Original Article

Assessment the prevalence of high-risk human papillomavirus types 16 and 18 in 15 to 45 years old women

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Received: 29 September, 2016, Accepted: 7 November, 2016

Abstract

Background: Cervical cancer is the fourth most common cancer in incidence and the first genital cancer in women around the world, which 95% of them are related to human papillomavirus (HPV) infections. The risk of cervical cancer increases 10-12 time in women with HPV infection. This study aim to evaluate the prevalence of high-risk HPV infections among 15-45 years old women.

Materials and Methods: This cross sectional study was carried out on 92 normal women who admitted at Semnan hygiene center and has 15-45 years old. Cervical samples were collected using Cytobrush cell collector and consequently DNA extraction was performed using commercial DNA extraction kit. Polymerase Chain Reaction (PCR) was done using HPV (GP5, GP6) universal primers accompanied by positive and negative control in each PCR run. In order to extracted DNA template quality control, actin gene used as housekeeping gene.

Results: In this investigation, study subjects age range found to be 15-45 with mean of 30±0.9 years old. HPV infection was not found in patient group. Thus, further approach in order to HPV16 and HPV18 types detection, was not performed. However, other studies represented low to moderate prevalence for HPV in some regions of Iran.

Conclusion: Cervical cancer is one the major health concern and the fourth most common cancer around the world. This cancer is more common in developing countries than developed countries due to lack of screening program. Regard to possible high prevalence rate of HPV virus and its association with cervical cancer, we suggest further determination of the HPV prevalence as well as planning in large-scale vaccination in high risk group.

Keywords: Human Papilloma Virus, Cervical cancer, Prevalence, HPV18

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Please cite this article as: Rezvani MR, Shams M, Sayaadi M, Beigi P, Shams M. Assessment the prevalence of high-risk human papillomavirus types 16 and 18 in 15 to 45 years old women. Arch Med Lab Sci. 2016;2(4):117-22.

Introduction

Regard to WHO report, cervical cancer is the fourth most common cancer among women and seventh most prevalent cancer among world's human population (1). Numerous studies has been reported that HPV is the first and most important agent causing cervical cancer (2, 3).Translatable HPV genome enters host cells and this process is necessary for creating and growth malignancy in those cells (4).The carcinogenicity of HPV is attributing to E6 and E7 viral gene which theirs modifier proteins interferes with P53 and Rb tumor suppressor proteins (5-7). In addition, E2 viral gene silencing is another factor for progress in HPV associated cervical cancer

malignancy (8). Knowledge on HPV association with High-grade squamous intraepithelial lesions and cervical cancer as well as pre-invasive lesion potential ability has been justify performing investigations on screening and treatment programs for further eliminating this viral infection (9). Cervical intraepithelial neoplasia (CIN) categorized to 3 different clinical stage follow as: CIN I as slight dysplasia, CIN II as moderate and CIN III as sever dysplasia (10).

The maximum incidence rate of cervical epithelial cells dysplasia is up to 35 years of age but the 45 is the second peak(11). The prevalence rate for cervical cancer found to be high in developing countries owing to lack of or weakness in screening program.

More than 82 percent of cervical cancer is in developed countries as well as more than 91 percent is in developing countries are associated with HPV (7). HPV genome existence in all degree of cervical cancer has been confirmed and women with HPV have several times increased risk for cervical cancer (12). Fifteen serotype are associated with cervical cancer out of 118 HPV known serotype (13,14). Some of these serotype including 16,18,31,33,39,45,51,52,56,58,59,68,73,82 HPV serotypes contributed to cause 90 percent of high grade CIN and cervical cancer (15).

HPV16 and HPV18 are most frequent serotype, which contributed to invasive cancer as well as CIN II and CIN III and found in 70 percent of women with these diseases (16,17). HPV 16 has been found in 75 percent of cervix, Vulva and anus carcinoma (18) and also is most common HPV serotype in women with normal cervical cytology (16, 17). HPV 16 and 18 attribute to 87 percent of invasive cervical cancer (ICC), 89 percent of squamous cell carcinoma (SCC) and 84 percent of high grade intraepithelial lesion (HSIL). Among theme HPV16 cause 56%, 55% and 45% and HPV 18 cause about 17%, 12% and 6% of those diseases respectively however these amounts is somewhat variable depend on geographic location (19). Risk of transmission through sexual contact is high in women (20-24). Many studies has been shown that existence of screening program for cervical cancer using conventional or PCR method can decrease morbidity and mortality in women specially women more than 30 years old (25). With respect to clinical importance of HPV specially types 16 and 18, this investigation aim to determine the prevalence rate for HPV 16 and 18 types in 92 normal Iranian women.

