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Differences on the prevalence of cervical HPV between Lithuania and Belarus

Case report

Zivile Gudleviciene*[1], Daiva Kanopiene[1], Janina Didziapetriene[1], Raisa Smolyakova[2], Ekaterina Gutkovskaya[2], Alexander Zhukovec[2], Galina Kostevich[2]

1 Institute of Oncology, Vilnius University, Santariskiu str. 1, LT-08660 Vilnius, Lithuania

2 N.N. Alexandrov National Cancer Center, Lesnoj 223040, Minsk region, Belarus

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Abstract: In some countries the cervical cancer incidence and mortality rates are much higher compared to the European average. The differences of HPV and its type prevalence between countries and regions influence cervical cancer incidence and mortality. Regarding the differences in cervical cancer incidence and mortality in Lithuania and Belarus, the aim of this study was to describe HPV infection level and HPVs type distribution among two study groups of patients with moderate or severe cervical intraepithelial neoplasia (CIN2-3) and cervical cancer. Our data shows that 74.2% [95% CI: 63.64÷84.76] of Lithuanian patients with cervical cancer and 85.6% [95% CI: 85.53÷92.85] of the study group with CIN2-3 were HPV positive, while in the study groups of Belarusian patients HPV infection was detected in 92.6% [95% CI, 74.25÷98.71] and 65.4% [95% CI, 44.36÷82.06] cases respectively. HPV 16 was the most prevalent type in Lithuanian as well as in Belarusian patients of the study groups. HPV 18 in Lithuanian patients of the study group with cervical cancer was identified in 10.2% [95% CI: 1.73÷18.67] and in the study group with CIN2-3 – in 2.6% [95% CI: 0.95÷6.15] of cases. HPV 18 was not detected in Belarusian patients of both groups.

Keywords: *HPV infection • Cervical intraepithelial neoplasia • Cervical cancer*

1. Background

Cervical cancer is the third most common cancer in women, and the seventh overall, with an estimated 530,000 new cases in 2008. More than 85% of the global burden occurs in developing countries, where it accounts for 13% of all female cancers. Overall, the mortality: incidence ratio is 52%, and cervical cancer is responsible for 275,000 deaths in 2008, about 88% of which occur in developing countries. Word-standardized incidence rate of cervical cancer is 15.2 cases per 100,000 women; mortality rate – 7.8 deaths per 100,000 women. In Europe incidence and mortality rates are lower – 10.6 and 3.3 cases per 100,000 women respectively (in EU countries – 9.0 and 3.0 per 100,000 women) [1].

Existing differences between European countries in cervical cancer incidence and mortality occur due to many different risk factors and depend on the screening program implementation [2]. It is well known that human papillomavirus (HPV) is associated with cervical cancer development [3-5]. The differences of HPV and its type prevalence between countries and regions influences cervical cancer incidence and mortality. High HPV infection rates feature in economically undeveloped countries. The infection is the most widespread among African women, especially in east Africa, and the least among South Asian women [6]. In Northern Europe approximately 8.0% of women in the general population

* *E-mail: zivile.gudleviciene@vuoi.lt*

are infected with HPV [7]. Thus, the cervical cancer incidence and mortality in several countries are much higher compared to the European average [8,9]. These differences were reported to comparing cervical cancer incidence and mortality in Lithuania and Belarus [10]. Therefore the aim of this study was to describe HPV infection level and HPV type distribution among two studies groups of Lithuanian and Belarusian patients with moderate or severe cervical intraepithelial neoplasia and cervical cancer.

2. Materials and methods

2.1. Study contingent

The study was performed during the period from 2011 March to October. In total 209 women were included in this study in both countries: 156 of whom cervical pathology (66 of cervical cancer and 90 with CIN 2-3) was diagnosed at the Institute of Oncology, Vilnius University (Lithuania) and 53 (27 with cervical cancer and 26 with CIN2-3) at the N.N. Alexandrov National Cancer Center (Belarus).

