Journal of Research in Medical and Dental Sciences
2018, Volume 6, Issue 2, Page No: 354-359

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Distribution of Human Papilloma Virus-16 *E7 oncoprotein* in Iranian Patients with Cervix Cancer

Ahmad Piroozmand[1,2]*, Fateme Jafariazad[3], Mohammad Niakan[4], Azad Khaledi[5,6], Hale Piroozmand[7]

[1]Department of Microbiology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
[2]Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan , Iran.
[3]MSc in Microbiology, Basic science, Shahed University, Tehran, Iran.
[4]Department of Microbiology, Medicine Faculty, Shahed University, Tehran, Iran
[5]Infectious Diseases Research Center, Kashan University of Medical Sciences, Kashan, IR Iran
[6]Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
[7]Veterinary Medical Student, Vetrinary Faculty, Islamic Azad University, Science and Research Branch, Tehran, Iran

DOI: 10.5455/jrmds.20186255

ABSTRACT

Cervical cancer is a common neoplasm of the female genital tract. Persistent infection with high-risk human papillomavirus (HPV), particularly HPV 16 is associated with cervical carcinoma due to the ability of producing the viral Oncoproteins (E6 and E7) can abrogate the cell cycle. This study aimed to determine the distribution of human papilloma virus-16 E7oncoprotein in Iranian patients with cervix cancer.Of the 150 paraffin blocks from patients with pre-cancerous lesions and cervical cancer of Tehran hospitals, Iran, 69 samples were confirmed by the pathologists as cervical cancer. Then, their DNAextracted using the phenol/chloroform method and to evaluate the HPV16 E7, primer related to E7 gene was used.Then, the data were analyzed using Chi-square test (Fisher exact test). The mean age of patients was 50±2.3 years. Most of the patients were from the age group of 41-51 years and all were married. Among total numbers of patients, 53.6% were positive for HPV16. The mostwomenwithcancer belonged to age-group 41-50, OCP consumer and women who got married under 18 years old.Results of the present study showedthat the HPV16 is one of the major genotypes that are associated with cervical cancer in Iranian patients which should be considered in prevention and vaccination programs. In addition, screening of a highly risky human papillomavirus, especially type 16 can be recommended for all patients suspected with cervical cancer using PCR of E7 that is accurate and effective.

Key words: Cervical Cancer, HPV 16, Oncoprotein E7

HOW TO CITE THIS ARTICLE: Ahmad Piroozmand, Fateme Jafariazad, Mohammad Niakan, Azad Khaledi, Hale Piroozmand, Distribution of Human Papilloma Virus-16 E7 *oncoprotein* in Iranian Patients with Cervix Cancer, J Res Med Dent Sci, 2018, 6 (2):354-359, DOI: 10.5455/jrmds.20186255

Corresponding author: Ahmad Piroozmand
Received: 10/01/2018
Accepted: 21/01/2018

INTRODUCTION

Human Papillomavirus (HPV) is the most commonly reported sexually transmitted infection and one of the main causes of cervical cancer. Cervical cancer is the third most common cancer in women all over the world [1]. The types of highly risky HPV are; 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 68, 69, 73 and 82 which can cause abnormal changes and susceptibility to incidence of cancer in the cervix and anogenitaland amongst, HPV16 plays a significant role followed by HPV18 in most countries [2]. Regarding the spectrum of cervical diseases, HPV-16 is steadily the most common HPV type causative to 50–55% of invasive cervical cancer cases, followed byHPV-18 and HPV-45, although at a lesser level [3].

Cervical cancer is considered to be the second most common cancer in women with breast cancer which it is common in Iran. Cervical invasive cancer is known as a preventable cancer owing to having a long period prior to invasion, the availability of appropriate screening programs and relatively effective and easy treatment of primary lesions [4]. Women who despite having a normal cytology suffered from high-risk types of HPV infection in the cervix, are at high risk during a multi-year period for a high-risk pre-invasive disease [5]. Despite the fact that infection with HPV is known to be a definitive cause of this cancer, insufficient information is available on the frequency and distribution of this infection in some populationsof some countries such as Iran [6].

The distribution and prevalence of the HPV virus also depends on the geographical area. In addition to the persistent infection with HPV, any factor that affects the DNA penetration of the HPV virus into the human genome, such as smoking, the use of contraceptive pills, low age, first sexual intercourse, immune deficiency, history of infectious syphilis, Chlamydia and multiple deliveries may create a lesion to develop into an invasivedisease form. This cancer generally occurs between the age of 30-55 years, but recently there are many reports of young women with HPV infection [7].