Methods

Patient and sample. In this study, 92 normal Iranian women who admitted to Semnan hygiene center were included. Study subjects age range was between 15-45 years. Cervical sample were collected using sterile Cytobrush cell collector according to standard protocol. Consequently, samples stored at -20ºC until subsequent use. In addition, ethical approval and study subjects consent were obtained and recorded in theirs medical records.

DNA extraction. In order to DNA extraction, the samples blended with 3 ml normal saline and centrifuge at 3000 g for 3 minutes twice. Supernatant discarded and the pellet used for subsequent extraction procedure. DNA extraction was performed using high yield DNA purification Kit (CinnaGen Molecular Biology and Diagnostic, Iran) according to the manufacturer instruction. Extracted DNA was evaluated by electrophoresis on 1% agarose gel.

Table1: Primers name and sequences.

Primer Name	Primer Sequence	Amplicon size (bp)
ß-ACTIN	Forward: GTGGGGCGCCCCAGGCACCA	535
	Reverse: CTCCTTAATGTCACGCACGATTTC	
HPV GP5, GP6	Forward: TTTGTTACTGTGGTAGATACTAC	150
	Reverse: GAAAAATAAACTGTAAATCATATTC	

Polymerase chain reaction:

1. Housekeeping gene. In order to DNA template quality control, Actin gene was used as housekeeping gene. PCR performed by Eppendorf thermal cycler, using specific primer against Actin gene (Table 1). Thermal cycling program was follow as : 94º C for 2 min followed by 25 cycles of 94º C for 30 sec, 60º C for 30 sec, 72º C for 30 sec and final extension at 72º C for 7 minutes. Eventually, 5µl of PCR product load into 1% agarose gel (Roche-Basel- Switzerland) with TAE 1X running buffer, then stained by GelRed and analyze by Gel Documentation System Transilluminator® (Thermo Fisher. USA)

3. PCR for HPV detection. Polymerase chain reaction was performed for each extracted DNA template using GP5 and GP6 universal primers against highly conserve HPV gene (table 1), (26). The thermal-cycling program was the Initial denaturation at 94º C for 5 min followed by 35 cycles of 94º C for 60 sec, 55º C for 60 sec, 72ºC for 40 sec and final extension at 72ºC for 10 minutes. In addition, positive and non-template control was used in each run then 5µl of PCR product load into 1% agarose gel beside 100bp DNA ladder and visualize by UV transiluminator®.

Results

In this investigation age range of the study subjects was between 15-45years with mean age of 30±0.9. Almost half of them were 25-35 years old (Figure 1). PCR was performed on extracted DNA using specific primer against actin gene and the result was excellent and represented the presence of actin gene in all samples (Figure 2). In addition, PCR carried out on extracted DNA using HPV specific primers. There was no positive sample for HPV among study subjects so PCR wasn't perform for HPV (types 16 and 18) detection. PCR accuracy confirmed by positive and negative (non-template) controls (Figure 3).

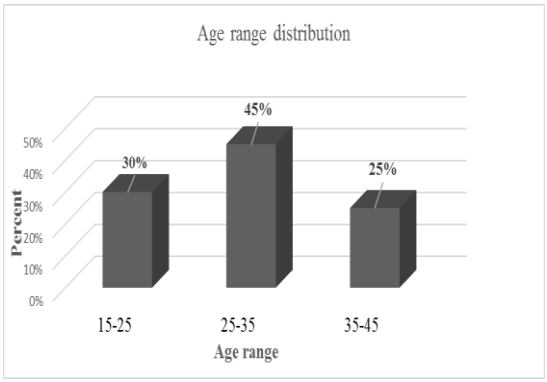

Figure 1. Age range distribution among study subjects.

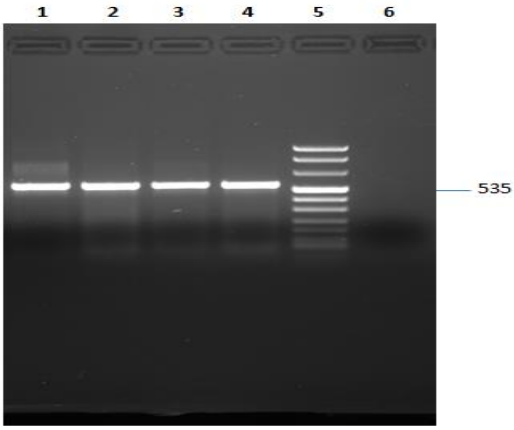

Figure 2. line 1-4 actin gene DNA amplification product on 1% agarose gel electrophoresis, line 5, 100 bp DNA ladder, line 6 non template control.

