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Seroepidemiological Evaluation of High-Risk Human Papillomavirus Types Among Married and Unmarried Iranian Women in Tehran, Iran

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Abstract

Background: Human papillomavirus (HPV) is a DNA virus that establishes productive infections only in keratinocytes of the skin or mucous membranes.

Objectives: This study aimed to determine the frequency of two high-risk genotypes of HPV among married and unmarried Iranian women.

Materials and Methods: This cross-sectional population-based study consisted of two groups of women: non-married girls referred for premarital counseling, and married women referred for pregnancy diagnosis. Blood samples were evaluated serologically with the ELISA method for HPV types 16 and 18.

Results: A total of 177 women (89 single and 88 married) were tested for HPV strains. The frequency of HPV type 16 in single women was significantly higher than in married women (66.3% vs. 40.9%, $P < 0.001$). The relative frequency of HPV type 18 was also significantly higher in single women than in married women (62.9% vs. 47.7%, $P < 0.001$). Moreover, HPV type 16 and 18 positivity was significantly associated with higher education levels in single women compared to married women ($P < 0.001$). Classification of HPV-infected women according to age revealed that the frequency of HPV type 16 was significantly higher in single women aged 25 - 35 years compared to married women ($P < 0.05$).

Conclusions: The results showed remarkable rates of high-risk HPV types (16 and 18) in the studied population, which can be a serious alert for public health. This result highlights the potential impact of prophylactic vaccines for future protection against high-risk HPV types in Iranian women.

Keywords: Seroepidemiological Study, Human Papillomavirus (HPV), Women, Vaccine, Iran

1. Background

Human papillomavirus (HPV) belongs to a large and diverse *Papillomaviridae* family, which contains non-enveloped small DNA viruses (1). HPV as a DNA tumor virus frequently causes epithelial proliferation and establishment of infections in the keratinocytes of the skin and mucosal surfaces (1, 2). Currently, HPV is known as one of the most common causes of sexually transmitted infections worldwide (3). So far, more than 100 HPV types have been identified, of which approximately 30 - 40 viral types are commonly associated with genital tract infections (4).

According to their association with cervical cancer and precursor lesions, HPV types have been subdivided into low-risk and high-risk categories (4). High-risk HPV types, such as HPV 16, 18, 31, 33, and 35, are mostly associated with cancer (5). HPV 16 and 18 are the most prevalent types reported worldwide, contributing to approximately 70% of cervical cancers, while other high-risk HPV genotypes are associated with more than 20% of cervical cancers globally (3, 6). These epidemiologic findings indicate the role of certain HPV types in cervical cancer development (7).

According to 2012 estimates, cervical cancer is the fourth most common cancer in women, with an estimated 528,000 new cases and 266,000 deaths globally each year (8). In Iran, with an incidence rate of 2.2 per 100,000 women annually, cervical cancer is the second most common malignancy in females (9). Moreover, the association of some HPV genotypes with other cancers, such as breast, head and neck, and lung cancers, has been evaluated in numerous investigations (2, 10). Prophylactic vaccines and routine cervical testing with Pap smears has resulted in a > 70% decrease in cervical cancers; however, reports still indicate HPV as a serious public health concern (3).

Numerous techniques are currently applied to identify HPV, ranging from consensus and type-specific PCR methods, real-time PCR assays, type-specific DNA in-situ hybridization, and detection of serum antibodies directed

against HPV epitopes (11). Although PCR-based detection of HPV is generally regarded as the standard method for establishing its presence, the selection of assays for clinical use will ultimately be influenced by concerns relating to sensitivity, specificity, reproducibility, cost, and feasibility (12). Several studies have indicated that the enzyme-linked immunosorbent assay (ELISA) method has desirable sensitivity and specificity, and its reproducibility is comparable to that of PCR-based assays (13-15).

2. Objectives

This study aimed to determined HPV prevalence among married and unmarried Iranian women. We also explored the distribution of HPV 16 and 18 among married and unmarried Iranian women, which can help in the planning of preventive policies, such as education and vaccination.

3. Materials and Methods

3.1. Study Design and Population

This cross-sectional population-based study was conducted for one year, in 2015, with a total of 180 women divided into two groups. The first group was 90 unmarried women referred for premarital counseling, and the second group was 90 married women referred for diagnosis of pregnancy. All subjects had been referred to one of three regional health centers, including Ershad, Resalat, and Dogmechi, affiliated with Shahid Beheshti University of Medical Sciences, Tehran, Iran. Due to lactescent serum, one of the unmarried subjects and two of the married subjects were excluded from the study. The study was approved by the university's ethics committee, and informed consent was obtained before sample collection.

