HUMAN PAPILLOMAVIRUS INFECTIONS IN A ROMANIAN AMBULATORY GYNECOLOGICAL WARD

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ABSTRACT

Background: Human papillomaviruses (HPV) are a large family of small double-stranded DNA viruses that infect squamous epithelia. Human Papillomavirus (HPV) high-risk (HR) types are the causal factor for cervical cancer and premalignant dysplasia. Taking into account the heterogeneity of HPV types across and within populations this study aims to assess the HPV frequency in Romanian women.

Results: We identified 21 different types of HR HPV, from 117 female patients of gynecological clinic at the Medical Centre in Bucharest, aged 16-59, included into a retrospective 22 month study, positive for the virus analyzed by LINEAR ARRAY HPV® assay. These women were infected by a single or multiple HPV types. HR types were found in 85,5% of women and the most frequent genotypes in order of decreasing frequency were16; 53; 18; 52; 31; 66, 73; 45, 58, 84; 33, 51; 35, 68, 82; 39, 59, 62; 56, 67; IS39. Differences in frequency of HR HPV types were found for presence of cervical lesions: HR- types 31, 52, 53, followed by HR-types16, 58, 68, 35, 51, 66, 73 in ASCUS; HR-types 53, 16, 33, 51, 52, 66, 18, 31, 58 in L-SIL and HR-types 16,18 in H-SIL, ASCH. Differences in frequency were found also for different HPV species and women age.

Conclusions: Compared with studies from other countries, our data indicated differences in frequency of HR-HPV type infection, an association of HR-types HPV 16, 52, 53, 31, 51, 58, 18, 33, 66 and cervical lesions, and a trend for distinct distribution of HPV types by age.

Key words: HPV-HR types, HPV frequency, cervical lesions

INTRODUCTION

HPV is the most frequent sexually transmitted disease in the world. HPV is involved in causing specific cutaneous lesions and preneoplastic and neoplastic lesions in female and male genital ducts. It is thus involved in producing various types of anogenital cancer: vulval, vaginal, cervical, anal, penile, as well as head and neck cancer. (1) Approximately 4% of all cancers are associated with HPV. (2)

The method of transmission is mainly sexually, vertically (mother-fetus), during labor and through contaminated, shared objects. The majority of infections are transitory, up to 70% receding in the first year and up to 90% in 2 years; 10-20% of the infections persist, allowing the evolution of preneoplastic lesions to cancer. (3)

According to the data provided by IARC (International Agency for Research in Cancer) GLOBOCAN 2008, Romania occupies the first place in Europe regarding the incidence of cervical cancer. Every year 3,402 new cases and 2,005 deaths are being registered because of this, cervical cancer being the third most frequent female genital neoplasm and the most frequent in women aged between 15-44 years.(4)

Romania is on the first place in Europe when it comes to mortality caused by cervical cancer which is 6.3-fold greater than the mean of European Union countries, according to the National Information Centre for the Prevention of Cervical Cancer (CNIPCCU, 2009).

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Cervical cancer is the second most frequent cancer in women worldwide, with an estimated number of approximately 530,000 new cases in 2008, around 86% of the cases being identified in developing countries; it is the second most common cause of mortality, accounting for approximately 275.000 of deaths in 2008. (4)

The worldwide prevalence of cervical HPV infection is approximately 10% (5); several studies in various countries show diverse estimated prevalence's of HPV infection: in USA ranging between 14% and above 90% (6,7,8); in South America – Venezuela, reaching approximately 90.1%. (9) The reason why such differences exist is related to the fact that certain studies report patients who are currently having an infection, while other studies report patients who have had a detectable HPV infection at some point in their life. (10,11) Another cause of this discrepancy is the diversity of requests for which testing was made. (12)

The prevalence of high-risk and low-risk cancer causing serotypes is approximately the same over time. (13) The prevalence of the infection decreases with age.

There are no data pertaining to the prevalence of HPV in the Romanian general population. Anyhow, for Eastern Europe – the region we are part of – it is estimated that 22.3% of the general female population have an HPV infection at some point in time. (4) High-risk cancer-causing serotypes are detected in 99% of cervical cancers. (14,15)

Within cancer-causing HPV serotypes, serotype 16 causes 50% of cervical cancers, and serotype 18 is involved in 10-20% of the cases. (16) Together, serotypes 16 and 18 cause over 65%-70% (17,18, 19,20) of cervical cancers. Serotype 16 is also found in most HPV-related vulvovaginal (21), penile, anal and head and neck cancers. (22) Serotype 18 has a role that is more important in the genesis of endocervical adenocarcinomas than in scuamocellular ones. (23,24)

