

Human papilloma virus 16/18 genotypes in patients with squamous cell carcinoma of cervix in northeast Iran

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ABSTRACT

Background: A relation has been established between infection with high-risk types of human papilloma virus (HPV) and development of cervical cancer. To estimate the risk of HPV infection for cervical malignancies, we conducted a case-control study in northeast Iran. **Materials and Methods:** This study was carried out on 123 paraffin embedded blocks with exact diagnosis of squamous cell carcinoma (SCC). A total of 100 cervical tissue specimens with normal histopathology product of hysterectomy were also used as control. Both groups were tested for the presence of HPV DNA and HPV 16/18 subtypes using PCR assay. **Results:** Large non-keratinising subtype of cervical carcinoma was the most frequent one (62.6%), followed by keratinising and small cell subtypes (27% and 10%, respectively). Overall prevalence of HPV infection in SCC of cervix was 34.2% (42 out of 123 cases). HPV 16 was the most common type in this group (21 cases, 17.1%), followed by HPV 18 (16 cases, 13%) and other subtypes (5 cases, 4.1%). In this study, overall prevalence of HPV infection in control group was 12% (including 3% HPV 16; 5% HPV 18 and 4% other subtypes). **Conclusion:** Although association of HPV 16/18 and SCC of cervix was relatively higher than control group, compared with the previous study, the association between cervical SCC and HPV infection was significantly lower in our study; and possibly, the other risk factors play a major role in carcinogenesis of cervical carcinoma in this region.

Key words: HPV 16/18, HPV, PCR, SCC of cervix

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INTRODUCTION

Squamous cell carcinoma (SCC) of cervix is one of the most frequent malignancies in women, and causes considerable morbidity. Indeed, cervical malignancies are the second most common cancer in women worldwide and the fifth most prevalent malignancy among Iranian females.¹⁻³ It is predicted that 607,402 new cases of cervical cancer and 320,832 attributable deaths will occur among women during 2015 worldwide.⁴ Based on World Health Organisation (WHO) annual report on 2010, Iran has a

population of about 25 millions women aged above 15 years and who are at risk of developing cervical cancer. Current estimates indicate that every year 643 women are diagnosed with cervical cancer and 286 die from the disease in Iran. Unfortunately, there is no exact data about the human papilloma virus (HPV) burden in the general population of Iran. However, rough estimations about cervical HPV infection in general population, based on the incidence of infection in the countries of this region (Southern Asia), is about 7.9% at a given time.⁵

So far, multiple factors have been evaluated to define the effect and role of them in cervical carcinogenesis. Factors including smoking, use of oral contraceptives, high parity, certain human leukocyte antigen (HLA) subtypes, immunosuppression, multiple partners and sexually transmitted diseases have been introduced as predisposing or risk factor of cervical carcinoma.⁶ HPV infection is the most notorious among them; as some studies have demonstrated that the persistence of HPV

Access this article online	
Quick Response Code:	Website: www.nigeriamedj.com
	DOI: 10.4103/0300-1652.144706

DNA is a necessary factor for the development of cervical SCCs. The association between certain HPVs, so-called high risk HPVs, and cervical cancer is well documented. HPV 16 and HPV 18 are the most important in cervical pathology. HPV 16 is detected in about 60% of cervical cancer cases, and HPV 18 accounts for 10% of cases.^{7,8} It seems there is no geographic variation in the distribution of HPV types.⁹

Currently, the most sensitive and reliable method for detection of HPV infection is PCR assay. Most laboratories use consensus primers, directed to a conserved L1 gene, and hence able to detect all mucosal HPV types. These primers includes GP5-GP6 set and MY09-MY11 set, which detect a region of L1 gene.^{10,11}

The objective of the present study was to evaluate the prevalence of HPV infection in cervical SCCs and to estimate the relative risk according to HPV 16/18 infection and cervical invasive SCC.

MATERIALS AND METHODS

This descriptive cross-sectional case-control study was performed on 123 formalin fixed paraffin embedded (FFPE) blocks of cervical tissue specimens with histopathologic diagnosis of cervical invasive SCC in molecular pathology laboratory, Ghaem Hospital, Mashhad, Iran. The FFPE blocks were retrieved from Mashhad educational hospitals (Qaem, Imam reza, and Omid's pathology department) archive from five recent years. Two histopathologists reviewed slides for confirming diagnosis and selecting the best paraffin blocks for DNA extraction and for assurance of consistency and quality of our materials. In parallel, 100 blocks of paraffin embedded cervical tissue specimens with normal histopathology and no tumoral tissue was used as the control group.

Cervical tissue specimens cut in 6 micro sections using separate disposable items such as gloves, blades and tubes to avoid any cross contamination between samples. To isolate DNA, at least 3 sections were deparaffinised by Xylene and dried tissue was incubated overnight at 56°C in a solution composed of 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 0.5% sodium dodecyl sulphate, 50 mM NaCl and 300 µg/mL of proteinase K. Proteinase K was inactivated at 95°C for 10 min. The samples were centrifuged for 3 min at 12,000 rpm and finally the supernatant was undergone extensive extraction by phenol/chloroform (1:1 ratio) to use for PCR. The reaction was performed in a 1.5 ml microtubes with re-suspended DNA, 20 mM Tris-HCl (pH 8.3), 8 mM MgCl₂, 7.5 mM DTT, 200 µM of each dNTP, 20 pmol of each primer and 0.25 U of Taq DNA polymerase. DNA concentrations were measured by nanodrop.

