda HPV -DNA was detected in 114 out of 186 invasive cervical cancer samples ,giving an overall HPV prevalence of 61.3% of the samples HPV 16 in 51 patients (44.7%). HPV 18 in 33 patients (28.8%), HPV 31 in 4 patients(3.5%), HPV 35 in 2 patients(1.8%), HPV 45 in 11 patients(9.6%), HPV 52 in 1 patient (0.9%). Infection with multiple types was seen in 3.6% cases. International studies also shows the same prevalence as our Indian studies showing the high risk HPV 16 and 18 being most commonly associated with cancer cervix. High risk genotypes like HPV 35, 52, 56 common in other parts of the country were not detected in our study.

These findings form a baseline for epidemiology of HPV in cervical cancer in our region. Additionally they represent the variation of high risk HPV according to geographic distribution. Knowledge of local genotypes helps to choose correct combination of HPV genotypes for vaccine production as well as inclusion in diagnostic kits.

Lack of uniform strategy which is able to identify at risk female population also limits the optimum management protocols. Although screening tests are available they suffer from variable sensitivity and specificity as well as the need for repeated testing.

Knowledge of the distribution of genotypes as per the geographic variation is important as to identify the vaccine with correct mix of HPV genotypes to be administered to the local population. The current data on the basis of which the vaccine has been marketed is mostly on the basis of large scale studies but on foreign population with a different ethnic and social profile.

Additionally, our study provides a baseline study before the HPV vaccination is initiated on a mass scale. This knowledge is important to monitor trends in distribution of high risk HPV in the post vaccination era on a broader aspect as well as on an individual case to case basis.

Before and after testing of patients for high risk HPV DNA following treatment of cancer cervix to detect the relapse or recurrence at much earlier period as compared to routine cytology or histology. Many studies of cervical disease, both cross sectional and prospective, have shown that specific HPV types predict the risk of progression to the high grade cervical intraepithelial neoplasia.

Genotyping could potentially provide information on individual risk stratification, therapeutic decisions, epidemiological studies and vaccine development. Current commercially available tests have been developed to detect the epidemiological studies that included people from all over the world. Adaptation of the assays to include HPV types according to their geographical distribution should be considered as a means of increasing test specificity.

Conclusion

Cervical cancer screening is now entering a new era in which we will increasingly rely on oncogenic HPV detection rather than the pleomorphic cellular changes caused by the infection. As we move from cytology based screening to HPV based screening, genotyping may prove useful in stratifying HPV positive women according to the risk of precancer and cancer to determine the appropriate clinical management strategy. However to achieve benefit to patients, the addition of HPV genotyping to cervical cancer screening must not be abused by excessive referrals to colposcopy and over treatment. Further more studies are required to establish the use of prophylactic vaccination against HPV which may help in preventing some of the cancer cervix cases as most of the patient show association of HPV.

Refrences

1. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). Summary report on HPV and cervical cancer statistics in India 2007. [Last Assessed on 2008 May 1]. Available from: http://www.who.int/hpvcentre .
2. Schiffman M, Castle PE, Jeronim J, Rodrigue AC, Wacholde S. Human papillomavirus and cervical cancer. Lancet. 2007; 370: 890–907.
3. Singh N. HPV and Cervical cancer - prospects for prevention through vaccination. Indian J Med

Paediatr Oncol. 2005; 26: 20–3.

4 Sowjanya AP,Jain M,Poli RU,Padma S,Das M,Shah KV.Prevalence and distribution of high risk human papilloma virus (HPV) types in invasive squamous cell carcinoma of the squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh ,India .BMC Infectious Diseases 2005; 5: 116-19.

5 Sarkar M, Konar H, Raut DK.Gynecological Malignancies: Epidemiological Characteristics of the Patients in a Tertiary Care Hospital in India.2012; 13: 6-2997.

6 Hubbard R.HPV Testing Methods.Arch Pathol Lab Med August 2003; 127: 940-46.

7 Ayinde OA, Omigbodun AO, Ilesanmi AO. Awareness of Cervical Cancer, Papanicolaou's Smear and Its Utilisation among Female Undergraduates in Ibadan. *Afr J Reprod Health* 2004; 8: 68-80.

8 Ferlay J, Bray F, Pisani P, Parkin DM . GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide 2004 IARC Cancer Base No. 5, version 2.0. Lyon: IARC Press.

9 WHO / ICO Information Centre on HPV and cervical Cancer (HPV information Centre).Human Papilloma virus and Related Cancers in India.Summary report 2010.

10 Pandey K and Bhogliwal in 2005 evaluated the influence of different risk factors for developing cervical carcinoma and identified that target population for mass surveillance.

11 Mohanty J,Mohanty BK.Risk factors for invasive carcinoma of cervix.Journal Obstet and Gynaecol India 2001; 21: 403-06.

12 Hammouda D,Munoz N,Herrero R,Arslan A,Bouhadef A,Oublil M,et al.Cervical carcinoma in Algeria :human papillomavirus and lifestyle risk factors.Int J Cancer.2005; 20: 483.

13 Cavalcanti SM,Deus FC,Zardo LG,Frugulhetti IC,Oliveira LH.Human papillomavirus infection and cervical cancer in Brazil:a retrospective study.Mem Inst Oswaldo Cruz.1996 Jul-Aug; 91(4): 433-40.

14 Franceschi S,Rajkumar T,Vaccarella S,Gajalakshmi V,Sharmila A,Snilders PJ,et al Huaman papillomavirus and risk factors for cervical cancer in Chennai,India:a case-control study.Int J Cancer 2003; 107: 127-133.

15 Gilham C,Varghese C,Gibson I,Peto J.HPV infection in a population based study in Kerala,India.23rd International Papilloma Virus conference and clinical workshop.2006.

16 Datta P,Patro RK,Bhatla N,Singh N.Prevalence of human papillomavirus infection in a cohort of young women in Delhi,India.2013; 33: 12-7.

17 Santos C,Munoz N,Klug S,Almonte M,Guerrero I,Alvarez M et al.HPV types and cofactors causing cervical cancer in Peru.British Journal of Cancer 2001; 85: 966-71.

18 Odida M,Sanjose S,Quint W,Bosch X,Klaustermeier J.Weiderpass E.Human papillomavirus type distribution in invasive cervical cancer in Uganda.BMC Infectious Diseases 2008; 8: 85.