As for the worldwide frequencies of HPV serotypes, data differ depending on the author and on data:

- globally, the incidences of most widely spread serotypes are the following: HPV 16- 50%, HPV 18- 14%, HPV 45- 8% (25) and HPV 31- 5%. HPV 16 is the most prevalent serotype. The prevalence of other types registers geographical variations, HPV 18 being most prevalent in South-Eastern Asia, and HPV 45 in Western Africa. In Central and Southern America the rarest types 39 and 59 can be found: (26);
- in Venezuela, serotypes 16 and 18 are most common, followed by serotypes 52, 33, 45 and 31 (9);
- in Brasil, the greatest frequency is that of serotypes 16, 18, 45, 58 and 66. (27)

According to GLOBOCAN 2008 data, following serotypes 16 and 18, the most common serotypes are the same throughout all regions of the world, and that is 31, 33, 35, 45, 52 and 58. (4)

The first vaccine which prevents infection with 4 HPV serotypes has been approved in 2006. (28)

PATHOGENESIS

HPV are viruses with a double-strand DNA which infect the stratified epithelium of the skin and the mucous membranes. About 200 (29,30) HPV serotypes were identified, differentiated by the genetic sequence of L1 external capside protein. Out of these, 15 are classified as high-risk cancer causing (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 73 and 82), 3 with probable risk cancer-causing (26, 53 and 66), and 12 with low-risk cancer causing (6,11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108). (31) From a phylogenetic point of view, high-risk cancer causing serotypes are divided in 2 groups, alpha -9, which include HPV 16 and related serotypes 31, 33, 35, 52 and 58, and alpha-7, which include HPV 18 and related serotypes 39, 45, 59 and 68. (32,33)

HPV infection occurs in the basal epithelial cells; HPV infection involves the presence of microabrassions in the genital epithelium. (2) During sexual intercourse the virus is stored in the basal membrane of the cervical epithelium. It is then taken over by the basal cells and once the squamos epithelium is mature, viral amplification develops. During this process, the virus remains hidden from the immune system of the host without any immune reaction. Almost half of the women infected with HPV do not develop detectable serum antibodies and they have a risk of reinfection with the same HPV serotype.(1)

Most of infections recede spontaneously, but in 10-20% of women infection persists; persistent infection is the most important risk factor in developing progenitor lesions of cervical cancer. (2)

The most clinically significant manifestation of HPV infection in the cervix is cervical intraepithelial neoplasia or CIN. CIN are genetically instable lesions, presenting a risk for progression to invasive cervical cancer equal to 30-40%. If left undetected and untreated, CIN II or CIN III can progress to cervical cancer in a few years or decades later

on. The mean duration of natural evolution to invasive neoplasm is approximately 13 years. (34)

The oncogenic properties of high-risk cancer causing serotypes lie in the presence of E6 and E7 genes and their inadequate expression in the dividing cells deregulates cellular division and differentiation. (2)

Infection with an HPV serotype does not prevent infection with another serotype. Of all sexually active women infected with mucous HPV, over 50% are infected with one or more virus serotypes. (35)

Future HPV testing can be used together with cytology or even as a primary method of screening to detect early preneoplastic cervical lesions, at least in the developed countries, proving to be superior to cytology when it comes to sensitivity and positive predictive value. (2)

OBJECTIVE

The objective of this retrospective study is to show the prevalence of cervical genital infections with high-risk cancer causing HPV serotypes in the gynecology ward of "VICTOR BABES" DTC over a period of 22 months (May 2009 – February 2011).

MATERIAL AND METHOD

Throughout the testing period, 251 patients were sampled for cervical secretion samples, which presented for tests upon request or with a recommendation for cytological modifications. The patients examined were aged between 16 and 59 years.

Patients live in an urban area and have a high socioeconomic status (HPV genetic typing is a costly test which is not included in the investigations reimbursed by the National Health Insurance House.)

All patients are sexually active with an intact uterus. All samples were examined with LINEAR ARRAY HPV genetic typing test, amplifying the target DNA by PCR technique and hybridizing the nucleic acids to obtain the individual qualitative detection (genetic typing) of 37 anogenital HPV types in the cervical cells sampled in liquid environment: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73(MM9), 81, 82(MM4), 83(MM7), 84(MM8), IS39 and CP6108.

Method – polymerization chain reaction (PCR) with colorimetric detection; the genetic typing is performed with a strip that contains specific pads in a linear position.

Reference values – undetectable HPV DNA; the detection limit of the working method is 120, 815, 1920 and 251 copies/mL for genotypes 16, 18, 31 and 45.