The study was performed by the support of Lithuanian and Belarusian Scientific Councils by the Bilateral Cooperation in Science and Technology Program for 2009-2013 years (No. in Lithuania TAP-19/2011 and TAP LB 04/2012 and No. in Belarus Б11ЛИТ-015 01/.2011). The study protocols, the Patients Information and Agreements forms were approved by the Vilnius Regional Committee of Biomedical Research (Lithuania, permission No. 158200-6-062-16) and by the Belorussian Regional Committee of Biomedical Research (Belarus, permission No. 34510-2-035). All women have signed the Patients Information and Agreements forms.

All women included in the study from the outpatient's clinic of Institute of Oncology, Vilnius University (Lithuania) or the clinic of N.N. Alexandrov National Cancer Center (Belarus) were examined by the gynecologist. Diagnosis – cervical cancer or CIN 2-3 – were confirmed by cytology and histology.

2.2. HPV detection and typing

All women with histologically confirmed diagnoses of cervical cancer or CIN 2-3 were tested for HPV. For this reason cervical scrapes were taken from the cervix using cervical brush, placed immediately in the transport buffer and transferred to the laboratory. HPV detection was performed using polymerase chain reaction (PCR).

In Institute of Oncology, Vilnius University (Lithuania), the DNA was extracted from all samples using GeneJet™ Genomic DNA Purification Kit (Thermo Scientific Fermentas Ltd., Vilnius, Lithuania). After DNA extraction all samples were tested for the beta-globin gene (internal control for the presence of DNA). HPV DNA detection was performed using two sets of general HPV primers: GP5+/GP6+ and PGMY09/11. The double HPV testing systems were used to ensure the precise HPV detection. Only cases with double positive or double negative HPV testing result were included in the analysis. As a positive control, the DNA extracted from HeLa and SiHa cells was used. The PCR was performed in 50 µl of PCR mix. DreamTaq™ Green PCR Master Mix containing DreamTaq™ DNA polymerase, optimized DreamTaq™ Green buffer, $MgCl_2$ and dNTPs was used (Thermo Scientific Fermentas Ltd).

HPV positive samples were tested for HPV genotypes. The in-house developed and optimized multiplex PCR-based systems were used with four sets of primers (from L1 gene) specific for sixteen HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82. As a positive control for HPV genotyping the recombinant plasmids containing HPV DNA of the respective HPV type were used. System relevance has been validated using clinical specimens with known HPV infection [11].

PCR products were analyzed under UV transilluminator after electrophoresis in 2% agarose gel stained with Atlas ClearSight DNA Stain (Bioatlas, Estonia). All results were documented by photoimaging and stored in the computer.

In N.N. Alexandrov National Cancer Center (Belarus) for DNA extraction was used DNA-Sorb-V (Russia). Hot start TagF real-time PCR with TagF DNA polymerase was used to detect HPV (AmpliSens HPV genotype Kit, Russia). PCR reaction was performed using 13 µl PCR mix and 5 µl DNA. Twelve oncogenic HPV types were investigated during RT-PCR (HPV 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59) followed by fluorescence hybridization using specific probes.

So, using two appropriate local HPV detection and typing systems HPV 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59 were typed in both countries. HPV 53 in Belarus and HPV 51, 66, 68, 73 and 82 in Lithuania were included additionally in the HPV typing systems.

2.3. Statistical analysis

Results are presented as mean ± standard deviation (SD) or percentage (%) with 95% confidence intervals (CI). The p-value of 0.05 was considered statistically significant.

3. Results

3.1. Women distribution by age

The study covered 209 women with cervical pathology. 156 of whom cervical pathology (66 – cervical cancer, 90 – CIN 2-3) were diagnosed at the Institute of Oncology, Vilnius University (Lithuania). The age average of cervical cancer patients was 52.8 ± 14.6 years. While CIN 2-3 was diagnosed for younger patients, in the group of women with severe intraepithelial cervical neoplasia the average age was 39.8 ± 9.8 years. However, in the groups of Belarusian patients the differences in distribution of patients by age were not found: the age average was 44.5 ± 3.6 year and 44.0, SD ± 7.5 year.

