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ORIGINAL ARTICLE

Human Papillomavirus Infections in Women With and Without Squamous Cell Abnormalities of the Uterine Cervix

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ABSTRACT

Background: Human papillomavirus infections are one of the most common sexually transmitted infections with viral aetiology. The aim of the study was to confirm the existence of an association between human papillomavirus infection and squamous cell abnormalities of the uterine cervix.
Methods: Cohort study, conducted in the period from January 2017 to June 2018 of 768 sexually active women, age groups of 20 to 59 years, divided into two groups: examined and control, who came to their annual gynaecological exam at University Clinic for Gynaecology and Obstetrics in Skopje. In all patients was done human papillomavirus-deoxyribonucleic testing. Human papillomavirus detection and typing was done using a polymerase chain reaction and reverse hybridisation.
Results: Data analysis showed an association between human papillomavirus infection and squamous cell abnormalities of the uterine cervix (p=0.00001). Human papillomavirus infection was detected in 22.91% of all patients, in 75.00% of patients with abnormal cervical cytology and in 12.50% of patients with normal cervical cytology. A single human papillomavirus infection was detected in 13.67% of all patients (in 59.66% of human papillomavirus positive patients). Mixed human papillomavirus infection was detected in 9.24% of all patients (in 40.34% of human papillomavirus positive patients). Human papillomavirus type 16 was the most common genotype with 40.91%.
Conclusion: This study confirmed that there is an association between human papillomavirus infection and squamous cell abnormalities of the uterine cervix and the young population under the age of 30 years is the most affected.

Key words: human papillomavirus, squamous cell abnormalities, uterine cervix.

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INTRODUCTION

Human papillomavirus (HPV) infections are one of the most common sexually transmitted infections with viral aetiology.[1] Epidemiological studies show that 50% of women are infected with HPV during the first two years of the onset of sex life, about 80% of sexually active women by the age of 50 will receive genital HPV infection and about 20% of women are infected with one or more HPV genotypes.[2] 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.[3] Young population of women, from 18 to 25, has the highest rate of HPV infection. After the 25th year of life, the incidence of HPV infection is reduced to reach its second highest level after 45 years of life.[4] The most common risk factor for squamous cell abnormalities of the uterine cervix is infection with HPV, especially with high-risk HPV genotypes. Only persistent, high-risk HPV infection represent a major risk factor for squamous cell abnormalities of the uterine cer-

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vix.[5] Deoxyribonucleic acid (DNA) from HPV has been found in 99.7% of cases of cervical carcinoma.[6] Detection of HPV can be done using two methods, the first one is direct hybridisation or in situ hybridisation, and the other one is amplification or polymerase chain reaction (PCR).[7]

The aims of the study were: to confirm the existence of an association between HPV-DNA infection and squamous cell abnormalities of the uterine cervix; to detect and typing HPV genotypes, which are the most common causes of squamous cell abnormalities of the uterine cervix; to determine the prevalence of HPV-DNA infection and to determine the most affected age group.

METHODS

Design of the study: The study is a cohort study.

Material: The material consists of 768 patients aged 20 to 59, divided into two groups: examined and control.

The examined group included 128 sexually active women with an abnormal cervical cytological finding, i.e. a finding of a Papanicolaou (PAP) test that showed a squamous cell abnormalities of the uterine cervix. The control group included 640 sexually active women with a normal cervical cytology finding, i.e. a PAP test.

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

Period of realisation: The study was conducted in the period from January 2017 to June 2018.

Location of the study: The study was conducted at the University Clinic for Gynaecology and Obstetrics in Skopje, the Republic of North Macedonia.

Methods of examination: In all women we have done the human papillomavirus-deoxyribonucleic acid (HPV-DNA) testing.

Cytological analysis: All samples for cytological analysis were taken using the Thin Prep PAP test and were analysed in the cytological laboratory at the University Clinic for Gynaecology and Obstetrics in Skopje by a cytopathologist. Cytological results were classified according to the revised Bethesda classification[8,9] such as: atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells - cannot e clude a high-grade squamous intraepithelial lesion (ASC-H); low-grade squamous intraepithelial lesion, LSIL (cervical intraepithelial neoplasia grade 1, CIN 1); high-grade squamous intraepithelial lesion, HSIL (cervical intraepithelial neoplasia grade 2, CIN 2, cervical intraepithelial neoplasia grade 3, CIN 3, carcinoma in situ, CIS) and invasive squamous cell carcinoma (ISCC).

