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The Prevalence of High-risk Human Papillomavirus Type 16 and 18 in Women in Rasht-Iran

Gholam Reza Pourseify (PhD)[1], Marzieh Mehrafza (PhD)[2*], Azadeh Raoufi (PhD)[3], Zahra Nikpouri (PhD)[2], Marjan Askari (PhD)[4], Elmira Hosseinzadeh (PhD)[3], Pegah raffia (PhD)[3], Kimiya Razeghian (PhD)[5], Ahmad Hosseini (PhD)[6]

[1] PhD Student of Genetic, Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran
[2] Fellowship of Infertility and IVF, Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran
[3] Graduated, Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran
[4] Assistant Professor, Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran
[5] General Practitioner, Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran
[6] Professor, Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran

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ABSTRACT

Background & aim: Cervical cancer is one of the leading causes of cancer death among females. Human papillomavirus (HPV) is the most important risk factor for cervical cancer. The aim of the present study was to explore the prevalence of high-risk human papillomavirus types 16 and 18 in women who undergo HPV test.

Methods: In this descriptive epidemiological study, which was conducted in Mehr Medical Institute, Rasht, Iran from 2019 to 2020, two cervical samples were obtained from each of 301 patients for cytological and real-time PCR evaluation. Genotyping the samples was carried out using the Real-Time PCR technique. Different genotypes were divided into the following groups: 16 and 18 genotypes, other high risk genotypes, possibly low risk and high risk genotypes.

Results: The prevalence of HPV types in the study participants with a mean age of 33.4± 6.5 (18-61) years were 36.5% (n=110). HPV16 and 18 were detected in 28 (25.7%) and 7 patients (6.4%), respectively. Histopathological findings among HPV positive and negative participants were similar. HPV distribution according to women´s age was: group 1 (20-24.9 years, 47%), group 2 (25-29.9 years, 42.6%), group 3 (30-34.9 years, 40.4%), group 4 (35-39.9 years, 27.6%) and group 5 (40≤ years, 28.3%).

Conclusion: The general percentage of HPV positive patients in the local area can be compared to the previous literature. The study includes updates on the prevalence and type of HPV distribution between women of Guilan province in Iran.

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Introduction

Infection with human papillomavirus (HPV) is the greatest risk factor for cervical cancer, as the second most common type of cancer in women worldwide. Epidemiological studies have shown that cervical cancer is still the leading cause of cancer death between women (1). There are more than 80 different serotypes of HPV that are divided into low risk and high risk groups due to their oncological potential (2).

Primary viral oncogenes play significant role in the development of cancer. Following persistent infection with oncogenic high-risk HPV, the viral genome can attach to the host DNA. This integration of viral genome disrupts the regulatory function of viral oncogenes (3). Synergism of viral oncogenes leads to abnormal cell proliferation and eliminates p53-dependent apoptotic pathways (4).

Epidemiological studies are crucial for assessing the distribution and prevalence of HPV infection in the general population. About 10% of women worldwide with normal cytological findings have a

* *Corresponding author:* Marzieh Mehrafza, Fellowship of Infertility and IVF, Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran. Tel: 00989111310227; Email: marzieh.mehrafza@gmail.com

detectable HPV infection that varies depending on the HPV test technology, sample size, age groups, and geographical area (5). HPV16 is the most common oncogenic genotype in the most regions of the world. HPV18 and other oncogenic genotypes such as 31, 39, 52, 56 and 58 are the most prevalent after 16. The results of the recent study by the International Agency for Research on Cancer (IARC) assessment of HPV carcinogenicity revealed that twelve HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are the most prevalent subtypes in the entire female population worldwide and classified as Group 1 "carcinogenic to humans" (6).

In our country, Iran, studies have been conducted in different geographical areas that have evaluated the prevalence of this virus (7-9). Generally, according to the latest systematic review and meta-analysis the prevalence of HPV among Iranian women with cervical infection is 38.6% (10). Regarding the prevalence of HPV in women in the north of the country (11), only one study has been conducted in which the prevalence of different genotypes has not been studied. Evaluating exact prevalence of HPV genotypes in Iran is essential for managing and treatment of this disease. This study aimed to explore the prevalence of high-risk human papillomavirus types 16 and 18 in women in Rasht-Iran from 2019 to 2020.

Materials and Methods

The current study was a descriptive epidemiological research that evaluated the proportion of patients with HPV referred to Mehr Medical Institute, Rasht, Iran, during 2019-2020. The study was approved by Institutional review board. Inclusion criteria were as follow: patients aged 14 to 59 years and HPV typing using real time-PCR. The study population was all women who underwent HPV typing test. Three hundred and one women were included in the present study. Patients without histopathological findings were excluded from the study. Informed consent was obtained from all participants following the demonstration of the study.

