



## SEXUAL BEHAVIOURS AND HUMAN PAPILLOMAVIRUS GENOTYPING AMONG BOSNIAN WOMEN

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### ABSTRACT:

**Purpose:** Youth represents the most vulnerable population, mainly due to their lifestyle derived from risky sexual behaviours. The aim of the study was to identify specific HPV genotypes correlating with cervical smear cytologic abnormalities, well as sociodemographic/behavioural characteristics and history of sexually transmitted diseases in Bosnian women age up to 30 years.

**Methods:** The longitudinal study was performed at the Department of Microbiology School of Medicine University of Sarajevo and Institute for Public Health dr. Andrija Stampar, Zagreb, Croatia from June 2017 to January 2018. Thirty (30) cervical smears were taken from tested females were screened using COBAS assay for HPV DNA testing. Routine Pap screening was performed in the Public Institution Department for Health Care of Women and Maternity of Sarajevo Canton. A self-administered questionnaire was applied.

**Results:** A total of 30 sexually active women with abnormal cytological reports were enrolled in the study. The mean age was 26 IQR (20.75, 29.0) year. The overall prevalence of HR HPV was 66.7 %. Women age of  $\leq 25$  years had a positive association with positive results of HPV testing, OR=1.91. Consumption of alcohol, cigarettes had a strong positive association with positive HPV testing.

**Conclusions:** The data obtained from this study indicate that HPV 16 is the most common HPV type found in the cervical specimens among young Bosnian women, followed by HPV 18. HPV DNA testing using as a screening test should be considered in the development of cervical cancer prevention programs in Bosnia and Herzegovina.

**Keywords:** Sexual behaviour, human papilloma-virus, risk factors, condom,

### INTRODUCTION:

Anogenital infections caused by human papillomaviruses (HPVs) are sexually transmitted and represent one of the most prevalent sexually transmitted diseases (STDs) worldwide.

Cervical cancer has been recognized as a leading cause of female mortality with approximately 528.000 new cases per year, being responsible for the death of 266.000 women each year [1].

The majority of identified HPVs are found in genus *Alphapapillomavirus* where differences are based on genotyping variation, epithelial tropism, species specificity and oncogenic potential respectively. Nearly 200 distinct HPV types have been found. Depending on the prevalence of HPV types in cervical cancer and its precursors, HPV types are classified as „low risk“ (LR) and „high risk“ (HR). In a recent systemic review, the median duration of any HPV detection was 9,8 months, and HR HPV (9,3 months) persisted longer than LR HPV (8,4 months); HPV-16 persisted longer (12,4 months) than HPV-18 (9,8 months) [2].

These viruses cause infections at cutaneous and mucosal sites, sometimes leading to the development different kind of warts; including skin, plantar, flat and anogenital warts, laryngeal papillomas, and several cancers, including those of the cervix, vulva, penis and anus, and a subset of head and neck cancers. Over 40 types infect the female anogenital region and may lead to precursor cervical lesions, cervical intraepithelial neoplasia (CIN) and cervical cancer.

Of these, more than 10 are significantly associated with the progression of CIN to cervical cancer. Therefore, HPV-types 16, 18, 31, 35, 29, 45, 51, 52, 56, 58, and 59 are classified as carcinogenic to humans [3]. The most of HR-HPV types are phylogenetically related to either HPV-16 (carcinogenic types 31, 33, 35, 52, and 58) or HPV-18 (carcinogenic types 39, 45, and 59 and probably carcinogenic 68).

Transmission of anogenital HPV is largely and predominantly sexual. During intercourse, micro-abrasions occur in the genital mucosa and keratinized epithelial tissues, allowing HPV to invade and infect basal keratinocytes. Condoms, which do not cover external epithelial sites harbouring HPV, offering partial protection from infection, particularly with consistent and correct use [4].