3.2. Prevalence of HPV and its types in Lithuania and Belarus

Among 66 of the Lithuanian patients with cervical cancer 74.2% [95% CI, 61.76÷83.87] of the study population were HPV positive. Surprisingly, the percent of infected patients with severe cervical intraepithelial neoplasia was higher: among 90 of the studied patients HPV was detected in 85.6% [95% CI, 76.20÷91.78] (p=0.059). However, HPV infection is predominant at 92.6% [95% CI, 74.25÷98.71] of Belarusian patients with cervical cancer. There were 65.4% [95% CI, 44.36÷82.06] (p=0.018) of another group of Belarusian women with severe cervical intraepithelial neoplasia infected by HPV (Figure 1). The data in Fig. 1 shows the distribution of HPV infection by the studied groups.

According to HPV types, the majority of Lithuanian as well as Belarusian patients of the study groups were infected with HPV type 16 (Fig. 2). In Lithuanian cervical cancer group HPV16 was confirmed for 48.5% of patients [95% CI, 36.14÷–61.013], in CIN 2-3 group – for 50.0% of patients [95% CI, 39.35÷60.65] (p=0.49). Whereas in Belarus cervical cancer patients it was detected for 74.1% [95% CI, 53.41÷88.13], and in the cases of CIN 2-3 – for 57.7% [95% CI, 37.19÷76.03] (p=0.17).

In the study group of Lithuanian women with cervical cancer the other most frequent types were HPV 18 (10.2%, n=5), HPV 39, 45, 56, 59 (4.1%, n=2 of each type) and in the study group of CIN 2-3 – HPV 33 (10.4%, n=8), HPV 31 (9.1%, n=7), HPV 58 (6.5%, n=5), HPV 18 (2.6%, n=2) (Fig. 3). The following HPV types were identified in the studies groups of Belarusian patients: in cervical cancer cases there were HPV 11, 31, 33, 35, 45 and 51, in CIN 2-3 cases – HPV 11, 33, 52 and 58 (Fig. 4). We would like to notice, that low risk HPV types were not investigated in the groups of Lithuanian women. The other most common HPV types (except HPV 16) between Lithuanian and Belarusian women were only HPV 45 in the case of cervical cancer and HPV 31, 33 and 58 in the case of CIN 2-3. It is important to notice, that HPV 18 was not identified in the both groups of Belarusian patients. On the other hand, for 6 Lithuanian patients cervical adenocarcinoma was diagnosed. 2 of these cases were HPV negative. In the remaining 3 HPV positive cases of adenocarcinoma HPV 16, 18, 45 were stated, in one case HPV type was not specified (HPVX). In Belarusian patient's cervical adenocarcinoma was diagnosed in 1 case which was HPV negative. In remaining 26 cases squamous cell carcinoma was diagnosed.

4. Discussions

The highest annual world-standardized mortality rates from cervical cancer are currently reported in the new European Union member states [8]. In the Lithuanian population cervical cancer still is in the leading position in cancer statistics and epidemiology. Every year approximately 500 new cases of cervical cancer are diagnosed in Lithuania and approximately 250 of them died from this pathology [12]. Whereas the incidence and standardized mortality rate from cervical cancer in Belarus is lower. In Fig. 5 are shown standardized mortality rates of cervical cancer in Belarus and European Europe.

It is well recognized that persistent infection of high-risk (oncogenic) HPV is one of the most important risk factor for cervical cancer development. Compared to the global or European average, a higher HPV prevalence was detected in previous Lithuanian studies: HPV in cervical cancer patients was detected in 92.0% and in 23.6% of healthy women [13,14]. In Belarusian studies HPV prevalence was stated in 78.6% of women with CIN2-3 [15] and in 27.5% of healthy women with various risk factors [16]. So, both countries show a high level of HPV infection in the healthy population in comparison with European average.

