Intriguing Results Regarding the Prevalence and Distribution of HPV Types in Women with Cervical Cancer in a Romanian Population

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Introduction
Cancer of the uterine cervix is the 5th most deadly type of female cancer [1]. It is clearly demonstrated that HPV infection is the necessary, but not the sufficient cause of most cervical cancers [2]. The association between cancer of the uterine cervix and HPV is unique because no other human cancer is dependent on a single infectious factor for its development [3]. Worldwide, HPV types 16 and 18 accounts for approximately 70% of cervical cancer cases with other high-risk types, such as HPV-45, HPV-31, HPV-33, and HPV-52, being responsible for the remaining cases [4,5].

The incidence is highest in developing countries. Little is known about viral and epidemiologic factors in Romania, a country with a high cervical cancer incidence. Few studies have been published [6-9], and they provide little evidence, due to the small sample size and their focus on cytologic abnormalities and pre-invasive cervical lesions, but not in invasive cervical cancer. Thus, the present study is aimed at providing baseline information about HPV prevalence and type distribution in a Romanian population with cervical cancer.

Methods
Study population and clinical specimens
The samples included in this study comprised 80 histologically confirmed invasive cervical cancer fresh specimens of previously non-irradiated Romanian women. These samples were collected at the First Obstetrics and Gynaecology Clinic of Târgu Mureș, a tertiary university hospital, between September 2015 and October 2016. The Ethics Committee of the hospital approved the study protocol and the University of Medicine and Pharmacy of Târgu Mureș supported the study by a research grant. Sampling tubes PreservCytTM and AmpliLute Liquid Media Extraction Kit are indispensable parts of the test. All specimens were collected by cervical brush and stored at +4 °C until analysed.

HPV genotyping
Detection consisted in genomic DNA extraction from cervical epithelial cells, PCR amplification of target DNA, hybridization of PCR products with oligonucleotide probes on solid strips and colorimetric identification of hybridization products, according to the manufacturer’s guidelines (Roche Molecular). The Master Mix reagent contains a pool of HPV biotinilated primers for the amplification of DNA from 37 HPV genotypes (including 13 high risk genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and the human β-globin gene as a control for cell adequacy, nucleic acid extraction, and PCR efficiency. The PCR denaturated amplicons were then hybridized and detected using the recommended LA protocol. The LA HPV genotyping strips were manually interpreted using the HPV reference guide provided.

Data analysis
All data of the HPV genotype-specific prevalence was analyzed, calculated, and presented as percentage. A chi square test and a logistic regression were performed to try to find a correlation between HPV type, stage of disease or histologic type. Statistical analyses for P values were performed using 2×2 contingency tables, with two-tailed P values calculated by using the Fisher exact test. All p values of <0.05 were considered statistically significant.

Results
General characteristics
The patients’ mean age at the time of diagnosis was 49.48 years (between 28 and 68 years old). The majority of histological diagnoses were squamous cell carcinoma (75 out of 80 patients, 93.75%), with 4 adenocarcinomas (5%) and 1 neuroendocrine carcinoma. Forty-one patients (51.25%) were in stage I (Ia1-Ib2), 24 (30%) in stage II, 14 (17.5%) in stage III and 1 (1.25%) was staged IVa.
HPV prevalence and type distribution

Of the 80 samples analyzed, 10 cases (12.5%) were negative for HPV-DNA. HPV-DNA prevalence was 87.5%. Most of HPV-DNA positive cervical cancer patients harboured single infections (87.14%); 7 patients had 2 HPV types (10%); and in 2 patients, 3 different HPV types were discovered (2.86%). HPV16 was the most detected type, with 72.5% relative contribution as single (58 patients) or multiple infection (2 cases of double infection and two patients harbouring 3 different HPV types). Other 2 HPV double infection harboured HPV 31 associated with HPV 39 and 73. Interestingly, HPV18 was identified only in one biopsy (1.3%). More high risk HPV types as single infection were found in 2 cases- 2.7% (HPV 31, 33, 39, 45, 53, 58, 62, 68); other single high risk HPV types were detected in only one woman -1.3% (56, 73, 82, 84). One low risk HPV (type 6) were identified.

