HPV Cervical Cancer Screening. An Analysis Over HPV Markers Between Worldwide Statistics and Romanian Reality

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The aim of the paper is to present our results on HPV types among adult women diagnosed with invasive cervical cancer and HPV types. All the patients have been diagnosed with CIN 2+. The cross-sectional survey was conducted at the Department of Obstetrics and Gynecology from the County Emergency Hospital Timisoara, Romania, during January 1, 2008, up to December 31, 2010. HPV determination was made using DNA amplification by the Polymerase Chain Reaction (PCR) technique and nucleic acid hybridization detection of 37 anogenital HPV genotypes.

Keywords: HPV infection, incidence of cytological and histological confirmation by biopsy, cervical cancer

A report by the World Health Organization 2010 provides key statistics about HPV infection and cervical cancer in the world, issues presented in detail in this chapter.

Human papillomavirus (HPV) has been found to be associated with several types of cancer: cervical, vulvar, vaginal, penile, anal, and oropharyngeal [1]. Certain types of HPV can produce local cellular changes of the cervix uteri epithelium, HPV. In time, it is possible to appear precancerous lesions and after that invasive cervical carcinoma. Cervical cancer is the second most common cancer in women worldwide [2], while the second cause of death among gynecological cancers and the seventh cause of cancer in women is occupied by ovarian cancer [14], being known that excessive angiogenesis is mainly a feature of malignant development, but also represents an important component for other pathologic conditions [15]. In Romania is the highest incidence and mortality rates from Europe [3,4]. Using the cervical screening programs, the number of invasive cervical cancer decreased in developed countries. More sensitive Pap smear screening methods combine Pap test with high risk HPV determination which identified more women at high risk for cervical cancer [5-7, 11-13]. The association of the other etiological factors (e.g. herpes simplex virus type 2 infections, cigarette smoking, vaginal douching, nutrition, and use of oral contraceptives) can increase the chance to appear the cancerous disease [8]. Using Pap test as screening for the cervix uteri cancer can identify abnormal changes at the cervical cells. On the other hand, a variety of tests have been created to identify the type of HPV and other investigations to identify associated infections are important to apply a better management in the cases at identified risks, while the anamnesis and the therapy are also important in the management [18, 19].

The aim of the paper is to present our results on HPV types among adult women diagnosed with invasive cervical cancer and HPV types among adult women diagnosed with CIN 2+.

Experimental part

Materials and methods

The cross-sectional survey was conducted at the Department of Obstetrics and Gynecology from the County Emergency Hospital Timisoara, Romania, during January 1, 2008, to December 31, 2010. The study began and, to some extent, was financially supported from a European grant run in the early part of the study period [9]. The survey was approved by the appropriate institutional review boards. From the patients with conventional Pap smear undertaken in our department in the studied period, there were selected the patients with abnormal changes. These patients followed other supplementary investigations to confirm a diagnosis and to identify the possible associated risk factors. HPV determination was made using DNA amplification by the *Polymerase Chain Reaction* (PCR) technique and nucleic acid hybridization detection of 37 anogenital HPV genotypes.

The tested HPV types were the following:

-HR-HPV (High-Risk HPV): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68;

-LR-HPV (Low-Risk HPV): 6, 11, 42, 43, 44;

-Other HPV: 26, 40, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108.

The analyzed sample consisted of 105 women. The inclusion criteria for the study were the macroscopic pathological aspects in the cervix or even cervical cancer, condilomatosys or recurrent vulvo-vaginitis. Data were processed using the software package SPSS v17. In the statistical inference and applied statistical testing, the levels

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of confidence and statistical significance were 95% and 5%, respectively. For testing normality of distribution for numerical values (i.e. the age, actually) the Shapiro-Wilk test was employed. The Mann-Whitney U test and Kruskal-Wallis test were employed as non-parametric alternatives for one-way analysis of variance in case of two or more independent samples, respectively. The Chi-square test and Fisher exact test were applied in testing the association between binomial data (e.g. presence vs absence of a medical condition).