A positive result includes communication of the HPV genotypes present in the sample.

The cytologic tests were performed in two ways: conventional and, since 2010, in liquid environment.

The classification of cytologic smears has been performed in conformity with the recommendations of the Ministry of Health, also taking into account Bethesda 2001 recommendations. (36,37)

The statistical processing of data for the prevalence of infection with various HPV serotypes was performed with Fisher test for p and OR, using Wolf approximation for 95% CI.

Kruskal-Wallis (Nonparametric ANOVA) nonparametric test was used for the distribution by age groups of high-risk cancer causing HPV serotypes.

RESULTS

Of all 251 tested patients, 135 were found to be HPV negative and 117 HPV positive.

Of all 117 patients detected with HPV infection, 17 (14,5%) were infected with serotypes without an oncongenic risk and 100 (85,5%) had serotypes with an oncogenic risk.

We identified 31 HPV serotypes out of which 18 were high-risk cancer causing serotypes (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 62, 67, 68, 73, 82, IS 39), 3 serotypes classified as having a potential oncogenic risk (53, 66 si 84) and 10 serotypes (6, 11, 42, 54, 55, 61, 72, 81, 83 and CP6108) without an oncogenic risk.

The patients were infected with one or several HPV serotypes.

The global frequency of high-risk cancer causing HPV serotypes, either alone or in combination, was 85,5% (100/117, Fisher test, p<0,0001-es; OR=1556,3, CI:92,428-26205), and that of serotypes with a low oncogenic risk was 14,5% (17/117, Fisher test, p<0,0001-es; OR=47,189, CI:2,803-794,57); their distribution is presented on descending basis in the following table:

Table 1

HPV/HR	p-Fisher test	OR;95%CI – using Wolf approximation
16-19,6% (23/117)	<0,0001-es	67,392;4,041-1124
53-14,5% (17/117)	<0,0001-es	47,189;2,803-794,57
18-12,8% (15/117)	<0,0001-es	40,980;2,422-693,41
52-8,5% (10/117)	0,0004-es	26,470;1,533-457,15

HPV/HR	p-Fisher test	OR;95%CI – using Wolf approximation
31-7,6% (9/117)	0,0008-es	23,728;1,365-412,55
66,73-6,8% (8/117)	0,0019-vs	21,037;1,200-368,79
45,58,84-5,9% (7 /117)	0,0042-vs	18,394;1,038-325,85
33,51-5,1% (6/117)	0,0093-vs	15,798;0,8797-283,71
35,68,82-4,2% (5/117)	0,0206-s	13,249;0,7242-242,37
39,59,62-3,4% (4/117)	0,0452-s	10,744;0,5720-201,84
56,67-2,5% (3 /117)	0,0987-nqs	8,284;0,4231-162,17
HPV/LR		
6-8,5% (10/117)	0,0004-es	26,470;1,533-457,15
61-5,9% (7/117)	0,0042-vs	18,394;1,038-325,85
42-5,1% (6/117)	0,0093-vs	15,798;0,8797-283,71
81,CP6108-4,2% (5/117)	0,0206-s	13,249;0,7242-242,37
11-2,5% (3/117)	0,0987-nqs	8,284;0,4231-162,17

HR – serotypes with a high oncogenic risk; LR – serotypes with a low oncogenic risk; es – extremely significant; vs – very significant; s – significant; nqs – not quite significant

In our study, the prevalence of high-risk cancer causing HPV serotypes is the following in descending order: 16; 53; 18; 52; 31; 66, 73; 45, 58, 84; 33, 51; 35, 68, 82; 39, 59, 62; 56, 67; for the serotypes with a low oncogenic risk the distribution in descending order is the following: 6, 61, 42, 81, CP 6108, 11.

We did not compute the prevalence of HPV serotypes that was not statistically significant (of all high-risk cancer causing HPV serotypes we found IS39 strain in one case and of all serotypes with a low oncongenic risk we found HPV 54 and 55 in 2 cases, HPV 72 and 83, each for one case).

In 41,8% of cases (49/117; Fisher test, p<0,0001-es, OR=195,83, CI:11,890-3225,5) multiple associations have been found as follows:

- 11,1% – association with low risk HPV and increased risk HPV (13/117; Fisher test, p< 0,0001-es, OR=35,CI:2,056-596,16);
- 22,2% – association of 2 HPV serotypes (26/117; Fisher test, p<0,0001- es, OR=78,486, CI:4,720-1305);
- 10,2% – association of 3 HPV serotypes (12/117; Fisher test, p<0,0001- es, OR=32,109, CI:1,878-548,93);
- 5,9% – association of 4 HPV serotypes (7/117; Fisher test, p=0,0042 – vs, OR=18,394, CI:1,038-325,85).