HPV-DNA testing: Samples from the cervical biopsy for HPV-DNA testing were taken and analysed at the University Clinic for Gynaecology and Obstetrics in Skopje at the Laboratory for HPV testing. HPV detection and typing were used to test the use of multiple polymerase chain reaction (Multiplex PCR) and reverse hybridisation. The results of the HPV DNA test were analysed and demonstrated based on the finding of the presence or absence of DNA from HPV and the specified genotype.[10] 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 55°C, 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 55°C. 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 primers for beta globin PC04 and GH20 was first made. Three pairs of primers were used, common to a

larger number of HPV types: degenerate primers My09/My11 and CPI/CPII G and Gp5+/6+. The samples were carried through all reactions with primers specific to high-risk and low-risk HPV genotypes. The third step was genotyping by using reverse hybridisation. It is a method that is based on the hybridisation of specific DNA probes that are immobilised on nitrocellulose or nylon tapes. It is a set of primers (SPF 10) with aim-propagation of the L1 gene on the viral DNA. The product of amplification with SPF promers 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 immobilised as parallel lines on membrane strips. After hybridisation 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: Data were analysed by a specific software for data-bases (Excel). Statistical analysis of the established statistical series was made with the statistical program Statistical Package for Social Sciences (SPSS), version 23.0. The structure of statistical series with attribute sings was analysed with determining the proportions and rates. The structure of numerical signs was analysed by determining the measures of central tendency (arithmetical 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 and Fisher exact test. Testing the differences between the comparing groups (their distributions, arithmetic environments and proportions) was done using Student's t-test and Chi-square test. Statistical significance was defined as a p value <0.05.

RESULTS

The distribution of patients by age groups is shown in Table 1. According to the t-test, the percentage difference between the average ages between the two groups was statistically non-significant with p>0.05 (Table 1).

HPV-DNA infection was detected in 22.91% of all patients, in 75.00% of patients with abnormal cervical cytology and in 12.50% of patients with normal cervical cytology. There was a statistically significant relationship between the modalities of HPV-DNA positive and HPV-DNA negative between the examined and the control group (chi-square test=67.1329, p<0.00001, p<0.05). Data analysis showed: an association between HPV-DNA infection and squamous cell abnormalities of the uterine cervix (Chi-square test=235.8722, p=0.00001, p<0.05); an association between the oncogenic potential of the virus and squamous cell abnormalities of the uterine cervix (Fisher exact test=0.0027, p<0.05); an increase in the presence of HPV-DNA infection in parallel with an increase in the cytological grade of cervical lesion (Chi-square test=10.7682, p=0.029296, p<0.05), and an association between the oncogenic potential of the virus and cytological grade of cervical lesion (Chi-square test=16.6967, p=0.000044, p<0.05) (Table 2).

The incidence of HPV-DNA infection was:

Table 1: Distribution of patients by age groups

Age group	Normal Cytology (n=640) n(%)	Abnormal cytology (n=128) n(%)
20-29	130 (20.31)	28 (21.87)
30-39	220 (34.38)	38 (29.69)
40-49	180 (28.12)	30 (23.44)
50-59	110 (17.19)	32 (25.00)
Average age	39.34	40.50
Standard deviation	9.70	10.85
Student's t-test: p<0.05 (p=0.4722, t=0.7204, 95%CI: -2.01-4.32)		

n=number of patients

40.51% in patients aged 20-29 years; 21.32% of 30-39 years; 15.24% of 40-49 years; 17.61% in patients aged 50-59 years. According to the t-test, the percentage difference between incidences of viral infection between the examined and the control group was statistically significant for $p<0.05$ ($p=0.0002$, $t=8.0032$, 95% CI: 43.9084-82.5816) (Table 3).

A single HPV-DNA infection was detected in 13.67% of all patients (in 59.66% of HPV-DNA positive patients). The most common was single HPV-DNA infection with high-risk HPV: 57.39%. Mixed HPV-DNA infection was detected in 9.24% of all patients (in 40.34% of HPV-DNA positive patients). The most common co-infection was high risk - high-risk HPV: 18.18%. According to the t-test, the percentage difference between the modalities of single and mixed HPV-DNA infection between the examined and the control group was statistically non-significant for $p>0.05$ ($p=0.7821$, $t=0.3157$, 95% CI: -88.7874 -102.8474) (Table 4).

Single HPV infection was most frequent in patients over 40 years of age (73.68%), while mixed HPV infection was most frequent in patients under 30 years of age (53.12%) (Table 5).

