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ТКИВНА ДЕТЕКЦИЈА И ТИПИЗАЦИЈА НА ХУМАН ПАПИЛОМА ВИРУСОТ КАЈ ЖЕНИ СО СКВАМОЗНИ ИНТРАЕПИТЕЛНИ ЛЕЗИИ И СКВАМОЗЕН ИНВАЗИВЕН КАРЦИНОМ НА ГРЛОТО НА МАТКАТА

TISSUE DETECTION AND TYPISATION OF HUMAN PAPILLOMAVIRUS IN WOMEN WITH SQUAMOUS INTRAEPITHELIAL LESIONS AND SQUAMOUS INVASIVE CARCINOMA OF THE CERVIX

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Abstract

Introduction. The most common risk factor for intraepithelial lesions and cervical carcinoma is infection with human papillomavirus (HPV), especially with high-risk HPV genotypes. Only persistent, high-risk HPV infections represent a major risk factor for intraepithelial lesions and cervical cancer. The aims of the study were: detection and typisation of HPV genotypes, which are the most common causes of intraepithelial lesions and cervical cancer, determination of the correlation between HPV infection and histopathological diagnosis, and the correlation between the grade of lesion of the cervix and oncogenic potential of the virus as well as determination of the most affected age group of patients.
Methods. This cross-sectional study included 100 sexually active patients with an abnormal Pap test at the age from 20 to 69 years (39±10.77), and was conducted at the University Clinic of Gynecology and Obstetrics in Skopje and University Clinic of Radiotherapy and Oncology in Skopje in the period from January 2014 to August 2014. In all patients colposcopic cervical biopsy was made with endocervical curettage for histopathological analysis and cervical biopsy for detection and HPV typisation. HPV detection and typisation were done using polymerase chain reaction (PCR) and reverse hybridization.
Results. HPV DNA was detected in 81.0% (81/100) of the examined women. The relationship between the prevalence of high-risk and low-risk HPV DNA genotypes was 72.0%:9.0%. The frequency of high-risk HPV DNA genotypes ranged from: 54.5% (12/22) in productive HPV infection-mild dysplasia, 86.4% (19/22) with moderate dysplasia, 91.2% (21/23) in severe dysplasia to 100% of squamous cell carcinoma in situ (6/6) and invasive squamous cell carcinoma (5/5). Mixed HPV infection was detected in 19.0% (19/100) of all patients, in 23.5% (19/81) of HPV DNA positive patients. The most common HPV DNA genotypes, in descending order, were HPV 16 (43.2%), HPV 31 (28.4%), HPV 18 (14.8%), etc. The highest frequency of HPV infection was found in patients under 30 years of age.
Conclusion. There was an association between HPV infection and squamous intraepithelial lesions and squamous invasive carcinoma of the cervix. There was a correlation between the grade of cervical lesion and the oncogenic potential of the virus.
The results of this study may be useful for building a national strategy in the fight against cervical cancer.

Key words: intraepithelial lesions, human papilloma virus (HPV), HPV typisation, polymerase chain reaction (PCR), reverse hybridization

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Апстракт

Вовед. Најчест ризик-фактор за појава на интраепителните лезии и карциномот на грлото на матката е инфекција со хуман папилома вирус (ХПВ), особено со високо-ризичните генотипови на ХПВ. Само перзистентните, високоризични ХПВ инфекции претставуваат главен ризик-фактор за појава на интраепителните лезии и карциномот на грлото на матката. Целите на студијата се: детекција и типизација на генотиповите на ХПВ кои се најчести причинители на интраепителните лезии и карцином на грлото на матката, одредување на корелацијата меѓу ХПВ инфекцијата и хистопатолошката дијагноза, како и корелацијата меѓу степенот на лезија на грлото на матката и онкогениот потенцијал на

