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Short Communication

Analysis of HPV Genotypes and Liquid-Based Cervical Cytology: Results from a Tertiary Academic Center in Northwestern Turkey

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SUMMARY: High-risk human papilloma virus (hrHPV) plays an important role in cervical cancer. The aim of this study was to investigate the distribution of HPV genotypes in the region and to correlate it with liquid-based-cytology (LBC) and colposcopic biopsy results. Furthermore, the potential relationship between HPV infections and bacterial vaginosis (BV) was investigated. HPV genotypes were determined using real-time PCR. LBC, biopsies, and BV examinations were performed by the Pathology and Cytology. Consecutive cervical specimens of 409 women who underwent both cytology and HPV-DNA tests were included in the study. A total of 172 (42.1%) patients were positive for HPV-DNA; of these, 107 (26.2%) had hrHPV. The most common HPV genotypes were HPV 59, 16, 33, 52, and 51, at 16.6%, 15.9%, 13.4%, 13.4%, and 8.9%, respectively. Epithelial cell abnormality was detected in 11.5% of LBC test results. The genotypes of HPV 33, 56, 66, and 68 were found at a higher rate in patients with epithelial cell abnormalities than in those with no detected abnormalities. Bacterial vaginosis was found in 24 patients (5.9%). HPV-DNA positivity was observed to be statistically higher in patients with BV than in those without BV.

Cervical cancer is the fourth most frequent cancer in women worldwide and the ninth in Turkey, representing 7.5% of all female cancer deaths globally (1). However, the disease prevalence in Mediterranean countries (Portugal, Spain, Italy, Croatia, Greece, and Turkey) appears to be highly variable (2), possibly due to differing mortality rates, implementation of vaccination programs, and application of HPV and cytology screening tests to provide early diagnosis. The aim of this study was to determine the most common HPV genotypes in our province in Turkey, to determine the relationship between epithelial cell abnormality (ECA) and hrHPV genotypes, and to investigate the potential link between BV and HPV infection.

The Research Ethics Committee of Düzce University Hospital approved the study protocol (decision number: 2019/80). Cervical brush samples were taken consecutively from patients who had been admitted to the gynecology and obstetrics outpatient clinic. HPV-DNA was detected, and the genotypes were determined. DNA was extracted using a magnetic-bead based method. Validation of isolation was achieved by adding an internal control at the extraction stage, according to the manufacturer's recommendations. For each of 409 samples, real-time PCR was implemented by the molecular microbiology laboratory using the Bosphore HPV Detection Kit v1 (Anatolia Geneworks, Istanbul, Turkey), which qualitatively identifies the most common HPV genotypes (6, 7, 11, 13, 16, 18, 31, 33, and 45) and the Bosphore hrHPV-Genotyping-Kit v1 (Anatolia), which can distinguish hrHPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

Cervical brushes and biopsy specimens taken at the same time from the patients were examined by the Pathology and Cytology Department. Cytological samples were prepared as liquid-based/Papanicolaou-stained slides, and biopsy materials were stained by hematoxylin and eosin for each case, while Ki-67, p16, and HPV immunohistochemical staining was performed when necessary. Liquid-based preparations were reported according to the Bethesda System for reporting cervical cytology 2014 criteria of 2014. The morphologic criteria for diagnosis of BV were visible squamous cells covered by a layer of coccobacilli on a clean background.

Binomial and Chi-square (post-hoc Bonferroni test) tests were used for comparisons between the ratios. Pearson's Chi-square (post-hoc Bonferroni test), Fisher-Freeman Halton (post-hoc Bonferroni test), and Fisher's exact tests were used to investigate the relationships between categorical variables. Odds ratios (ORs) with their 95% confidence intervals (95% CI)

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Table 1. Distributions of the patients' age groups, HPV-DNA, cytology and BV results

		n	Total (*n* = 409) %
Age groups	< 25	15	3.7
	25-34	127	31.1
	35-44	149	36.4
	45-54	89	21.8
	> = 55	29	7.1
HPV-DNA	Negative	237	57.9
	lrHPV	65	15.9
	hrHPV	107	26.2
	Single hrHPV	68	16.6
	Multiple hrHPV	39	9.5
Cytology (LBC)	Normal	362	88.5
	ASC-US	29	7.1
	LSIL	12	2.9
	ASC-H	2	0.5
	HSIL	4	1.0
BV	Positive	24	5.9
	Negative	385	94.1

Abbreviations: lrHPV, low-risk HPV; hrHPV, high-risk HPV; BV, bacterial vaginosis; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion.