In our neighbor country Latvia the overall HPV prevalence in healthy women was similar (26.2%) whereas in Russia it was higher (33.4%) [17]. In the same study analyzing HPV prevalence among women with cervical lesions HPV detection increased according to the cytological abnormality and lesion grade: from 27% in normal samples to 77% in cancer.

In another study among young women (15-23 years of age) with various risk factors (sexually transmitted diseases) in the Polish population 56.5% of them were

Figure 1. HPV distribution by the studied groups.

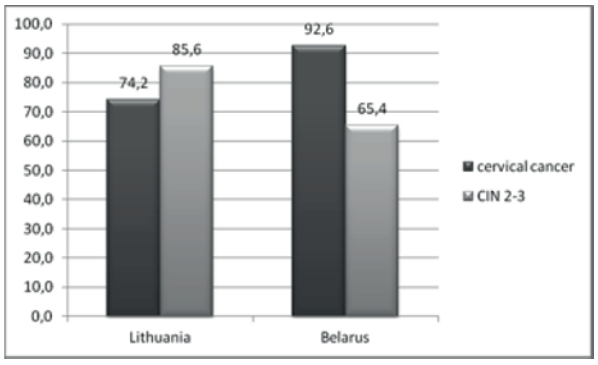


Figure 2. Identification of HPV 16 in the studied groups.

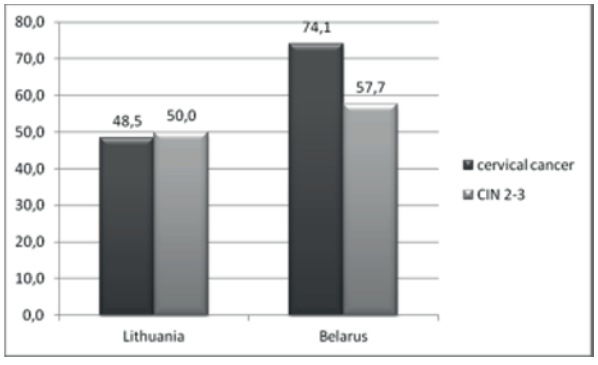
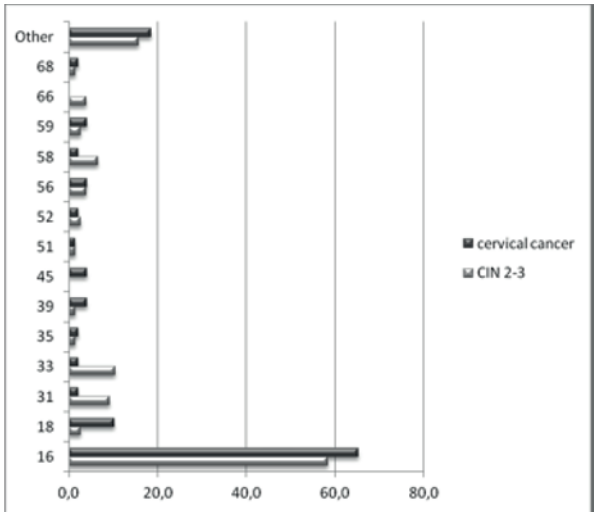


Figure 3. HPV types identification in the groups of Lithuanian patients.



infected with HPV, of which 40.4% by the oncogenic types. The most common HPV type was 51, 16 and 31, as well as 52, 53 and 18 [17]. So, the differences in the prevalence of HPV among neighbor countries in the same region are detected. On the other hand, the different HPV detection systems, different inclusion criteria were used in all reported studies what could influence

Figure 4. HPV types identification in the groups of Belarusian patients.