In 2 cases (1,7%) association of 5 HPV serotypes and in one case (0,85%) association of 6 serotypes have been discovered, both statistically insignificant.

In 2 cases (1,7%) 2 low-risk HPV serotypes were associated (statistically insignificant).

As for the associations, the most frequent high-risk cancer causing serotypes found in associations are:

- HPV 16,18 and 53, each in 10 cases (10/49; Fisher test, p<0,0001- es, OR=72,038, CI: 4,126-1257,6);
- HPV 52 and 66, each in 8 cases (8/49, Fisher test, p<0,0001- es, OR=55,506, CI:3,135-982,88);
- HPV 73 in 7 cases (7/49, Fisher test, p<0,0001- es, OR=47,824, CI:2,674-855,43);
- HPV 31, 33, 51 and 58, each in 6 cases (6/49, Fisher test, p=0,0003- es, OR=40,494, CI: 2,234-734,01);
- HPV 45 and 82 in 5 cases (5/49, Fisher test, p=0,0011- vs, OR=33,494, CI:1,815-618,25);
- HPV 62, 68 and 84, each in 4 cases (4/49, Fisher test, p=0,0046 – vs, OR=26,802, CI: 1,415-507,85);
- HPV 56 in 3 cases (3/49, Fisher test, p=0,0180 – s, OR=20,398, CI:1,033-402,63).

According to the phylogenetic classification, we noticed the increased prevalence in associations of serotypes included in alpha-9 group (16, 31, 33, 35, 52 and 58) and serotypes not included in alpha-9 or alpha-7. (53, 66, 73, 84)

In infections with serotypes with a low oncogenic risk, the most commonly seen in associations are:

- HPV 6 in 6 cases (6/49, Fisher test, p=0,0003 – es, OR=40,494, CI:2,234-734,01);
- CP6108 in 5 cases (5/49, Fisher test, p=0,0011 – vs, OR=33,494, CI:1,815-618,25);
- HPV 81 in 4 cases (4/49, Fisher test, p=0,0046 – vs, OR=26,802, CI:1,415-507,85);
- HPV 11, 42 and 61, each in 2 cases (2/49, Fisher test, p=0,0699 – nqs, OR=14,263, CI: 0,6721-302,70);
- HPV 54 in one case – statistically unsignificant.

The mean age in the 117 patients infected with HPV serotypes was 30.21 years, ranging between 16 and 58 years.

The distribution of various HPV serotypes by age groups is shown in the Table 2.

The highest overall prevalence of HPV serotypes infection (with high and low oncogenic risk) was 52.1% (61/117, Fisher test, p<0,0001- es, OR=294,98, CI:17,920-4855,7) in the group age

Table 2

1HPV	HPV	<20 years	21-30 years	31-40 years	41-50 years	51-60 years	No. of pat.
	16	0	8	4	1	0	13
	18	1	2	2	0	0	5
	31	0	0	2	0	1	3
	39	0	1	1	0	0	2
	45	0	1	1	0	0	2
	52	0	1	1	0	0	2
	53	0	3	3	1	0	7
	55	0	0	1	0	0	1
	56	0	1	0	0	0	1
	58	0	0	1	0	0	1
	59	0	1	1	1	0	3
	62	0	0	1	0	0	1
	66	0	4	0	0	0	4
	67	0	0	0	1	0	1
	68	0	0	1	0	0	1
	73	0	1	0	0	0	1
	81	0	0	1	0	0	1
	83	0	0	1	0	0	1
	84	0	2	1	0	0	3
	6	0	3	1	0	0	4
	11; CP6108	0	2	0	0	0	2
	42	0	1	1	1	1	4
	54	0	0	1	0	0	1
	61	0	1	3	0	0	4
2 HPV	51, **CP6108**	0	1	0	0	0	1
	16,33	0	0	1	0	0	1
	16,39	0	1	0	0	0	1
	16,45	0	1	0	0	0	1
	16,51	0	0	1	0	0	1
	16,73	0	1	0	0	0	1
	16,82	0	2	0	0	0	2
	16,84	0	1	0	0	0	1
	18,45	0	1	0	0	0	1
	18,51	0	0	1	0	0	1
	18,62	0	1	0	0	0	1
	18,73	1	0	0	0	0	1
	31,45	0	1	0	0	0	1
	31,53	0	0	1	0	0	1
	31,59	0	1	0	0	0	1
	35,53	0	1	0	0	0	1
	39,68	0	0	1	0	0	1
	45,84	0	0	1	0	0	1
	53,68	0	0	0	1	0	1
	53,**81**	0	2	0	0	0	2
	6,18	0	1	0	0	0	1
	6,62	0	1	0	0	0	1
	67,73	0	0	1	0	0	1
	16,18	0	1	0	0	0	1
	6, CP6108	0	0	1	0	0	1
	54,61	0	0	1	0	0	1
3 HPV	**11**,68, **CP6108**	1	0	0	0	0	1
	11,73, IS39	1	0	0	0	0	1
	18,**55**,**81**	0	0	0	1	0	1
	31,**42**,51	0	1	0	0	0	1
	31,66,67	0	0	1	0	0	1

