Vijeću Medicinskog fakulteta

Na osnovu Odluke Vijeća Medicinskog fakulteta o formiranju Komisije za doktorske studije, broj:1457 od 16.06.2015.godine, a u skladu sa tačkom 3.8 Vodiča za doktorske studije UCG - Centra za doktorske studije, nakon razmatranja ispunjavanja formalnih uslova za ocjenu doktorske disertacije i poštujući princip kompetentnosti, Komisija za doktorske studije dostavlja Vijeću Medicinskog fakulteta

INICIJALNI PRIJEDLOG
Sastava Komisije za ocjenu doktorske disertacije

I. DOKTORAND: **Dr med Milena Lopičić**

Naziv doktorske disertacije: **"Uloga faktora rizika i infekcije humanim papilomavirusima u nastanku skvamoznih cervikalnih intraepitelnih lezija"**

II. U skladu sa članom 38 Pravila doktorskih studija, doktorand dr med Milena Lopičić ispunjava uslove za ocjenu doktorske disertacije.

III. Komisija za ocjenu doktorske disertacije:

- **Prof. dr Aleksandra Vuksanović Božarić**, redovna profesorica Medicinskog fakulteta Univerziteta Crne Gore - predsjednik

- **Prof. dr Gordana Mijović**, redovna profesorica Medicinskog fakulteta Univerziteta Crne Gore – mentor, član

- **Prof. dr Aleksandra Knežević**, redovna profesorica Medicinskog fakulteta Univerziteta u Beogradu, član

KOMISIJA ZA DOKTORSKE STUDIJE

Prof. dr Filip Vukmirović

UNIVERZITET CRNE GORE

VIJEĆU MEDICINSKOG FAKULTETA

Komisiji za doktorske studije

PODGORICA

PREDMET: **Zahtjev za ocjenu doktorske disertacije**

Poštovani,

U skladu sa Pravilima studiranja na doktorskim studijama Univerziteta Crne Gore, podnosim zahtjev za ocjenu doktorske disertacije pod nazivom:

„Uloga faktora rizika i infekcije humanim papilomavirusima u nastanku skvamoznih cervikalnih intraepitelnih lezija".

Završetkom doktorske disertacije i objavom rada u časopisu sa SCI/SCIE liste, koji sadrži djelove sopstvenih istraživanja sprovedenih u okviru izrade doktorske disertacije, ispunila sam uslove za njenu predaju.

Ovim putem se obraćam Komisiji za doktorske studije Medicinskog fakulteta da inicira prijedlog Komisije za ocjenu doktorske disertacije.

Uz zahtjev prilažem:

- Pismenu saglasnost mentora
- Štampani primjerak doktorske disertacije (7 primjeraka)
- Fotokopiju radova objavljenih kao rezultat doktorske teze
- Biografiju i bibliografiju
- CD sa cjelokupnim sadržajem doktorske disertacije u PDF formatu, kao i radove na kojima je zasnovana doktorska disertacija
- Pisanu izjavu o autorstvu (Prilog 1 iz Uputstva o oblikovanju doktorske disertacije).

S poštovanjem,

Podnosilac:
Milena Lopičić
dr med. Milena Lopičić

U Podgorici, dana 20.03.2024. godine

UNIVERZITET CRNE GORE

MEDICINSKI FAKULTET

Na osnovu Odluke Senata Univerziteta Crne Gore, br. 03-1642/2 od 09.07.2019. godine, imenovana sam za mentora za izradu doktorske disertacije kandidata dr med. Milene Lopičić. U fazi predaje doktorske disertacije na pregled i ocjenu, u skladu sa Pravilima doktorskih studija Univerziteta Crne Gore, dajem:

SAGLASNOST

Saglasna sam da kandidat, dr med. Milena Lopičić, može predati doktorsku disertaciju pod nazivom „Uloga faktora rizika i infekcije humanim papilomavirusima u nastanku skvamoznih cervikalnih intraepitelnih lezija“ na pregled i ocjenu.