HPV-DNA typing identified a total of 20 HPV-

Table 2: Distribution of HPV-DNA infection according to cytological diagnosis

HPV-DNA testing	All samples (n=768)	Normal cytology (n=640)	CYTOLOGICAL DIAGNOSIS							
			Abnormal cytology							
			ASC-US (n=13)	ASC-H (n=7)	LSIL (n=31)	HSIL (n=56)			ISCC (n=21)	TOTAL (n=128)
						CIN 1 (n=31)	CIN 2 (n=20)	CIN 3 (n=21)	In situ SCC (n=15)	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
HPV-DNA negative	592 (77.09)	560(87.50)	7 (53.85)	3 (42.86)	9 (29.03)	4 (20.00)	4 (19.05)	3 (20.00)	2 (9.52)	32(25.00)
HPV-DNA positive	176 (22.91)	80(12.50)	6 (46.15)	4 (57.14)	22(70.97)	16(80.00)	17(80.95)	12 (80.00)	19 (90.48)	96(75.00)
High-risk positive	133 (75.57)	60(9.38)	0 (0)	3 (42.86)	12(38.71)	11(55.00)	17(80.95)	12 (80.00)	18 (85.71)	73(57.03)
Low-risk positive	11 (8.59)	0(0)	4 (30.77)	1 (14.28)	4 (12.90)	2 (10.00)	0 (0)	0 (0)	0 (0)	11 (8.59)
High and low -risk positive	72 (40.91)	60(9.38)	2 (23.08)	0 (0)	6 (19.35)	3 (15.00)	0 (0)	0 (0)	1 (4.76)	12 (9.37)

n=number of patients; ASC-US: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ISCC: invasive squamous cell carcinoma; CIN: cervical intraepithelial neoplasia; HPV-DNA: human papillomavirus deoxyribonucleic acid.

Table 3: Distribution of HPV-DNA infection by age group in 768 patients

Age group	NORMAL CYTOLOGY			ABNORMAL CYTOLOGY			Total HPV-DNA positive by age group
	HPV-DNA positive (n=80)	HPV-DNA negative (n=560)	TOTAL (n=640)	HPV-DNA positive (n=96)	HPV-DNA negative (n=32)	TOTAL (n=128)	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
20-29	40 (30.77)	90 (69.23)	130 (20.31)	24 (85.71)	4 (14.29)	28 (21.87)	64 (40.51)
30-39	30 (13.64)	190 (86.36)	220 (34.37)	25 (65.79)	13 (34.21)	38 (29.69)	55 (21.32)
40-49	10 (5.56)	170 (94.44)	180 (28.13)	22 (73.33)	8 (26.67)	30 (23.44)	32 (15.24)
50-59	0 (0)	110 (100)	110 (17.19)	25 (78.12)	7 (21.88)	32 (25.00)	25 (17.61)

n=number of patients; HPV-DNA: human papillomavirus deoxyribonucleic acid.

DNA genotypes, of which 15 were high-risk (HPV DNA-16, -18, -31, -33, -35, -39, -45, -52, -53, -56 , -58 -59, -66, -68, and -73) and 5 low-risk (-6, -11, -40, -42 and -61). The prevalence of 20 HPV-DNA genotypes in single and mixed HPV-DNA infections according to cytological diagnosis is shown in Table 6. Among high-risk HPV-DNA genotypes, HPV-16 was the most common (40.91%). Among the low-risk HPV-DNA genotypes, the most common was HPV-6 (7.96%), HPV-16 was most common in patients with high-grade squamous intraepithelial lesion and invasive squamous cell carcinoma, while HPV-6 in patients with low-grade squamous intraepithelial lesion (Table 6).

DISCUSSION

HPV infections are widespread among people, occur with almost every person in both sexes at a certain period of their life, are usually short-lived, asymptomatic, spontaneously disappearing and only a small percentage of them need treatment.[11] Seventy-five percentage of the sexually active population, in the course of their lives, was in contact with one or more HPV genotypes.[12] Depending on the geographical affiliation, the study population and the method used, the frequency of HPV genotypes in various cervical lesions varies considerably. In 1996, the World Health Association recognized the importance of HPV for cervical cancer.[6]

Table 4: Distribution of single and mixed HPV-DNA infections in 176 HPV-DNA positive patients