вирусот и одредување на најафектираната возрасна група на пациентки.
Методи. Студија на пресек (cross-sectional study), спроведена во периодот од јануари 2014 година до август 2014 година на 100 сексуално активни пациентки, со абнормален ПАП тест, на возраст од 20 до 69 години (39±10,77), на ЈЗУ Универзитетски клиники за гинекологија и акушерство и за онкологија и радиотерапија во Скопје. Кај сите пациентки беа направени колпоскопска цервикална биопсија со ендоцервикална киретажа за хистопатолошка анализа и цервикална биопсија за детекција и ХПВ типизација. ХПВ детекција и типизација беше направена со помош на полимераза верижна реакција (PCR-polymerase chain reaction) и реверзна хибридизација.
Резултати. ХПВ ДНК беше детектирана кај 81,0% (81/100) од испитуваните жени. Односот меѓу преваленцијата на високоризични и нискоризични ХПВ ДНК генотипови беше 72,0%:9,0%. Фреквенцијата на високоризични ХПВ ДНК генотипови се движеше од: 54,5% (12/22) кај продуктивна ХПВ инфекција-лесна дисплазија, 86,4% (19/22) кај умерена дисплазија, 91,2% (21/23) кај тешка дисплазија до 100% кај ин ситу сквамозен карцином (6/6) и инвазивен сквамозен карцином (5/5). Мешана ХПВ инфекција беше детектирана кај 19.0% (19/100) од сите пациентки, односно кај 23,5% (19/81) од ХПВ ДНК позитивните пациентки. Најзастапени ХПВ ДНК генотипови, по опаѓачки редослед, беа ХПВ 16(43,2%), ХПВ 31 (28,4%), ХПВ 18(14,8%) итн. Највисока фреквенција на ХПВ инфекција е најдена кај пациентките под 30-годишна возраст.
Заклучок. Постои асоцијација помеѓу ХПВ инфекцијата и сквамозните интраепителни лезии и сквамозниот инвазивен карцином на грлото на матката. Постои корелација меѓу степенот на лезија на грлото на матката и онкогениот потенцијал на вирусот.
Резултатите од оваа студија можат да бидат корисни за градење национална стратегија во борбата против цервикалниот карцином.

Клучни зборови: интраепителни лезии, хуман папилома вирус (ХПВ), ХПВ типизација, полимераза верижна реакција (ПВР), реверзна хибридизација

Introduction

Worldwide, cervical cancer is ranked at the third place in frequency of all cancers, with more than half a million new cases each year and covers 8.8% from all cases of malignant neoplasms in women [1].

According to the latest data from GLOBOCAN, Europe, Macedonia is on the second place, with 31.4 higher incidence, after Romania, nearly tripled greater incidence than the average European, which is 10.6 of every 100 000 [1,2].
Various forms of intraepithelial lesions precede the appearance of the cervical cancer, which include a lot of progressive morphological changes, from productive HPV infection-mild dysplasia to carcinoma in situ [3].
The most common risk factor for intraepithelial lesions of the cervix and for cervical cancer is infection with human papillomavirus (HPV), particularly in high-risk types of HPV [4,5].
Only persistent, high-risk HPV infections represent a major risk factor for intraepithelial lesions of the cervix [6].
DNA from HPV has been found in 99.7% of cases of cervical carcinoma [7]. Human papillomaviruses are a very diverse group of viruses. They show affinity for squamous epithelium and cause infections with distinctive, unusual flow, which is often subclinical, with possible rare but serious consequences [8-10].
HPV infection is the most common sexually transmitted disease, with viral etiology [11]. Epidemiological studies show that 50% of women are infected with HPV during the first two years of beginning the sexual life [12-14].
However, the exact prevalence is difficult to be determined, because of the large number of asymptomatic and subclinical infections, as well as difficulty in distinguishing relapses and reinfections [15-17]. An estimated 20% of women are infected with one or more HPV types. The infection usually disappears spontaneously in a few months, and only 1-2% of these women will develop clinically relevant intraepithelial lesions or cervical carcinoma [13].
There are different classifications of HPV: by genetic similarity, by oncogenic potential and by affinity for certain tissues. According to genetic similarity, they are distributed into different genotypes. Until today, more than 150 HPV genotypes are known, and new ones are being discovered on a daily basis. But only about 40 of them have affinity for anogenital epithelium [18]. According to the oncogenic potential, they are divided into high-risk and low-risk. The following genotypes are included in the high-risk ones: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, and low-risk are: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 [19]. The prevalence of genotype varies depending on the geographical regions. In Europe and North America, HPV 16 is still the most common high-risk genotype [20]. Detection of HPV can be done using two methods, the first one is direct hybridization or in situ hybridization, and the other one is amplification or polymerase chain reaction (PCR) [21].
The aims of the study were: detection and typisation of HPV genotypes, which are the most common causes of intraepithelial lesions and cervical cancer, determination of correlation between HPV infection and histopathological diagnosis, also correlation between the grade

of lesion of the cervix and oncogenic potential of the virus and determination of the most affected age group of patients.

