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ORIGINAL ARTICLE

Comparison of External and Internal Site Genital Sampling to Detect High-Risk HPV DNA in Women

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SUMMARY

Background: **Human papillomavirus (HPV), the most common pathogen causing sexually transmitted diseases worldwide, is also an oncogenic virus. Due to the inadequacy of serologic tests in the diagnosis of HPV, NAATs (nucleic acid amplification tests), such as PCR (polymerase chain reaction), represent the gold standard today. Endocervical brush sampling in women has been successfully used for HPV genotyping for many years.**
The aim of this study was to determine the diagnostic efficacy for PCR HPV genotyping of multi-site samples taken simultaneously with a swab from the external genitalia in addition to endocervical brush sampling in women applying for Hr-HPV screening.
Methods: **This study included 105 asymptomatic patients who came to the Gynecology Polyclinic of the University of Health Sciences Tepecik Training and Research Hospital between February 2023 and June 2023 for control purposes. Both the samples taken with a brush from the cervical area and the samples taken with a swab from the external genital area were sent to a screening laboratory for testing for Hr-HPV DNA. The samples were analyzed by real time PCR.**
Results: **The success rate of positive detection of swab samples of HPV type 16 was significantly higher, with a difference of 17.6% ($p < 0.0001$). For HPV type 18, the swab sample had a significantly higher positive detection rate, with a difference of 60.0% ($p = 0.002$).**
Conclusions: **The results of this study show that HPV genotyping from the external genital area can be performed as an alternative to cervical sampling in sexually active women to increase the reach of a screening program.**
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INTRODUCTION

Cervical cancer is the second most common type of cancer in women worldwide. Nearly 99% of cervical cancers are associated with infection by high-risk genotypes of the human papillomavirus (hr-HPV) [1]. Cervical cancer can be prevented primarily with HPV vaccines and secondarily with screening programs.
Comprehensive cervical cancer cytology screening pro-

grams have been shown to be effective in reducing both the incidence and mortality of cervical cancer [2]. However, despite a significant reduction in the incidence of cervical cancer, cytology screening programs also provide false-negative results, and their sensitivity varies between 30% and 87% [3].

Evidence of the high diagnostic performance of the HPV test led to changes in the joint screening guidelines of the American Cancer Society (ACS), the American Society for Colposcopy and Cervical Pathology (ASCCP), and the American Society for Clinical Pathology (ASCP). In 2012, HPV testing along with cytology (co-testing) was recommended every 5 years as a method of screening for cervical cancer in women over the age of 30 [4].

Since serological tests are insufficient for the diagnosis of HPV, nucleic acid amplification tests such as the polymerase chain reaction (PCR) have become the gold standard [5]. PCR is also valuable, because it can detect multiple genotypes in a single sample. In addition, there have been significant developments in sampling for the detection of HPV DNA in women with HPV infection over the past two decades. Endocervical brush sampling has been successfully used for HPV genotyping in women for many years. However, the vaginal swab has been shown to have similar efficacy to the cervical swab and/or brush for genital HPV DNA testing in women, and it has even been suggested that self-collected vaginal swabs may be the first choice for screening programs due to their convenience and low cost [6].

However, the studies in question only compared cervical and vaginal sampling for HPV DNA testing. For men, the accepted sampling method is to take swab samples from the penis at multiple sites. Swab samples are taken from the squamous epithelial layer of the penile shaft, the glans penis, the coronal sulcus, and the scrotum for PCR testing [7]. The efficacy of swab samples from the external genitalia in HPV genotyping in women is unknown.

The aim of this study was to determine the diagnostic efficacy of simultaneous collection of multiple samples with an endocervical swab and an external genital swab for PCR HPV genotyping in women presenting for hr HPV screening.

MATERIALS AND METHODS

Study population

This prospective cross-sectional study was conducted on 105 asymptomatic patients attending the gynecology outpatient clinic of Health Sciences University Tepecik Training and Research Hospital for screening between February and June 2023.

Inclusion criteria for participation were sexually active women aged 25 - 40 years.

Brush samples from the cervical area and swab samples from the external genital area were sent to a screening laboratory for hr-HPV DNA testing.

Clinical specimen collection

Two samples were collected from each patient to investigate the efficacy of external genital sampling in HPV DNA screening in women.

The first was a standard cervical sample obtained from the portio and cervical canal. After insertion of the vaginal speculum, the sample was collected using a cervical brush (Evalyn brush, validated with the Roche Cobas 4800 hr-HPV test) and transferred to a ThinPrep sample collection container (Hologic Inc., Marlborough, MA, USA).