Regarding the burden of STDs, youth represents the most vulnerable population category, mainly due to their lifestyle derived from sexual risky behaviours. Bosnia and Herzegovina and other countries in the Region facing the following problems that contribute to the spread of STDs, which are including: irregular condom usage, casual sex partners, an inadequate level of knowledge about prevention of infections and perception of sexuality in general.

The aim of the study was to estimate the frequency of HR-HPV infection among Bosnian women with abnormal cytology report under the 30 years old using COBAS HPV typing assay and correlate the identified HPV-types with certain sociodemographic/behavioural characteristics and history of sexually transmitted diseases.

According to incomplete official data and surveillance of HPV prevalence and cervical cancer control, efforts should be made to improve cervical cancer screening program and prevention strategy in general, where the target group are younger women being screened for cytological examination.

Thus, it is important and essential to incorporate and establish HPV DNA typing in clinical settings and screening environment in purpose to evaluate the prevalence of HPV-types and to monitor the impact on cervical cancer control in the population of BiH. Furthermore, our goal was to assess whether STD-related risk-taking behaviours among young women with abnormal cytological report in Bosnia and Herzegovina are related to each other and what are the personal, family or socio-demographic factors influencing them.

## **MATERIAL AND METHODS:**

### **Study population**

The longitudinal study was performed at the Department of Microbiology School of Medicine University of Sarajevo and Institute for Public Health dr. Andrija Stampar, Zagreb, Croatia from June 2017 to January 2018.

Thirty (30) women from Bosnia and Herzegovina were enrolled in the study to participate and to investigate HPV infection well as sociodemographic/behavioural characteristics and history of sexually transmitted diseases. Cervical cytology reports and HPV test results were collected. Women were eligible if they under the age of 30 with abnormal Pap smear cytology.

### **Cytological examination**

Thirty cervical smears were taken, and routine Pap screening was performed in the Public Institution Department for Health Care of Women and Maternity of Sarajevo Canton, in Sarajevo. Smears were obtained from the cervix of each participant with cytobrush for routine Pap screening. It is consisted of endocervical and ectocervical mu-

cus samples (squamocolumnar junction), collected with Ayre's spatula and endocervical brush. The cell smear was held in a glass slide, which was fixed with alcohol and forwarding for staining using conventional the Papanicolaou staining. Degree of cervical abnormalities was interpreted according to the Bethesda system for reporting cervical or vaginal cytologic diagnoses revised in 2001 [5] as following: the atypical squamous cell of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL), and high grade squamous intraepithelial lesion (HGSIL). Cytology report from the Pap smear results was used to examine the relationship between HPV infections and histologically diagnosed cervical lesions.

Written participant consent was not necessary because each cervical sample is accompanied by the Laboratory request forms, which have to be signed and approved by the practising physician responsible for the verbal participant consent. The handling and publication of participants data in this study were strictly in accordance with the Declaration of Helsinki DoH/Oct2008, including confidentiality and anonymity.

### **The Questionnaire**

All participants completed a self-administered questionnaire based on socio-demographic data, sexual behaviours and history of sexually transmitted diseases. The following instruments were used and modified [6, 7]: Teenage Sexual Health and Behaviour Questionnaire (Jomeen and Whithfield, 2010), and Youth Risk Behaviour Survey (Centres for Disease Control and Prevention, 2011). A self-administered questionnaire was applied among tested women consisting of two parts. The first part examined the socio-demographic characteristics, and the second part examined sexual behaviours as well as the history of STDs. The questionnaire contained 26 questions; 7 questions in the first part and 19 in the second part. The sociodemographic variables analyzed were: age, education, a period of the first menarche, number of delivery and abortion. The second part determined perception about knowledge, awareness, sexual behaviours and sexual experience, history of sexually transmitted diseases, and condom use. The young women filled out the written based questionnaire form by themselves within 15 minutes.