Material and methods

This was a cross-sectional study conducted in the period from January 2014 to August 2014. The material is represented by 100 patients treated in the outpatient settings of the University Clinic of Gynecology and Obstetrics and University Clinic of Radiotherapy and Oncology in Skopje.

Criteria for inclusion

The study included sexually active patients with an abnormal cervical cytology finding (abnormal PAP test).

Exclusion criteria

The study did not include: pregnant women, women with previous surgery on the cervix (cervical conization, carbon dioxide laser vaporization and total abdominal hysterectomy) and also previous abnormal cytological and histopathological findings of the cervix.

Methods of examination

All 100 patients underwent colposcopic cervical biopsy with endocervical curretage for histopathological analysis and cervical biopsy for detection and HPV typisation.
All samples of cytology were taken by using Thin Prep Pap test and analyzed in the Cytology laboratory of the University Clinic of Gynecology and Obstetrics in Skopje by a cytopathologist. Cytological results were classified according to the revised Bethesda classification (Zerat, 2002; Solomon *et al*. 2002) [22,23]: atypical cells with undetermined significance (ASCUS), squamous intraepithelial lesion of low grade-LSIL (CIN 1), squamous intraepithelial lesion of high grade -HSIL (CIN 2, CIN 3, CIS) and invasive squamous cell carcinoma.
Samples for histopathological analysis were taken at the University Clinic of Gynecology and Obstetrics in Skopje and were treated at the University Clinic for Radiotherapy and Oncology in Skopje, at the Department of Histopathology Clinical Cytology, by an experienced pathologist. According to the morphology determined in bioptic samples, cervical findings were characterized as: normal finding (non-specific cervicitis), productive HPV infection (flat condyloma, cervicitis virosa)-mild dysplasia, moderate and severe dysplasia, in situ squamous cell carcinoma and invasive squamous cell carcinoma.
Samples from the cervical biopsy for detection and HPV typisation were taken and treated at the University Clinic of Gynecology and Obstetrics in Skopje, at the Laboratory for HPV typisation.

The first step in HPV testing was the isolation of DNA from the collected cells from the cervical biopsies. For isolation of DNA series of three paraffin cuts were prepared. Cuts were incubated in 1 ml of xylene, 5 minutes at 55ºC, and centrifuged at 10 000 G for five minutes at room temperature. The same procedure was repeated two more times. After careful removal of the remains of xylene, the samples were briefly incubated twice in 1 ml of 100% ethanol, and centrifuged for 5 minutes at room temperature. After removal of ethanol, short drying followed in air and incubation overnight in buffer with freshly added proteinase K at 55ºC.
The second step was the detection of DNA in HPV by using polymerase chain reaction (PCR). To verify the quality and integrity of the isolated DNA, actually of a present inhibitor, for each sample a reaction of multiplication of the specific beginners (primers) for beta globin PC04 and GH20 was first made. Three pairs of beginners (primers) were used, common to a larger number of HPV types: degenerate beginners My09/ My11 and CPI/CPII G and Gp5/6+. The samples were carried through all reactions with starters (primers) specific to high-risk and low-risk HPV genotypes.
The third step was genotyping by using reverse hybridization. It is a method that is based on the hybridization of specific DNA probes that are immobilized on nitrocellulose or nylon tapes. It is a set of beginners (SPF 10) with aim-propagation of the L1 gene on the viral DNA. The product of amplification with SPF beginners is the size of 65 bp, and allows detection of 25 new genotypes. Denatured biotinylated PCR products are hybridized with specific oligonucleotide probes that are immobilized as parallel lines on membrane strips. After hybridization and washing with streptavidin, alkaline phosphatase is added, which binds to the biotinylated hybrids formed previously. Incubation with BCIP (5-bromo-4-chloro-3-indolyl-phosphate)/NBT (nitro blue tetrazolium) chromogen gives purple precipitate and the results are interpreted visually.

Statistical Analysis

Statistical data were entered into a specific software database (Excel). Statistical analysis of the established statistical series was conducted with the statistical program SPSS.
The structure of numerical signs was analyzed by determining the measures of central tendency (arithmetic mean) and measures of dispersion (standard deviation).
Analysis of the relationship (the existence of association) between two sets of attribute variables was performed using the chi-square test.
Statistically significant data were defined as a p value <0.05.