Mentor:

U Podgorici, 20.03.2024. godine

prof. dr Gordana Mijović

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RESEARCH ARTICLE

Distribution of vaccine-related high-risk human papillomaviruses and their impact on the development of cervical dysplasia in women in Montenegro

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ABSTRACT

Cervical cancer (CC) is the third leading cause of death in women in Montenegro. Human papillomavirus (HPV) is the causative agent of CC however, HPV genotype distribution varies across regions. This study examined the distribution and impact of vaccine-related high-risk (HR)-HPVs on the development of cervical dysplasia in Montenegrin women. A total of 187 women who had a clinical indication for cervical biopsy were enrolled. Based on histopathological findings, women were classified into 2 groups, with and without dysplasia. HR-HPV was detected by real-time PCR. Twelve HR-HPV genotypes were detected in 40.6% of cervical samples. The 7 most prevalent HR-HPVs in order of decreasing frequency were HPV 16 (39.5%), 45 (23.7%), 31 (21.0%), 33 (17.1%), 18 (6.6%), 52 (6.6%), and 58 (6.6%), all of them are targeted by nonavalent vaccine. Vaccine-related HR-HPVs had a higher prevalence (92.1%) than the other HR-HPVs detected in HR-HPV–positive samples. Among HR-HPV–positive women, HPV 16 and 33 were more common in women with dysplasia than in those without dysplasia (HPV 16: 28.9 vs 7.2%; HPV 33: 11.8 vs 3.6%). HPV 16 was the most common HR-HPV genotype in cervical samples, followed by HPV 45, 31, 33, 18, 52, and 58. HPV 16 and 33 were shown to be associated with the development of cervical dysplasia. These results indicate that prophylactic nonavalent vaccine can potentially prevent approximately 90% of HR-HPV infections and 60% of cervical dysplasia cases in Montenegrin women.

KEYWORDS

human papilloma virus, HR-HPV infection, dysplasia, HPV vaccine

INTRODUCTION

Invasive cervical cancer (CC) is among the most common malignancies in women. According to GLOBOCAN data from 2018, cancer of the cervix uteri is the fourth most common cancer among women worldwide, with an estimated 569.847 new cases and 311.365 deaths each year [1]. More than 85% of the global burden is diagnosed in developing countries, where CC accounts for 13% of all female cancers [2].

CC represents an important public health issue in Montenegro [3]. It was the fourth most common female cancer (excluding non-melanoma malignant skin neoplasms) and fourth leading cause of death among women in Montenegro in the 2013 Registry of Malignant Neoplasms of Montenegro [4]; and is the second most common female cancer and third leading cause of death (age-standardised incidence and mortality rates by world standard population of 26.2/100,000 and 10.5/100,000, respectively) according to 2020 GLOBOCAN statistics [5].

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Genital human papillomaviruses (HPVs) are sexually transmitted and approximately 630 million people worldwide are infected [6]. HPV is the causative agent of CC [7–9]. The International Agency for Research on Cancer has identified 15 α-types as high-risk (HR) genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, 66, 68, 73, and 82) [10]; collectively, these account for >90% of all cases of CC [11].

A prophylactic HPV vaccine is a potential tool for eradicating CC. There are currently 3 such vaccines that have been approved by the United States Food and Drug Administration: a bivalent HPV vaccine containing virus-like particles (VLPs) of the HR-HPV 16 and 18 genotypes (Cervarix, produced by GlaxoSmithKline); a quadrivalent HPV vaccine that includes VLPs of HPV 16 and 18 along with the 2 low-risk (LR) genotypes HPV 6 and 11 (Gardasil, produced by Merck); and a nonavalent HPV (9vHPV) vaccine containing VLPs of 7 HR genotypes—namely, HPV 16, 18, 31, 33, 45, 52, and 58—plus HPV 6 and 11 (Gardasil9, produced by Merck) [12, 13]. Although Gardasil9 has been approved in Montenegro since 2019 (www.cinmed.me), the HPV vaccination program has yet to be implemented.

