

## Identifying Human Papilloma Virus (HPV) in Women with Genital Warts by Multiplex-PCR Method

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### Abstract

**Background:** Many studies have shown that some types of Human Papillomavirus (HPV) play an important role in cervix cancer which has been identified in 99% of cervix cancers worldwide. This study aimed at identifying the frequency of high-risk types of this virus in cervical lesions among patients referred to Al-Zahra Hospital, Rasht.

**Materials and Methods:** Forty five vaginal and cervical swap samples of women with genital warts were tested considering the presence of DNA of 14 common HPV types by using 3 series of specific PCR.

**Results:** Out of 45 vaginal swap samples of women suffering genital warts, 37 samples (82.2%) were reported with 95% CI (70.6-93.83) considering the presence of HPV. The frequency distribution of HPV types in 45 patients with genital wart were as follows: 5 cases (13.5%) with HPV35, 4 cases (10.8 %) with HPV16, 4 cases (10.8 %) with HPV31, 3 cases (8.1 %) with HPV33, 3 cases (8.1 %) with HPV52, 3 cases (8.1 %) with HPV58, 2 cases (5.4 %) with HPV45, 1 case (2.7 %) with HPV18, 1 case (2.7 %) with HPV59, and 1 case (2.7 %) with HPV39.

**Conclusion:** Inasmuch as samples of our study have been collected from one of the most important women-referring medical centers (Al-Zahra Medical and Teaching Center) in Guilan province, thus the results can be used for screening, management, and vaccination of the target population against the common types of virus in Guilan.

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## Introduction

Cervical cancer is the second cause of mortality in the world. In this type of cancer, more than any other type, the effect of prevention, early diagnosis and treatment is obvious on mortality reduction [1]. Since 1970, human papilloma virus (HPV) was introduced as the most important and best-known environmental cause of this cancer [2]. This virus is transmitted sexually and although more than 200 different types have been recognized, molecular studies have shown that only 15 types (52, 56, 58, 59, 68, 73, 82, 16, 18, 31, 33, 35, 39, 45, 51 types) of it called high-risk types are carcinogenic [3]. However, other predisposing factors such as having multiple sexual partners, immune deficiency syndrome, oral contraceptives and smoking are other causes of cervical cancer [4, 5]. By clarifying the fact that high-risk types of HPV cause 99% of cervix cancer and early diagnosis and treatment of these infections can prevent the lesions to become cancerous, one can easily notice the importance of diagnosis in screening programs and routine clinical practices [6-8]. Limitations of serological diagnosis methods (Pap smear) of cervix cancer led to developing more sensitive methods [9]. Moreover, culture and serological methods are not reliable and sensitive for HPV diagnosis [10]. Molecular techniques such as PCR in affected tissues can be helpful [11]. In recent years, various methods such as RFLP, reverse hybridization and

direct sequencing have been used to determine the genotypes of the virus [12]. However, using specific PCR for each type is a relatively simple and economical method to determine HPV types [13, 14]. Although, types 16, 18, 31, and 33 are introduced as cancer-causing types around the world, they are not always the most common ones [14]. Thus, it seems necessary to separately identify HPV types in each population so as to use them in effective screening program, management and vaccination of the target population against the common types of virus in that community. To accomplish this goal, 14 high-risk types of cervix lesions among the abovementioned patients were evaluated by multiplex PCR technique for the first time in Rasht.

## Materials and Methods

In this study, 45 women with genital warts were studied who had referred to Al-Zahra Hospital, Rasht for vaginal examination since 2010 October to 2011 April. Sampling was done by a gynecology assistant by using sterile swab of vagina and cervix of all patients under sterile conditions, and then samples were placed in 1ml of transport solution (PBS) and quickly transferred to molecular laboratory. After homogenization in transport solution, swabs of vaginal discharge were kept in