Results

The study included 100 patients, aged 20 to 69 years (39±10.77) of whom: 24 (24%) were aged 20-29 years, 32 (32%) aged 30-39 years, 26 (26%) aged 40-49 years, 16 (16%) aged 50-59 and 2 (2%) aged 60-69 years. The distribution of HPV infection according to cytology diagnosis is shown in Table 1.

Table 1. Distribution of HPV infection according to cytology (PAP) diagnosis

		Cytology (Pap) diagnosis													
		ASCUS (n=9)		CIN 1 (n=31)		CIN 2 (n=37)		CIN 3 (n=17)		In situ squamous carcinoma (n=4)		Invasive squamous carcinoma (n=2)		Total (n=100)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
HPV infection	HPV DNA Negative	3	(33.3)	6	(19.4)	9	(24.3)	1	(5.9)	0	(0)	0	(0)	19	(19.0)
	HPV DNK Positive	6	(66.7)	25	(80.6)	28	(75.7)	16	(94.1)	4	(100)	2	(100)	81	(81.0)
	H-R HPV DNK Positive	4	(44.5)	21	(67.7)	25	(67.6)	16	(94.1)	4	(100)	2	(100)	72	(72.0)
	L-R HPV DNA Positive	2	(22.2)	4	(12.9)	3	(8.1)	0	(0)	0	(0)	0	(0)	9	(9.0)

Legend: **ASCUS**-atypical squamous cells of undetermined significance; **CIN**-cervical intraepithelial neoplasia; **H-R HPV DNA**-high-risk human papillomavirus deoxyribonucelic acid; **L-R HPV DNA**-low-risk human papillomavirus deoxyribonucelic acid

Table 2. Distribution of HPV infection according to histopathological diagnosis

		Histopathological diagnosis													
		Normal finding (n=22)		Productive HPV infection-Mild dysplasia (n=22)		Moderate dysplasia (n=22)		Severe dysplasia (n=23)		In situ Squamous carcinoma (n=6)		Invasive Squamous carcinoma (n=5)		Total (n=100)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
HPV infecton	HPV DNA Negative	12	(54,5)	4	(18,2)	2	(9,1)	1	(4,4)	0	(0)	0	(0)	19	(19,0)
	HPV DNA Positive	10	(45,5)	18	(81,8)	20	(90,9)	22	(95,6)	6	(100)	5	(100)	81	(81,0)
	H-R HPV DNA Positive	9	(41,0)	12	(54,5)	19	(86,4)	21	(91,2)	6	(100)	5	(100)	72	(72,0)
	L-R HPV DNA Positive	1	(4,5)	6	(27,3)	1	(4,5)	1	(4,4)	0	(0)	0	(0)	9	(9,0)

Legend: **H-R HPV DNA**-high-risk human papillomavirus deoxyribonucelic acid; **L-R HPV DNA**-low-risk human papillomavirus deoxyribonucelic acid

Analysis of data showed an increase in the presence of HPV infection in parallel to increasing grade of cytological lesions of the cervix.

The distribution of HPV infection in correlation with the histopathological analysis is shown in Table 2.

HPV DNA was detected in 81.0% (81/100) of the examined women. Analysis of the data showed a rise in HPV infection, along with increasing the grade of cervical lesion.

Data analysis demonstrated association between HPV infection and emergence of squamous intraepithelial lesions and squamous invasive cervical cancer (chi-squared test = 23.156, $p < 0.00001$, $p < 0.05$).

The relationship between the prevalence of high-risk and low-risk HPV DNA was 72%:9% (88.9%:11.1% between HPV DNA positive cases).

Analysis of data showed an increased presence of high-risk HPV DNA in parallel to increasing the grade of lesion.

Data analysis showed an association between the oncogenic potential of the virus and the grade of cervical lesion (chi-square test = 11.943, $p = 0.018$, $p < 0.05$).

The frequency of HPV infection according to age groups in all patients is shown in Figure1.

Mixed HPV infection was detected in 19.0% (19/100) of all patients (23.5% of the HPV DNA positive patients). The most common co-infection was high-risk-high risk HPV: 16.1% (13/81) (Table 3).

