Original Article

The Fundamental Place of Pap Test in Iran, Does Primary HPV-Genotyping Seem Cost-Effective in Replace? A Cohort Study

Abstract

Background: Human papillomavirus (HPV) is a known risk factor for cervical cancer, and currently, primary HPV typing is recommended for screening instead of cervical cytology. However, there are limited studies on the prevalence of HPV in Iran. **Methods:** This cross-sectional study evaluated the liquid-based cervical smears of 700 women with no history of HPV vaccination and cervical dysplastic disease from 2017 to 2020 in Isfahan, Iran. Here, we compare the prevalence of HPV genotypes using COBAS with Pap smear cytology results in evaluating the most appropriate cervical cancer screening test. **Results:** The prevalence of HPV infection was 23.3%, including 8.7% with HPV 16/18 and 14.6% with other HR (high-risk) HPVs. In cytology reports, 8 out of 16 individuals with high-risk lesions were negative for any type of HPV; on the other hand, there were 129 HR HPV-positive patients out of 570 negative or low-risk Pap smear results. **Conclusions:** It assumed that there is no superiority for HPV genotyping over cytology or vice versa in detecting high-risk patients for cervical cancer; as only 26.8% of women with HPV show abnormal cytology; and from those with normal cytology, 17.9% were positive for HR HPV.

Keywords: *Human papillomavirus viruses, mass screening, Pap smear, uterine cervical neoplasms*

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Introduction

Cervical cancer is the most common gynecological cancer in women and the fourth leading cause of cancer death in developing countries. According to the latest global statistics in 2018, 570,000 new cases and about 311,000 deaths due to cervical cancer have been recorded, with Asia having the largest share.[1] The World Health Organization (WHO) announced 917 cases of cervical cancer in Iran, more than half of which died due to advanced disease in 2019.[2] Unfortunately, only 48% of Iranian women have participated in cervical cancer screening, and more than half of the newly diagnosed cases are those who have not been screened.[3-7] Human papillomavirus (HPV) is the leading risk factor in developing nearly all cervical cancers, which infected near half of sexually active women.[8,9]

According to the WHO statistics, an increasing trend of all cancers is predicted by 2040, especially in developing countries with low screening and diagnostic resources.[10,11] Although prior study revealed the low cost value for the implementation of national HPV vaccination in Iran, recent data seem controversial.[6,12] As a middle-income county, Iran falls in the category of countries with less than 30% coverage of HPV vaccination. Therefore, it is necessary to focus on screening programs and define the most valuable and effective methods based on local situations and the high mortality rate of cervical cancer in comparison with its incidence.[2,13-16] There are two standard screening methods for cervical cancer: A Pap smear test and HPV genotyping, which detects the HPV virus but is incapable of finding cellular changes. The 2019 American society of cancer prevention (ASCCP) guidelines suggest that HPV testing is more valuable than cytology in identifying cervical cancer.[17] The FDA approved the COBAS HPV test as the first DNA-based-HPV test for early cervical cancer screening. One of the advantages of this system is the reduction in repetitive tasks, the production of hazardous environmental waste and reducing costs by eliminating the need for additional identifiers.[18]

Iran's national screening program for cervical cancer includes a nonmandatory Pap smear examination every 5 years

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for 30-50-year-old women. However, there is no governmental funding support for HPV screening. To planning the most appropriate preventive and screening program, the present study examines the prevalence of the HPV infection and its genotype in unvaccinated women based on COBAS and compares it with cervical cytology reports in Iran.

Methods and Materials

In this cross-sectional study, we evaluate the liquid-based cervical smears of women with no history of HPV vaccination and cervical dysplastic disease, who were referred to oncologic department for cervical cancer screening with Pap smear and HPV test, in Isfahan, Iran. Data were gathered retrospectively from 2017 to 2020. All samples which had results of Pap smear ± cytology was included in the study. Samples that do not have the age of the patient or the results of the Pap smear test were excluded.