1HPV	HPV	<20 years	21-30 years	31-40 years	41-50 years	51-60 years	No. of pat.
	51,53,66	0	1	0	0	0	1
	52,53,66	0	0	0	1	0	1
	52,56,58	0	1	0	0	0	1
	52,73,**81**	0	1	0	0	0	1
	52,**61**,73	0	1	0	0	0	1
	6,18,73	0	1	0	0	0	1
	6,45,62	0	1	0	0	0	1
4 HPV	18,31,33,53	0	0	1	0	0	1
	18,53,72,84	0	1	0	0	0	1
	33,35,52,58	0	1	1	1	0	3
	52,58,68, **CP6108**	0	1	0	0	0	1
5 HPV	16,**42**,51,82,84	0	1	0	0	0	1
	6,56,66,82, **CP6108**	0	1	0	0	0	1
6 HPV	33,35,52,58,**61**,82	0	0	0	1	0	1
Bold-serotypes LR		4	61	40	10	2	117

ranging from 21 to 30 years, followed by 34.1% (40/117, Fisher test, p<0,0001- es, OR=141,62, CI:8,582-2337) in the age group ranging from 31 to 40 years. We found a prevalence of 8.5% for the age group ranging from 41 to 50 years (10/117, Fisher test, p=0,0004 – es, OR=26,470, CI:1,533-457,15); prevalence was 3.4% for ages under 20 years (4/117, Fisher test, p=0,0452 – s, OR=10,744, CI:0,5720-201,84) and 1.7% (2/117, Fisher test, p=0,2146 – ns) for ages over 50 years.

The comparison between infections with high oncogenic risks in patients included in the study showed an extremely significant difference in the distribution by age groups (Kruskal-Wallis test, Nonparametric ANOVA, p<0,0001- es, KW= 55,199)

Dunn's Multiple Comparisons Test

	Mean Rank	
Comparison	**Difference**	**P value**
<20 years vs. 21-30 years	-44.075 ***	P<0.001
<20 years vs. 31-40 years	-39.550 ***	P<0.001
<20 years vs. 41-50 years	-13.275 ns	P>0.05
<20 years vs. >50 years	4.900 ns	P>0.05
21-30 years vs. 31-40 years	4.525 ns	P>0.05
21-30 years vs. 41-50 years	30.800 **	P<0.01
21-30 years vs. >50 years	48.975 ***	P<0.001
31-40 years vs. 41-50 years	26.275 *	P<0.05
31-40 years vs. >50 years	44.450 ***	P<0.001
41-50 years vs. >50 years	18.175 ns	P>0.05

Separate comparison of alpha-7 (HPV18, 39, 45, 59, 68 and 70) and alpha-9 (HPV16, 31, 33, 35, 52, 58 and 67) infections with serotypes with high oncogenic