As HPV genotype distribution varies across regions and countries, there are geographic differences in the incidence and mortality of CC [14]. As such, data on the distribution of HR-HPV types included in available prophylactic HPV vaccines (i.e., vaccine-related HR-HPV genotypes) are important for decision-making within the vaccination program.

The aim of the present study was to evaluate the distribution of vaccine-related HR-HPVs and their impact on the development of cervical dysplasia in women in Montenegro. This research was conducted on female population of Montenegro and it provides comprehensive scientific evidence for the development of a national CC prevention program.

MATERIALS AND METHODS

Sample collection

The study was conducted at the Clinical Centre of Montenegro, Podgorica from 2012 to 2018, and included 187 women with a clinical indication for cervical biopsy. According to institutional protocols, indications for cervical biopsy were an abnormal Pap test (III a, III b, or IV) and/or abnormal colposcopy finding. Prior to biopsy, cervical swabs for HR-HPV detection were obtained from each subject. All women participated voluntarily and signed an informed consent form before sample collection. Cervical samples were collected from 2012 to 2018.

HR-HPV sample collection, DNA extraction, and genotyping

Cervical swabs for HPV testing were collected using a cytobrush (Kito-Brush; Kaltek, Padova, Italy). Samples were placed in ThinPrep Pap Test PreservCyt Solution (Cytyc Corporation, Boxburgh, MA, USA) in a 20-mL vial and stored at −70 °C. DNA extraction and genotyping were performed at the Centre for Medical Microbiology, Institute of Public Health of Montenegro.

After sample dissolution, the solution was vortexed and 1–10 mL of each sample (5 mL clear; 3 mL cloudy) was transferred to a sterile 1.5-mL plastic tube and centrifuged at 1,300 rpm for 12 min. The supernatant was aspirated with a Pasteur pipette (3 mL), and the precipitate was used for DNA extraction. DNA was isolated using the DNA-Sorb-A extraction kit (REF K-1-1/A; Sacace Biotechnologies, Como, Italy) according to the manufacturer's instructions.

Qualitative detection and genotyping of 12 HR-HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) was performed using the HPV High-Risk Typing Real test (Sacace Biotechnologies), which is based on multiplex real-time PCR amplification, in 4 reaction tubes per sample. Each tube contained primers directed against regions of 3 HPV types with the human β-globin gene used as the internal control. A 20-µL volume of total nucleic acid extract per sample was used in 4 reactions (8 µL master mix and 5 µL eluate for a total of 13 µL of each of 4 PCR mixtures). As proof of the validity of the test, we relied on the certificate provided by the manufacturer.

Biopsy specimen processing and analysis

Biopsy samples were embedded in paraffin and cut into sections that were stained with haematoxylin and eosin according to standard procedures. Histopathological analysis was independently performed by 2 pathologists without prior knowledge of patients' clinical data and HPV status. According to the severity of the cervical lesion, patients were classified into 2 groups: women with dysplasia who had histologically confirmed cervical intraepithelial neoplasia (CIN) 1/2/3 or carcinoma in situ and women without dysplasia.

Statistical analysis

Statistical analyses were performed using SPSS v.23.0 software (SPSS Inc., Chicago, IL, USA). All data were categorical. Descriptive data are expressed as a percentage of a group for discrete measures. The Pearson's chi-squared test was used to analyse all data. The association between observed parameters and histopathological status of the cervix was analysed by univariate and multivariable binary logistic regression. The probability of HPV in the examined groups is expressed as a negative predicting value (NPV) and positive predicting value (PPV) with 95% confidence intervals (CIs). For all statistical analyses, $P < 0.05$ was considered significant.

RESULTS

This study enrolled 187 women with an average age of 42.97 ± 10.56 years (range: 19–74 years). The subjects were classified into 2 groups according to histopathological status of the cervix: women with dysplasia (study group) and women

Table 1. Distribution of women by histopathological status

Histopathological status (%)	N (%)
Without dysplasia	111 (59.4%)
CIN 1	39 (20.9%)
CIN 2	11 (5.9%)
CIN 3	24 (12.8%)
CIS	2 (1.1%)
Total	187 (100%)

Abbreviations: CIN, cervical intraepithelial neoplasia; CIS, carcinoma in situ.

without dysplasia (control group) (Table 1). CIN 1 (20.9%) and CIN 3 (12.8%) were the most frequently diagnosed grades of neoplasia in the study group.