Type of HPV-DNA infection	Total HPV-DNA positive (n=176)	CYTOLOGICAL DIAGNOSIS								
		Normal cytology (n=80)	Abnormal cytology							
			ASC-US (n=6)	ASC-H (n=4)	LSIL (n=22)	HSIL (n=45)			ISCC (n=19)	TOTAL (n=96)
					CIN 1 (n=22)	CIN 2 (n=16)	CIN 3 (n=17)	In situ SCC (n=12)		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Single	105 (59.66)	50 (62.50)	1 (16.67)	1 (25.00)	10 (45.45)	8 (50.00)	12 (70.59)	10 (83.33)	13 (68.42)	55 (57.29)
Single high-risk	101 (57.39)	50 (62.50)	0 (0)	0 (0)	8 (36.36)	8 (50.00)	12 (70.59)	10 (83.33)	13 (68.42)	51 (53.12)
Single low-risk	4 (2.27)	0 (0)	1 (16.67)	1 (25.00)	2 (9.09)	0 (0)	0 (0)	0 (0)	0 (0)	4 (4,17)
Mixed	71 (40.34)	30 (37.50)	5 (83.33)	3 (75.00)	12 (54.55)	8 (50.00)	5 (29.41)	2 (16.67)	6 (31.58)	41 (42.71)
Mixed high-risk-high risk	32 (18.18)	10 (12.50)	1 (16.67)	1 (25.00)	3 (13.64)	6 (37.50)	4 (23.53)	2 (16.67)	5 (26.32)	22 (22.92)
Mixed high-risk-low risk	31 (17.61)	20 (25.00)	3 (50.00)	1 (25.00)	3 (13.64)	2 (12.50)	1 (5.88)	0 (0)	1 (5.26)	11 (11.46)
Mixed low-risk-low risk	8 (4.55)	0 (0)	1 (16.67)	1 (25.00)	6 (27.27)	0 (0)	0 (0)	0 (0)	0 (0)	8 (8.33)

n=number of patients; ASC-US, atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ISCC: invasive squamous cell carcinoma; CIN: cervical intraepithelial neoplasia; HPV-DNA: human papillomavirus deoxyribonucleic acid.

Table 5: Distribution of single and mixed HPV-DNA infections by age group in 176 HPV-DNA positive patients

Age group	NORMAL CYTOLOGY			ABNORMAL CYTOLOGY			All HPV-DNA positive (n=176)
	Single HPV-DNA infection (n=50)	Mixed HPV-DNA infection (n=30)	TOTAL (n=80)	Single HPV-DNA infection (n=55)	Mixed HPV-DNA infection (n=41)	TOTAL (n=96)	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
20-29	20 (50.00)	20 (50.00)	40 (50.00)	10 (41.67)	14 (58.33)	24 (25.00)	64 (36.36)
30-39	20 (66.67)	10 (33.33)	30 (37.50)	13 (52.00)	12 (48.00)	25 (26.04)	55 (31.25)
40-49	10 (100)	0 (0)	10 (12.50)	14 (63.64)	8 (36.36)	22 (22.92)	32 (18.18)
50-59	0 (0)	0 (0)	0 (0)	18 (72.00)	7 (28.00)	25 (26.04)	25 (14.21)

n=number of patients; HPV-DNA: human papillomavirus deoxyribonucleic acid.