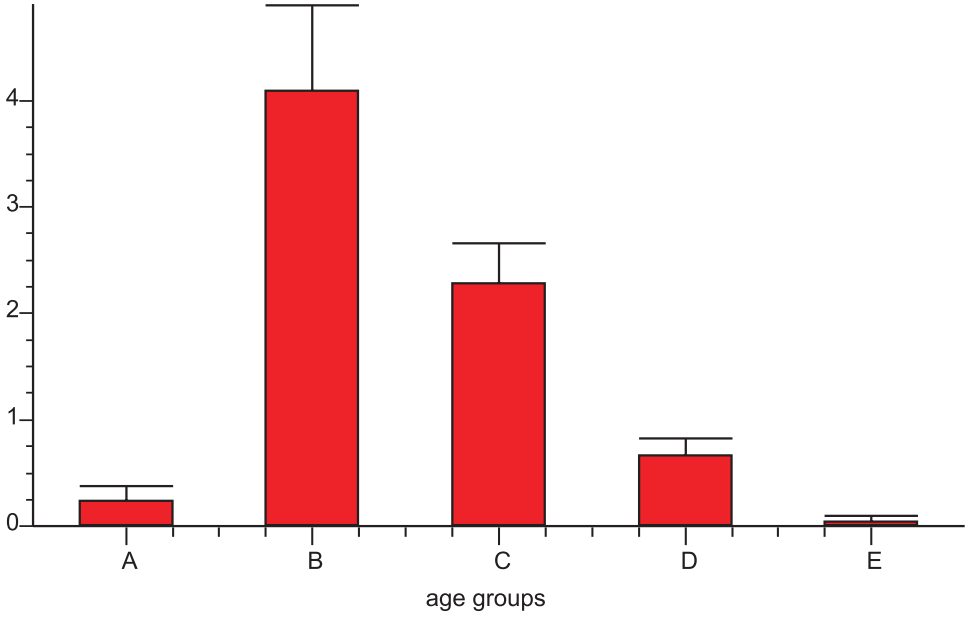

Figure 1. *Comparison between infections with high oncogenic risks by age groups*

risk and infections with other highly oncogenic types (53, 66, 73, 84) showed a significantly different distribution by age groups (Kruskal-Wallis test, Nonparametric ANOVA, p=0,0127 – s, KW=12,728).

Separate comparison of infections with serotypes with high oncogenic risk, with low oncogenic risk and associated ones does not show a significantly different distribution by age groups (Kruskal-Wallis test, Nonparametric ANOVA, p=0,0624 – nqs, KW=8,948).

As for serotypes with low oncogenic risk, we also found a significantly different distribution by age groups (Kruskal-Wallis test, Nonparametric ANOVA, p=0,0038 – vs, KW=15,498). (Figure 2)

Of all 117 cases with HPV infection, 29,9% (35/117) did not show cytological modifications, and 26,4% (31/117) showed cytological modifications as follows: 9,4% (11/117) – ASCUS cytological smears; 11,1% (13/117) – L-SIL cytological smears; 3,4% (4/117) – H-SIL cytological smears; 2,5% (3/117) – ASCH cytological smears.

No cytological data is available for 43,5% (51/117).

The frequency of serotypes with high oncogenic risk associated with modified cytology is the following: 25,8% (8/31) for HPV 16; 19,3% (6/31) for HPV 52 and 53; 12,9% (4/31) for HPV 31, 51 and 58; 9,6% (3/31) for HPV18, 33 and 66.

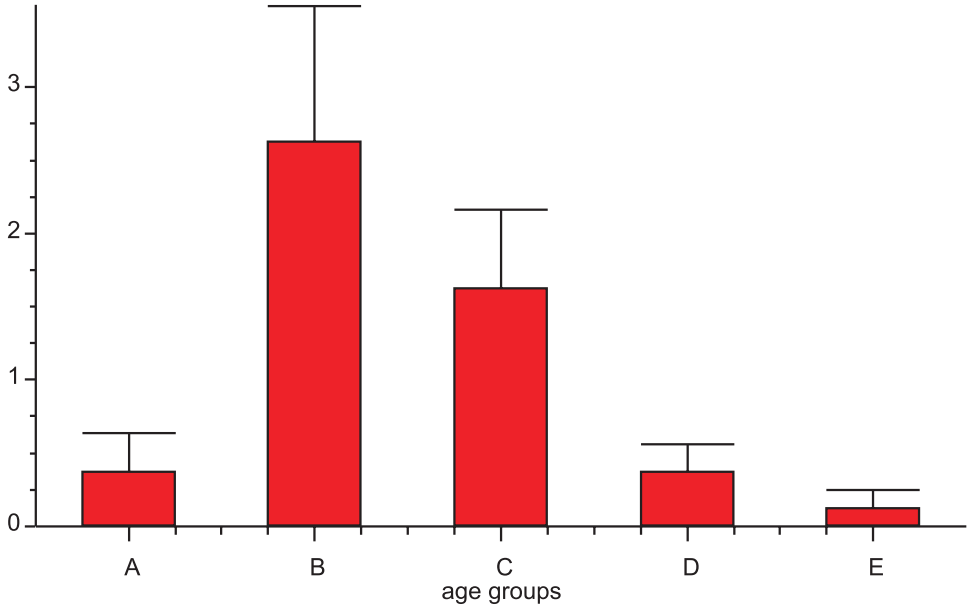

Figure 2. *Comparison between serotypes with low oncogenic risk by age groups*

Table 3

1 HPV	HPV	ASCUS	LSIL	HSIL	ASCH	NLIM	NR PAC
	6					1	1
	16	2	1		1	2	6
	18					1	1
	31	1	1			1	3
	39					1	1
	42		1			2	3
	45					1	1
	52		1			1	2
	53	1	2			3	6
	59		1				1
	61					1	1
	66	1	1				2
	67					1	1
	68					1	1
	81					1	1
	83		1				1
	84					2	2