Distribution of HR-HPV infections

The 12 HR-HPV genotypes included in the diagnostic test were detected in 76/187 (40.6%) women (Fig. 1A). The dominant genotype was HPV 16 (39.5%), followed by HPV 45 (23.7%), HPV 31 (21.0%) and HPV 33 (17.1%) (Fig. 2). HPV 16 was detected at a significantly higher frequency than the other genotypes ($P = 0.000$). The detection rate of at least 1 of the 7 HR-HPV genotypes targeted by the 9vHPV vaccine (HPV 16, 18, 31, 33, 45, 52, and 58) was 70/76 (92.1%). The overall prevalence of infection with HR-HPVs targeted by the 9vHPV vaccine was significantly higher than that of infection with other HR-HPV genotypes ($P = 0.000$) (Fig. 1B).

The genotyping results showed that HR-HPVs were present as single and multiple infections. Single HR-HPV infection was observed in 55/76 women (72.4%) infected with HR-HPV, whereas 21/76 women (27.6%) had a multiple HR-HPV infections (Fig. 1C). Single infection occurred at a significantly higher frequency than multiple infections ($P = 0.000$).

Distribution HR-HPV genotypes according to histopathological status

The overall prevalence of HR-HPV was 59.2% in women with dysplasia and 27.9% in those without dysplasia (Fig. 3). HR-HPV infection was significantly associated with cervical abnormalities.

No statistically significant difference was observed in the frequency of cervical dysplasia between women with single vs multiple HR-HPV infections, $P = 0.414$ (Table 2). In both