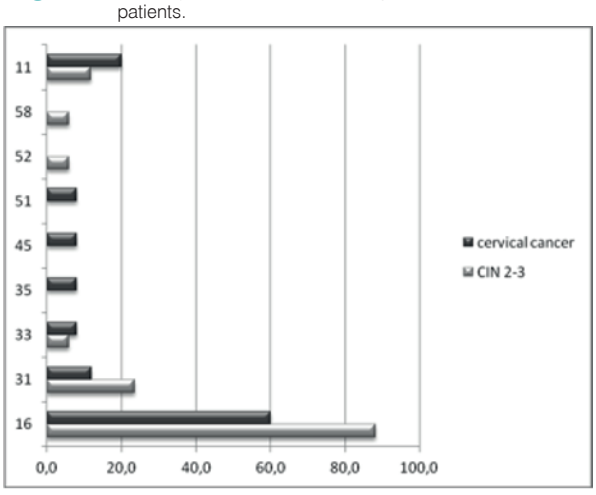
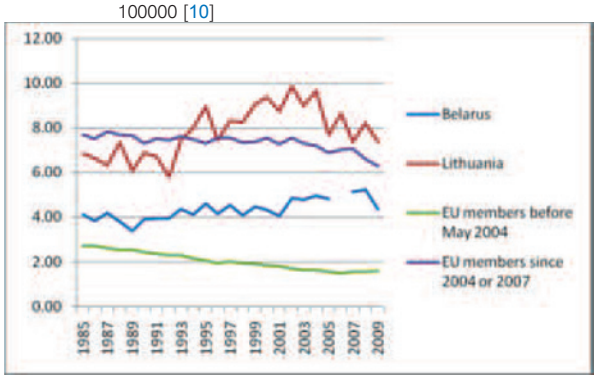


Figure 5. Standardised mortality rates, cervical cancer, 0-64, per 100000 [10]



the different results as well. The International Agency for Research on Cancer (IARC) has provided the different level of HPV infection in cervical cancer samples depending of the study type, country or region of country (approximately from 77 to 100%) [18].

In our study we have introduced the data about HPV infection level and HPV type distribution among two studies groups of Lithuanian and Belarusian patients with severe cervical intraepithelial neoplasia and cervical cancer. Belarus has lower mortality from cervical cancer compared with Lithuania, where 92.6% of the studied Belarusian patients with cervical cancer were positive for HPV, while HPV infection in the same group of Lithuanian patients was 74.2%. The level of HPV infection in Belarusian patients with CIN 2-3 was lower (65.4%) in comparison with Lithuanian patients with the same pathology (85.6%). In many studies in the world HPV prevalence in the cervical cancer cases is stated more than 90-99% [18]. However, in some populations it was stated less HPV prevalence in comparison with the world. Only 77% of HPV prevalence in cervical cancer cases was stated by the Kulmala SM [16] in the

Table 1. Distribution of HPV types across cervical intraepithelial neoplasia and cervical cancer

HPV type	Cervical cancer Frequency in %			CIN 2-3		
	Lithuania	Belarus	World [17]	Lithuania	Belarus	World [17]
High risk						
16	48.5	74.1	54.3	50.0	57.7	45.0
18	10.2	0.0	12.6	2.6	0.0	7.1
Other high risk						
31	2.0	12.0	4.2	9.1	23.5	8.8
33	2.0	8.0	4.3	10.4	5.9	7.2
35	2.0	8.0	1.0	1.3	0.0	4.4
39	4.1	-	0.4	1.3	-	1.1
45	4.1	8.0	4.2	0.0	0.0	2.3
51	1.3	8.0	0.6	1.3	0.0	2.9
52	2.0	0.0	2.5	2.6	5.9	5.2
56	4.1	-	0.7	3.9	-	3.0
58	2.0	0.0	3.0	6.5	5.9	6.9
59	4.1	-	0.8	2.6	-	1.5
66	0.0	-	-	3.9	-	-
68	2.0	-	0.5	1.3	-	1.0
Low risk						
6	-		0.6	-		1.9
11	-	20.0	0.3	-	11.8	1.3
Other	18.4	-	-	15.6	-	-

