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ORIGINAL RESEARCH

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Comparison of biopsy results between two groups of cytology-negative HPV 16/18 and other types of high-risk HPV positive patients

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Abstract

Cervical cancer is the fourth most common cancer among women worldwide. It is believed that Human Papilloma Virus (HPV) is responsible for 100% of cervical cancers. 200 HPV genotypes have been identified to date, of which 13–15 are high-risk HPV genotypes infecting the genital area. 218 females suffering from high-risk HPV infection and showing a negative cytology test were selected in a retrospective cross-sectional study and divided into two groups: 1. HPV 16/18 (121 women) and 2. Other high-risk HPV (OHrHPV) (97 women). The demographic and clinical data were collected from Motahari clinic, Shiraz University of Medical Sciences, between September 2020 and January 2023. The collected data were analyzed using IBM SPSS software version 26. Data analysis was carried out using chi-square, *t*-test, and Mann-Whitney, and $p < 0.05$ was defined as being statistically significant for all the aforementioned tests. The mean age for the HPV 16/18 and OHrHPV groups were 35.27 ± 7.698 and 36.58 ± 8.756, respectively. The most prevalent HPV genotype was HPV type 16 (n = 96) in the population, followed by HPV type 18 (n = 25) and HPV type 31 (n = 17). The HPV 16/18 group had 15 high-grade colposcopy results, while only four similar results were observed in the OHrHPV group (p value = 0.031). The most prevalent HPV genotype in patients with cervical intraepithelial neoplasia (CIN) 2 and CIN3 was HPV16. The cytology test failed to identify over 4% of the lesions in the OHrHPV group. Direct referral for colposcopy in the OHrHPV group results in the identification of missed diagnosed lesions and lost to follow-up patients.

Keywords

Uterine cervical neoplasms; Human papillomavirus viruses; Colposcopy; Papanicolaou test

1. Introduction

With an anticipated 604,000 registered cases and 342,000 deaths in 2020, cervical cancer is the fourth most prevalent cancer in women worldwide. In 2020, approximately 90% of new cases and deaths occurred in low-income and middle-income countries [1].

Human Papilloma Virus (HPV) is believed to be responsible for more than 95 percent of cervical cancers [2]. Of the more than 200 HPV genotypes identified to date, around 40 HPV genotypes infect the genital region, of which 13–15 of the high-risk HPV genotypes are believed to be carcinogenic. The high risk HPVs are 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 [3, 4]. Based on worldwide data, HPV 16 and 18 are responsible for over 70% of cervical cancers, while six other genotypes (HPV 31, 33, 35, 45, 52 and 58) are responsible for the additional 20% [5]. The risk of cervical squamous cell carcinoma is 435 times higher in HPV 16 and 248 times higher in HPV 18 infected individuals as compared to non-infected individuals [6]. Many studies have demonstrated that HPV infection profiles vary substantially geographically [7–10].

Based on the American Cancer Society guidelines, cervical cancer screening should begin at the age of 25 with primary HPV testing every five years and continue until the age of 65. If a primary HPV test is unavailable, women aged 25–65 strongly recommend screening with co-testing (HPV testing plus cytology test) every five years or a cytology test every three years [11].

The main objective of cervical cancer screening is to reduce the incidence, mortality, and treatment-related morbidity by identifying treatable abnormalities and precancers (cervical intraepithelial neoplasia (CIN) grades 2 and 3, and adenocarcinoma *in situ*) [11]. Untreated CIN2 has a 5% chance of turning into a cancerous lesion, while CIN3 has a higher probability ranging from 12–31% [12]. Previous studies in different regions stated that the screening failed to diagnose

CIN2 and CIN3 in patients infected with high-risk HPVs other than types 16 and 18 [3, 13, 14].

Prior studies mainly concentrate on the risk of cervical cancer among patients suffering from HPV type 16 and 18. In this regard, the present investigation was carried out to compare cervical cancer risk among two high-risk HPV-positive groups with normal cytology: 1. HPV 16/18 and 2. Other high-risk HPV (OHrHPV) and to compare the results of co-test and colposcopy biopsy. To the best of our knowledge, the current study is a forerunner in investigating the aforementioned risk comprehensively.

2. Method

2.1 Study population

The current retrospective study included high-risk HPV-positive cytology-negative females who voluntarily accepted colposcopy to further investigate cervical cancer at Motahari Clinic, affiliated with the Shiraz University of Medical Sciences, between September 2020 and January 2023. Individuals were informed of the risks of HPV genotyping, cytology screening, and colposcopy.

The inclusion criteria consisted of 1—Being High-risk HPV positive; 2—Having normal cytology results; 3—No sex within 72 hours; 4—Informed consent; 5—Understanding the risk of colposcopy and cervical biopsy.