Table 2. The distribution rates of each hrHPV genotype according to cytology and BV (*n* = 107 patients)

	Cytology (LBC)			BV			
hrHPV genotypes[1)]	Normal (*n* = 127)	Abnormal (*n* = 30)		Negative (*n* = 146)	Positive (*n* = 11)		Total (*n* = 157)
	n (%)	*n* (%)	*p*	*n* (%)	*n* (%)	*p*	*n* (%)
HPV16	21 (16.5)	4 (13.3)	0.512	23 (15.7)	2 (18.2)	0.651	25 (15.9)
HPV18	3 (2.4)	0 (0)	–	3 (2.1)	0 (0)	0.999	3 (1.9)
HPV31	1 (0.8)	1 (3.3)	–	1 (0.7)	1 (9.1)	0.114	2 (1.3)
HPV33	17 (13.4)	4 (13.3)	0.284	21 (14.4)	0 (0)	0.625	21 (13.4)
HPV35	2 (1.6)	1 (3.3)	–	3 (2.1)	0 (0)	0.999	3 (1.9)
HPV39	6 (4.7)	2 (4.3)	0.231	7 (4.8)	1 (9.1)	0.386	8 (5.1)
HPV45	2 (1.6)	1 (3.3)	–	3 (2.1)	0 (0)	0.999	3 (1.9)
HPV51	14 (11)	0 (0)	0.385	13 (8.9)	1 (9.1)	0.577	14 (8.9)
HPV52	18 (14.2)	3 (10)	0.722	19 (13)	2 (18.2)	0.353	21 (13.4)
HPV56	6 (4.7)	4 (13.3)	0.019	10 (6.8)	0 (0)	0.999	10 (6.4)
HPV58	5 (3.9)	0 (0)	0.999	5 (3.4)	0 (0)	0.999	5 (3.2)
HPV59	23 (18.1)	3 (10)	0.999	23 (15.7)	3 (27.2)	0.189	26 (16.6)
HPV66	3 (2.4)	4 (13.3)	0.004	6 (4.1)	1 (9.1)	0.347	7 (4.4)
HPV68	6 (4.7)	3 (10)	0.073	9 (6.2)	0 (0)	0.999	9 (5.7)

Abbreviations: BV, bacterial vaginosis; hrHPV, high-risk HPV.
[1)]: hrHPVgenotypes were detected more than once in some patients (co-infection).

were calculated. The SPSS-22 program was used for statistical analysis, with $p < 0.05$ considered statistically significant.

Of the 409 patients included in the study, HPV-DNA was negative in 237 (57.9%) of the patients, while 65 (15.9%) patients were found positive for low-risk HPV (lrHPV) and 107 patients (26.2%) for hrHPV (Table 1). The most commonly observed hrHPV genotypes were HPV59 (16.6%), 16 (15.9%), 33 (13.4%), 52 (13.4%), and 51 (8.9%) ($p < 0.001$).

Cytological examination revealed epithelial cell abnormalities in 47 (11.5%) LBC-samples (Table 1).

Table 3. Liquid-based cytology and HPV-DNA results in biopsy-detected dysplasia cases (n = 14)

Biopsy	PAP smear	HPV-DNA genotype
CIN I	ASC-US	16
CIN I	HSIL	33, 52, 68
CIN I	ASC-H	Negative
CIN I	LSIL	Negative
CIN I	LSIL	Negative
CIN I	LSIL	33
CIN I	LSIL	33
CIN I	ASC-US	31
CIN II	HSIL	lrHPV (6, 7, 11, 13)
CIN II	ASC-US	Negative
CIN II	LSIL	Negative
CIN II	Normal	59
CIN III	HSIL	33, 55
CIN III	ASC-US	39, 56, 68

Abbeviations as follows: ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesions; ASC-H, atypical squamous cells cannot exclude HIS; HSIL, high-grade squamous intraepithelial lesions; CINI, low-grade squamous intraepithelial neoplasia; CINII-III, high-grade squamous intraepithelial neoplasia.

The ASC/SIL ratio was 1.7/1. A total of 362 (88.5%) samples tested as negative (normal), 29 (7.1%) as ASC-US, 12 (2.9%) as LSIL, 2 (0.5%) as ASC-H, and 4 (1%) as HSIL. Genotypes HPV56 and 66 were found more frequently in patients with ECA than in those with negative (normal) smears ($p < 0.05$) (Table 2).