Table 6: The prevalence of HPV-DNA genotypes according to cytological diagnosis

HPV-DNA genotype	Type of HPV-DNA infection	All HPV-DNA positive (n=176)	CYTOLOGICAL DIAGNOSIS								
			Normal cytology (n=80)	Abnormal cytology							
				ASC-US (n=6)	ASC-H (n=4)	LSIL (n=22)	HSIL (n=45)			ISCC (n=19)	TOTAL (n=96)
							CIN 1 (n=22)	CIN 2 (n=16)	CIN 3 (n=17)	In situ SCC (n=12)	
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
16	Single	45 (25.57)	20 (25.00)	-	-	1 (4.54)	4 (25.00)	9 (52.94)	5 (41.67)	6 (31.58)	25 (26.04)
	Mixed	27 (15.34)	10 (12.50)	1 (16.67)	2 (50.00)	1 (4.54)	3 (18.75)	3 (17.65)	3 (25.00)	4 (21.05)	17 (17.71)
18	Single	3 (1.70)	-	-	-	-	-	-	-	3 (15.79)	3 (3.12)
	Mixed	17 (9.66)	10 (12.50)	-	-	1 (4.54)	1 (6.25)	2 (11.76)	-	3 (15.79)	7 (7.29)
31	Single	8 (4.55)	-	-	-	-	1 (6.25)	3 (17.65)	3 (25.00)	1 (5.26)	8 (8.33)
	Mixed	7 (3.98)	-	-	-	1 4.54)	1 (6.25)	1 (5.88)	2 (16.67)	2 (10.53)	7 (7.29)
33	Single	2 (1.14)	-	-	-	-	-	1 (5.88)	1 (8.33)	-	2 (2.08)
	Mixed	5 (2.84)	-	1 (16.67)	-	-	2 (12.50)	-	-	2 (10.53)	5 (5.21)
35	Single	1 (0.57)	-	-	1 (25.00)	-	-	-	-	-	1 (1.04)
	Mixed	14 (7.95)	10 (12.50)	-	1 (25.00)	1 (4.54)	1 (6.25)	1 (5.88)	-	-	4 (4.17)
39	Single	10 (5.68)	10 (12.50)	-	-	-	-	-	-	-	-
	Mixed	1 (0.56)	-	-	-	1 (4.54)	-	-	-	-	1 (1.04)
45	Single	7 (3.98)	-	-	-	-	1 (6.25)	-	2 (16.67)	4 (21.05)	7 (7.29)
	Mixed	2 (1.14)	-	-	-	-	-	-	-	2 (10.53)	2 (2.08)
52	Single	1 (0.57)	-	1 (16.67)	-	-	-	-	-	-	1 (1.04)
	Mixed	14 (7.95)	10 (12.50)	-	-	1 (4.54)	1 (6.25)	2 (11.76)	-	-	4 (4.17)
53	Single	-	-	-	-	-	-	-	-	-	-
	Mixed	1 (0.57)	-	1 (16.67)	-	-	-	-	-	-	1 (1.04)
56	Single	2 (1.14)	-	1 (16.67)	-	1 (4.54)	-	-	-	-	2 (2.08)
	Mixed	3 (1.70)	-	1 (16.67)	-	1 (4.54)	-	1 (5.88)	-	-	3 (3.12)
58	Single	20 (11.36)	20 (25.00)	-	-	-	-	-	-	-	-
	Mixed	1(0.57)	-	-	-	-	1 (6.25)	-	-	-	1 (1.04)
59	Single	-	-	-	-	-	-	-	-	-	-
	Mixed	2 (1.14)	-	-	-	2 (9.09)	-	-	-	-	2 (2.08)
66	Single	2 (1.14)	-	-	-	2 (9.09)	-	-	-	-	2 (2.08)
	Mixed	1 (0.57)	-	-	-	1 (4.54)	-	-	-	-	1 (1.04)
68	Single	-	-	-	-	-	-	-	-	-	-
	Mixed	13(7.39)	10 (12.50)	-	-	1 (4.54)	1 (6.25)	1 (5.88)	-	-	3 (3.12)
73	Single	-	-	-	-	-	-	-	-	-	-
	Mixed	2 (1.14)	-	-	-	1 (4.54)	-	-	1 (1.88)	-	2 (2.08)
6	Single	1 (0.57)	-	1 (16.67)	-	-	-	-	-	-	1 (1.04)
	Mixed	13 (7.39)	1 (1.25)	4 (66.67)	3 (75.00)	4 (18.18)	-	1 (5.88)	-	-	13 (13.54)
11	Single	1 (0.57)	-	-	-	1 (4.54)	-	-	-	-	1 (1.04)
	Mixed	8 (4.54)	-	-	-	8 (36.36)	-	-	-	-	8 (8.33)
40	Single	-	-	-	-	-	-	-	-	-	-
	Mixed	2 (1.14)	-	-	-	2 (9.09)	-	-	-	-	2 (2,08)
42	Single	2 (1.14)	-	1 (16.67)	-	1 (4.54)	-	-	-	-	2 (2.08)
	Mixed	2 (1.14)	1 (1.25)	-	-	-	-	-	-	1 (5.26)	1 (4.76)
61	Single	-	-	-	-	-	-	-	-	-	-
	Mixed	4 (2.27)	-	2 (33.33)	1 (25.00)	-	-	1 (5.88)	-	-	4 (4.17)

n=number of patients; ASC-US: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ISCC: invasive squamous cell carcinoma; CIN: cervical intraepithelial neoplasia; HPV-DNA: human papillomavirus deoxyribonucleic acid.