### **Indicators**

Socio-economic status was measured by 3-point scales ranging from (1) worse than others in the environment to (3) better than others in the environment. The question of when you got the first menarche was followed by the scaled answers (1) < 12 years to (4) ≥16 years. Response regarding first sexual intercourse was coded by a 7-point scale ranging from (1) <14 years to (7) > 18 years. Frequency of condom use was accessed by a 2-point scale ranging from (1) never to (2) always. Responses regarding the number of sexual partners were collapsed into categories: 1-2 partners, 3 or more partners.

Answers about the age of last sexual partner were split into two categories: older than subject or in the same ages. Use of condoms on last sexual intercourse was tested with yes or no question type. Question for testing consuming the cigarettes, alcohol and drugs were categorized from

(1) yes to (4) no. Sexual intercourse with a partner with STD, previous history of STD including symptoms as well were split into two categories: yes and no.

### HPV genotyping

Roche Cobas HPV test (Roche Molecular Systems, Pleasanton CA) was used for HPV DNA typing. The Cobas HPV test can detect 14 high-risk HPVs. This HPV DNA testing method separately detects HPV 16 and HPV 18 and a pool of 12 other high-risk (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) [8].

Roche Cobas HPV test is an automated qualitative in vitro test for the detection of HPV DNA in patient specimens which utilizes amplification of target DNA by the polymerase chain reaction (PCR) and nucleic acid hybridization for detection of 14 [9]. HR-HPV types in a single analysis. The test specifically identifies HPV 16 and HPV 18 while concurrently detecting the rest of the high-risk types (31, 33, 35, 45, 51, 52, 56, 58, 59, 66, and 68) at clinically relevant infection level.

### Statistical analysis

Basic standard methods of descriptive statistics quantitatively describing or summarizing features of a collection data were applied. Odds ratios (ORs) were calculated to estimate the association between HPV infection and each of the potential categorical risk factors (demographic, sexual behaviour and history of sexually transmitted infections) using Open Epi Version 3. ([www.Openepi.com](http://www.Openepi.com)).

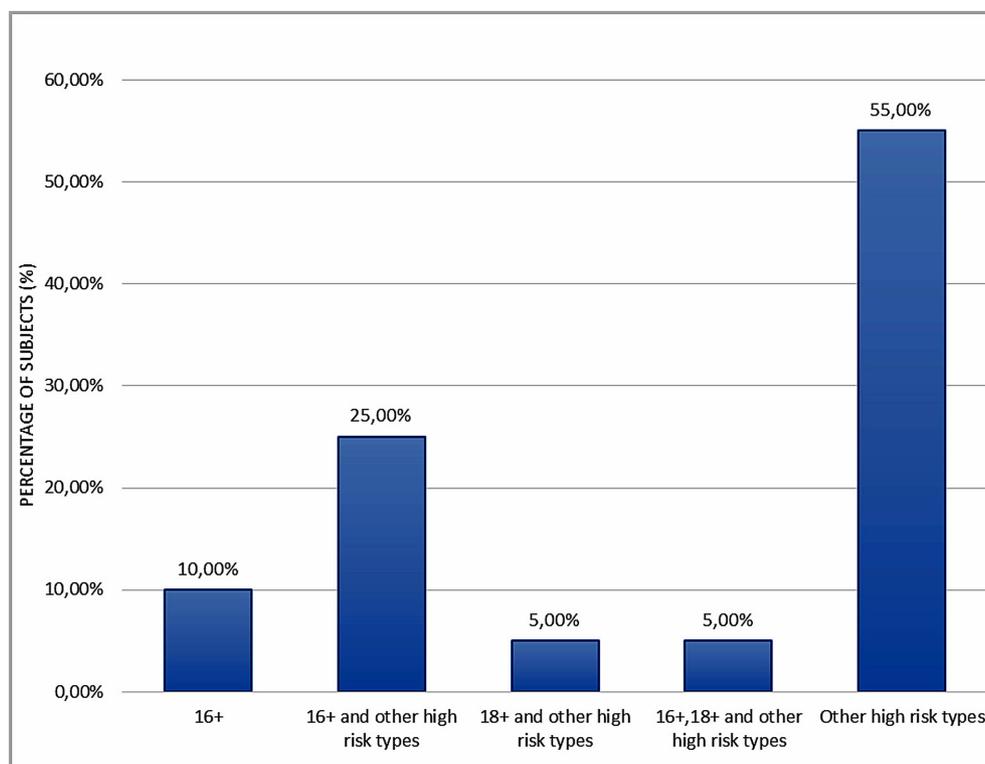
### RESULTS:

The overall prevalence of HR HPV infection among Bosnian women with abnormal cytology report 30 years old and younger using COBAS HPV DNA typing assay was 66.7% (table 1). Distribution of prevalence of different high-risk HPV types is shown in table 1. The other high-risk HPV types were the most prevalent detected in 36.7% women with abnormal cytology, then HPV 16 and other high-risk types detected in 16.7% participants (graph. 1).

**Table 1.** Prevalence of HPV types among Bosnian women with abnormal cytology under 30 years (n=30)  
*HR HPV- high-risk human papillomavirus*

HPV types	Number	Percent (%)
16+	2	6.7%
16+ and other high-risk types	5	16.7%
18+ and other high-risk types	1	3.3%
16+, 18+ and others high risk types	1	3.3%
Others high-risk HPV types	11	36.7%
Overall (all HR HPV types)	20	66.7%
Negative	10	33.3%

**Graph. 1.** Distribution of the proportion of different high-risk HPV types of infection among HPV positive women



The median age of 30 studied women was 26 IQR (20.75, 29.0) year. According to questionnaire answers about half of them 16 (53.3%) finished gymnasium as high school. Seventeen participants (17/65.7%) had their first menstruation between the ages of 12 and 14. Twenty-seven (27/90.0%) never gave birth and also had abortion of child. More than half of women (16/53.3%) have had the first sexual intercourse in the ages over 18 years. Regarding safe sex practices, more than half of them, 16 (53.3%) used condom in the first coitus. Fourteen (46.7%) had one or two

lifetime male partners, and 10 (33.3%) three or more lifetime male sex partners. Two thirds participants, 23 (76.7%) are in relationship with permanent partner and 9 (30.0%) of them have been used condom rarely (< 30%) in the last 12 months. Seven young women (23.3%) have had sexual intercourse with the casual partner and 4 (57.1%) of them used a condom in the last coitus.

Fourteen participants (46.7%) used alcohol sometimes, and 17 (56.7%) were currently smokers (table 2).

**Table 2.** Demographic and sexual behavioural risk factors for genital infection of HPV among women with abnormal cytology (n=30)