The exclusion criteria consisted of 1—No consent; 2—Abnormal cytology result; 3—Low-risk HPV; 4—Multiple HPV infections; 5—Incomplete medical records.

2.2 HPV genotyping

HPV direct flow CHIP kit (HPVP019L, Master Diagnóstica, Granada, Spain) was utilized for HPV genotyping, which can detect 18 high-risk or putative high-risk genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82MM4) and 18 low-risk genotypes (6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84 and 89) [15].

2.3 Cytology screening

Liquid-based pap test was used for cervical screening, and only the normal results were selected for further evaluation. The Bethesda system, 2014 was utilized to categorize the specimens [16].

2.4 Colposcopy

C100A colposcope (Ecleris, Medley, FL, USA) was utilized to evaluate the lesion region. Punch cervix biopsies were taken from aceto-white areas, erosion areas, abnormal vessels, or suspicious areas. Four random punch biopsies were taken in the 16/18 group, even without any abnormal areas visible. The samples were taken at 1–2 mm depth, and preserved in 10% formaldehyde. Then, endo-cervix biopsies were taken using a sharp curette.

2.5 Statistical analysis

All patients were anonymized and given identification codes. Statistical analysis was carried out using IBM SPSS statistics (ver. 26, IBM corp., Armonk, NY, USA). The mean and standard deviation (SD) for continuous variables and the number and percentage for categorical variables were dully obtained. A Chi-square test was used to compare the categorical variables, and the Kolmogorov-Smirnov test was applied to evaluate whether the continuous variables were normally distributed. Parametric tests, such as the independent *t*-test, were used to compare the variables with normal distribution. Non-parametric tests, such as the Mann-Whitney and Kruskal-Wallis tests, were further utilized to compare variables that did not have a normal distribution. A p-value of < 0.05 was considered statistically significant.

3. Results

Colposcopy was carried out for 476 women infected with high-risk HPV genotypes with normal cytology tests during the period mentioned above. Fifty-seven women were excluded due to incomplete medical records, and 201 were excluded due to multiple HPV infections (more than one HPV genotypes in one individual). The remaining 218 women who suffered from high-risk HPV were enrolled in this study. The population was divided into two groups. Group 1—HPV 16/18 (n = 121) and group 2—OHrHPV (n = 97). The mean age for the HPV 16/18 was 35.27 ± 7.698, while the mean age for the OHrHPV group was 36.58 ± 8.756. Mean gravida and parity for the HPV 16/18 group were 1.53 ± 0.537 and 1.30 ± 0.413, respectively. However, similar measures for the OHrHPV group were 1.47 ± 0.653 and 1.24 ± 0.405, respectively. There was no statistically significant difference between groups in terms of age and parity (p value = 0.349 and p value = 0.196, respectively). Two groups differed statistically in terms of gravida (p value = 0.04).

The most prevalent kind of HPV infection was HPV type 16 (n = 96) in the population, followed by HPV type 18 (n = 25) and HPV type 31 (n = 17). The complete list of HPV infections is listed in Table 1.

TABLE 1. Complete list of HPV infections.

HPV type	Number of patients	Percentage
16	96	44.0
18	25	11.5
31	17	7.8
51	13	6.0
52	13	6.0
53	9	4.1
59	8	3.7
35	7	3.2
56	7	3.2
66	6	2.8
33	5	2.3
39	5	2.3
45	3	1.4
68	3	1.4
58	1	0.5
Total	218	100

HPV: Human Papilloma Virus.

Table 2 consists of exocervix biopsy results for both groups. There were 15 high-grade colposcopy results in the HPV 16/18 group, while there were four in the OHrHPV group. There was a statistically significant higher number of high-grade colposcopy results in the HPV 16/18 group (p value = 0.031). All of the endocervix colposcopy results were normal, except for one case from the HPV 16/18 group, which was CIN3. There was no statistically significant difference between groups regarding endocervix colposcopy results (p value = 0.369).

The most prevalent HPV genotype in patients with CIN2 and CIN3 is HPV16. A complete list of HPV genotypes and high-grade colposcopy results (CIN2 and CIN3) are listed in Table 3.

4. Discussion

The American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines mandate colposcopy for HPV16 and/or 18 infections, but OHrHPV infections merely undertake periodic follow-up if the cytology test is negative; however, the false negative rate of the cytology test appears to be high, and the annual follow-up based on the ASCCP guidelines will significantly elevate the rate of missed diagnosed of high-grade squamous interepithelial lesions (HGSIL) among the OHrHPV group [17].

