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Original Article

Health Management and Policy Section

The Prevalence of Human Papilloma Virus (HPV) in Women using Liquid Base Pap Smear in Rasht, Northern of Iran

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ABSTRACT

Background: HPV is one of the most common sexually transmitted infections. However, little is known about its prevalence in the female population in Rasht, Northern of Iran. The aim of this study was to find the incidences of HPV viruses in high-risk women in Rasht by wet Pap smear from 2010 to 2015.

Materials and Methods: This cross-sectional study investigated HPV prevalence and its genotype distribution among 103 apparently healthy and non- healthy women with abnormal cells in pap exam. DNA samples were extracted by boiling and phenol - chloroform methods, then used as template for amplifying of specific fragment of HPV genome by PCR using GP5+ / GP6+ primers. PCR products were electrophoresed in 1.5% agarose gel (Roche, Germany) containing Sybrsafe. DNA ladder (Roche Co, Germany) was used to detect the molecular weights of observed bands under UV lamp.

Results: Overall, 4/98 women (4.08%) with normal cells and 1/5 women (20%) with abnormal cells were positive for at least one of the high risk HPV types in wet Pap smear. The most HPV infection was found in 26 to 39-year-old individuals.

Conclusion: We evidenced a moderate prevalence of HPV infection but needs to be given more attention because in apparently healthy women also, HPV infection was observed. Health officials should conduct the study and wider screening of this infection occurring in this province. Screening for this infection must be recommended in this region.

Keywords: GP5+/GP6+, PCR, Rasht (Guilan province, Northern of Iran)

INTRODUCTION

Cervix carcinoma (CC) is a leading cause of mortality in women but these rates have significantly reduced over the time in developed countries due to successful cervical screening programs using the Papanicolaou test [1,2], aimed at early detection of cytological abnormalities [3]. Infection with an oncogenic or high-risk human papillomavirus (HPV) genotype is the major causative factor for development infection [4,5].

According to the World Health Organization and the National Institutes of Health, cervical cancer is the first cancer caused by the virus and approximately 93% of cervical cancers are positive for HPV virus [6]. Although more than 200 different types of HPV have been identified, epidemiologic molecular studies have shown that 15 types of HPV (16 18 31 33 35 39 45 51 56 58 59 68 73 and 82) are at high risk for cervical cancer. HPV virus that causes genital warts, but also, other types of infection is associated with carcinoma. Now, in America after seeing abnormal cells in Pap smears, molecular HPV (PCR) is required. Cervical cancer in America is fourth most common cancer in women after breast cancer malignancy, colorectal and endometrial cancer. Molecular orientation of HPV is coming down with a Pap test and were followed up to about 50% of patients with ASCUS and 80% of patients with L-SIL (Low grade Squamous Intra epithelial Lesion) a type of cancer-causing HPV have been follow-up for cancer incidence [7]. Little information on prevalence of HPV infection in Iran is available. It has been estimated that young women are most infected with this virus. Shin HR and colleagues have reported that the prevalence of HPV infection in sexually active women 20-74 y old in South Korea was 10.4% [8]. On the other hand, Zhao et al., in China, found oncogenic subtypes in 23.6% of middle-aged women [9]. Epidemiological data shows that High-risk oncogenic genital HPV- 16 and 18 are the most frequently detected types in CCs worldwide [10]. According to above mentioned study, the prevalence of HPV infection varies in different geographical region. So, the aim of this study was to determine the prevalence of HPV infection in women in Rasht, Northern of Iran.

MATERIALS AND METHODS

A cross sectional study was conducted between 2010 to 2011 in the city of Rasht and 103 married women were screened. Wet PAP was prepared from Razi pathobiology laboratory. Among 103 wet PAP, 98 samples were normal and 5 had abnormal cells. All the samples were subjected for DNA extraction.

DNA extraction

Genomic DNA from cell suspension was extracted using standard Proteinase K digestion, phenol chloroform extraction and ethanol precipitation method. The quality and concentration of DNA was measured by Nano-Drop 2000 device at a wavelength of 260/280 nm. Briefly, SDS and Proteinase K added to cell suspension and incubated at 55°C for 2 h. Then, 5 M NaCl added and extracted twice with equal volumes of phenol- chloroform (1:1): IAA and once with chloroform: IAA. RNase A added and incubated for one hour at 37°C. Then, DNA was precipitated by adding 1.5 volumes of 100% Ethanol. Finally, the content of tube was centrifuged briefly to pellet the DNA and resuspended the pellet in H_2O.