		Positive			Negative			OR
		N	R%	K%	N	R%	K%	
Age	≤ 52	9	75,00%	45,00%	3	25,00%	30,00%	1,91
	>25	11	61,10%	55,00%	7	38,90%	70,00%	
High school	Medicine	5	100,00%	25,00%				3,33
	Others	15	60,00%	75,00%	10	40,00%	100,00%	
First menstruation	≤ 14	17	73,90%	85,00%	6	26,10%	60,00%	3,78
	>14	3	42,90%	15,00%	4	57,10%	40,00%	
The number of births	0	18	66,70%	90,00%	9	33,30%	90,00%	1
	1	2	66,70%	10,00%	1	33,30%	10,00%	
Number of abortions	≥ 1	3	100,00%	15,00%				1,76
	0	17	63,00%	85,00%	10	37,00%	100,00%	
First intercourse	<18 years	5	100,00%	25,00%				3,33
	≥ 18 years	15	60,00%	75,00%	10	40,00%	100,00%	
Use of protection during intercourse	No	12	85,70%	60,00%	2	14,30%	20,00%	6
	Yes	8	50,00%	40,00%	8	50,00%	80,00%	
Number of partners	3+ partners	10	62,50%	50%	6	37,50%	60,00%	0,88
	1-2 partners	10	71,40%	50,00%	4	28,60%	40,00%	
long-lasting relationship	No	6	85,70%	30,00%	1	14,30%	10,00%	3,86
	Yes	14	60,90%	70,00%	9	39,10%	90,00%	
Use of contraception	No	6	50,00%	30,00%	6	50,00%	60,00%	0,29
	Yes	14	77,80%	70,00%	4	22,20%	40,00%	
Usage of condoms during last intercourse	No	18	66,70%	90,00%	9	33,30%	90,00%	1
	Yes	2	66,70%	10,00%	1	33,30%	10,00%	
Unknown partner	Yes	6	85,70%	30,00%	1	14,30%	10,00%	3,86
	No	14	60,90%	70,00%	9	39,10%	90,00%	
Usage of condom with unknown partner	No	5	71,40%	25,00%	2	28,60%	20,00%	1,33
	Yes	15	65,20%	75,00%	8	34,80%	80,00%	
Age of last partner	Same age	6	75,00%	30,00%	2	25,00%	20,00%	1,71
	Older	14	63,60%	70,00%	8	36,40%	80,00%	
Alcohol	Yes	12	85,70%	60,00%	2	14,30%	20,00%	6
	No	8	50,00%	40,00%	8	50,00%	80,00%	
Cigarettes	Yes	11	84,60%	55,00%	2	15,40%	20,00%	4,89
	No	9	52,90%	45,00%	8	47,10%	80,00%	

<b>Drugs</b>	<i>Yes</i>	<b>2</b>	100,00%	10,50%				<b>0,94</b>
	<i>No</i>	<b>17</b>	68,00%	89,50%	<b>8</b>	32,00%	100,00%	
<b>Sexual intercourse with partner with STD</b>	<i>Yes</i>	<b>3</b>	75,00%	15,80%	<b>1</b>	25,00%	10,00%	<b>1,69</b>
	<i>No</i>	<b>16</b>	64,00%	84,20%	<b>9</b>	36,00%	90,00%	
<b>Sexual transmitted disease</b>	<i>Yes</i>	<b>9</b>	69,20%	52,90%	<b>4</b>	30,80%	40,00%	<b>1,69</b>
	<i>No</i>	<b>8</b>	57,10%	47,10%	<b>6</b>	42,90%	60,00%	
<b>Symptoms</b>	<i>Yes</i>	<b>10</b>	83,30%	66,70%	<b>2</b>	16,70%	25,00%	<b>6</b>
	<i>No</i>	<b>5</b>	45,50%	33,30%	<b>6</b>	54,50%	75,00%	

As is shown graph. 1, among HPV-positive women, 55.0% had infection with others high-risk oncogenic HPV types, and 25% had infection with HPV 16+ and other high-risk types 25%.

Demographic and behavioural characteristics of women with abnormal cytology reports were examined for their associations with HPV positivity (table 2)

Patient age of  $\leq 25$  years had positive association with positive results of HPV testing, OR=1.91. Also women who went to Medical school also had positive association with positive result of HPV. Patients who had their first period under or in 14th year of life had OR=3.78 to have positive HPV test. Number of births did not had any association with HPV results. Women who had abortions had positively of with positive HPV. Early first intercourse had significant positive association with HPV finding, OR=3.33. Not using protection during intercourse gave 6 times higher chances of HPV infection. Female patients who did not

have long lasting relationship had odds ratio of 3.86 to have HPV infection than those in long lasting relationship. Use of protection during sexual intercourse had protective role, OR=0.29. Having sexual intercourse with "stranger" gave OR=3.86 to get HPV infection, while sexual intercourse with stranger and condom usage gave OR=1.33. Consummation of alcohol, cigarettes had strong positive association with positive HPV testing. Sexual intercourse with partner who had STD gave OR=1.69 changes to get HPV infection or some other STD. Having symptoms of genital infection is 6 times more frequent in patients with HPV.