In most cases, HPV infections do not produce special symptoms and they resolved spontaneously. Of course, persistent infection with specific types of HPV such as types 16 and 18 cancause to precancerous lesion. If untreated, it may progress to cervical cancer, in which thesymptoms of cervical cancer appear only in advanced steps, that the symptoms are: irregular, intermenstrual (between periods) or abnormal vaginal bleeding after sexual intercourse; back, leg or pelvic pain; fatigue, weight loss, loss of appetite; vaginal discomfort or odourous discharge [8].

E6 and *E7* are the early expressing proteins and the primary oncoproteins of high-risk HPV complicated in human epithelial cell immortalization and transformation [9].The carcinogenicity of high-risk genotypes of HPV depends on E6 and E7 oncoproteins [10, 11]. Inhibition of *E6* and *E7* expression in cervical cancercells can suppresses cell growth and consequently induces apoptosis [12]. Therefore, *E6* and *E7* of HPV arethe ideal targets for diagnostic aims and therapeutic vaccines design.Identifying the DNA virus is the suitable accurate method for screening the cervical cancer, which can prevent of cancer by the timely detection of the cancerous formed lesions. Since the E7 gene is one of the most important anticoagulants in the cancerous process, identifying this oncogene also helps to screen patients who are infected with high risk types of HPVcausingthecertain types of cancer.The aim of this studywas to identify the HPV16 virus through E7 region of its genome with PCRtechnique and accurate diagnosis of people are at high risk for cervical cancer with HPV16 virus.

MATERIALS AND METHODS

This study was performed on cervical cancer samples. All samples were collected from the archives of Mirza Kuchak Khan, Imam Khomeini and Pars hospitals of Tehran, Iran. The samples were used as paraffin blocks which were confirmed previously by pathologists. The number of samples (A95% confidence interval) and the error rate (0.12) with formula:
(p (1-p) / d2 n = z2α / 2), a total of 69 samples were calculated and selected. When sampling, the patients' demographic information such as patients age, marriage age, history of taking OCPpills and tobacco were collected through study thepatients' record or using the designed questionnaires.

DNA Preparation

Using microtome, samples were taken withthickness 10 micrometers and sterilized in 2 ml Ependorftubes. To remove paraffin from the specimens, they were placed in warm zeolin (56 ° C) and then washed twice with 90% ethanol. After high-speed centrifugation, samples were kept in a digestion buffer at 37 °C overnight, and in a shaker/incubator for4hat 56 °C. This buffer contains Tris-HCl [pH 7.5] 50 mM, EDTA 10 mM, NaCl 50 mM, 0.5%SDS, and Proteinase K of 2 mg/ml. Then by placing the samples at 95 ºC, Proteinase K was inactivated and DNA extracted using phenol/chloroform extrac*tion* kit[13]. Then the purity and concentration of extracted DNA was determined using NanoDrop spectrophotometer.

Investigating the presence of human papillomavirus 16 (HPV16) DNA using the PCR technique

The primer sequences used to amplifying the E7 region of HPV16genome is shown in Table 1. The program used for amplifying the desired genefragment was as: 1 cycle for 5 minutes at 95 °C, then 10 cycles (95 ° C, 30 s; 57 °C, 1 min; 72 °C, 1 min) and 25 cycles (95 ° C, 30 s; 48 °C, 30 s; 72 °C, 1 minute); then 1 cycle at 72°C for 5 minutes; and hold it at 4 °C. To carry out the PCR reaction, 3 µl of the extracted DNA in 30 µl of the reaction volume was amplified. The reaction volume consisted of; 1.5 µl of each primer, 0.6 µl of polymerase enzyme, and the remainingwas master mix (dNTPs, MgCl2, 10X buffer) and distilled water.ThenPCR products were electrophoresed on a 1.5% agarose gel at 100 volts for 40 minutes and then stained usingEthidium bromideon a UV device. The target fragmentwas 196 bp, and Marker 100 bp (Qiagen) was used. In the positive control tube, the extracted DNA was a sample that already positive for HPV16 (using a positive specimen from the University of Tehran lab) and was used of distilled water as a negative control.

Table 1: Primers used in PCR reaction for detection of HPV16 E7

Amplified region	Primer	Amplified length	Sequences
HPV16 E7	Pr.591-620	196bp	5'ATA TAT GTT AGA TTT GCA ACC AGA GAC AAC 3'
	Pr.786-762		5'GTC TAC GTG TGT GCT TTG TAC GCA C 3'

Data analysis

Data were analyzed using Chi-square test (Fisher exact test). And P-values <0.05 were considered statistically significant.