Among 24 patients with cytopathologically detected BV, 9 (37.5%) were positive for high-risk HPV and 7 (29.2%) were positive for low-risk HPV, while no HPV-DNA was detected in 8 patients (33.3%) ($p = 0.034$). Of the 385 patients without BV, 229 (59.5%) were HPV-DNA negative, while 58 (15.1%) were low-risk HPV positive and 98 (25.5%) were hrHPV positive. The odds of HPV-DNA positivity were 2.9- fold higher in patients with BV than in those without BV (OR = 2.9, 95% CI = 1.2–7.0, $p = 0.012$). The distribution rates of each hrHPV genotype according to BV status are shown in Table 2.

With approval of the clinician, biopsy specimens were obtained from 18 patients. Squamous dysplasia was detected in 14 (78%) of these samples (low grade: 8, high grade: 6) (Table 3).

The World Health Organization (WHO) estimated 570,000 new cases of cervical cancer in 2018, representing an estimated 311,000 deaths (7.5%) of all female cancer deaths worldwide (1). According to data from the Cancer Department of Health Ministry, cervical cancer incidence ranked 9th, at 2.3%, among all female cancer cases in Turkey (3). Epithelial cell abnormality rates measured by cytology may vary in different studies. In the largest multi-center study published in Turkey, from a total of 354,725 smears, the total number of ECA was 18,020, and the overall ECA rate was 5.08%, ranging between 0.3% and 16.64% among centers. The atypical squamous cell/squamous intraepithelial lesion rates varied within the range of 0.21–13.94%, with an average of 2.61%. It was concluded that there had been an increase in the overall ECA rate in Turkey and that there were great differences among centers in terms of quality assurance measures (4). Another study, carried out in northwestern Turkey, retrospectively analyzed the results of HPV-DNA (Qiagen Hybrid Capture) and immunocytochemical HPV antibody staining (Ultra Vision Quanto detection) of gynecological smears, and observed their compatibility with histologic diagnosis. The percentage of smears exhibiting ECA was 3.4% and the rate of ASC/SIL was 1.89% (5), similar to our rate of 1.7.

Genotypes 33, 35, 45, 52, 56, 58, 59, and especially genotype 16 are frequently encountered in cervical neoplasms worldwide (6–10). In our study, various genotypes of hrHPV were found in 26.2% of the patients and ranked by frequency as HPV59, 16, 33, and 52. In addition, HPV56 and 66 were found at higher rates than other genotypes in patients with abnormal cervical cytology. Although HPV16/HPV18 were detected most frequently in the literature, the common presence of HPV59, 52, 51 and higher correlation rates of HPV56 and 66 with ECA in this study and HPV51 in another study (10) suggests that genotypes may show regional differences. In our study, cytology was reported as negative for satisfactory cellularity in the HPV59 positive patient. The HPV-DNA was negative in 5 (36%) out of 14 patients with dysplasia in the biopsy specimen, suggesting that HPV genotyping tests and cytology are better performed as complementary co-tests. The discrepancies may be due to several reported parameters, such as the episodic nature of HPV infections, L1 deletions, molecular biology of early invasive lesions, or the variety of sampling methods (11).

The American Society of Colposcopy and Cervical Pathology (ASCCP) and American College of Obstetricians and Gynecologists (ACOG) recommend that women aged 30–65 years should be screened with a co-test and cytology every five years. In patients younger than 30 years of age, HPV infections may be transient and, therefore, screening via cytology only is recommended every three years (12).

Our cytology results were found to be negative in 21 (84%) of 25 HPV16-positive patients and in 3 (100%) HPV18-positive patients. These patients require follow-up by repeat HPV-DNA and cytology. The limitation of the study is that the tests were not repeated in these patients.

Eubiotic microbiota are being investigated for their potential role in delayed clearance of HPV infection. In our study, we observed an association between BV and HPV infection, since the presence of BV in HPV-DNA positive cases was statistically higher than that in HPV-DNA negative cases, and the risk of HPV-positivity was increased 2.9-fold ($p = 0.012$). These findings are consistent with those of other studies reporting such a link from Paraguay, Brazil, and Finland (13–15).

To conclude, considerations of regional differences in hrHPV genotypes in designing vaccines may lead to broader protection. In addition, because BV is common in the dynamic ecosystem of vaginal flora, more data from longitudinal studies are needed on the role of BV-predicted HPV persistence, implying that treatment

of asymptomatic BV in hrHPV infections might be supported.

Conflict of interest None to declare.

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