From each female, two cervical samples were acquired, one in phosphate-buffered saline (PBS) for molecular evaluation and the other in fixator for cytological evaluation; respectively.

In order to perform the Pap smear correctly, individuals were asked to refrain from sexual intercourse and stop taking any vaginal medication for 48 hours before the test. A plastic spatula was used to take the sample from outside and inside of the cervix. Immediately after spreading the samples on the slide, 96% ethanol was used to fix the samples. The slides were stained by Papanicolaou method and studied by a pathologist with ×400 and ×1000 magnification. According to the Bethesda system, the results are divided into the following: normal, negative for intraepithelial lesions or malignancy, reactive changes & repair, epithelial and glandular cell abnormality.

DNA extraction was performed based on 4 step procedure (lyse, bind, wash and elute) of the QIAGEN protocol (B) during 1 month of sample collecting. All procedures were done based on manufacturer's instruction entitled Spin Protocol of the QIAamp DNA Mini Kit (Qiagen Inc. Valencia, CA). The volume of 200µl of starting material utilized was used. The product DNA was washed in sterile double distilled H2O (50µl) and stored at −20 ◦C until use.

Real-time polymerase chain reaction (PCR) method was performed for the analysis of HPV typing on each pop smear samples. For internal control, Actin gene was used as housekeeping gene. PCR steps were performed for each DNA sample using GP5 and GP6 primers against conserved regions of the HPV gene. Different types of genotypes were divided into one of the following groups:

High risk: 16, 18

Other High risk types: 31, 33, 35, 39, 45, 51, 52, 56,59,66,68

Possibly high risk: 26, 53, 67, 70, 73, 82

Low risk: 6, 11, 40, 42, 43, 44, 55, 83

The statistical analysis was done using SPSS version 21 (Chicago, IL, USA). Descriptive statistics were used for data analyzing. Simple Chi square test was used for categorical variables. P-value less than 0.05 was considered as significant.

Results

A total of 301 women with a mean age of 33.4± 6.5 (18-61) years were included in the present study. The prevalence of HPV types in the study participants were 36.5% (n=110).

Table 1. HPV genotype distribution among infected women

HPV type	N (%)
Low risk	16 (14.55)
Possibly high-risk	16 (14.55)
High risk viral type	27 (24.55)
Possibly high risk + High risk viral type	6 (5.45)
High risk viral type + Low risk	6 (5.45)
Possibly high risk + Low risk	3 (2.73)
Low risk + High risk viral type + Possibly high risk	3 (2.73)
HPV 16	8 (7.27)
HPV 16+ HPV 18	2 (1.82)
HPV 16 + Low risk	3 (2.73)
HPV 16 + Possibly high risk	2 (1.82)
HPV 16 + High risk viral type	2 (1.82)
HPV 16 +Possibly high risk + Low-risk	4 (3.64)
HPV 16 + High risk viral type	2 (1.82)
HPV 16 + High risk viral type + Low risk	1 (0.91)
HPV 16 + High risk viral type + Possibly high risk + Low risk	3 (2.73)
HPV 16 + High risk viral type + Possibly high risk	1 (0.91)
HPV 18	3 (2.73)
HPV 18+High risk viral type	1 (0.91)
HPV 18 + High risk viral type + Possibly high risk	1 (0.91)

Among infected patients, 61.8% (n=68) had one type of HPV and the rest (38.2%, n=42) showed more than one type of HPV infection. The HPV genotypes distributions among infected women were presented in table 1.

HPV16 and 18 types infection were found in 28 (25.7%) and 7 patients (6.4%) indicated, respectively and two patients (0.6%) showed multiple-type infection for hpv16 + hpv18.

Table 2. HPV distribution according to age of infected women

	group 1	group 2	group 3	group 4	group 5	P-value
HPV positive	8/17(47%)	26/61(42.6%)	40/99(40.4%)	21/76(27.6%)	13/46(28.3%)	0.2

Group 1 (20-24.9 years) Group 2 (25-29.9) Group 3 (30-34.9) Group 4 (35-39.9) Group 5 (40≤)

HPV distribution according to women´s age was presented in table 2: group 1 (20-24.9 years), group 2 (25-29.9), group 3 (30-34.9), group 4 (35-39.9) and group 5 (40≤) (p=0.2).