RESULTS

The mean age of patients was 50±2.3 years. The minimum and maximum ages of them were 30 and 89 years, respectively. Most of the patients were from the age group of 41-51 years and all were married. 81.8% of the patients had marriage age of 18 years or lower which 40.9% of those were infected by HPV16, also 65.9% of patientswere consumers of contraceptive pills, which 27.2% had HPV16, and 18.1% of cases were smokers (cigarrete-Hookah),also, of which 13.63% were infected with HPV16. More details are abstracted in Table 2.

According to the results of the PCR reaction, 53.6% of the patients were infected with HPV16 virus (Figure 1).

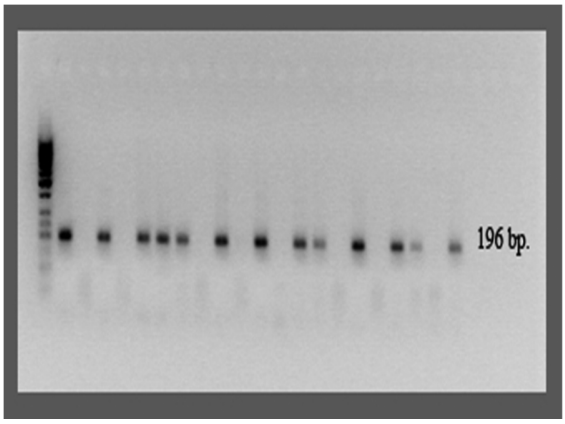

Figure1: Gel electrophoresis for PCR product of E7 gene of HPV16. Left to the right: Line 1; ladder 100 bp, line 2; related with positive control, line 3; negative control, lines 4-14 are associated with positive samples for E7 gene of HPV16

Table 2. Distribution frequency of HPV16 based on the risk factors of cervix cancer

Variables groups		No of HPV Negative N (%)	No of HPV Positive N (%)	Groups N (%)	Total of patients (%)	P value
Age groups	20-30	12(17.39)	0(0)	17.39	69(100)	0.01
	31-40	5(7.24)	1(1.45)	8069		
	41-50	12(17.39)	10(14.45)	31.88		
	51-60	10(14.49)	4(5.80)	20.29		
	60>	7(10.14)	8(11.59)	21.37		
Marriage age	≤18	18(40.90)	18(40.90)	81.8	44(100)	0.4
	>18	6(13.63)	2(4.54)	18.18		
Smoker	yes	1(25)	12(27.27)	27.52	44(100)	0.1
	no	20(45.45)	11(25)	45.70		
OCP consumer	yes	17(38.63)	12(27.27)	65.9	44(100)	0.4
	no	7(15.90)	8(18.20)	34.1		

DISCUSSION

Since cytological screening is not sufficientto detect carcinogenesis alone, as well as studies have shown that people with cervical cancer are infected with HPV, by identifying high-risk types of the papilloma virus that are the main cause of cervical cancer and timely treatment, the progress of the lesions can be prevented from becoming cancerous.

In the present study, a total of 69 samples (53.6%) were infected with HPV16, which is close to global results [14]. In line with our study, a comprehensive review study showed that themajority of HPV-positive tumors contained the ''highrisk'' HPV types 16 (40.0%)[15]. According to studies conducted by the International Center for Cancer Research in different countries of the world, more than half of the cervical cancers have been infected with the HPV 16 virus [16-19]. The extent and distribution of the virus depends on the geographical area and there may be differences in the prevalence of different genotypes of the human papillomavirus in different regions. For example, in Mozambique genotypes 35 and 58, and in the Pacific region, HPV58 is more prevalent[20, 21]. A study conducted by Munoz et al in 1995 on the effect of the geographical situation on the prevalence of HPV and the development of cervical cancer showed that among 1000 samples collected from 22 countries, 93% of the tumors were infected with the virus and the prevalence of the HPV16 genotype was about 50% and it was the dominant genotype among all countries except Indonesia[3]. In Iran, various studies carried out about this issue, including a study conducted by a Hamkar and et al., in Mashhad, has reported the prevalence of HPV16 about 50.6%,but, in the studies of Farjadian and *et al.,* in southern Iran, only 26.7% of the samples were positive for it [22, 23] A study conducted by Pavai and et al., in Hungary in 2006, reported the prevalence rate of HPV16, 14% [24], which is in contrast to our results, while another one that carried out by Varnai and et al., in Germany presented the prevalence 66.6%, which is in line with results of current study. This difference in reporting of prevalence of HPV16 possibly referred to the difference in geographical regions, and also due to use of paraffin blocks instead of fresh tissue samples [25].

