## Original Article

# Frequency of high risk human papillomavirus types in squamous cell carcinoma of cervix among women

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## Abstract

**Objective:** To determine the frequency of high-risk subtypes of Human papillomavirus (HPV) 16, 18, 31 and 45 in squamous cell carcinoma of cervix (SCC) among women.

**Methods:** A descriptive study was conducted in the department of Molecular Genetics Laboratory, Ziauddin University Hospital, North Nazimabad, Karachi, Pakistan. A total of fifty formalin-fixed paraffin embedded samples (blocks) of SCC of cervix were collected from two Pathology laboratories through convenience sampling. These blocks were analyzed for presence of HPV and its subtype. DNA was extracted by QIAamp DNA Kit and amplification was done by Polymerase chain reaction (PCR). General primers were used for HPV DNA and HPV genotyping for 16, 18, 31 and 45 was done by using a standard kit.

**Results:** HPV was detected in nine (18%) out of fifty paraffin embedded tissues of squamous cell carcinoma of cervix. Out of the 9 cases that were positive, five (55.6%) were infected with HPV16, while in the rest of the positive samples, the genotype could not be identified in four (44.4%). HPV was not determined in majority (82%) of the samples.

**Conclusion:** This study concluded that HPV might not be the major cause of SCC of cervix. There could be other causes among women leading to prevalent HPV types that the study did not look into due to limitations (JPMA 60:193; 2010).

## Introduction

Cervical cancer is the second most common cancer in women throughout the world after breast cancer.[1] In developing countries, approximately 500,000 new cases of cervical cancer are diagnosed annually with an increase in incidence by 10-folds and 250,000 deaths per annum.[1,2] According to the American Cancer Society, it was estimated that in the US 11,070 new cases and 3870 deaths due to cervical cancer have been reported in 2008.[3] It is claimed to be a multifactorial disease in which several environmental and genetic factors play an important role.[4,7] Human Papillomavirus is considered to be the most important risk factor in the development of cervical cancer[8] and sexual transmission is the predominant route of HPV infection.[9] Transitional zone of cervix is the most common site of cervical cancer and it is most susceptible to the carcinogenicity of HPV. More than 100 subtypes of HPV have been detected by different techniques like PCR.[10] HPV is classified into its subtypes on the basis of their oncogenic potential into high risk subtypes and low risk subtypes. The high risk subtypes are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 while low risk subtypes are 6, 11, 40, 42, 44, 54, 61, 70, 72, 81.[11] Early (E) proteins, particularly E6 and E7, of high risk subtypes of HPV 16 and 18, play an important role in the development of cervical cancer. E6 inactivates p53 gene and E7 inactivates Rb (Retinoblastoma) gene, these are tumour suppressor genes, inactivation of these genes causes uncontrolled proliferation of cells.[12]

Many studies have been done on the prevalence of HPV in cervical cancer and its type-specific distribution in the world. These studies have shown that high-risk subtypes of HPV (particularly HPV 16 and 18) are the most common among squamous cell carcinoma of cervix.[13,14] Evidences showed that the prevalence of HPV 16 and 18 in cervical cancer are high in the Western countries and China.[15,16] In Asia, a comparative data indicated that HPV 16 and 18 are more prevalent in India, Srilanka and Bangladesh,[17] additionally this study concluded that HPV-16 and 18 were

detected in 80% of patients with cervical cancer in India. Increased development of cervical cancer has been associated with other risk factors, which are responsible for interaction between host and virus which increases the susceptibility of HPV infection. These factors are early age of sexual contact, multiple sexual partners, increased parity[7] poor sexual hygiene, prolonged use of oral contraceptives,[4,5] smoking[6] and altered immune status of the patients.[7] In resource constraint settings, low coverage of Papanicolaou (Pap) smears and limited contact with the healthcare system, poor laboratory services, and lack of follow up for abnormal Pap smear results can be regarded as additional factors.