Table 3. Frequency of histopathology finding among HPV positive and negative participants

Pap smear report	Normal	Negative for intraepithelial lesions or malignancy	Reactive changes & repair	Epithelial Cell Abnormality	Glandular	Negative for intraepithelial lesions or malignancy + Reactive changes & repair	p-value
HPV positive	50	0	34	0	0	5	0.824
HPV negative	81	1	60	0	0	5	

Cytological findings of participants were presented in table 3: normal, negative for intraepithelial lesions or malignancy, reactive changes & repair, epithelial cell abnormality, glandular table 3. There was no significant difference among groups (p=0.824).

Discussion

The prevalence of HPV16 and HPV18 were 25.7% and 6.4% respectively which were lower compared with the world prevalence (12, 13). In this study, 36.5% of women were positive for HPV infection. This result was consistent with a systematic review and meta-analysis findings conducted in 2017 showed that the prevalence

rate of HPV types was 38.6% among Iranian women with cervical infections (10).

We have indicated that the HPV prevalence reach its highest point in females <35 years old, especially, females less than 25 years have shown the peak prevalence at 47%. The highest rates of HPV infection in women worldwide have been shown in females <25 years old (14).

The global HPV prevalence among females without cervical lesions is about 11-12%, which the severity of cervical lesions (cervical dysplasia and invasive cervical cancer) can increase such prevalence up to 90% (12). HPV16 subtype has a greater potential to develop cervical lesions as compared to other subtypes; so it also has a greater chance of being found during routine cytology-based cervical screening (15, 16).

In the present study, no patients indicated epithelial or glandular cell abnormality and cytological findings did not show any correlation with HPV results. Cervical cytology screening is a very specific test for high-grade premalignant lesions or cancer but, even if the quality of collection and proliferation, stabilization, smear maintenance, and reporting by well-trained technicians and cytopathologists are good, it has moderate sensitivity. Cytological screening failure to estimate cervical cancer and a false negative rate for invasive cancer of up to 50% have been indicated (17).

There is an international consensus as "high-risk" genetic variants, including genotypes 16,18,31,33,35,39,45,51,52,56,58,59, and 66 can lead to cervical cancer. However, Infection with low-risk genotypes 6 and 11 can cause changes in cervical tissue or low grade cervical disease and genital warts (18). The HPV virus can`t be grown easily in tissue culture. As a result, DNA sequence analysis can be used to identify HPV genotypes. Two diagnoses used to identify HPV genotypes are PCR and in situ hybridization. Considering the fact that the PCR-based methods used by majority of studies can only detect a subset of HPV types, Many "HPV-negative" cases may be infected by other undetectable types of HPV. However, detection of cervical HPV biopsies using these cytology-related methods could potentially improve screening for cervical cancer.

Several studies have been conducted on the prevalence and distribution of HPV in different provinces of Iran. In a study that examined the prevalence of high risk HPV types 16 and 18 in healthy Iranian women aged 15 to 45 years in Semnan province, HPV infection was not found in any of the participants (19). In another study that reported the prevalence of human papillomavirus in 100 cervical biopsy specimens in Mazandaran province, HPV DNA was observed in 78.6% of cervical cancers, 64.3% of dys/metaplasia and 9% of normal cases. HPV types 16 and 18 were observed in 60.6% of cases who were positive for HPV-positive cervical carcinoma (20).

Generally, the prevalence of HPV varies slightly between different geographical areas. Two-thirds of invasive cervical cancers are associated with HPV types 16 or 18. However, more than sixteen other HPVs were associated with invasive cervical cancer, the most common of which were 45, 31, 33, 58, and 52. HPV 15 and 16 types are clearly the predominant types in Asian women with and without cervical neoplasia. In invasive cervical cancer, the prevalence of HPV16 is reported to be 52%.(21).

Because the HPV genotyping test in this study was performed by real time PCR method and the signals were read using four different fluorescent dyes, this approach has a higher accuracy, sensitivity and detection power compared to older methods of molecular HPV detection (22) and it was the strength of this study, which, the results can be used in the development of medical strategies to prevent the spread of this virus. The weakness of present study was that, it was not possible to access patients' demographic information especially risk factors, so the effect of many demographic parameters were not evaluated.

Conclusion

The general percentage of HPV positive patients in the local area can be compared to the previous literature. The study includes updates on the prevalence and type of HPV distribution among women of Guilan province. HPV DNA testing has a greater sensitivity than cytology-based cervical screening and can be performed in cervical screening program. We suggest detecting exact HPV genotypes, for designing protection and treatment against this virus.

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Conflicts of interest

Authors declared no conflicts of interest.

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