Cervical intraepithelial neoplasia grade 1 (CIN1) was detected in 13 patients (table 3.). Out of that, 7,69% had HPV 16, the same percentage was without detected HPV infection. Other HR had 53.85% of CIN 1 patients. Combination of HPV 16 and other HR had 15.38% of patient with CIN 1.

**Table 3.** HPV prevalence by cytological abnormalities in Bosnian women

Variables	Description	16+		Neg.		O+		O+, 16+		O+, 16+, 18+		O+, 18+	
		N	%	N	%	N	%	N	%	N	%	N	%
<b>Atypical squamous cells (ASC)</b>	Positive	0	0	3	37,5	4	50	1	12,5	0	0	0	0
<b>Undetermined significance (ASC-US)</b>	Positive	0	0	3	42,9	4	57,1	0	0	0	0	0	0
<b>Squamous intraepithelial lesion (SIL)</b>	Positive	1	7,1	2	14,3	8	57,1	1	7,1	1	7,1	1	7,1
<b>CIN 1/Dysplasia levis</b>	Positive	1	7,69	1	7,69	7	53,85	2	15,38	1	7,69	1	7,69
<b>CIN 2/Dysplasia media</b>	Positive	0	0	0	0	0	0	1	50	1	50	0	0
<b><i>Bacillus vaginalis</i></b>	Positive	0	0	7	43,8	8	50	0	0	1	6,3	0	0
<b>Mixed flora</b>	Positive	1	11,1	3	33,3	4	44,4	1	11,1	0	0	0	0
<b>Fungi</b>	Positive	1	33,3	1	33,3	0	0	0	0	1	33,3	0	0
<b><i>Gardnerella vaginalis</i></b>	Positive	1	33,3	0	0	1	33,3	1	33,3	0	0	0	0
<b>HPV-related changes</b>	Positive	0	0	4	30,8	6	46,2	3	23,1	0	0	0	0
<b>Inflammation</b>	Positive	1	7,1	4	28,6	6	42,9	2	14,3	1	7,1	0	0
<b>Parakeratosis</b>	Positive	0	0	1	20	3	60	0	0	1	20	0	0
<b>Dyskeratoses</b>	Positive	0	0	0	0	2	66,7	0	0	1	33,3	0	0
<b>Hyperkeratosis</b>	Positive	1	12,5	2	25	3	37,5	1	12,5	0	0	1	12,5

Atypical squamous cells were found in 8 patients, out of 50% had other HR HPV types, while negative HPV were found in 37.5% of females. Inflammation was presented in 14 patients, out of 42.9% had other HR types, while 7.1% had HPV 16+, and in 28.6% of them, results for HPVs were negative.

#### DISCUSSION:

The main goal of this study was to achieve insights into the sociodemographic profile and sexual behaviour of tested young Bosnian women with abnormal cytological report, as well as to demonstrate the frequency of HPV DNA genotyping. The peak incidence of HPV infections occurs in younger adults than 25 years old. These group of viruses facing oncogenic potential, where HPV-16 and HPV-18 are found more frequently in cervical carcinomas. Epidemiological studies emphasize that mentioned HR-HPVs are responsible for more than 70% of all cervical cancers, with type 16 being most common. Widespread use of the Papanicolaou smear has led to an essential decrease in deaths regarding cervical carcinomas. Testing algorithms include reflex testing of abnormal Pap smears, co-testing, or primary HPV screening. Studies have indicated that primary HPV screening might be the preferred method in most settings environment [8].

According to our results, the overall prevalence of HR HPV infection among Bosnian women with abnormal cytology report 30 years old and younger using COBAS HPV typing assay was 66.7%. The most of HR HPV positive could be correlated to age and cervical diagnosis of the tested women. Out of that, HPV-16 was detected in 2 (6.7%) of tested women; following with 16.7% in category of HPV-16 and others HR-HPV, and 3.3% of HPV 18 and others HR-HPV. Other HR-HPV types were detected in 11 (36.7%) cases. The most frequent type in this age category was HPV-16, found in 8 (26.7%) of samples, which was to be expected.