Results and discussions

The study sample included 105 subjects, whose general characteristics are presented in table 1. Overall, the percent of the DNA-HPV positives in the studied sample (i.e. among those with a positive cytology, included in study) was 35.2% (37/105), with a 95%CI of (26.3%; 45.2%). Only two subjects were HPV vaccinated, both with HPV16 (single HPV infection), with no history of sexually transmitted diseases, aged 26 and 27, respectively.

Table 2 show the distribution of the HPV positive cases across the High-Risk and Low-Risk HPV types, respectively.

The most frequent agent was HPV16, with16 subjects out of 37 having this type, so leading to a percent of 43.2% with 95%CI (27.5%; 60.4%). The total High-Risk HPV positivity among the DNA-HPV positives was 83.8% (i.e. 31/37), with 95%CI (67.3%; 93.2%). In contrast, the Low-Risk HPV positivity among the DNA-HPV positives was 21.6% (i.e. 8/37), with 95%CI (10.4%; 38.7%).

We investigated a hypothetical relationship between the Pap smear results and the DNA-HPV positivity, but no significant association was found between the two. Similarly, the relationship between the history of cervical cancer and the DNA-HPV positivity was investigated, but did not reach the statistical significance as only eight patients in total had cervical cancer (table 3).

Total number of subjects	105
Age in years [mean ± std. dev.]	35.03 ± 12.01
min – max (Q1 – Q2 – Q3)	18 – 79 years (26 – 31 – 42)
≤ 24 years [freq (%)]	21 (20%)
25 – 44 years [freq (%)]	63 (60%)
45 – 59 years [freq (%)]	17 (16%)
≥ 60 years [freq (%)]	4 (4%)
DNA-HPV positives [freq (%)]	37 (35.2%)
HR-HPV [freq (%)]	31 (29.5%)
LR-HPV [freq (%)]	8 (7.6%)
Multiple HPV infection [freq (%)]	10 (9.5%)
History of cervical cancer [freq (%)]	8 (7.6%)
Pap smear results	
C I [freq (%)]	6 (5.7%)
C II [freq (%)]	72 (68.6%)
C III [freq (%)]	26 (24.8%)
C V [freq (%)]	1 (1%)

Table 1

GENERAL CHARACTERISTICS OF THE ANALYZED SAMPLE. FOR THE AGE (I.E. NUMERICAL VALUES) THE MEAN, STANDARD DEVIATION, MAXIMUM, MINIMUM, AND THE QUARTILES (Q1, Q2, Q3) ARE PROVIDED. FOR THE BINOMIAL VARIABLES INDICATING THE PRESENCE OF A MEDICAL CONDITION, THE FREQUENCIES AND PERCENTS ARE PROVIDED

Table 2

THE FREQUENCIES OF THE HIGH-RISK HPV TYPES AMONG THE DNA-HPV POSITIVES. THE FREQUENCIES OF THE LOW-RISK HPV AND OTHER TYPES AMONG THE DNA-HPV POSITIVES (37 SUBJECTS IN TOTAL)

HR-HPV	HPV16	HPV18	HPV31	HPV33	HPV39	HPV51	HPV59	HPV66	HPV68
freq	16	4	7	4	1	1	1	4	2
%	43.2%	10.8%	18.9%	10.8%	2.7%	2.7%	2.7%	10.8%	5.4%

LR-HPV	HPV6	HPV11	HPV42	Other
freq	3	2	3	4
%	8.1%	5.4%	8.1%	10.8%

Table 3

IN THE FIRST PART WE PRESENT THE RELATIONSHIP BETWEEN THE PAP SMEAR RESULTS AND THE DNA-HPV POSITIVITY. THE PERCENT'S ARE CALCULATED FROM THE ROW TOTALS. IN THE LAST PART WE PRESENT THE RELATIONSHIP BETWEEN THE HISTORY OF CERVICAL CANCER AND THE DNA-HPV POSITIVITY. THE PERCENT'S ARE CALCULATED FROM THE ROW TOTALS