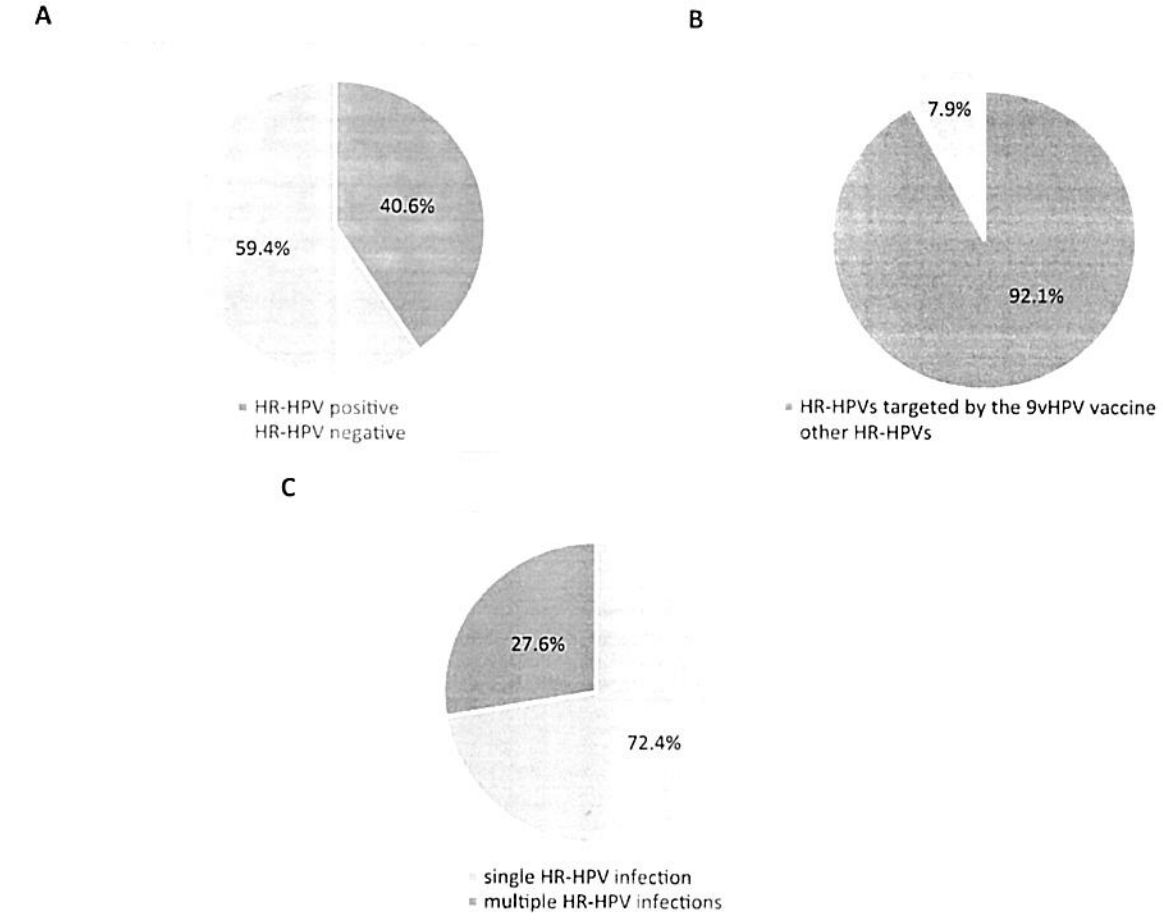


Fig. 1. Distribution of HR-HPV infections. **A.** Prevalence of HR-HPV infection. **B.** Prevalence of infections with HR-HPVs targeted by the 9vHPV vaccine. **C.** Single and multiple HR-HPV infections

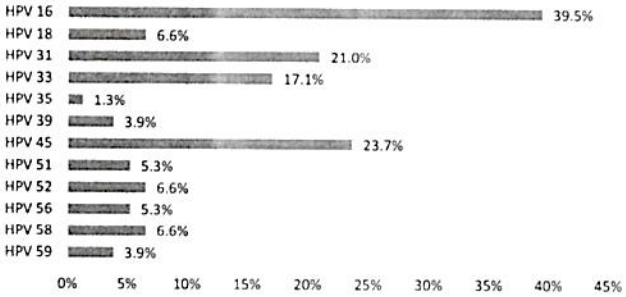


Fig. 2. Frequency of HR-HPV genotypes among HR-HPV–positive women in Montenegro

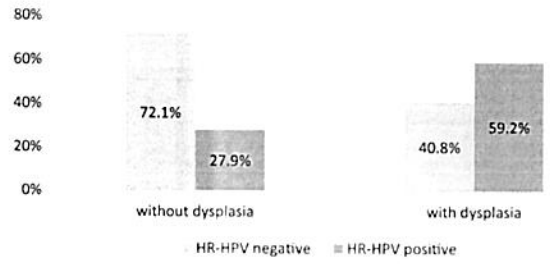


Fig. 3. Distribution HR-HPV infections according to the histopathological status of women in Montenegro

the study and control groups, single infection was more common than multiple infections (women with cervical dysplasia: $P = 0.039$; women without cervical dysplasia: $P = 0.002$).

Analysis of the distribution of HR-HPV genotypes according to the histopathological status of the cervix revealed significantly higher frequencies of HPV 16 and 33 in the study group than in the control group (HPV 16: 28.9% vs 7.2%, $P = 0.000$; HPV 33: 11.8 vs 3.6%, $P = 0.030$). The rate of infection with at least 1 of the 7 HR-HPV genotypes targeted by the 9vHPV vaccine was significantly higher in the study group than in the control group ($P < 0.000$) (Table 2).

Logistic regression analysis was carried out to identify risk factors for cervical dysplasia in women infected with HR-HPV. Infection with HR-HPV and with HPV 16 or 33 were identified as risk factors and were included in the multivariate logistic regression model. Among these, only HPV 16 infection was an independent risk factor for cervical dysplasia ($P = 0.049$); women with HPV 16 infection were about 2.7 times more likely to develop the condition (95% CI: 01.098–7.700) (Table 3).

Predictive value of HR-HPV infection status for the development of cervical dysplasia according to histopathological status of the cervix

PPVs and NPVs for each of the HR-HPV genotypes detected in infections and for HR-HPV genotypes targeted by the 9vHPV vaccine were determined by comparing genotype frequencies in women with vs without dysplasia.