It was not found any statistical correlation between the HPV genotype, patients' age, histology of the tumour or stage of disease.

Discussion

The present study has shown the prevalence of HPV genotypes in a Romanian population with invasive cervical cancer, based on PCR. Our results are intriguing, with a high prevalence of HPV 16 (72.5%) and 10 other high risk HPV type, with a low prevalence (1.3-2.7%) for each one. HPV 18 was detected in only one patient, despite the presence of 4 adenocarcinoma and one neuroendocrine carcinoma histologies. This high variety of HPV types among Romanian population is quite specific, compared to other recently published data.

Multiple infection was detected in 12.85% of women in our study, which is extremely high compared with 1.2% as described in a worldwide study by Munoz [10]. In other studies, multiple infections were reported in 1.7-52.2% of the cases [11-15]. Yet, in areas with a high overall prevalence of HPV in the normal population, the percentage of multiple infections in the same group seems to be higher [16].

The HPV prevalence in cervical cancer patients differs substantially in other studies performed in different world regions. Regarding other European countries, a pooled analysis study performed in Italy [17] included 574 invasive cervical cancers from 3 different major studies performed in Rome, Milano and Central and Southern Italy comprising the whole genetic pool of the country. HPV 16 genotype was the most detected type, in single (69.3%) and in co infections with other genotypes (30.7%), in all squamous cell carcinoma and adenocarcinomas. Whereas in the cited study HPV 18 type was the second most common genotype linked with invasive cervical carcinoma (23.3%), we can observe that HPV 18 positive infections (2.13%) are still low numbers in the Romanian population. This trend is confirmed also with other HPV high risk types: 45, 31, 58, 33.

In studies performed in Portugal [14] and Croatia [11], HPV prevalence was 97.9%, 92.5% respectively. In another research performed in Northern Ireland [18], HPV was isolated in 92.2% of squamous cell carcinomas and 64.3% of adenocarcinoma patients, respectively. Most of squamous cell carcinomas histologies (81.3%) had only one HPV type detected.

In women from Israel with cervical cancer [19], HPV was positive in 96.5%, and high risk HPV types were detected in 93%. The most common HPV types was 16 (57.4%), followed by type 45 (9.6%), and type 18 (7.8%). Multiple HPV types were seen in 0.9% of patients.

The HPV prevalence in cervical cancer patients ranged from 84.5% to 97% in researches performed in different world regions [20-26], but the prevalence of different HPV types were extremely different between countries. In all studies, HPV 16 was the most prevalent type (36.8-76.7%), followed by HPV18 (8.5-35.4%), HPV35 (8.7%), HPV45 (6.8-17.2%), HPV33 (3.4-4.0%) and HPV52 (2.2-10.3%). The prevalence of HPV 16 is higher in Europe, South America and China compared to other Asian countries; by contrary, HPV 18, 45 and 52 is more frequent in South East Asia compared to the rest of the world.

A very interesting result derived from this study: 12.5% (10 patients) of the study population where diagnosed with invasive cervical carcinoma with a negative HPV genotyping result. In a study performed on data from 17 European countries, comprising 3162 cases, Tjalma et al. [27] concluded also that 8.2% of invasive cervical carcinoma where HPV negative.

Interestingly, similar to our research, single low risk HPV infection was also found in some studies [19,21].

Conclusion

Because of substantial geographical variation in the HPV genotype distribution, data regarding HPV type-specific prevalence for a particular country are mandatory for providing baseline information to estimate effectiveness of currently implemented cervical cancer prevention strategies including HPV-DNA testing-based screening and HPV vaccination. This study provides important baseline data for improving the acceptance of HPV vaccination in Romania.

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References