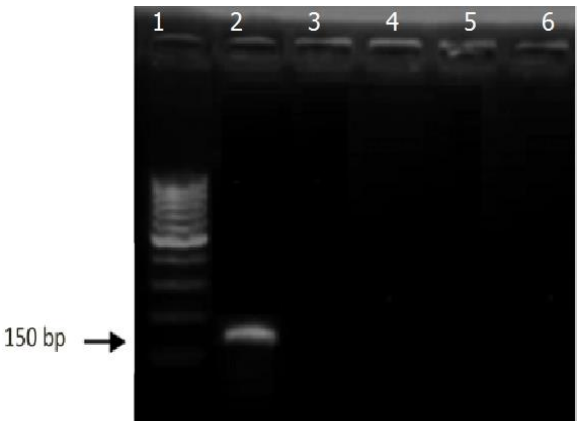

Figure 3. HPV DNA amplification, line 1 100bp DNA ladder, line 2 positive control, line 3-6: PCR on sample from study subjects. 1% Agarose gel electrophoresis with 1X TAE running buffer was used for gel electrophoresis.

Discussion

Cervical cancer is one of the most common cancer in term of incidence rate and cause of morbidity. According to WHO report, 528000 of new cervical cancer case has been diagnosed in 2012 around the world, which 85% of them were in developing countries (1). Regard to previous studies, the risk factors for cervical cancer were included:

having young age at first sexual contact, history of Chlamydia or herpes simplex infection, smoking, immune system failure, multiple sexual partner, familial background, having oral contraceptive pills and HPV infection (27, 28). Approximately, 95% of cervical cancer related to high risk HPVs. In endemic area, at least 80% of women infected by HPV until 50 years old. In general, about 14-35% of women infected by HPV viruses but 80-90% of them were recovered within 2 years (29).

This investigation aim to determine the prevalence rate for high risk HPV included type 16 and 18 among 15-45 Iranian women in Semnan province. Our study illustrated that there was no infected individuals who infected by HPV.

A Canadian study in 2000, determine prevalence rate for HPV among 1004 high risk women whom had 15-49 years old using Hybrid Capture II (HCII) ,PCR and genotyping methods and found the prevalence of 24% for HPV among 20-24 years women (30). In this study, sample number has been very much more than our study and they use combined test for HPV detection. With respect to mentioned reasons and existence of some risk factors in theirs study group as well as religious beliefs and social behaviors of people in Semnan province, our result is reasonable and justified.

A study in 2003 on 22089 women with age range of 20-90 years represented the prevalence rate of 5.2 % for HPV among them and the selected method was Pap smear (31). Sample size in our study was much lower than their study and age range was narrower either. For example most of them were 45% were 35-45 and 30% 15-25 years old, respectively.

In an investigation by Tarkowski on 312 girls who has mean of 16 years old with history of 2 years sexual activity and 4 different sexual partners, the prevalence of HPV found to be 64% using PCR method (32).

In another study was performed by Altugu in Turkey, HPV prevalence evaluated among 148 women with pyrogenic endocervicitis using HC II method showed to be 5.4%. About 40 % and 16.2% of them had been using intrauterine devices (IUD) and oral contraceptive, respectively (33).

A study in Argentina showed the prevalence of 43% and 60% for HPV in rural and Guarani Indians women respectively using PCR which had been associated with the age in firs sexual contact, number of sexual partner and smoking (34).

In a study by Bagheri et al, in Tajrish hospital located in Tehran, Iran, showed prevalence of 36.5% and 5% for HPV among 59 patients with transitional cell carcinoma and 20 normal individuals respectively. The patients' age was from 18-81 years and method for detection was PCR method (35). High prevalence in this study in patients group may due to increase in HPV copy number by the time. Only one sample (5%) in the control group was positive (35).

A study in Shiraze city, Iran, on 101 women with cervical cancer, evaluated the prevalence of HPV using PCR method and found to be 87% among them (36).

In another study in Tehran, Iran, on 851 women who has 18-65 years, the prevalence of HPV showed to be 31.1% using Pap smear, PCR and RFLP which 7.3% and 2.8% of them are type 16 and 18 HPV respectively (2).

Our result disagreement with other studies may be owing to difference in sample size, age range and theirs risk factor for HPV as well as religious beliefs and social behaviors.

Conclusion

In conclusion with respect to HPV clinical importance and high prevalence in some area of Iran, we suggest performing further investigation on determining exact prevalence of HPV in Iran for eliminating and early treatment of this disease . In addition, we suggested large-scale vaccination for HPV in high risk group in Iran.

Conflicts of Interest

None.

Acknowledgment

Assistance of Fatemeh Shobeirian in providing samples is deeply appreciated.

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