Table 2. Distribution HR-HPV genotypes according to the histopathological status of women in Montenegro

HR-HPV infection	Histopathological status		*P*
	Without dysplasia	With dysplasia	
Multiplicity			
Single infection	24 (77.4%)	31 (68.9%)	0.414
Multiple infection	7 (22.6%)	14 (31.1%)	
HR-HPV genotype			
HPV 16	8 (7.2%)	22 (28.9%)	0.000*
HPV 18	2 (1.8%)	3 (3.9%)	0.372
HPV 31	7 (6.3%)	9 (11.8%)	0.184
HPV 33	4 (3.6%)	9 (11.8%)	0.030*
HPV 35	1 (0.9%)	0 (0%)	0.407
HPV 39	1 (0.9%)	2 (2.6%)	0.355
HPV 45	9 (8.1%)	9 (11.8%)	0.395
HPV 51	1 (0.9%)	3 (3.9%)	0.157
HPV 52	1 (0.9%)	4 (5.3%)	0.069
HPV 56	3 (2.7%)	1 (1.3%)	0.520
HPV 58	1 (0.9%)	4 (5.3%)	0.069
HPV 59	3 (2.7%)	0 (0%)	0.149
HR-HPVs targeted by 9vHPV vaccine	27 (24.3%)	43 (56.6%)	0.000*

Data are shown as n (%).
*$P < 0.05$ (chi-squared test).
Abbreviations: HPV, human papilloma virus; HR-HPV, high-risk human papilloma virus.

Table 3. Differences in risk of HPV infection between women with vs without cervical dysplasia evaluated by multivariate logistic regression analysis

Risk factor	Multivariate logistic regression model	
	Exp B (95% CI)	*P*
HR-HPV infection	2.001 (0.918–4.361)	0.081
HPV 16	2.746 (1.098–7.700)	0.049*
HPV 33	2.152 (0.563–8.225)	0.262

*$P < 0.05$.
Abbreviations: Exp B, relative risk, CI, confidence interval; HPV, human papilloma virus; HR-HPV, high-risk human papilloma virus.

Based on the obtained PPVs and NPVs, 61.4% (PPV) of women infected with HR-HPVs targeted by the 9vHPV vaccine were predicted to develop dysplasia (95% CI: 49.0–72.8%), while 71.8% (NPV) of those without infection with these HR-HPVs were unlikely to develop the condition (95% CI: 62.7–79.7%) (Table 4).

DISCUSSION

Montenegro is a country with 620,029 inhabitants (www.monstat.org/cg/). In this study, it took a long time to collect samples of women who were indicated for a cervical

Table 4. Likelihood difference of HR-HPV infection as a predictor of cervical dysplasia according to the histopathological status of the cervix

HR-HPV infection	NPV	PPV
HPV 16	0.656 (0.576–0.730)	0.733 (0.541–0.877)
HPV 18	0.599 (0.524–0.671)	0.600 (0.147–0.947)
HPV 31	0.608 (0.531–0.682)	0.562 (0.299–0.802)
HPV 33	0.615 (0.538–0.688)	0.692 (0.386–0.909)
HPV 35	0.591 (0.517–0.663)	0.000 (0.000–0.987)
HPV 39	0.598 (0.523–0.669)	0.667 (0.094–0.992)
HPV 45	0.604 (0.526–0.678)	0.500 (0.260–0.740)
HPV 51	0.601 (0.526–0.673)	0.750 (0.194–0.994)
HPV 52	0.604 (0.529–0.676)	0.800 (0.284–0.995)
HPV 56	0.590 (0.515–0.682)	0.250 (0.006–0.806)
HPV 58	0.604 (0.529–0.676)	0.800 (0.284–0.995)
HPV 59	0.587 (0.512–0.659)	0.000 (0.000–0.806)
HR-HPVs targeted by the 9vHPV vaccine	0.718 (0.627–0.797)	0.614 (0.490–0.728)

Abbreviations: HPV, human papilloma virus; HR-HPV, high-risk human papilloma virus; NPV, negative predictive value; PPV, positive predictive value.

biopsy and to identify those with histopathological confirmed dysplasia. However, as CC is the third leading cause of death among Montenegrin women and the fact that it took 6 years to collect a sufficient number of samples indicate that it is necessary to intensify efforts to strengthen the national CC prevention program.