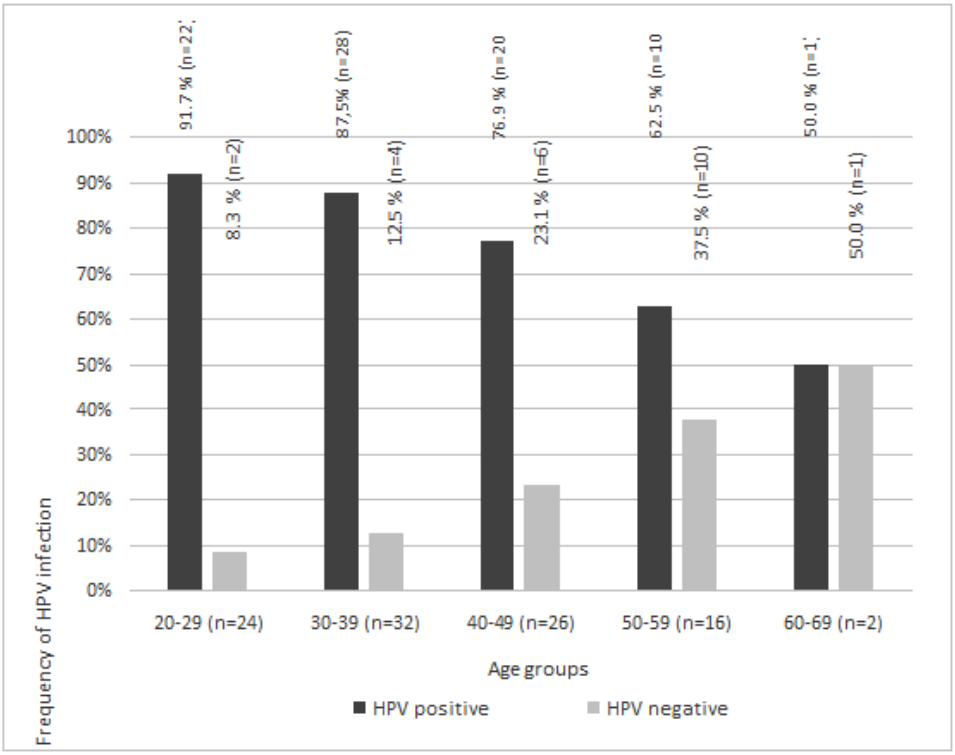

Fig. 1. Single HPV infection was detected in 62.0% (62/100) of all patients (76.5% of the HPV DNA positive patients). The most common was single HPV infection with high-risk HPV: 72.8% (59/81)

Table 3. Distribution of single and mixed HPV infection compared to histopathological diagnosis

		Histopathological diagnosis													
		Normal finding (n=10)		**Productive HPV infection-Mild dysplasia (n=18)**		**Moderate dysplasia (n=20)**		**Severe dysplasia (n=22)**		**In situ Squamous carcinoma (n=6)**		**Invasive Squamous carcinoma (n=5)**		**Total (n=81)**	
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Type of infection	**Single infection**	5	(50.0)	12	(66.7)	15	(75.0)	20	(91.0)	6	(100)	4	(80.0)	62	(76.5)
	Single H-R HPV	5	(50.0)	10	(55.6)	14	(70.0)	20	(91.0)	6	(100)	4	(80.0)	59	(72.8)
	Single L-R HPV	0	(0)	2	(11.1)	1	(5.0)	0	(0)	0	(0)	0	(0)	3	(3.7)
	Mixed infection	5	(50.0)	6	(33.3)	5	(25.0)	2	(9.0)	0	(0)	1	(20.0)	19	(23.5)
	Mixed H-R–H-R HPV	4	(40.0)	2	(11.1)	5	(25.0)	1	(4.5)	0	(0)	1	(20.0)	13	(16.1)
	Mixed H-R-L-R HPV	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
	Mixed L-R-L-R HPV	1	(10.0)	4	(22.2)	0	(0)	1	(4.5)	0	(0)	0	(0)	6	(7.4)

Legend: **H-R HPV –** high-risk human papillomavirus; **L-R HPV –** low-risk human papillomavirus

The freqency of single and mixed HPV infection by age of patients (n = 81) is shown in Figure 2

Data analysis showed that mixed HPV infections were most frequent in patients under the age of 30(36.4%; 8/22). HPV typisation revealed 11 HPV genotypes, of which 9 were high-risk (HPV DNA 16, 18, 31, 33, 45, 52, 56, 58 and 59) and two low-risk (6, 11). The prevalence of HPV 11 DNA genotypes in single and mixed HPV infection compared to the histopathological diagnosis is shown in Table 4.