In Pakistan, the epidemiological data regarding common malignant tumours among males and females have been collected by different institutes at national level. This data revealed that between 1960-1964, cervical cancer was the second most common malignant tumour, it became the 5th most common between 1977-1988.[18] Another study showed that from1992 to 2001, it became the 9th most common malignancy among females.[19] Karachi Cancer Registry (KCR) and Jinnah Postgraduate Medical Centre (JPMC) have collected local data, which showed that cervical cancer is the 3rd and 4th most common malignancy among females, respectively.[20] Unfortunately, this represents only the registered cases, those patients who were either not diagnosed or unable to reach the diagnostic and treatment facilities have not been included. Information on HPV prevalence and its type-specific distribution is scanty among Pakistani women. Limited research on this topic has been conducted due to social barriers.

Cervical cancer is a preventable disease. Prophylactic and therapeutic vaccines are available in the developed countries. A study revealed that these vaccines are highly effective[21] and if HPV is considered to be the major etiological factor in the Pakistani females, these vaccines can be introduced for Pakistani population as well.

The aim of the study was to determine the frequency of high-risk subtypes of HPV (16, 18, 31and 45) in squamous cell carcinoma of cervix among women.

## Methods

This descriptive study was planned at Ziauddin University (ZU), Clifton and conducted at the Department of Molecular Genetics Laboratory, Ziauddin University Hospital, North Nazimabad, Karachi, Pakistan, from April 2008 to December 2008. All available (fifty) formalin-fixed paraffin embedded samples (blocks) of squamous cell carcinoma of cervix (SCC) were collected from two Pathology laboratories, 'The Laboratory', Saddar and 'Dr. Yasmeen Syed Laboratory', Malir through convenience sampling. The blocks were recruited from January 2003 to November 2008. Only the formalin-fixed paraffin embedded samples (blocks) of females with diagnosed cases of SCC were included. All samples were re-confirmed by an expert histopathologist. The study population was mixed and there was no pre- selection of age, race and ethnicity. The limitation of the study samples was no access to the history and related factors could be collected. The study was approved by Ethics Review Committee of Ziauddin University.

The DNA was extracted by QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) as per Manufacturer's instruction. In the step 1, Presence of human genomic DNA was verified by amplification of 268 bp (base pair) fragment of β-globin gene by using SyberMix primers on Rotorgene 3000A (Corbett Research).

For primary PCR amplification, Real time PCR Rotorgene 3000A (Corbett Research) Deionized water was used as a negative control. After completion of the primary PCR amplification, a small amount of the My09/My11 PCR products were used for the nested PCR using Primers GP5+/GP6+, by using the same temperature protocols.

Amplified products were analyzed on 2% agarose gel, which was stained with ethidium bromide and the identification of amplified product was done on the basis of predicted fragment size.

Seegene (Seeplex Genotyping Kit) were used for HPV genotyping. Then purpose genotyping by using a kit is to maintain quality control. This kit contains probes of HPV genotypes 6, 11, 16, 18, 31 and 45.

Microsoft excel was used for data entry and analysis. The results for the categorical variables were presented as frequencies and percentages.

## Results

The age range of women for the selected samples was between 25 and 90 years. Table-1 shows that HPV was detected in 9 (18%) out of fifty paraffin embedded tissues of squamous cell carcinoma of cervix while HPV was not

**Table-1: HPV status in patients with squamous cell carcinoma of cervix (2003-2008).**

| Age (years) | Total no. of Patients(n) | HPV Positive(%) | HPV Negative(%) |
|---|---|---|---|
| 20-29 | 02 | 00 | 02 |
| 30-39 | 10 | 02 | 08 |
| 40-49 | 18 | 02 | 16 |
| 50-59 | 10 | 03 | 07 |
| 60-69 | 07 | 00 | 07 |
| 70-79 | 01 | 01 | 00 |
| 80-89 | 01 | 01 | 00 |
| 90 > | 01 | 00 | 01 |
| Total | 50 | 09(18%) | 41(82%) |

**Table-2: HPV genotyping in patients with squamous cell carcinoma of cervix.**

| HPV (Any type) | HPV 16 | HPV 18 | HPV 31 | HPV 45 | Unknown |
|---|---|---|---|---|---|
| 09 | 05(55.6%) | 0 (0%) | 0(0%) | 0(0%) | 04(44.4%) |

detected in remaining 41 (82%). Table-2 shows the HPV genotyping for the cases that were found to be positive for HPV. It revealed that out of the positive cases, 5 (55.6%) were infected with the HPV16, while in the remaning 4 (44.4%) positive samples, the genotype could not be identified as only