In the current study, an attempt was made to collectthe samples randomly from all parts of Iran. Human papillomavirus infection is the most important risk factor for cervical cancer, but other factors such as aging, young age marriage, smoking and OCP are prone to cervical cancer [26]. In original, occurrence of cervical cancer over time is result from a combination of different risk factors along with persistent HPV infection[26]. According to the results of our study, the majority of cancer patients with HPV 16 infection are in the age group of 41-51 years, which is similar to other studies [27], therefore, it is necessary the people be screened from the very beginning of youth because the cancer is a long-term process [28]. Although the majority of patients with cervical cancer (infected with HPV16) were those who married at the age of under 18 years, but in this study, the Chi-square test did not show a significant relationship, which may be due to low sample size and lack of access to personal information of all patients. However, the current study has some limitations, for example; due to the lack of cooperation of some patients, only demographic data (age of marriage, smoking, taking OCP) were available to 44 of them.

CONCLUSION

Sincethe genotype 16 of the human papillomavirus is considered to be the most risky cause of cervical cancer in most parts of the world;here the aim was to detect this virus in samples of Iranian patients, so that, if necessary, the general state of the country for the programingof vaccinations isclearer. Our results showed that the papillomavirus genotype16 was a dangerous cause of cervical cancer in Iran, and it should be considered in prevention and vaccination programs. In addition, screening of a highly risky human papillomavirus, especially type 16, can be recommended for all cervical cancer by PCR. Identification of high-risk HPV strains based on E7 from its genome for screening can be accurate and effective. It is recommended that those at risk, in addition to performing a Pap smear test, should also be candidate for a PCR test for HPV. With this technique, various types of human papillomavirus can be identified in patients and even seemingly healthy people and the use of this system can help to epidemiological studies of the HPV virus in community. In addition, the factors such as smoking, OCP pills, early age marriage, and etc., can contribute to this subject, but due to the low sample size, there is no significant relationship with chi-square test. It is

hoped that in future more studies with more sample sizes will be conducted.

Acknowledgements
We would like to thank and appreciate the efforts of the personnel of Mirza Kuchak Khan, Imam Khomeini and Pars hospitals, and the authorities of Kashan University of Medical Sciences, and Department of Microbiology, Faculty of Basic Sciences, Shahed University, Tehran, Iran.

Conflicts of interest
None declared.

CONCLUSION

The results of this study showed that sexual educational programs as film for cardiac patients can enhance sexual function and quality of life of these patients.

Considering the importance of sexual issues in cardiac patients and its consequences and shortage of educational in this regard, nurses need to enhance their counseling role, provide the best solution to solve patient problems and improve their sexual function and quality of life using sexual counseling programs. Sexual educational is recommended in the hospital and should be continued persistently after discharge from the hospital. Sexual educational through CD-ROM allows everyone to benefit from this package regardless of their education and temporal constraints. It is also suggested that follow-up tests be conducted at different intervals after the education

REFERENCES

1. Chen Z, Jing Y, Wen Q, Ding X, Wang T, Mu X, Chenzhang Y, Cao M. E6 and E7 Gene Polymorphisms in Human Papillomavirus Types-58 and 33 Identified in Southwest China. PloS one. 2017; 12(1):e0171140.
2. Takehara K, Toda T, Nishimura T, Sakane J, Kawakami Y, Mizunoe T, Nishiwaki M, Taniyama K. Human papillomavirus types 52 and 58 are prevalent in uterine cervical squamous lesions from Japanese women. Pathology Research International. 2011;2011.
3. Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shan KV. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. JNCI: Journal of the National Cancer Institute. 1995; 87(11):796-802.
4. Bruni L, Diaz M, Castellsagué M, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. Journal of Infectious Diseases. 2010; 202(12):1789-99.
5. Dadkhah F, Alishah N. A comparative study on colposcopy directed biopsy and Pap smear tests in patients with an abnormal Pap smear. Iranian Journal of Pathology. 2006; 1(1):13-16.
6. Ili CG, Brebi P, López J, García P, Leal P, Suarez E, Roa JC. Genotyping of human papillomavirus in cervical intraepithelial neoplasia in a high-risk population. Journal of Medical Virology. 2011; 83(5):833-7.
7. Collins Y, Einstein MH, Gostout BS, Herzog TJ, Massad LS, Rader JS, Wright J. Cervical cancer prevention in the era of prophylactic vaccines: a preview for gynecologic oncologists. Gynecologic Oncology. 2006; 102(3):552-62.
8. Leaver D, Labonte G. HPV and Cervical Cancer. Radiation Therapist. 2010;19(1):27-45.
9. Rampias T, Sasaki C, Psyrri A. Molecular mechanisms of HPV induced carcinogenesis in head and neck. Oral Oncology. 2014; 50(5):356-63.
10. Kraus I, Molden T, Holm R, Lie AK, Karlsen F, Kristensen GB, Skomedal H. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. Journal of Clinical Microbiology. 2006; 44(4):1310-17.
11. Fields B, Knipe D, Howley P. Ovid technologies Inc. Fields' virology. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2007.
12. Chang JT, Kuo TF, Chen YJ, Chiu CC, Lu YC, Li HF, Shen CR, Cheng AJ. Highly potent and specific siRNAs against E6 or E7 genes of HPV16-or HPV18-infected cervical cancers. Cancer Gene Therapy. 2010; 17(12):827-36.
13. Lesnikova I, Lidang M, Hamilton-Dutoit S, Koch J. Rapid, sensitive, type specific PCR detection of the E7 region of human papillomavirus type 16 and 18 from