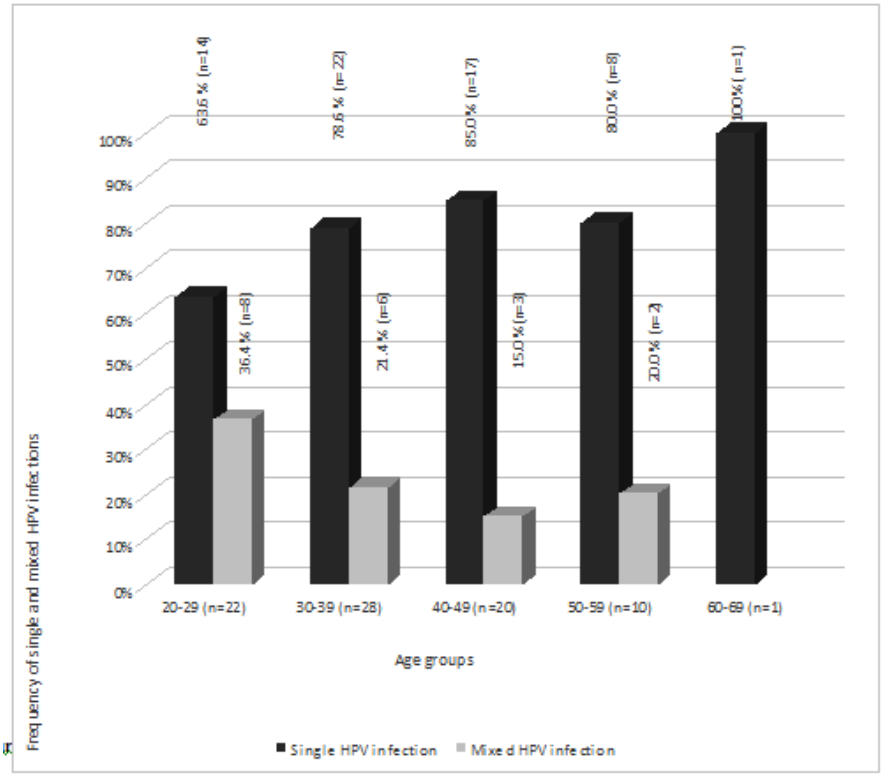

Fig. 2. The frequency of single and mixed HPV infections according to age groups in 81 patient

Tabela 4. The prevalence of HPV DNA genotypes in correlation to histopathological diagnosis

		Histopathological diagnosis													
		Normal finding (n=10)		Productive HPV infection-mild dysplasia (n=18)		Moderate dysplasia (n=20)		Severe dysplasia (n=22)		In situ Squamous carcinoma (n=6)		Invasive squamous carcinoma (n=5)		Total (n=81)	
HPV genotype	Type of infection	n	%	n	%	n	%	n	%	n	%	n	%	n	%
16	Single	0	(0)	1	(5.5)	9	(45.0)	13	(59.1)	4	(66.6)	3	(60.0)	30	(37.0)
	Mixed	2	(20.0)	1	(5.5)	1	(5.0)	0	(0)	0	(0)	1	(20.0)	5	(6.2)
18	Single	0	(0)	2	(11.1)	3	(15.0)	2	(9.1)	1	(16.7)	1	(20.0)	9	(11.1)
	Mixed	0	(0)	0	(0)	3	(15.0)	0	(0)	0	(0)	0	(0)	3	(3.7)
31	Single	4	(40.0)	4	(22.2)	2	(10.0)	5	(22.7)	0	(0)	0	(0)	15	(18.5)
	Mixed	2	(20.0)	1	(5.5)	4	(20.0)	0	(0)	0	(0)	1	(20.0)	8	(9.9)
33	Single	1	(10.0)	1	(5.5)	0	(0)	0	(0)	1	(16.7)	0	(0)	3	(3.7)
	Mixed	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
45	Single	0	(0)	2	(11.1)	0	(0)	0	(0)	0	(0)	0	(0)	2	(2.5)
	Mixed	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
52	Single	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
	Mixed	1	(10.0)	0	(0)	1	(5.0)	1	(4.5)	0	(0)	0	(0)	3	(3.7)
56	Single	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
	Mixed	1	(10.0)	0	(0)	1	(5.0)	1	(4.5)	0	(0)	0	(0)	3	(3.7)
58	Single	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
	Mixed	1	(10.0)	1	(5.5)	0	(0)	0	(0)	0	(0)	0	(0)	2	(2.5)
59	Single	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
	Mixed	1	(10.0)	1	(5.5)	0	(0)	0	(0)	0	(0)	0	(0)	2	(2.5)
6	Single	0	(0)	2	(11.1)	1	(5.0)	0	(0)	0	(0)	0	(0)	3	(3.7)
	Mixed	1	(10.0)	4	(22.2)	0	(0)	1	(4.5)	0	(0)	0	(0)	6	(7.4)
11	Single	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
	Mixed	1	(10.0)	4	(22.2)	0	(0)	1	(4.5)	0	(0)	0	(0)	6	(7.4)