1 HPV	HPV	ASCUS	LSIL	HSIL	ASCH	NLIM	NR PAC
2 HPV	16,18			1			1
	16,33		1				1
	16,51				1		1
	16,82					1	1
	16,84			1			1
	18,45			1			1
	18,51		1				1
	18,73					1	1
	31,45					1	1
	31,53	1					1
	39,68					1	1
	45,84					1	1
	51, **CP6108**					1	1
	53,68	1					1
	6,18					1	1
	6,62					1	1
	6, CP6108					1	1
	67,73			1			1
3 HPV	18,**55**,**81**					1	1
	31,**42**,51	1					1
	51,53,66		1				1
	52,56,58				1		1
	52,73,**81**	1					1
	53,**61**,73					1	1
	6,18,73					1	1
	6,45,62					1	1
4 HPV	33,35,52,58	1	1			1	3
	52,58,68, **CP108**	1					1
5 HPV	16,**42**,51,82,84					1	1
	6,56,66,82, **CP108**					1	1
Bold-serotypes LR		11	13	4	3	35	Total = 66

We notice the high frequency that HPV serotypes with high oncogenic risk of group alpha-9 and those not included in alpha-7 and alpha-9 have in the modified cytological smears.

As for the unmodified cytological smears we found the following frequency of HPV serotypes: 20% (7/35) for HPV 6; 14,2% (5/35) for HPV 18; 11,4% (4/35) for HPV 16, 45, 53 and 84; 8,5% (3/35) for HPV 42, 73, 82 and CP6108; 5,7% (2/35) for HPV 31, 51, 52, 61, 62, 68 and 81; 2,8% (1/35) for HPV 33, 35, 56, 66 and 67.

For each cytological modification we have the following frequency on a descending basis:

- in ASCUS cytological smears: HPV 31, 52 and 53 – 3/11; HPV 16, 58 and 68 – 2/11; HPV 35, 51, 66, 73, 81 and CP6108 – 1/11;
- in L-SIL cytological smears: HPV 53 – 3 /13; HPV 16, 33, 51, 52 and 66 – 2/13; HPV 18, 31, 58 and 83 – 1/13;
- in H-SIL cytological smears: HPV 16 and 18-2/4;
- in ASCH cytological smears: HPV 16 – 2/3.

The prevalence of infection associated with cytological modifications for serotypes with high oncogenic risk belonging to group alpha-7 (HPV 18, 39, 45, 59, 68 and 70) and alpha-9 (HPV 16, 31, 33, 35, 52, 58 and 67) is statistically significant (Fisher test, p=0,0382 – s, OR=0,2338,CI:0,05831-0,9371 for alpha-7, and p<0,0001 – es, OR=12,037, CI:3,735-38,787 for alpha-9). We did not find a statistically significant prevalence for serotypes not included in groups alpha-7 and alpha-9 (HPV 53, 66, 73, 84) (Fisher test, p=0,7886 – ns).

The comparison of the distribution of serotypes with high oncogenic risk associated to cytological modifications did not show a statistically significant difference between groups alpha-7, alpha-9 and other serotypes (Kruskal-Wallis test, Nonparametric ANOVA, p=0,0897 – nqs, KW=6,499).

DISCUSSIONS

We found the following prevalence of serotypes with high oncogenic risk in our study, throughout the analyzed period, in the female population that requested HPV serotypes testing, with or without an oncogenic risk: 19,6% (23/117) for serotype 16, the most common worldwide, 14,5% (17/117) for serotype 53 and 12,8% (15/117) for serotype 18, second most common worldwide; the prevalence was under 10% for the other 14 serotypes with high oncogenic risk, as showed below on a descending basis: 52; 31; 66, 73; 45, 58, 84; 33, 51; 35, 68, 82; 39, 59, 62; 56, 67; 83, IS39.

Serotypes 31 and 45, most common worldwide, following 16 and 18, are to be found in 7,6% (9/117), and 5,9% (7/117) of the cases.

High prevalence of 3 serotypes classified as having a possible oncogenic risk, 53 (14,5%-17 /117), 66 (6,8%-8 /117) and 84 (5,9%-7 /117) is to be noted.

Comparing our study with another study, also performed in Bucharest, which found the following strains involved in cervical infections with HPV serotypes with high oncogenic risk – shown on a descending basis: 16,18,31 şi 51(38), we notice that both studies include serotypes 16 and 18, both considered most common worldwide (serotype 16 is the most frequent).