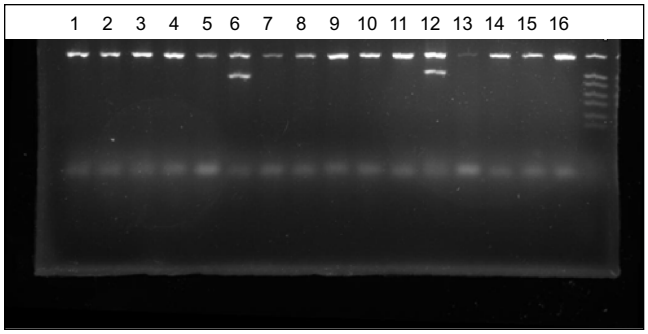


**Diagram: Gel electrophoresis of amplified HPV DNA ; Shows that PCR primer for β- globin is positive in all samples (1-16), ladder shows bands of HPV subtypes 16, 18, 31, 45, 6 and11 (from top to bottom) and HPV 16 positive bands are seen in lane 6 and 12.**

Figure: HPV Genotyping From Seegene Kit.

16, 18, 31and 45 were analyzed in this study. The limited budget did not allow the authors to look for other possible HPV subtypes.

## Discussion

The present study was carried out to identify which subtype is more frequent among the Pakistani population. It showed that out of fifty samples only nine were positive for HPV and only five of these had HPV 16 while in the other four HPV positive samples, the subtype could not be identified since the kit used in the study had probes of 16, 18, 31 and 45 only. On comparison with the previous work done in Pakistan, only two studies were reported with HPV genotypes in cervical cancer.[21,22] Saeed Khan and colleagues revealed that HPV were detected in 59 out of 60 cases, in which 56 showed presence of HPV 16, only one was positive for HPV 18 and in the remaining two samples HPV subtypes could not be determined.[21] In another study by Anwar and colleagues, HPV 16 and 18 were detected in 17% of the non-neoplastic specimens and in 69% of cervical cancers from Pakistan, while the Japanese samples were 19% and 68% positive for HPV, respectively.[22]

This study indicated that HPV16 is most common among all positive samples but there could be other causes for squamous cell carcinoma in the women tested or probably a different subtype of HPV might be prevalent in Pakistani population. There can be other causes of SCC of cervix among the Pakistani population, which the study did not look into due to limitations. A multicenter study done by IARC,[23] showed that there is an increased detection of high risk HPV in women who had first sexual contact at younger age.

Pap smear is being offered in most government and private hospitals. It is a recognized tool for screening, which shows cervical cytology, but it has its own drawbacks. This test is prone to errors at different levels, resulting in a high number of false negative results.[24] Pap smear screening fails to detect HPV, which is considered to be a causative agent of abnormal cytology of cervix and cervical carcinoma. It is not a common practice in Pakistan to carry out HPV screening. Type specific distribution of HPV and determinants of cervical cancer have not been studied adequately in a Pakistani population. A major reason for this are the social taboos associated with all matters pertaining to sex and sexually transmitted disease. A local study showed that most of the patients present at advance stages of cervical cancer.[25] The high rate of clinical presentation of patients in the advanced stages can be attributed to the lack of public awareness regarding cervical cancer screening programmes. In addition to Pap smear, screening of women once or twice during their lifetime using a high sensitivity test such as HPV genotyping would be more logical and effective in a low resource country.

The age group in this study ranged between 25-90 years indicating a very wide range in the prevalence of squamous cell carcinoma, which is all the more alarming, suggesting an even greater need for the development of screening programmes.

The limitation of the study is that the complete history of the patients, their ethnic groups, socioeconomic status is not known.

## Conclusion

This study concluded that HPV16 is the most common among all HPV DNA positive cases but there could be other causes for squamous cell carcinoma in the women tested or probably a different subtype of HPV prevalent in the studied population. HPV genotyping in resource constraint settings can be considered only in high risk women or those who can afford. Large scale educational and screening programmes should be encouraged in the different settings to decrease frequency of cervical cancer.

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