PCR

All extracted DNA were subjected for amplifying of a 150 bp fragment of HPV genome by PCR using automatic thermo cycler (Eppendorf Personal 5332, Germany).

PCR reaction were performed to amplify a 150 bp fragment in a final volume of 25 µL containing 2.5 µL 10x buffer, 0.75 µL $MgCl_2$ (50 mM), 2.5 unit Taq DNA polymerase (Cinnagene CO., Iran), 0.5 µL dNTP (10mM) (Cinnagene Co., Iran), 20 Pico mole from each primers

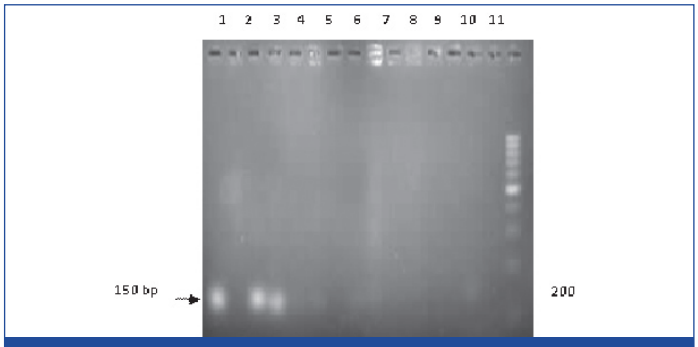
GP5+ 5′ TTT GTT ACT GTG GTA GAT ACT AC 3′ and GP6+ 5′ GAA AAA TAA ACT GTA AAT CAT ATT C 3′ and 1 µL from genomic DNA as a template. Amplification was performed with pre-incubation (94° for 4 min), followed by 35 cycles of denaturation (94° for 30 s), annealing (50°C for 30 s), extension (72°C for 30 s) and a final extension (72°C for 5 min). PCR products were electrophoresed in 2% agarose gel (Roche, Germany) containing Sybrsafe. DNA ladder (Roche Co, Germany) was used to detect the molecular weights of observed bands under UV lamp.

RESULTS

The mean age of all 103 married women was 30 ± 7.8 y. HPV genome was found in clinically normal group and a group that includes the Mocha cell disease ASCUS, ILI were 4 of 98 (4.08 %) and 1 of 5 (20%) respectively [Table/Fig-1,2].

Groups of women with	HPV	Age (Years)		Total (n)
		20-25 (n)%	26-39 (n)%	
Normal cell	+	1 (1.02%)	3 (3.06%)	4
	_	49 (50%)	45 (45.91%)	94
Abnormal cell	+	0	1 (20%)	1
	_	0	4 (80%)	4
				103

[Table/Fig-1]: Distribution of HPV infection among women with clinically normal and women with abnormal cells based on age



[Table/Fig-2]: Gel electrophoresis of 150 bp fragment of HPV genome.
Lane 1: Positive control. Lane 2: Negative control. Lane 3 and 4: positive samples. Lanes 5 to 15: negative samples, Lane 16: DNA Ladder (100 bp)

DISCUSSION

Our study was the first survey on prevalence of HPV infection in women in Rasht, Northern of Iran. In our study, prevalence of HPV infection in women with normal and abnormal cell Pap smear was 4.08% and 20% age between 26-39 y old women but, the HPV prevalence in USA among 14-59 y old women was 26.8% [11]. Also, the prevalence of HPV infection in different age's group of women is 4% to 19.3% in different countries [12-15]. Other studies in Asia, reported the prevalence of HPV infections approximately 10%, 10.9%, 6.3% and 23.6% in South Korea, Southern Vietnam, Thailand and China respectively [8,9,16]. Given that similar work was performed in Tehran University of Medical Sciences and abnormal Pap specimens has been reported in 5 to 6 percent [17]. Prevalence of HPV infection in Rasht is higher than above mentioned HPV prevalence especially in normal pap smears and similar to Hijjaj et al., study in Bahrain which was 11% [18].

Because of relation between cervical cancer and HPV infection and lack of adequate information about different types of HPV in population of women in this region of Iran, further studies are needed to identify unknown genotypes other than 16, 18 in women, and also the prevalence of HPV genotypes in men in Rasht.

CONCLUSION

A moderate prevalence of HPV infection was evidenced in this study but needs to be given more attention as the infection was observed in apparently healthy women also. This information will increase our understanding of the natural history of HPV infection and assist in the development of effective screening programs. Health officials should conduct the study and wider screening of this infection occurring in this province.

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