After obtaining smeared cell slides for a liquid-based cytology test, the remaining cell samples on the cytobrush were stored at room temperature for further HPV analysis. Roche COBAS® HPV test 4800 (Roche Molecular Systems, Pleasanton, CA), approved by the United States Food and Drug Administration, was used for HPV DNA typing. The COBAS® HPV test uses the amplification of target DNA by the polymerase chain reaction (PCR) and nucleic acid hybridization and can detect 14 high-risk HPVs. This HPV DNA testing method separately detects HPV 16 and HPV 18 and a pool of 12 other HR HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

We categorize patients based on HPV genotyping results into five groups: Negative, positive HPV-16, positive HPV-18, positive HPV-16 or 18 and other types, and positive for other HR-HPVs. The Pap smear result was categorized as normal (including negative result, atrophy, or mild inflammation), moderate to severe inflammation, low-risk malignant cells including atypical squamous cell with undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), and high-risk malignant cells (high-grade squamous intraepithelial lesion (HSIL)).

Data were analyzed using SPSS software version 22 (IBM Corp. Armonk, New York, USA). To describe continuous and categorical variables, mean ± standard deviation (SD) and frequency were used. The Chi-square was used to compare the frequencies between groups. The significance level was set at 0.05.

The ethics code is IR.MUI.MED.REC.1399.1047 and dated on 2021/02/15.

Results

This cross-sectional study included 700 cervical smear specimens from 700 healthy women. The mean (± SD) age of the study population was 37.3 (±8.62), of which 19 (2.7%) were under 25 years old and 4 (0.6%) were above 65 years old.

The prevalence of HPV infection was 23.3%, including 8.7% with HPV 16 and/or 18 and 14.6% with other HR HPVs. The distribution of HPV genotypes in the study population and age groups is provided in Table 1. Considering positive HPV genotypes, the frequency of HR HPV decreases in older age groups; 42.1% of women under 25 years old and only 6.8% of women in the age group of 55–64 years old were positive for HR HPVs [Supplementary Figure 1].

From 700 samples in our study, 586 samples were also examined for cytology, in which malignant cervical cells, including ASCUS, LSIL, and HSIL, were reported in 113 (19.3%) cases. HPV was detected in 18.7% of patients with normal cytology and 23.5% of those with moderate to severe inflammation. The HPV prevalence in low-risk and high-risk individuals was 32.0% and 50.0%, respectively. The prevalence of other HR HPVs in all Pap smear categories except the high-risk category is higher than the prevalence of HPV 16 or 18. In cytology reports, 16 individuals were reported as HSIL, from which eight individuals were negative for any type of HPV; four of them were positive for HPV 16, one for HPV 18, and two for other HR HPVs [Table 2].

In the age group of 25–34 years old, the prevalence of HPV in three groups of negative, low-risk, and high-risk cytology was 26.3%, 34.2%, and 71.4%, respectively (*P* value, 0.02). Details on the prevalence of HPV in different cytology categories are presented in Table 3.

Discussion

Cervical cancer is the leading cause of young age mortality in Asian countries. Declining the incidence and mortality of cervical cancer is a global goal that could achieve by manipulating HPV infection persistence, as the leading

Table 1: Distribution of HPV genotypes based on age categories

	Whole sample *n*=700	Age group *n* (%)					
		Under 25 *n*=19	25-34 *n*=291	35-44 *n*=248	45-54 *n*=109	55-64 *n*=29	65 and higher *n*=4
Negative	537 (76.7)	11 (57.9)	214 (73.5)	189 (76.2)	92 (84.4)	27 (93.1)	4 (100)
HPV 16 or 18 with or without other types	61 (8.7)	2 (10.5)	27 (9.3)	21 (8.5)	10 (9.2)	1 (3.4)	0
Other high-risk HPVs	102 (14.6)	6 (31.6)	50 (17.2)	38 (15.3)	7 (6.4)	1 (3.4)	0