countries of former Soviet Union. The reasons for this, discussed in this paper, might be that the HPV analysis was done from cervical swap samples, not from the biopsy. This might explain the failure to detect some of these infections. For HPV testing in the Lithuanian patients cervical swabs were used. So, it also could impact the less HPV detecting in these specimens. On the other hand – these all countries are neighboring countries. This geographical location also could influence the same (and sometimes lower compared with the world) HPV prevalence. Other reasons for differences in HPV prevalence – different age of women examined in both countries. In our study the differences were stated in the age of studied women. In Lithuania all women attending for the treatment were included in the study. According our data we can make the assumption, that precancerous lesions in Lithuania were diagnosed for younger patients and cancer affects older women. Whereas in the Belarus women included in this study were matched according the age. It also could impact the different HPV prevalence in both countries and studied groups.

Further we could discuss about small number of patients in the studied groups. However, in both cases our data are similar to the findings of other studies [15,18].

Regarding HPV typing the most prevalent type in Lithuanian as well as in Belarusian patients was HPV 16, but the frequency of HPV 16 distribution was different in all cases (from 48.5 to 74.1%). The frequency of HPV 16 and other HPV type distribution across cervical intraepithelial neoplasia in comparison with literature data [18] is shown in the Table 1. HPV18 was not detected in the Belarusian patients. The main reason could be the insufficient number of cases analyzed in this study and different HPV typing systems used. On the other hand HPV18 is more more likely to cause cervical adenocarcinoma. In the Belarusian study group only 1 cases of HPV negative adenocarcinoma was diagnosed. However, the results concerning distribution of HPV types in comparison with literature data showed that the common HPV types between Lithuanian and Belarusian patients were HPV 16 and 45 in case of cervical cancer and HPV 16, 31, 33 and 58 – in the cases of severe cervical intraepithelial neoplasia.

This study showed some differences in the HPV prevalence and cervical cancer incidence and mortality in two neighboring countries – Lithuania and Belarus. Cervical cancer screening program in Lithuania was started in 2004 year. In Belarus the cervical cancer screening program does not exist. Women are tested

using opportunistic screening when they attend to the clinic. Even situation of cervical cancer incidence is slightly better comparing to the situation in Lithuania. However, these incidences are higher comparing to the European Union [10]. During the 8 years (from 2004 to 2012) since the screening program was implemented in Lithuania, the situation in cervical cancer incidence was not changed. It is still one of the highest in the Europe. So, government of both countries must improve or start a screening program. Regarding the improvement of the situation, HPV vaccination could be started in both countries. Both vaccines against HPV 16 and HPV 18 could be implemented in Lithuania and Belarus to decrease incidence and mortality from cervical cancer.

5. Conclusions

In the study group of Belarusian patients with cervical cancer HPV infection level was higher in comparison with Lithuanian cancer patients, while HPV infection was found rather frequently in the group of Lithuanian patients with CIN 2-3.

1. HPV 16 was the most common HPV type both in Lithuanian as well as Belarusian patients with cervical cancer and severe cervical intraepithelial neoplasia, but the frequency of HPV 16 distribution was different.
2. The other common HPV type in Lithuanian and Belarusian women was HPV 45 in cervical cancer patients and HPV 31, 33 and HPV 58 in CIN 2-3 patients. HPV 18 type was identified only in Lithuanian women.

6. Acknowledgements

The study was performed by the support of Lithuanian and Belarusian Scientific Councils by the Bilateral Cooperation in Science and Technology Program for 2009-2013 years (No. in Lithuania TAP-19/2011 and TAP LB 04/2012 and No. in Belarus Б11ЛИТ-015 01/.2011).

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