		Pap smear results				
		C I	C II	C III	C V	Total
DNA-HPV	0	6 (8.8%)	47 (69.1%)	14 (20.6%)	1 (1.5%)	68
	1	0	25 (67.6%)	12 (32.4%)	0	37
Total		6 (5.7%)	72 (68.6%)	26 (24.8%)	1 (1%)	105

		History of cervical cancer			Statistical testing
		0	1	Total	Fisher-exact test, p=0.127
DNA-HPV	0	65 (95.6%)	3 (4.4%)	68	OR=3.34
	1	32 (86.5%)	5 (13.5%)	37	95% CI (0.61 ; 22.88)
Total		97 (2.4%)	8 (7.6%)	105	did not reach statistical sig.

Table 4

THE POSSIBLE ASSOCIATIONS BETWEEN AGE AND HPV INFECTIONS. STATISTICAL SIGNIFICANCE FOR THE APPLIED TESTING WAS MARKED WHEN ATTAINED

		N	Age in years		Statistical testing	Figure reference
			mean ± std.dev.	*min – median - max*		
DNA-HPV	0	68	35.78 ± 11.77	19 – 33.5 – 79	Mann-Whitney U test p=0.252	Figure 1 Table 5
	1	37	33.65 ± 12.48	18 – 31 – 74		
Multiple HPV infection	0	77	35.66 ± 12.16	19 – 32 – 79	Kruskal-Wallis test p=0.006**	Figure 2 Table 5
	1	18	37.56 ± 11.91	19 – 33 – 64		
	2+	10	25.6 ± 5.91	18 – 23.5 – 38		
Cervical cancer	0	97	34 ± 11.27	18 – 31 – 79	Mann-Whitney U test p=0.008**	Figure 3 Table 5
	1	8	47.5 ± 14.49	24 – 49.5 – 74		
** = very significant statistical differences						

As the sexual behavior changed over the last 20 years in Romania, the possible associations between age and HPV infections were investigated. Table 4 shows the syntheses of the descriptive analysis, together with the statistical testing.

As presence of abnormal changes in the Pap smear was an inclusion criterion for the present study, the observed overall rate for DNA-HPV in the study sample (i.e. among subjects with a positive cytology) was 35.2% (37/105), i.e. lower than values around 55% – 89% found in literature [10].

Although there seemed to be an association between the history of cervical cancer and the DNA-HPV positivity, the statistical significance was not attained most probably due to the overall low rate of subjects with cervical cancer within the study sample (fig. 1-3). This aspect should be considered in sample size calculation when designing further studies.