The second sample was obtained by swabbing three different sites in the external genital area: the inside of the labia minora, the fourchette (at the posterior junction of the labia minora), and the perineum (Figure 1a, 1b, and 1c). These sites were chosen because condyloma accuminata lesions occur there most frequently [8]. To obtain samples, polyester swabs (Dacron) were moistened with isotonic saline solution and then rubbed back and forth 2 - 3 times at each target site. After swabbing, these samples were also transferred to ThinPrep specimen collection containers (Hologic Inc., Marlborough, MA, USA).

All samples were taken in the gynecological outpatient clinic by the same specialist. They were then transported via cold chain to the Medical Microbiology Laboratory for real-time PCR analysis for HPV DNA using a Cobas 6800 instrument (Roche Diagnostics, Indianapolis, USA) according to the manufacturer's instructions. Oncogenic HPV types 16 and 18 were reported separately, while other hr-HPV types (HPV 31, 33, 35, 45, 51, 56, 58, 59, 66, and 68) were reported without differential genotyping. Both samples obtained from each patient were analyzed simultaneously.

Ethical approval

The study was approved by the ethics committee of our college (approval number: 06/2023), and written informed consent was obtained from all participants. The study protocol was designed in accordance with the ethical guidelines of the Declaration of Helsinki.

Statistical analysis

Statistical analyses were performed using IBM SPSS® Version 26 software (2019 release, IBM Corp., Armonk, NY, USA). The conformity of the continuous variables to the normal distribution of the data was examined by using analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were performed using mean, standard deviation, median, and range for normally distributed variables. Categorical variables were analyzed using frequency and percentages. Nominal (present/absent) scores related to HPV genotyping in the materials received (real-time PCR results for HPV-16, HPV-18, other hr-HPV, and overall HPV status) were compared by using Pearson’s or Fisher’s exact chi-squared tests.

Phi/Cramer's V nominal-nominal correlation analysis was used, and Cohen’s kappa test was applied to mea-

sure the reliability of the agreement between the two sampling methods. Pearson's or Fisher's exact chi-squared test was used to compare detection rates between the brush and swab samples. An online diagnostic test evaluation calculator (MedCalc) was used to determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy [9]. A p-value less than 0.05 was considered significant.

RESULTS

The mean age of the patients in the study was 32.4 ± 6.0 years (median: 31, range: 25 - 50 years). The positive detection rates for HPV types and overall HPV diagnosis based on the results of the PCR tests of the two samples (cervical brush and external swab samples) were evaluated.

The external swab sample was significantly more successful in detecting HPV-16, with a 17.6% higher positive detection rate ($p < 0.0001$). Correlation analysis of positive detection with the cervical brush and the external swab sample showed a good level of correlation (Phi/Cramer's V = 0.892) and agreement (Cohen's κ = 0.887).

The external swab sample was also significantly more effective in detecting HPV-18, with a 60.0% difference in positive detection rate ($p = 0.002$). For HPV-18, the cervical brush and external swab had moderate correlation (Phi/Cramer's V = 0.623) and agreement (Cohen's $\kappa = 0.559$).

For other HPV types, the positive detection rate was 30.0% higher ($p < 0.0001$) when external swabs were taken, and there was a good correlation (Phi/Cramer's V = 0.791) and agreement (Cohen's $\kappa = 0.769$).

The positive detection rate for HPV in general was also 25.0% (n = 10) higher with the external smear method ($p < 0.0001$). Correlation analysis showed a good level of correlation (Phi/Cramer's V = 0.806) and agreement (Cohen's $\kappa = 0.788$) (Table 1) (Figure 2).

DISCUSSION

Many countries are developing new guidelines for the introduction of HPV testing for cervical cancer screening in basic screening programs [4]. However, women who avoid screening are still at risk of developing cervical cancer [10]. Increasing participation rates is critical to reducing the incidence of cervical cancer. In current practice, samples are collected by physicians, which can contribute to cultural barriers and make it difficult for women to participate in screening programs [11]. Therefore, self-sampling may help to reach people who do not participate in these screening programs [12]. In our study, both the external genital swabs and the cervical swabs were collected by a physician during the gynecologic examination, but the external genital samples can be easily self-collected.

Previous studies have indicated that vaginal HPV self-sampling has the potential to increase the coverage of cervical cancer screening without significantly compromising test accuracy [13]. These studies examined the concordance in hr-HPV detection between self-collected vaginal samples and physician-collected cervical samples [6]. However, there is no previous study that has addressed the role of multiple samples from the external genital area in HPV genotyping in women. To our knowledge, this study is the first in the literature to investigate the efficacy of external genital multiple specimens in HPV DNA testing.

In this study, we used polyester swabs for sampling. Polyester swabs with a plastic shaft are preferred for virological and nucleic acid-based tests. Together with nylon flocked swabs, they are commonly used in HPV DNA testing. Studies have shown that synthetic swabs have similar efficacy in detecting HPV DNA and are superior to cotton swabs [14].