Discussion

Seventy-five percentage of sexually active population is in contact with one or more HPV genotypes [24].
Early detection and treatment of intraepithelial lesions of the cervix can be crucial in the prevention of cervical cancer [25].
Depending on the geographical region, study population and the method used, the frequency of HPV genotypes in different cervical lesions varies considerably.
In this study, HPV was detected in 81.0% of the examined population. This high rate of HPV prevalence correlate with some previously published studies [26-29].
HPV 16 was the most common genotype (43.2%). Despite HPV 16, the most common genotypes were: HPV 31 (28.4%), HPV 18(14.8%), HPV 6(11.1%), HPV 11(7.4%), HPV 33,52,56 (3.7%) and HPV 45,58,59 (2.5%).
In the study of Stojanovska C *et al.* from 2009, which comprised 6988 patients, the following distribution of the most common genotypes was detected: HPV 16 (32.1%), HPV 31(14%), HPV 53(12.6%), HPV 18 (9.9%), HPV 58 (5%), etc. [30]. The study of Duvlis from 2000 including patients from Macedonia, detected the following distribution of the most common genotypes: HPV 16(27.5%), HPV 31(13.1%), HPV 66(10.3%), HPV 6 (9.4%), HPV 1 (8.4%), etc. [31].
In these three studies the most common types were HPV 16 and HPV 31, but there were deviations in the distribution of the remaining HPV genotypes.
This study found a significant association between presence of HPV infection with intraepithelial lesions and cervical cancer. The high percentage of high-risk HPV genotypes in severe dysplasia (91.2%) and squamous cell carcinoma in situ (100%), once again has confirmed the strong connection between the oncogenic potential of the virus and the development of intraepithelial lesions and cervical cancer. The relationship between high-risk and low-risk HPV genotypes was 72%: 9%. In the study of Garcia-Garcia this relationship was 79.8%:19.7% [32].
The high percentage of HPV 16 in severe dysplasia (59.1%), in situ squamous cell carcinoma (66.6%) and in invasive squamous cell carcinomas (80%), showing that the high-risk HPV 16 genotype is with the biggest oncogene potential.
Mixed HPV infection was found in 19.0% of the examined patients, 23.5% of HPV positive. This percentage of mixed infections is in agreement with the previously published studies of Vujosevic [33] and Milutin-Gasperov [34]. Comparing the results for mixed HPV infection is especially difficult because of the use of different cervical samples (biopsy or cytological). Tissue biopsies, which receive more efficient amplification and subsequently clarified gangs as a result of a higher concentration of DNA, are better than those isolated from cytobrash samples.
The highest frequency of HPV infection was found in patients under the age of 30 years (91.7%), while the lowest in patients over 60 years of age (50.0%). An inverse correlation between HPV infection and age of patients was detected, which corresponds with the results of some other studies [35-37]. In the group of patients under the age of 30 the highest frequency of mixed HPV infection (36.4%) was detected. This high frequency among young people can be explained by their sexual behavior or promiscuity.

Conclusion

This study showed that HPV infections, especially infections with high-risk HPV genotypes are in strong correlation with the occurrence of squamous intraepithelial lesions and squamous cervical cancer. Also, there is a correlation between the grade of cervical lesion and the oncogenic potential of the virus. The young population under the age of 30 years is the most affected and the HPV 16 is the most common genotype in our environment. The results of this study may be useful for developing a national strategy for the fight against cervical cancer.

Conflict of interest statement. None declared.

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