Basic data on the prevalence of cervical HPV infection in women in Montenegro before prophylactic vaccination against HPV would be useful because the local epidemiology of this infection has not yet been established. Thus, this study focused on the distribution of vaccine-related HR-HPV genotypes and their impact on the development of cervical dysplasia in Montenegrin women preceding the development of a national HPV vaccination program.

The global prevalence of HPV infection among females is 32.1%, with higher infection rates in developing countries (42.2%) than in developed countries (22.6%). In almost all European countries, the HPV prevalence was below 30%; however, the prevalence was higher in Eastern Europe (28.9%) compared to that of in Western Europe (3.7%) [15].

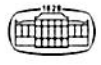
In our study, 12 HR-HPV genotypes were detected in 40.6% of cervical samples from Montenegrin women, which is a similar prevalence rate as in Bulgaria (38.8%) [16] but higher than that of reported in other studies from the region: 29% in Romania (15 HR-HPV) [17], 15.5% in Serbia (the same 12 HR-HPVs as in this study) [18], and 13.1% in Kosovo (14 HR-HPVs, according to United Nations Security Council resolution 1244 of 1999) [19].

The distribution of HPV genotypes varies across populations and geographic regions [20]. Data on the distribution of HPV genotypes in CC or CIN are available from a small number of studies conducted in Eastern Europe [21]. In our study, 187 cervical samples of Montenegrin women were screened for the presence of 12 HR-HPVs. We showed that the 7 most prevalent HR-HPV genotypes in descending order were HPV 16, 45, 31, 33, 18, 52, and 58, which are all targeted by the 9vHPV vaccine. Their overall prevalence was 92.1% of HR-HPV–positive samples or 37.4% of all women in the study, which was higher than the overall prevalence of other detected HR-HPV genotypes. In a study of women in Bosnia and Herzegovina, the frequency of the same 7 genotypes was 68% [22], which is lower than the frequency among women in Montenegro.

In the present study, the most common genotype was HPV 16 (39.5%). This percentage of HPV 16 prevalence corresponds to previously published result in 2012 (36.8%) [23]. Some studies conducted in countries surrounding Montenegro including Republic of North Macedonia, Albania, and Serbia reported a similar prevalence of HPV 16 among women (40.9, 41.0, and 42.9%, respectively) [24, 25, 18]. In recent studies from 14 European countries (mostly in northern and western Europe), HPV 16 was the most prevalent HPV genotype, detected in 29.8% of HPV-positive samples (range: 19–43%) [26]. The second most frequent genotype in this study in HR-HPV–positive women was HPV 45 (23.7%); this is also the second-ranked genotype in Serbia, with a frequency of 15% [18]. In contrast, other countries in Europe reported a low frequency of this genotype (2.2% in Croatia and 0.5% in Belgium) [27, 28]. Similar to HPV 16 and 18, the HPV 45 is thought to have a greater potential for malignant transformation and host immune evasion than other highly oncogenic genotypes [29]; therefore, women infected with HPV 45 require careful monitoring. The third most common genotype in our cohort was HPV 31, found in 21% of HR-HPV–positive women. Other studies have shown that HPV 31 was one of the most common HR genotypes. As in Montenegro, HPV 31 was the third most common genotype in Serbia [18] and Bulgaria (10.7 and 12.2%, respectively) [16]; however, in Croatia [30], Bosnia and Herzegovina [22], and Kosovo [19] it was the second most common genotype (12.6, 14.3, and 15.4%, respectively).