3.2. Sampling and Diagnostics

Under aseptic conditions, 2 - 5 ml samples of venous blood were obtained from all participants and transported to the reference laboratory of the infection research center of Mofid hospital. Sera were tested for IgG antibodies against HPV 16 and HPV 18 using ELISAs specific to each HPV type. The ELISA tests were performed with the Human HPV 16 and 18 IgG E7 p- ELISA kit (Cat No. E 2042 Hu, E 2023 Hu) according to the manufacturer's instructions. A titer of IgG antibodies greater than 250 IU/mL was considered as positive by the manufacturer.

3.3. Statistical Analysis

Analysis was performed with SPSSTM software, version 21.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency. Values were expressed as the mean $\pm$ standard deviation (continuous variables) or percentages of the group (categorical variables). Chi-square or Fisher's exact tests was used to estimate any statistical association for quantitative variables, and t-tests were used to compare the means. $P < 0.05$ was regarded as significant relevance.

4. Results

A total of 89 single and 88 married women participated in the present study. The mean and range of age, geographical distribution, and education levels of participants are shown in Table 1.

The results showed that the mean age of the single women was significantly lower than that of the married women ($P < 0.001$), while education levels were significantly higher in the single women compared to the married women ($P < 0.001$).

Of the 177 examined women, the frequencies of HPV types 16 and 18 were 53.8% (n = 95) and 55.4% (n = 98), respectively. The frequency of HPV 16 in single women was significantly higher than in married women (66.3% vs. 40.9%, respectively; $P < 0.001$). The relative frequency of HPV 18 was significantly higher in single women than in married women (62.9% vs. 47.7%, respectively; $P < 0.001$). Moreover, HPV 16 and 18 positivity was significantly associated with higher education levels in single women compared to married women ($P < 0.001$). Classification of HPV-infected women according to age revealed that the frequency of HPV 16 was significantly higher in single women aged 25 - 35 years compared to married women ($P < 0.05$). Despite the higher relative frequency of HPV 18 in single women aged < 35 years compared to married women, the differences were not statistically significant. The full results of the serological examinations for all participants are presented in Table 2.

The relative frequency of HPV 16 positivity with regard to number of pregnancies in married women was as follows: no pregnancies, 15/38; one pregnancy, 12/23; two pregnancies, 4/14; three pregnancies, 2/8; four pregnancies, 2/4; and five pregnancies, 1/1. The rates for HPV 18 positivity in these patients based on number of pregnancies were: no pregnancies, 17/38; one pregnancy, 14/23; two pregnancies, 5/14; three pregnancies, 3/8; four pregnancies 2/4; and five pregnancies, 1/1. There were no statistically significant differences between rates of HPV 16 and HPV 18 with regard to the number of pregnancies in married women.

Table 1. Demographic Information of Single and Married Women Screened for HPV[a]

Evaluated Factors	Single Group Total, n = 89	Married Group Total, n = 88	Significance Level
Age, y, Mean ± SD	27 ± 4.6	36.7 ± 8.6	P < 0.001
Range	15 - 44	20 - 62	
Geographical distribution			P = 0.9
Resalat	29 (32.6)	30 (34.1)	
Dogme Chi	30 (33.7)	27 (30.7	
Ershad	30 (33.7)	31 (35.2)	
Education level			P < 0.001
Academic	83 (93.3)	56 (63.6)	
Non-academic	6 (6.7)	32 (36.4)	

[a]Values are expressed as No. (%) unless otherwise indicated.

Table 2. Clinical Characterization and Association of Related Risk Factors with HPV Genotypes[a]

Evaluated Factors	Single Group Total, n = 89	Married Group Total, n = 88	Significance Level
Anti-HPV antibody type 16, Mean ± SD	357.2 ± 134.7	275.8 ± 166	P < 0.001
Range	17.5 - 519.3	43.9 - 1000	
Anti-HPV antibody type 16 positivity	59 (66.3)	36 (40.9)	P < 0.001
Anti-HPV antibody type 18, Mean ± SD	296.3 ± 117.3	284.4 ± 179.2	P = 0.6
Range	25 - 410.1	25 - 1000	
Anti-HPV antibody type 18 positivity	56 (62.9)	42 (47.7)	P = 0.04
Anti-HPV antibody type 16 positivity based on education level			
Academic	56 (62.9)	21 (23.9)	P < 0.001
Non-academic	3 (3.4)	15 (17)	P = 1.0
Anti-HPV antibody type 18 positivity based on education level			
Academic	53 (59.6)	17 (19.3)	P < 0.001
Non-academic	3 (3.4)	25 (28.4)	P = 0.3
Anti-HPV antibody type 16 positivity based on age group			
15 - 25	20 (22.5)	3 (3.4)	P = 0.4
25 - 35	39 (43.8)	19 (21.6)	P = 0.03
35 - 45	0	9 (10.2)	ND*
45 - 55	0	4 (4.5)	ND*
55 - 65	0	1 (1.1)	ND*
Anti-HPV antibody type 18 positivity based on age group			
15 - 25	19 (21.3)	2 (2.3)	P = 0.2
25 - 35	37 (41.6)	22 (25)	P = 0.3
35 - 45	0	12 (13.6)	ND*
45 - 55	0	5 (5.7)	ND*
55 - 65	0	1 (1.1)	ND*