First, the extracts were checked for DNA quality by amplification of the human β-Actin gene. In all samples,

HPV detection was performed by GP5+/GP6+ primers [Table 1]; and eventually, positive cases for common HPV DNA assay were tested for identification of HPV 16 and HPV 18 subtypes using primers for HPV 16 and HPV 18 specific target sequences.¹¹⁻¹³ General HPV positive cases with HPV 16/18 negative results were of low risk type, as confirmed by Genpack®.

Forty cycles of amplification were done using Applied Biosystems thermocycler. Standard PCR protocol consisted of a denaturation step at 94°C for 30 s, followed by a primer annealing step at 50°C for 1 min, and a chain elongation step at 72°C for 30 s for each cycle. Eventually, the amplification product was analysed by 2% agarose gel electrophoresis.

RESULTS

A total of 123 patients with SCC of cervix were included in this study; mean age was 49.8 years (ranging from 25 to 80 years) with a peak in the 40-50 years age group (35.8%). The most frequent SCC subtype was large non-keratinising type (77 cases, 62.6%) followed by keratinising (33 cases, 27%) and small cell type (13 cases, 10.4%). HPV DNA was found in 42 out of 123 patients (34.1%) and 12 out of 100 cases (12%) in controls [Table 2].

High risk HPV infection was significantly higher in SCC cases than controls (odd ratio = 4.9; 95% CI =2.18-11.22) including 21 HPV 16 positive in cases compared with 3 in controls (odd ratio = 6.65; 95% CI = 1.92-22.03) and 16

Table 1: Characteristics of primers used in this study

Primer	Sequence (5'-3')	Target	Amplimer length (bp)
β-ActinF	TTCCAGCCTTCCTTCTGG	β-Actin	225
β-ActinR	TTGCGCTCAGGAGGAGCAAT		
GP5	TTTGTACTGTGGTAGATACTAC	L1	150
GP6	GAAAAATAAACTGTAAATCATATTC		
E6F	TCAAAGCCACTGTGTCCTG	HPV16	692
E6R	CGTGTTCTTGATGATCTGCA		
HPV-18F	ACCTTAATGAAAAACCACGA	HPV 18	450
HPV18-R	CGTCGTTGGAGTCGTTCTCG		

Table 2: Frequencies of HPV 16 and 18 in squamous cell carcinoma of cervix

HPV type	Squamous cell carcinoma (SCC) subtype			Total
	Large non-keratinizing type	Keratinizing type	Small cell type	
High risk HPV	31 (83.8%)	4 (10.8%)	2 (5.4%)	37
HPV 16	18 (85.7%)	3 (14.3%)	0	21
HPV 18	13 (81.3%)	1 (6.3%)	2 (12.5%)	16
Low risk HPV	3 (60%)	2 (40%)	0	5
Total	34	6	2	42

HPV 18 positive in cases compared with 5 in controls (odd ratio = 2.84; 95% CI = 1.00-8.05).

Low risk HPV DNA was found in 5 of 123 cases of SCC compared with 4 of 100 in controls. There was no significant difference between cases and controls in low risk HPV infection (odd ratio = 1.01; 95% CI = 0.26-2.89).

DISCUSSION

It has been shown that some viruses associate with the pathogenesis of various benign lesions and some malignant neoplasms such as cervical cancer.¹⁴⁻¹⁷ Currently, there are increasing evidences that confirm the multi-factorial nature of carcinogenesis through different molecular biologic pathways. Many studies overemphasise on the role of high risk HPV infection in the development and progression of cervical carcinoma, considering the existence of this entity without HPV infection impossible or exceptional.¹⁸⁻²⁰

The overall frequency of HPV infection in our study group was 34.2% (30.1% considering high-risk type HPVs). This figure is much lower than what was reported in Mazandaran province, Iran, (78.6%),²¹ Brazil (76%),²² South Korea (90.3%)²³ and other countries ranging over at least 65% of cases.^{11,24-27}

This significant discrepancy may be, at least to some extent, due to formaldehyde fixation effect on PCR product result, especially for products less than 200 bp (including GP5/GP6), and eventually false negative results.^{11,22} Therefore, it is possible that if fresh specimens had been examined, HPV positive cases might have been higher. Moreover, recent studies have suggested the possibility of false negative results mainly in high grade squamous intraepithelial neoplasia and invasive SCC, and detection of HPV DNA by multiple methods or multiple primers may be conclusive and lead to a higher rate results.^{7,23}

In contrast, multiparity and especially the age at first intercourse are common risk factors of cervical carcinoma and HPV infection simultaneously. Therefore, HPV positive cases with cervical SCC would be seen more frequent in regions with this high risk sexual habit. The other possible reason is the lower frequency of HPV infection in this region, which may cause overriding of the other risk factors effect over the HPV infection effect in carcinogenesis statistically.

CONCLUSION

Although association of HPV 16/18 and SCC of cervix was relatively higher than control group (odd ratio = 4.9; 95% CI = 2.18-11.22); compared with the previous study, the association between cervical SCC and HPV infection was significantly lower in this study; and other risk factors may play a major role in carcinogenesis of cervical carcinoma in this region.

ACKNOWLEDGEMENTS

This study was the result of a residency thesis and financially supported by the research Vice Chancellor of Mashhad University of Medical Sciences. The authors would like to thank them and also Mr. Mahmoud Bagheri for performing PCR.

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How to cite this article: Ayatollahi H, Homaei-Shandiz F, Kooshyar MM, Tabatabaee-Yazdi SA, Mehrjerdian M, Jafarian AH, *et al.* Human papilloma virus 16/18 genotypes in patients with squamous cell carcinoma of cervix in northeast Iran. *Niger Med J* 2014;55:495-8.

Source of Support: Nil, **Conflict of Interest:** None declared.

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