Table 2: Distribution of HPV genotypes based on cervical cytology reports

Pap smear reports	Total *n*=586	HPV genotypes					*P*
		Negative	HPV 16	HPV 18	HPV 16 or 18 and other HR	Other types HR	
NL (mild inflammation or negative or atrophy)	112 (19.1)	91 (81.3)	8 (7.1)	0	4 (3.6)	9 (8.0)	0.01*
moderate to severe inflammation	361 (61.6)	276 (76.5)	12 (3.3)	0	14 (3.9)	59 (16.3)	
Low-risk malignant cells (ASCUS, LSIL)	97 (16.6)	66 (68.0)	6 (6.2)	0	6 (6.2)	19 (19.6)	
High-risk malignant cells (HSIL)	16 (2.7)	8 (50.0)	4 (25.0)	1 (6.3)	1 (6.3)	2 (12.5)	

ASCUS=atypical squamous cell with undetermined significance, HSIL=high-grade squamous intraepithelial lesion, HPV=human papilloma virus, HR=high-risk, LSIL=low-grade squamous intraepithelial lesion. *Chi-square

Table 3: Prevalence of HPV in different cytology categories based on age groups

	Age categories	Pap smear reports			*P*
		Negative for malignant cells (normal, inflammation, or atrophy)	Low-risk malignant cell (ASCUS, LSIL)	High-risk malignant cells (HSIL)	
HPV genotype	Under 25 yr				0.3*
	Negative	5 (45.5)	5 (71.4)	-	
	Positive	6 (54.5)	2 (28.6)	-	
	25-34 yr				0.02*
	Negative	143 (73.7)	25 (65.8)	2 (28.6)	
	Positive	51 (26.3)	13 (34.2)	5 (71.4)	
	35-44 yr				0.1*
	Negative	133 (78.2)	21 (63.6)	5 (71.4)	
	Positive	37 (21.8)	12 (36.4)	2 (28.6)	
	45-54 yr				0.03*
	Negative	66 (86.8)	11 (73.3)	-	
	Positive	10 (13.2)	4 (26.7)	1 (100)	
	55-64 yr				0.5*
	Negative	18 (90)	3 (100)	-	
	Positive	2 (10)	-	-	
	65 yr and above				-
	Negative	2 (100)	1 (100)	1 (100)	
	Positive	-	-	-	

*Chi-square

risk factor, by using effective screening or vaccination.[13] The results of COBAS-HPV genotyping show that 23.3% of unvaccinated women were infected by HR HPV. Based on the results of our study, there is no superiority for HPV genotyping over cytology or vice versa in detecting high-risk patients for cervical cancer, as only 26.8% of women with HPV show abnormal cytology, and from those with normal cytology, 17.9% were positive for HPV 16 or 18.

According to the last cancer epidemiologic reports in Iran, the estimated incidence rate of cervical cancer infection and mortality is about 2.3 and 1.2 in 100000, respectively. Although the rate of infection is almost low, the mortality rate is significant.[2,14-16]

Screening with one method had a rare but high risk of delay in the timely detection of cervical cancer.[19,20] In our study, high-grade dysplasia was detected in 50% of women with a negative HPV test. This percentage is unneglectable as they would be missed if only HPV genotyping was done. Though not all cases of HSIL would develop into cervical cancer, the rapid progression potential of these lesions has been confirmed and require a timely and aggressive approach.[21]

In this regard, a Turkish study revealed the presence of some unclassified HPV infections in patients with abnormal cytology. At the same time, numerous cases of positive HR HPV with normal cytology were detected.[22] Su Y *et al*. showed the presence of HPV infection in only 13.5% of patients with any abnormal cytology, although they did not differentiate between low and high-risk cytology.[23] The study of Kovacevic *et al*.[24] demonstrated the possibility of high-grade lesions despite normal HPV. It concluded that there is no guarantee for detecting all high-risk patients with a single screening method. They also find a probable correlation between other types of HPV, even the noncarcinogenic subtypes, with cancer progression.