[a]Values are expressed as No. (%) unless otherwise indicated.
*ND, not determined due to small sample size.

5. Discussion

Currently, HPV is still an important topic due to reports indicating rapidly increasing rates of these infections. Two prophylactic HPV vaccines are currently approved for the prevention of high-risk HPV types. Gardasil is a quadrivalent vaccine for HPV types 6, 11, 16, and 18, and Cervarix is a bivalent vaccine against HPV types 16 and 18 (16, 17). Epidemiological data from regional studies on the distribution of HPV types in women with or without malignancy is crucial for predicting the impact of these vaccines (17). The present study reports that the prevalence of serologi-

cal positivity for HPV types 16 and 18 among healthy women in northern Iran (Tehran) are 53.8% and 55.4%, respectively.

The results of the present study for the frequency of HPV types 16 and 18 are consistent with previous reports from other regions in Iran and worldwide, which indicate a high proportion of these HPV genotypes in the general population compared to other high-risk HPV types (18-21). However, compared to studies applying cytological or DNA-based methods for identification of HPVs among healthy women, the rates of HPV types 16 and 18 in the present study were relatively high. Previously, Khodakarami et al. from Tehran in 2011, Zandi et al. from Bushehr in 2010, and Safaei et al. from Shiraz in 2010 reported that the prevalence of HPV types 16 and 18 among healthy Iranian women ranged from 2% - 3.1% and 0% - 1.5%, respectively (22-24). Beyond the HPV genotyping methods, different explanations may exist for such differences between our results and those of the mentioned studies, for example the number of asymptomatic patients in our study population. Interestingly, the results on HPV prevalence among Iranian cervical cancer patients are the closest to ours. Previous reports showed that the rates of HPV types 16 and 18 in Iranian cervical cancer patients ranged from 28.5% - 85.7% and 3% - 53.2%, respectively (18). Another explanation may be related to the reliability of IgG antibodies as a marker for recent HPV infection, since IgG serological positivity may result from previous or transient infections. In this regard, studies on the natural history of HPV infections have demonstrated their transient nature in young women (16). Although certain HPV types, such as HPV 16, are associated with higher rates of persistence, it has been documented that HPV 16 may clear by more than 70% after two years (16).

With regard to age classification, we observed a higher rate of serologic positivity for HPV 16 and 18 in younger women (< 35 years old) in both of the groups. Shafaghi et al. reported that among healthy women attending regular gynecological visits in Tehran, the highest frequency of HPV occurred in young women and decreased with age (9). Moreover, in agreement with our results, age-specific HPV prevalence rates worldwide are the highest in women younger than 35 years of age (17).

Concerning the effectiveness of HPV vaccinations, two key points must be considered. First, HPV vaccines are most effective when administered to HPV-naive women (16), who can be efficiently identified with serological assays (25, 26). Second, the U.S. Food and Drug Administration has approved Gardasil for use in girls and women aged 9 - 26, which is consistent with the higher prevalence of HPV in younger women (16, 17).

In summary, despite the limitations, we showed remarkable rates of high-risk HPV genotypes 16 and 18 in the studied population. Such a high frequency of these genotypes is a serious public health concern, since HPV 16 and 18 together account for a high proportion of HPV malignancies. This result highlights the potential impact of prophylactic vaccines for future protection against high-risk HPV types in Iranian women. However, further studies with wider sample sizes and age distributions, especially in schoolgirls, are recommended for reaching a comprehensive conclusion.

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Footnotes

Authors' Contribution: Study concept and design, Abdollah Karimi, Aliakbar Abedini; sampling, Zari Gholinejad; practical work, Somayeh Shamsy; drafting of the manuscript, Abdollah Karimi, Aliakbar Abedini; study supervision, Abdollah Karimi, Roxana Mansour Ghanaie.

Conflicts of Interest: None declared.

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