In the current study, the most prevalent HPV infection was HPV 16 (43%), followed by HPV 18 (11.2%). The Addressing the Need for Advanced HPV Diagnostics (ATHENA) study, which screened over 25,000 women using liquid-based cytology and HPV detection, indicated that HPV16 was the most common genotype, followed by HPV52, 31 and 18 [18]. A study by Bruni *et al*. [19] (2010) stated that HPV types 16, 18, 31, 52 and 58 are among the ten most prevalent genotypes among over one million cytology-negative populations in five continents. Another study by Dorsun *et al*. [20] indicates that the two most pervasive high-risk HPV genotypes were HPV 16 and 18.

The researchers found that the rate of CIN2 is equal for HPV 18, 31, 52 and 53. The positive predictive value for CIN2+ in OHrHPV genotypes is relatively high among individuals in populations with negative cytology. HPV 33, 51, 58, 59 and 18 demonstrated comparable positive predictive values for CIN2+ in populations with negative baseline cytology [14].

In the current study, the researchers found that the frequency of CIN 2 and CIN 3 colposcopy result in the HPV 16/18 group is significantly higher than in the OHrHPV group. The risk associated with HPV is proportional to viral virulence and host vulnerability. Hence, the pathogenicity of a specific HPV is determined by its genotype and prevalence in the community. The majority of cervical lesions in HPV-infected individuals indicated the pathogenicity of the virus. The pathogenicity increases as the prevalence of cervical dysplasia increases. Previous literature stated that the strongest pathogenicity was related to HPV 16, followed by HPV 18 [17].

The risk of CIN2 and CIN3 in OHrHPV was 3.9%. The rate of high-grade dysplasia (CIN2 and CIN3) among 49 cytology-negative OHrHPV-positive patients was reported as being 4% in a study by Vural *et al*. [13], which was similar to the current study; however, the study population of the present study is higher than that carried out by Vural *et al*. [13]. In another study by Koyuncu *et al*. [21], the rate of HGSIL after performing a colposcopy among 604 OHrHPV patients was 6.2%. A higher study population may be the reason for the higher rate of high-grade lesions. Aydin *et al*. [14] reported that the rate of CIN 2 and CIN 3 among 97 OHrHPV women was 8.3%. A study by Aydoğmuş and Aydoğmuş was carried out in 2019 showed that the colposcopy results showed that 15.6% of the 77 cytology-negative OHrHPV patients were HGSIL [22]. A further reason for such results could be attributed to the distinct healthcare programs of the countries [23]. For instance, a study was conducted by the Vrije Universiteit Medical Centre-Saltro laboratory in the Netherlands indicates that individuals who followed up with a cytology test in the 0, 6th and 18th months had a relevant negative predictive value [24]. However, since the procedure is patient-dependent, a significant proportion of patients (28–33%) were lost to follow-up in the trials [25]. In another study by Thrall *et al*. [26], the loss to follow-up rate was almost 50%.

The possible risks of immediate referral for colposcopy are

TABLE 2. Exocervix biopsy results for both groups.

Group	Low-grade		High-grade		p value
	Normal (%)	CIN1 (%)	CIN2 (%)	CIN3 (%)	
HPV 16/18	48 (39.7)	58 (47.9)	6 (5.0)	9 (7.4)	0.005
OHrHPV	25 (25.8)	68 (70.1)	3 (3.1)	1 (1.0)	
HPV 16/18	106 (87.6)		15 (12.4)		0.031
OHrHPV	93 (95.9)		4 (4.1)		

HPV: Human Papilloma Virus; OHrHPV: Other high-risk HPV; CIN: cervical intraepithelial neoplasia.

TABLE 3. HPV genotypes and high-grade biopsy results.

Biopsy	HPV type					Total
	16	18	31	52	53	
CIN2	5 (55.6)	1 (11.1)	1 (11.1)	1 (11.1)	1 (11.1)	9
CIN3	7 (70)	2 (20)	1 (10)	0	0	10

HPV: Human Papilloma Virus; CIN: cervical intraepithelial neoplasia.

a rise in patient anxiety and the possibility of complications during the surgery [27].

5. Conclusions

In conclusion, the researchers found that HPV 16 and 18 are the most frequent genotypes in the population of the area where the study was carried out. The risk of CIN2 and CIN3 is higher among the population infected with HPV 16 and 18. Despite the lower risk of CIN2 and CIN3 in the OHrHPV group, the cytology test failed to recognize nearly 4% of the lesions. Direct referral for colposcopy in the OHrHPV group could result in the lower missed diagnosed lesion and loss to follow-up patients.

6. Limitations

One of the most important limitations of the study is the retrospective design, which results in data limitation. The researchers highly recommend collecting data related to cervical cancer risk factors (other than HPV) for future studies.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

FSN, MH, SMAA, ZA, MAJ and SMH—designed, performed the study, and wrote the manuscript together for publication.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethics committee of Shiraz University of Medical Sciences approved the study design (Ethics code: IR.SUMS.REC.1401.679). All methods were carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects and/or their legal guardian(s) for participation in the study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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