In this study, HPV-DNA infection was detected in 75.00% of patients in the investigated group and in 12.50% of patients in the control group. This relatively high percentage of HPV-DNA infection in patients with squamous cell abnormalities of the uterine cervix corresponds with some previously published studies; in the study of Mazarico et al. from 2012, HPV-DNA infection was detected in 73.20% of women with squamous cell abnormalities of the uterine cervix,[13] while in the study of Pista et al. from 2013, HPV-DNA infection was detected in 77.4% of study women.[14] The detected prevalence of 12.50% of HPV-DNA infection of the uterine cervix in patients with a normal cytological finding in our study is in accordance with the estimated global prevalence of 10-12%, with variations of 1-26% depending on the geographical affiliation and age.[15] The British meta-analysis of Anderson et al. since 2013, women with normal cervical cytological findings have shown a prevalence of HPV-DNA infection of 12.00%.[16] An estimat-

ed prevalence of 11.2% in women with normal cervical cytology is shown in the Chinese study of Bao et al. from 2008, worked on 2 902 women.[17] An identical prevalence (11.1%) is shown in the Chilean study of Balanda et al. from 2014-2015.[18]

In this study, a significant association was found between the presence of HPV-DNA infection and the incidence of squamous cell abnormalities of the uterine cervix (p=0.00001). The high percentage of high-risk HPV-DNA genotypes in severe dysplasia (80.95%), and in situ squamous cell carcinoma (80.00%) and invasive squamous cell carcinoma (90.48%), once again confirms the strong association between the oncogene potential of the virus and the development of squamous intraepithelial lesions and squamous invasive carcinoma of the cervix (p<0.05). The relationship between high-risk and low-risk HPV-genotypes in HPV-DNA positive patients was 75.57%:8.59%. In the Spanish study of Garcia-Garcia from 2010, that ratio was 79.80%:19.70%.[19]

The highest frequency of HPV-DNA infection was found in patients under 30 years of age (40.51%), while the lowest in patients aged 40 to 49 (15.25%). Our results correspond to the results of some previously published studies: in the Italian study of Agodi et al. from 2009, the highest frequency of HPV-DNA infection was detected in patients under the age of 25 (73.2%);[20] American study of Evans from 2006 showed the highest frequency of HPV-DNA infection in patients under the age of 30 (96.00%; 458/475);[21] an identical percentage of HPV-DNA infection (96.00%), also showed the study of Hariri et al. from 2012, worked on 3 058 American women aged 18 to 39.[22]

In this study, mixed HPV-DNA infection was found in 9.24% of all patients, in 40.34% of HPV-DNA positive. This percentage of prevalence of mixed HPV-DNA infections corresponds to previously published studies of: Sandri et al. from 2009 (43.00%),[23] Cuschieri et al. from 2001 (43.30%),[24] Vujosevic et al. from 2012 (42.00%).[25]

In the group of patients under 30 years of age, the highest frequency of mixed HPV infection was detected (53.12%). These high frequencies in the young population can be explained by their sexual behavior, that is, promiscuity. In a Serbian study by Kovacevic G. et al. from 2016, performed in students from the University of Novi Sad, mixed HPV infection was detected in 49.4%.[26] Relatively high rates of mixed HPV infection among the young population were also detected in the Bulgarian studies of Grozdanovin 2014 (63.00%)[27] and Kovachev in 2013 (53.00%),[28] as well as in the Romanian study of Moga in 2014 (54.93%).[29]

HPV-16 was the most common genotype with 40.91%. In the retrospective study of Andonovska in 2014, performed in 7,411 women, the following distribution of the most common ge otypes was detected: HPV-16 (23.39%), HPV-31 (10.68%), HPV-53 (10.60%) and HPV-18 (6.19%).[30] In the study of Stojanovska et al. (2009), performed in 6,988 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.[31] At the same time, in the study of Duvlis in 2000, performed in patients from the Republic of North Macedonia, the following distribution of the most common genotypes was detected: HPV-16 (27.5%), HPV-31 (13.1%), HPV-66 (10.3%), HPV-6 (9.4%), HPV-18 (8.4%), etc..[32] In the four Macedonian studies, the most common HPV genotype was HPV-16, but there are deviations in the distribution of the other most common HPV genotypes. The high percentage of HPV-16 in severe dysplasia (70.59%), in *in situ* squamous cell carcinoma (66.67%) and invasive squamous carcinoma (52.63%), distinguishes HPV-16 as a high risk genotype with the highest oncogenic potential. It is confirmed by this study that: there is an association between HPV-DNA infection and squamous cell abnormalities of the uterine cervix; 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.

CONCLUSION

It is confirmed by this study that: there is an association between HPV-DNA infection and squamous cell abnormalities of the uterine cervix; 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.

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CONFLICT OF INTEREST

None.

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