Compared to cervical brush sampling, the positive detection rate with external genital swab sampling was 17.6% higher for HPV-16, 60% higher for HPV-18, 30% higher for other hr-HPV types, and 25% higher for hr-HPV in general. Correlation and agreement between the methods ranged from moderate to good.

No low-risk HPV types were examined in this study, which can be considered a limitation. However, considering that the most common sites for genital warts caused by low-risk HPV types are the same areas that we sampled in this study [10], we predict that low-risk HPV types can also be detected much more effectively than by sampling the cervix. This suggests that sampling from the external genital area alone may be a valid approach to screening for genital HPV infections.

Vulvar cancer is rare and accounts for about 4% of all genital cancers in women [15]. However, the incidence of vulvar cancer and vulvar intraepithelial neoplasia appears to have increased in recent decades, particularly in young women. Vulvar cancer can be prevented if diagnosed early. Topical treatment has been shown to be an effective alternative to surgical treatment of vulvar precancerous lesions [16].

HPV DNA has also been detected in a significant proportion of invasive vulvar and vaginal carcinomas and intraepithelial neoplasia [17].

The aim of all cancer screening tests is the early detection of cancer, if possible before symptoms appear. The earlier cancer is diagnosed, the easier it is to treat in most cases and the better the prognosis for the patient. Prophylactic vaccines against HPV infections have the potential to reduce the burden of cervical cancer and other HPV-associated cancers. These vaccines may reduce the risk of cancers associated with HPV, including vulvar and vaginal cancers [18]. However, their true therapeutic benefit remains unclear. Currently available HPV vaccines include a bivalent vaccine that targets types 16 and 18 (Cervarix®); a quadrivalent vaccine that targets types 6, 11, 16, and 18 (Gardasil®); and a multivalent vaccine that targets types 6, 11, 16, 18, 31, 33,

Table 1. The examination of differences, correlation, and compatibility between the swab and brush sampling tool.

HPV	Brush n (%)	Swab n (%)		Total	p-value	Phi/Cramer's V	Cohen's kappa coefficient
		N	P				
16	N	88 (100)	3 (17.6) *	91 (86.7)	< 0.0001	0.892	0.887
	P	0 (0)	14 (82.4)	14 (13.3)			
18	N	100 (100)	3 (60.0) *	103 (98.1)	*0.002*	0.623	0.559
	P	0 (0)	2 (40.0)	2 (1.9)			
Other high type	N	75 (100)	9 (30.0) *	84 (80.0)	< 0.0001	0.791	0.769
	P	0 (0)	21 (70.0)	21 (20.0)			
Any type of high-risk HPV	N	65 (100)	10 (25.0) *	75 (71.4)	< 0.0001	0.806	0.788
	P	0 (0)	30 (75.0)	30 (28.6)			

Phi/Cramer's V nominal-nominal correlation analysis was used and Cohen's Kappa test was applied to measure the reliability of the agreement. Pearson's or Fisher's exact chi-squared test was used to compare the success in diagnosing the disease between brushand swab samples. p < 0.05 was considered significant. N - negative, P - positive, * shows the reason for the significant difference.