temperature -20 °C. Separating DNA from vaginal discharge was performed based on extraction kit of DNG (Cinna Gen). Simultaneously with multiplex PCR method, extracted DNA was evaluated in terms of quantity ( $1.6 < OD < 1.9$ ) and quality by using primer pairs PCO3 (5'-ACACAACGTGTTCACTAGC-3') and PCO4 (5' CAACTTCATCCACGTTCAC-3') which proliferates a part of  $\beta$ -globbin gene. For toping of 14 types of HPV including 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 66 all samples were evaluated under three series of multiplex PCR based on specific kit primers of high risk HPV of Sacace Company of Italy. In first series PCR high-risk types, in second series mediate-risk types, and in third series low-risk types were assessed. Finally, the detection and photography of PCR products under UV light after electrophoresis of products for 45 minutes on Agarose gel 2% and 3% consisting of 0.5-1 mg in ml of ethidium bromide was performed.

## Results

Mean age of patients was  $31.8 \pm 7.9$  years (age range of 20-54). Presence of 723 bp band after PCR process about  $\beta$ -blobin displayed that the quality of all isolated DNA samples were good. Presence of various bands from 227 bp to 520 bp in the sample indicated presence of HPV (Fig. 1). Out of 45 vaginal swab samples of patients with genital warts, 37 samples (82.2%) with 95% confidence intervals (70.6-93.83) were reported to be positive and 8 cases (27.8%) negative considering the presence of HPV DNA. The frequency distribution of types of HPV in patients with genital warts include: 10 patients (27.0%) with 2 high-risk serotypes as simultaneous serotypes (35, 45), (16, 52), (18, 33), (59, 35), (18, 52), (39, 52), (18, 35), 5 patients (13.5%) HPV 35, 4 patients (10.8%) HPV 16, 4 patients (10.8%) HPV 31, 3 patients (8.1%) HPV 33, 3 patients (8.1%) HPV 52, 3 patients (8.1%) HPV 58, 2 patients (5.4%) HPV 45, 1 patient (2.7%) HPV 18, 1 patient (2.7%) HPV 59, 1 patient (2.7%) HPV 39. Based on our findings, the prevalence of HPV infection in women with genital warts, especially in Guilan, Rasht, is reported 82.2%.

## Discussion

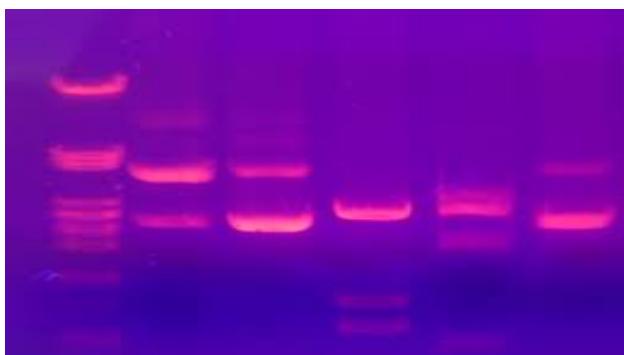
Cervical cancer is common around the world and considered as the most common type of cancer in developing countries. In terms of frequency, this cancer is the second most common cancer among women and annually more than 400,000 cases of this malignancy is reported in the world [16]. Many studies have demonstrated that some high-risk types of HPV play an important role in causing cervix cancer which has been identified in more than 99% of cervix cancers in the world [4]. Researchers remind many risk factors which cause this cancer. Based on many control studies so far, HPV infection is recognized as the main cause of developing neoplasia and malignance in epithelium of sexual organs, this especially becomes more evident when constant infection occurs due to the high-risk type. However, there

is no comprehensive statistics on prevalence oncogene of cervix in Iran [6].