We have the following distribution for the serotypes with low oncogenic risk, shown on a descending basis: 6, 61, 42, 81, CP6108,11, 54, 55, 72, 83. The low prevalence of serotype 11, second most common serotype worldwide, following serotype 16, is to be noted.

We also noted the high incidence of association between 2 or more serotypes with oncogenic risks out of 49 cases of associations, 26 of which were found with 2 serotypes, 12 with 3 serotypes and 7 with 4 serotypes.

According to the phylogenetic classification, we can note the increased prevalence in associations of serotypes included in group alpha-9 (16, 31, 33, 35, 52 and 58) and serotypes not included in group alpha-9 or alpha-7. (53,66,73,84)

The highest overall prevalence of HPV serotypes infection (with high and low oncogenic risk) was 52.1% in the age group ranging from 21 to 30 years, followed by 34.1 % in the age group ranging from 31 to 40 years. We found a prevalence of 8.5% for the age group ranging from 41 to 50 years; prevalence was 3.4% for ages under 20 years and 1.7% for ages over 50 years.

The comparison between the infections with the 21 serotypes with high oncogenic risk (also including the serotypes classified as having a potential oncogenic risk) identified in patients included in the study showed a significantly different distribution by age groups; separate comparison of infections with alpha-7 (HPV 18, 39, 45, 59, 68 şi 70) and alpha-9 (HPV16, 31, 33, 35, 52, 58 şi 67) high oncogenic serotypes and infections with other high oncogenic types (HPV 53, 66, 73, 84) showed a significantly different distribution by age groups.

The same significantly different distribution by age groups was also found in serotypes with low oncogenic risk.

Cytological data is available only for 66 patients out of the 117. According to the analysis of the existing data, we found that the prevalence of the infection associated with cytological modifications for serotypes with high oncogenic risk included in group alpha-7 (HPV 18, 39, 45, 59, 68 and 70) and alpha-9 (HPV 16, 31, 33, 35, 52, 58 and 67) is statistically significant.

We found no statistically significant prevalence for the infection associated with cytological modifications in serotypes not included in groups alpha-7 and alpha-9 (HPV 53, 66, 73, 84).

CONCLUSIONS

In our study, the prevalence of the infection with serotypes with high oncogenic risk is 85,5%, and 14,5% for serotypes with low oncogenic risk.

The frequency of HPV serotypes with high oncogenic risk is as follows (showed on a descending basis): 16; 53; 18; 52; 31; 66, 73; 45, 58, 84; 33, 51; 35, 68, 82; 39, 59, 62; 56, 67; IS 39.

As for the serotypes with low oncogenic risk, the frequency is as follows (showed on a descending basis): HPV 6, 61, 42, 81, CP6108, 11, 54, 55, 72, 83.

In 41,8% of cases we found multiple associations between two or more HPV serotypes, noting the increased prevalence in associations of serotypes included in group alpha-9 (16, 31, 33, 35, 52 and 58) and serotypes not included in group alpha-9 or alpha-7 (53, 66, 73, 84).

The highest prevalence of HPV serotype infections is in young patients, most of them with an age ranging between 21 and 40 years, and in our study the mean age was 30.21 years, ranging between 18 and 58 years.

The highest prevalence was 52,1% in the age group ranging between 21 and 30 years and 34,1% in the age group ranging between 31 and 40 years.

Analyzing the distribution of HPV serotype infections by age groups, on a separate basis for those

with a high oncogenic risk and low oncogenic risk, we found a significantly different distribution by age groups.

Separate comparison between infections with high oncogenic risk included in group alpha-7 (HPV 18, 39, 45, 59, 68 and 70) and alpha-9 (HPV 16, 31, 33, 35, 52, 58 şi 67) and infections with other highly oncogenic types, not included in these two groups (HPV 53, 66,73,84) showed significantly different distribution by age groups.

According to current data, the analysis of the prevalence of infections associated with cytological modifications for serotypes with high oncogenic risk, included in group alpha-7 (HPV 18, 39, 45, 59, 68 and 70) and alpha-9 (HPV 16, 31, 33, 35, 52, 58 and 67) is statistically significant.

We found no statistically significant prevalence for serotypes not included in groups alpha-7 and alpha-9 (HPV 53, 66, 73, 84).

Therefore, we have the following frequency of serotypes with high oncogenic risk for modified cytologies: HPV 16; 52, 53; 31, 51, 58; 18, 33 and 66.

The current results are the results of an analysis performed in a sample of 117 patients with cervical infections with HPV serotypes, with or without oncogenic risk; as the sample size of patients included in the study sample increases, we will communicate the results, as they can be different regarding the prevalence order of the serotypes that were found.

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