Similar findings were reported by other recent studies; 18 studies reported from many European countries; where is confirmed that HPV-16 is the most prevalent HPV type, found in 29.8% of HPV positive samples (range 19-43%) [10]. In Europe, HPV prevalence in invasive cervical cancer is 85.9%, in exfoliated cells of women with low-grade lesions in Pap smears is 67.8%, while in cervical samples of women with normal cytology the HPV prevalence is 8.1% [11-13]. Considering the prevalence of HPV in young women with different cytological report findings in this study, defined age group (less than 30 years) showed important influence on HPV positivity and the detection of cytological abnormalities. According to Akarolo-Anthony et al., the age group most affected by HPV infections were those aged less than 30 years [14].

There is limited data regarding the prevalence and

distribution of HPV types in Bosnia and Herzegovina. There were a few studies for BiH, about HPV prevalence related to cervical samples of women with normal and abnormal Pap smear. Mahmutovic et al. confirmed HPV prevalence for HR-HPV from 22.7%-54.5%, with the highest frequency in the group with abnormal Pap smear. Besides, the most prevalent HPV genotypes was HPV-16 [15]. Salimovic Basic et al. in the study from 2013. confirmed that the most prevalent HPV genotypes in BiH were HPV-16, 18, 31, 33, and 45, and persistent infections increased with severity of cervical cytology [16]. Similar to our results was a study conducted in France that showed HPV 16 to be the most prevalent, followed by HPV 53 and 31 [17].

Considering the high prevalence of HPV in tested Bosnian women with different cytologic findings aged 30 years old and less significant impact on HPV positivity and detection of cytological abnormalities has been observed. According to our findings, many studies confirmed that this age category of women is more likely to be carriers of significant cervical lesions [14, 18]. Our observed data shown that CIN 1 was detected in 13 of them, out of 7 had HPV 16, while others was without detected HPV infection. Among those diagnosed with inflammation, 10 were HPV positive. Marks et al. found a much higher prevalence (43.8%) of HPV infection among women diagnosed with inflammation, which is in concordance to our results [19].

In the second part of the study, the sociodemographic and behavioural characteristics of the tested women were analyzed, as well as issues related to sexually transmitted diseases through epidemiological self administered questionnaires (demographic data, sexual knowledge, attitudes and behaviours).

From the questionnaire, it is estimated that the fourteen (46.7%) women had one or two-lifetime male partners, and 10 (33.3%) three or four-lifetime male sex partners. Also, it is important to note that 9 (30.0%) of them have been used condom rarely (< 30%) in the last 12 months regarding other STDs. In relation to sociodemographic factors and HPV infection strong association has been found regarding consumption of alcohol and cigarettes, STDs and symptoms but not in concordance to other results [20] which can be explained by the small size of the sample.

#### CONCLUSIONS:

The data obtained from our study indicate that HPV 16 is the most common HPV type found in the cervical samples among tested women under the age of 30 with abnormal Pap smear in Bosnia and Herzegovina, as in most studies worldwide, followed by other HR-HPV. However, despite the small sample size, this data on the prevalence and distribution of HPV types in women with abnormal cytology report may contribute to increasing knowledge of the HPV epidemiology in Bosnia and Herzegovina.

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*Please cite this article as:* Mahmutovic-Vranic S, Radaslic VD, Aljicevic M, Abduzaimovic A, Joguncic A, Vraneš J. Sexual Behaviours and Human Papillomavirus Genotyping among Bosnian Women. *J of IMAB.* 2020 Jan-Mar;26(1):2898-2904. DOI: <https://doi.org/10.5272/jimab.2020261.2898>

Received: 03/06/2019; Published online: 11/02/2020



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