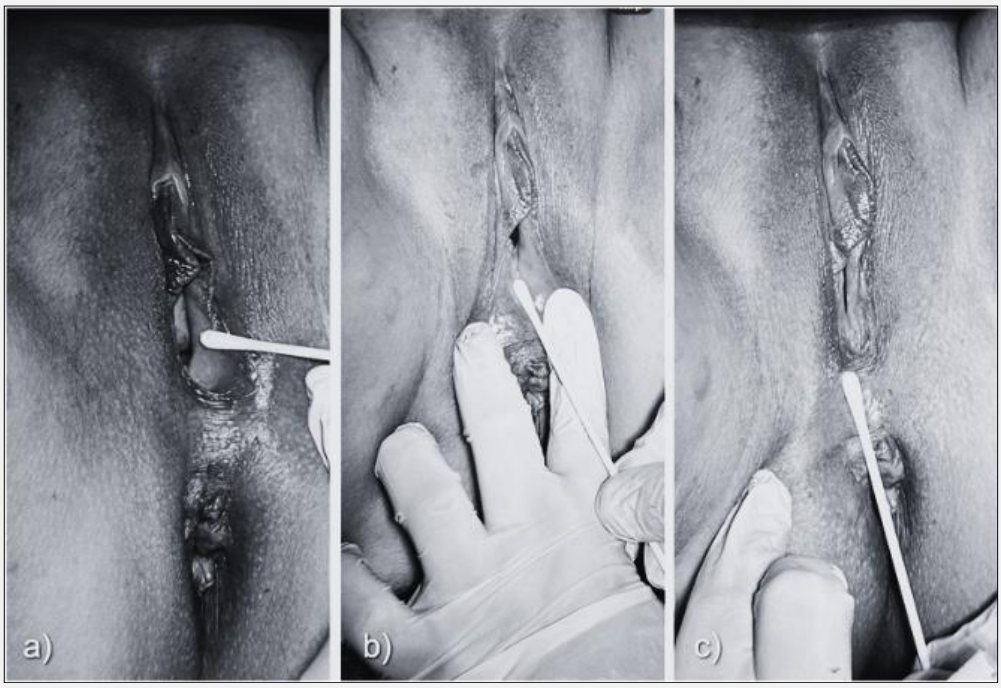

Figure 1. Sampling: (a) inside the labia minora, (b) fourchette, (c) perineum.

45, 52, and 58 (Gardasil 9®) [19].

Despite the proven efficacy of HPV vaccines, the burden of cancer and HPV-related diseases remains high. Recent studies have also shown an increase in cancers caused by HPV types not covered by vaccines [20]. In this study, we identified 14 different HPV types in a total of 105 patients. Types 16 and 18, which are among the best-known hr-HPV types, accounted for only 20% of all detected hr-HPV types. This can be seen as evidence that the vaccination campaigns are effective.

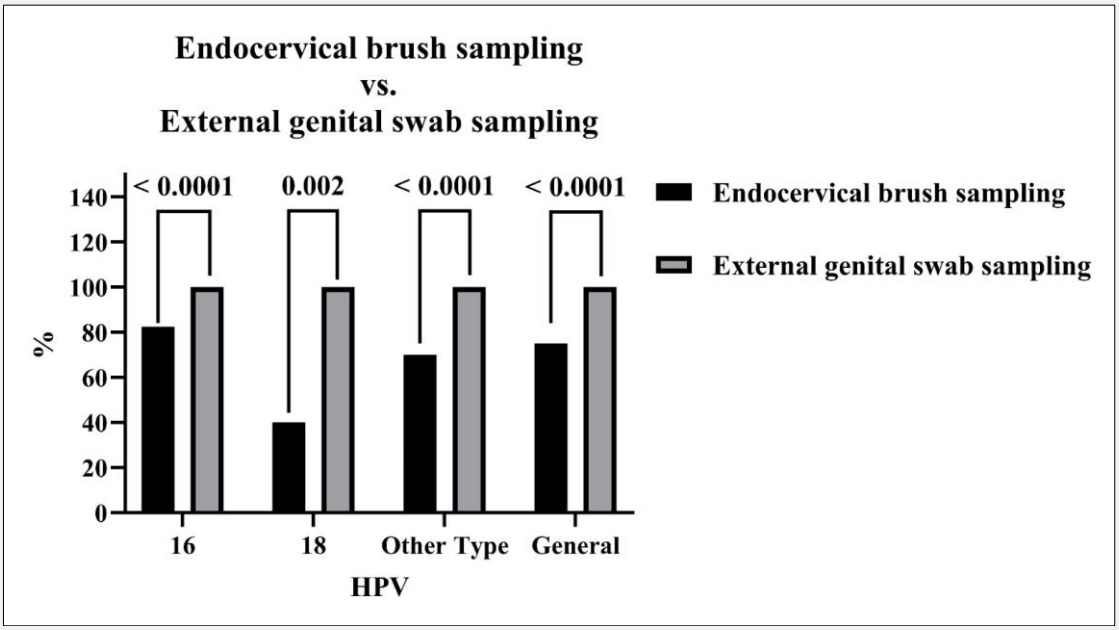

Figure 2. Comparison of positive PCR results with endocervical brush sampling & external genital swab sampling.

However, the diversity of HPV types observed in this study suggests, in our view, that future vaccines should cover many more HPV types.

Global type-specific HPV prevalence data for gynecologic cancers in women are needed. These data will help predict the future impact of prophylactic HPV vaccines at the population level. The results of our study demonstrate the importance of HPV screening for vulvar cancer.

The main limitation of this study is the small sample size. Larger studies are needed to corroborate our findings and provide further data to clarify outstanding questions.

In conclusion, the results of this study suggest that HPV genotyping by sampling from the external genital area can be performed as an alternative to cervical sampling in sexually active women to increase the reach of screening programs. Further large studies and histopathological data are needed to determine whether genotyping of HPV in the external genital area can be a useful alternative to cervical sampling in cervical cancer screening programs.

This study is important in that it will shed light and attention on cancer lesions that may occur in the vulvar area through HPV genotyping of swab samples from the external genital samples.

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Declaration of Interest:
The authors declare that they have no known competing financial interests or personal relationships that may have influenced the work reported in this article.

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