The occurrence of multiple HPV infections in HPV-positive women shows geographical variation in European countries (9–50%) [31]. The clinical significance of infection with multiple HPV genotypes is unclear, but it may be linked to disease progression [22, 32–34]. We found an increased incidence of multiple HPV infections in women with cervical dysplasia compared to those without dysplasia (31.1 and 22.6%, respectively); however, there was no association between multiple infections and dysplasia. This is supported by results from other studies [22, 30, 35]. In both the study and control groups, single infection was more common than multiple infections, but a different prevalence may be observed by expanding the spectrum of HR-HPV genotypes and testing for genotypes that are uncommon or have low oncogenic potential.

As expected, we found that the prevalence of HR-HPV infection was significantly higher in women with dysplasia than in controls (59.2 and 27.9%, respectively). HPV 16 and 33 were more common in the former group. In addition, the multivariate logistic regression analysis showed that HPV 16 infection was a risk factor for cervical dysplasia: women with HPV 16 infection had a 3 times higher risk of developing



this condition. Based on these results, we conclude that HPV 33 and especially HPV 16 play important roles in the development of precancerous cervical lesions in women in Montenegro. This is related to their high malignant potential and low clearance rate, which can induce changes in the cervical epithelium. A study of 44,102 women found that similar to HPV 18 and 31, clearance rates were low for HPV 16 and 33. Genotype-specific differences in clearance rate have been shown to be correlated with an increased risk of cervical lesion progression [36].

Our results showed that cervical infections with vaccine-related HR-HPV genotypes (16, 18, 31, 33, 45, 52, and 58) were more common in women with dysplasia than in those without dysplasia (56.6 and 24.3%, respectively). Based on these results, the prophylactic 9vHPV vaccine can potentially prevent approximately 90% of HR-HPV infections and 60% of cervical dysplasia cases in Montenegrin women.

The results of this study provide preliminary data on the prevalence and distribution of HR-HPV genotypes in cervical tissue as well as their impact on the development of cervical disorders. However, because of the small sample size and inclusion of only 12 HR-HPV genotypes, the observed prevalence may not represent that in the general population of women in Montenegro. Therefore, a larger study with an expanded range of HPV genotypes—including other HR as well as LR and uncommon genotypes—is needed.

In summary, a high prevalence of HR-HPV genotypes was detected in the cervical tissue of women in Montenegro. HPV 16 was the most common genotype, followed by HPV 45, 31, 33, 18, 52, and 58. HPV 16 and 33 likely play an important role in the development of precancerous cervical lesions. The currently used nonavalent HPV vaccine covers all of the most common HR-HPV genotypes detected in women in Montenegro.

It is important for each country to have strong evidence of the distribution of HPV genotypes in its population in order to implement an appropriate national cervical prevention program. The results obtained herein can contribute to the decision-making process for an immunisation program in Montenegro.

Conflict of interest: The authors have no conflict of interest to declare.

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Autor je jednog, a koautor sedam naučnih radova objavljenih u časopisima koji se nalaze u međunarodnim citatnim bazama, kao i više radova objavljenih u domaćim časopisima i prezentovanih na domaćim i međunarodnim kongresima.

Jedan je od autora nacionalne smernice dobre kliničke prakse, „Laboratorijska dojagnostika u kliničkoj bakteriologiji", čiji je izdavač Ministarstvo zdravlja Crne Gore.

Do sada je bila učesnik jednog nacionalnog i sedam međunarodnih naučno-istraživačkih projekata.

Sa ciljem usavršavanja iz oblasti rezistencije bakterija na antibiotike, bila je u dve studijske posete, u Zagrebu (Hrvatska) i Berlinu (Nemačka).

Udata je i majka troje dece.

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UNIVERZITET CRNE GORE

MEDICINSKI FAKLTET

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P O T V R D A

Potvrđuje se da je dr med Milena Lopičić , predala 7 primjeraka doktorske disertacije, pod nazivom **„ Uloga faktora rizika i infekcije humanim papilomavirusom u nastanku skvamoznih cervikalnih intraepitelnih lezija " dana 20.03.2024**.godine (Broj protokola 531) .

Potvrda se izdaje u svrhu pregleda i ocjene doktorske disrtacije.

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