Based on a study by Meloni *et al*.,[25] the rate of HPV infection among women with cytological diagnoses of ASCUS, LSIL, and HSIL was 37.3%, 60.1%, and 57.9%, respectively. In our study, the rate of HPV infection was 18.8% in normal

Pap smears, 32% in low-risk abnormalities (ASCUS, LSIL), and 50% in the HSIL group. In the present study, we found a high rate of HR HPV in patients with moderate to severe inflammation (23.5%), but there is not any consensus on the triage role of HPV in these cases.[26]

The present study demonstrated a higher proportion of HPV infection among patients under 35 years old, mostly younger than 25 years old, which shows the need for an educational program on sexually transmitted diseases. Although cervical cancer is not considered the most prevalent malignancy in our province, the significant transition in HPV prevalence due to changes in lifestyle behavior and socioeconomic factors ringing the alarm.[27]

Although the investigational study by the COBAS method considers one of the most valid methods of detecting high-risk HPV, especially in the primary setting, it does not permit recognition of non-HPV16 and 18 subtypes, which restricts the authors of the present study from analyzing the most prevalent subtype in infected cases. Based on our findings, the most prevalent subtype among infected cases was other HR HPVs with or without HPV 16 or 18. Although this finding was in line with the results of another study in North Iran,[28] it was inconsistent with two studies of other provinces that showed HPV genotypes 18 as the most dominant subtypes.[29,30] Our population's most common HPV genotypes were HPV16 in women with HSIL and other HR HPVs in low-risk cases (ASCUS and LSIL). This difference could be due to the sampling method and the study population.

Our study has some limitations. First, the retrospective design of the study limited us to gather different demographic variables and also to have enough sample size over all ages. Second, we collect our data from those who referred to the oncologic department for cervical cancer screening, which may introduce selection bias to our study. Finally, we do not have follow-up data on our study sample; hence, we cannot make a statement about the role of HPV infection in the outcome of patients.

The results of COBAS-HPV genotyping show that 23.3% of unvaccinated women were infected by HR HPV%, including 8.7% with HPV 16 and/or 18 and 14.6% with other HR HPVs. Based on the results of our study, there is no superiority for HPV genotyping over cytology or vice versa in detecting high-risk patients for cervical cancer and screening with one method had a risk of delay in the timely detection of cervical cancer. The high prevalence of HPV infection in unvaccinated women also reveals the need for studies on other prevention programs such as universal vaccination.

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Conflicts of interest

There are no conflicts of interest.

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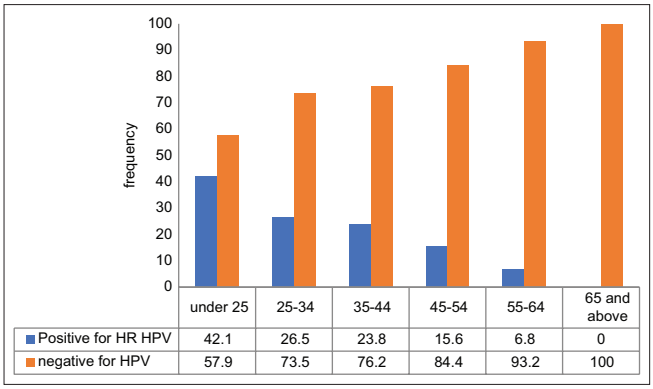

	under 25	25-34	35-44	45-54	55-64	65 and above
Positive for HR HPV	42.1	26.5	23.8	15.6	6.8	0
negative for HPV	57.9	73.5	76.2	84.4	93.2	100

Supplementary Figure 1: Frequency of high-risk HPV genotypes through age groups. HPV = Human pappiloma virus, HR = High-risk