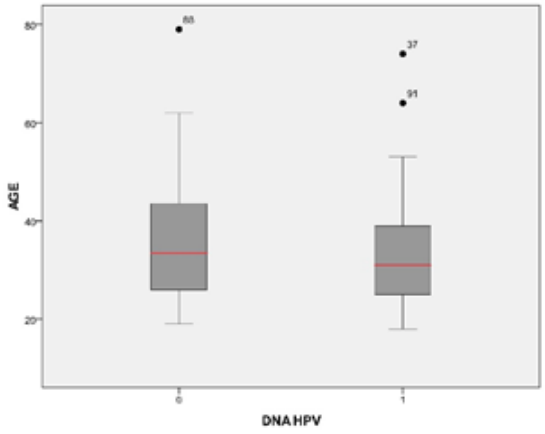

Fig. 1. Age distribution for the two groups of DNA-HPV positivity

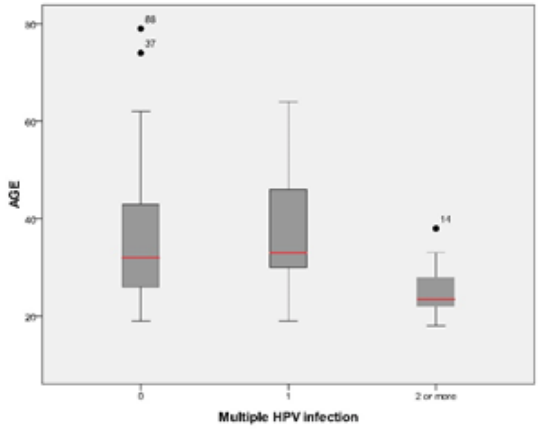

Fig. 2. Age distribution in the three groups considered for multiple HPV infection

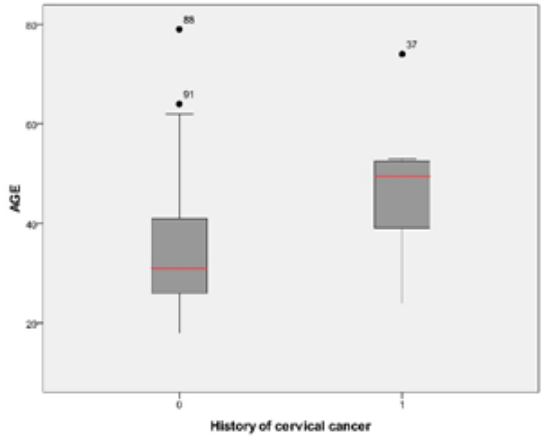

Fig. 3. Age distribution for the two groups of cervical cancer history

The results obtained data set is similar to the global data which is contained in the Bega Hospital, except that the prevalence of HPV is high recorded but can not be extrapolated to the general population of Romania Timis or the lot being unrepresentative, HPV type 16 is the most common virus [16]. Multiple HPV infection occurs in younger patients, and a major problem is the vaccination, which at the time the study was not included in the national campaign.

A summary of worldwide available statistics is an important part of our paper, pointing out the importance of HPV testing among women of different ages, to quantify the risk and early diagnose the pre-cancer lesions to avoid the not wanted mortality.

Missing data on HPV cervical cancer screening in our country underlined in our research are an alarm signal for the medical specialists and a trigger to new research screening campaigns.

Assessing the accuracy of diagnostic tests on HPV cervical cancer screening is a continuously updating research domain, due to the fact that there it is not a generally accepted gold standard test for primarily cervical cancer screening in the world. We can conclude that Papanicolau is the routine cytology test for screening, but PCR HPV DNA test wins territory as an additional test, together or replacing the cytology one [17].

A general accepted recommendation of international guidelines for cervical cancer screening in women is that screening should be performed from ages 21 to 65 years with cytology (Pap smear) every 3 years. For women ages 30 to 65 years who want to lengthen the screening interval, screening with a combination of cytology and human papillomavirus (HPV) testing every 5 years is recommended.

In Romania there is a National Centre to Inform about Cervical Cancer, answering the number 08008-00008. Even if here clinical trials on HPV and cervical cancer were not taking place, national media campaigns come to inform us about this topic.

Conclusions

From the patients with conventional Pap smear undertaken in our department in the studied period, there were selected the patients with abnormal changes. The most frequent agent was HPV16, with16 subjects out of 37 having this type, so leading to a percent of 43.2% with 95%CI (27.5% ; 60.4%). The total High-Risk HPV positivity among the DNA-HPV positives was 83.8% (i.e. 31/37), with 95%CI (67.3% ; 93.2%). In contrast, the Low-Risk HPV positivity among the DNA-HPV positives was 21.6% (i.e. 8/37), with 95%CI (10.4% ; 38.7%). So, we can conclude that the Papanikolau test is the routine cytology test for screening, but PCR HPV DNA test wins territory as an additional test, together or replacing the cytology one.

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