paraffin embedded sections of cervical carcinoma. Infectious Agents and Cancer. 2010;5(1):2.
14. Cattani P, Zannoni GF, Ricci C, D'Onghia S, Trivellizzi IN, Di Franco A, et al. Clinical performance of human papillomavirus E6 and E7 mRNA testing for high-grade lesions of the cervix. Journal of Clinical Microbiology. 2009; 47(12):3895-901.
15. McKaig RG, Baric RS, Olshan AF. Human papillomavirus and head and neck cancer: epidemiology and molecular biology. Head & Neck. 1998; 20(3):250-65.
16. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. International Journal of Cancer. 2007; 121(3):621-32.
17. Munoz N. Human papillomavirus and cancer: the epidemiological evidence. Journal of Clinical Virology. 2000; 19(1):1-5.
18. Silins I, Wang X, Tadesse A, Jansen KU, Schiller JT, Avall-Lundqvist E, et al. A population-based study of cervical carcinoma and HPV infection in Latvia. Gynecologic Oncology. 2004; 93(2):484-92.
19. Masumoto N, Fujii T, Ishikawa M, Mukai M, Ono A, Iwata T, et al. Dominant human papillomavirus 16 infection in cervical neoplasia in young Japanese women; study of 881 outpatients. Gynecologic Oncology. 2004; 94(2):509-14.
20. Naucler P, Da Costa FM, Ljungberg O, Bugalho A, Dillner J. Human papillomavirus genotypes in cervical cancers in Mozambique. Journal of General Virology. 2004; 85(8):2189-90.
21. Herrero R, Hildesheim A, Bratti C, Sherman ME, Hutchinson M, Morales J, et al. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. Journal of the National Cancer Institute. 2000; 92(6):464-74.
22. Farjadian S, Asadi E, Doroudchi M, Dehaghani AS, Tabei S, Kumar V, et al. High risk HPV types in southern Iranian patients with cervical cancer. Pathology & Oncology Research. 2003; 9(2):121-5.
23. Hamkar R, Azad TM, Mahmoodi M, Seyedirashti S, Severini A, Nategh R. Prevalence of human papillomavirus in Mazandaran province, Islamic Republic of Iran, 2002.
24. Pavai Z, Fule T, Horvath E, Mathe M, Pap Z, Denes L, Jung J. Comparative detection of high-risk HPV (16, 18, 33) in cervical bioptic material of county hospital of Tg. Mures. Romanian Journal of Morphology and Embryology. 2006; 47(3):229-34.
25. Varnai AD, Bollmann M, Bankfalvi A, Speich N, Schmitt C, Griefingholt H, et al. Predictive testing of early cervical pre-cancer by detecting human papillomavirus E6/E7 mRNA in cervical cytologies up to high-grade squamous intraepithelial lesions: diagnostic and prognostic implications. Oncology Reports. 2008; 19(2):457-65.
26. Luhn P, Walker J, Schiffman M, Zuna RE, Dunn ST, Gold MA, et al. The role of co-factors in the progression from human papillomavirus infection to cervical cancer. Gynecologic Oncology. 2013; 128(2):265-70.
27. Chew G, Cruickshank M, Rooney P, Miller I, Parkin D, Murray G. Human papillomavirus 16 infection in adenocarcinoma of the cervix. British Journal of Cancer. 2005; 93(11):1301.
28. Nojomi M, Gilani M, Erfani A, Mozafari N, Mottaghi A. The study of frequency of risk factors of cervical cancer among women attending general hospitals in Tehran, 2005-2006. Razi Journal of Medical Sciences. 2007; 14(56):189-95.