Laboratory findings on genome diagnosis of HPV in samples suggest that the prevalence of cancer-causing HPV in patients was 82.2% in this study which is consistent with findings of several studies in other areas of study. In a research in northern Iran (Mazandaran), the presence of HPV in cervix cancer samples is reported 81.4% [18]. Based on Farjadian et al. who studied 101 cases of cervix carcinoma in Southern Iran (Shiraz), 88 samples were HPV positive (87.1%) [19]. in a study by Jabbarpour et al. in Tabriz after studying 75 recorded samples in formalin, prevalence of HPV was reported 62% [6]. Then, the prevalence of HPV in center of Iran (Tehran) was 73% based on study by Keihani et al. in a population of 100 [1]. In southeastern Iran (Yazd), HPV prevalence was 75% based on Mahmoudi et al. [24]. This study shows that frequency of HPV infection in cervix cancer in Iran is considerable and comparable with other countries [23]. Prevalence in other countries is also consistent with our results. After evaluating 104 biopsies from women with cervix cancer, Gonzalez et al have shown the prevalence of HPV infection in these lesions 56.4%, [26]. Martina et al. studied 261 samples in terms of presence of HPV and declared that 234 of 261 samples (89.6%) were HPV positive [27]. Pap smear test which identifies lesions and outcomes of infection is used as a common screening test worldwide due to availability and low cost. But still false negative is seen in this test [20]. Since HPV infections are usually asymptomatic, there is a need to find ways to improve the process of screening [21]. Therefore, using this method along with cytologic methods can increase the ability and accuracy of clinical studies [19]. Further, determining the most common genotypes of the virus in a region is very important in epidemiological studies and developing vaccination programs against HPV [22]. Simultaneous infection with two high-risk serotypes was the most common one in this study. Type 35 was in the second place in terms of frequency and type 16 and 31 with same frequency in third place; serotypes 33, 52, 58, 45, 39, 59 and 18 had the lowest prevalence, respectively. In studies done in Mazandaran and Yazd, type 16 was found as the most common type in samples with cancer [18-24]. But in our study, the prevalence of type 35 in samples with HPV was more than types 16 and 31 (Table 1). The most common types of HPV found in Tabriz by Jabbarpour et al. was reported type 16 with a frequency of 64.5% followed by types 13, 18 and 33 with 3.6%, 11.22% and 1.6%, respectively among positive samples. Simultaneous infection with various types was witnessed in 15 samples (20%). In study by Mahmoudi et al. in Yazd, type 16 was the most common genotype observed in 70% of positive HPV samples followed by genotypes 18 (16.7%), 45 (6.7%), 33 (3.3%) and 73 (3.3%) [24]. Similarly in a study in Tehran by Nikan et al. the prevalence of serotype 11 was 13 (21.66%), that of serotypes 16 and 18 was 14 (23.33%), and prevalence of serotypes 31 and 33 was 15 (25%) [2].

**Table 1.** Different types of HPV prevalence among cases of genital warts in women

HPV types identified	16	18	31	33	35	39	45	52	58	59	Multi	Total
Number(%)	4(10.8)	1(2.7)	4(10.8)	3(8.1)	5(13.5)	1(2.7)	2(5.4)	3(8.1)	3(8.1)	1(2.7)	10(27.0)	37(100.0)



**Figure 1.** Discussion: electrophoresis of PCR products on Agarose gel 2% (Second series). M is indicative of 100 bp marker. 1 shows disease with simultaneous infections of serotype 52 (with 450 length band) and serotype 16 (with 680 length band). 2 show infection with of serotype 45 (with 500 length band).

In other studies done in different countries by the International Cancer Research (IARC), the most common types of high-risk cervical HPV which can infect cervix based on prevalence are as follows [29]: HPV 16 (53%), HPV 18 (15%), HPV 45 (9%), HPV 31 (6%), HPV 33 (3%). This difference in pattern seems to be more due to differences in studied populations i.e. the population of Mazandaran and Tehran follows a pattern similar to the global one, whereas southern regions of Iran with a different pattern. Though, ultimate conclusions necessitate further research. According to the study by Jabbarpour et al. [6], these results can strengthen the

assumption of different frequency of circulating types in a population from other communities.

The extent of individual and multiple infections was respectively 78% and 22%. These results can be used in developing medical strategies for simultaneous targeting of specific and multiple types of this virus [25]. Due to the fact that samples of our study were gathered from one of the most important referral center for women (Al-Zahra Hospital) in Guilan Province, thus the results can be used for the first time for screening, management and finally vaccination of target group against common types of viruses in Guilan.

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## Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

## Conflict of Interest

The authors declare no conflict of interest.

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