

Risk Factors for the Development of Cervical Intraepithelial Neoplasia

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Abstract: Introduction: Cervical intraepithelial neoplasia is a precursor to cervical cancer. Approximately 1.5 per 1,000 women in developed countries are diagnosed with CIN 2/3 annually, with the highest incidence among women aged 25 to 29 years, at 8.1 per 1,000 women. Goal of this research is to examine the presence of risk factors in patients with developed cervical intraepithelial neoplasia. Subjects and methods: The study included all female subjects treated at the Clinic for Gynecology and Obstetrics of the KCUS. The study is based on the analysis of prospectively collected relevant data obtained from 100 HPV genotyped female patients. In the experimental part of the study, the subjects were divided into two groups of fifty subjects each as follows: Group I (n = 50): patients of reproductive age who were HPV genotyped. Group II (n = 50): patients of perimenopause and menopause who were HPV genotyped. Results: The largest number of respondents were born in the 1980s (n=9). The oldest respondent was 65 years old, while the youngest was 23 years old. The largest number of respondents started sexual relations at the age of 18 (n=18), then at the age of 20 (n=17). The largest number of respondents had two partners each, 25%, then one 20%, three 18%. A total of three respondents used contraceptive pills, of which two from group I and one from group II, and two used an intrauterine device as a form of contraception, i. e. spiral, one from each group. Of the 100 subjects included in this study, 13 were negative for human papillomavirus, and 87% were positive. The results of cervical biopsy in group I showed that the largest number of subjects, 38% (n=20), had a pathohistological diagnosis of CIN III, followed by 28% (n=14) of CIN II and 10% (n=5) of CIS. In group II, the largest number of subjects also had a pathohistological diagnosis of CIN III, 40% (n=20), CIN II 20% (n=10) and CIS 12% (n=6). Conclusion: We conclude that risk factors are extremely present in patients with cervical intraepithelial neoplasia and that it is necessary to implement a public health campaign to raise awareness about the presence and prevention of risk factors.

Keywords: risk factors, cervical intraepithelial neoplasia

1. Introduction

Cervical intraepithelial neoplasia is a precursor to cervical cancer. Approximately 1.5 per 1,000 women in developed countries are diagnosed with CIN 2/3 annually, with the highest incidence among women aged 25 to 29 years, at 8.1 per 1,000 women (1).

The main risk factors associated with the development of cervical intraepithelial neoplasia (CIN) and cervical cancer are: infection with high - risk human papillomavirus, age, genetics, smoking, childbirth, microbiome and infections, use of oral contraceptives and diet. Some researchers have highlighted that adolescents may be at increased risk of squamous intraepithelial lesions (SIL), due to differences in the biological maturity of the immune system and cervix. In addition, cervical ectopy and metaplastic changes in the transformation zone from puberty onwards may increase the susceptibility of the cervix to HPV infection in adolescents (2). High parity, combined with increasing age, also plays a significant role in the progression of SIL (3). Genetic polymorphisms in various genes are associated with the risk of developing various cancers. In research, polymorphic variants of different human leukocyte antigen (HLA) molecules have been shown to be associated with the development of cervical cancer (4).

There is evidence that dietary folate and beta - carotene deficiency may be a cofactor in the development of cervical squamous cell dysplasia (5). Use of oral contraceptives for more than five years increases the risk of cervical cancer two to fourfold, even after excluding other factors (6).

Cigarette smoking has been identified as a cofactor for HPV infection, and a positive correlation between cigarette smoking and cervical cancer has been demonstrated. The effect of smoking is explained by the action of nicotine, which is found in the cervical mucus of women, and acts to reduce local immunity to HPV infection. HPV prevalence is associated with active smoking, especially with the intensity of smoking. The association with smoking, which appears to be dose - dependent and disappears after smoking cessation, was confirmed in a study that restricted the analysis to HPV - positive women (7).

In women who have never had sex, cancer occurs extremely rarely. Women who have had sex before the age of 20 have a 2.5 - fold increased risk of developing cervical lesions. Women who have had sex with three to five partners have an eight - fold increased risk of developing cancer (8).

Recent studies have shown that the microbiota plays an important role in the development of various types of cancer, suggesting its involvement in various carcinogenic mechanisms. Gynecological malignancies have also been linked to the constitution of the microbiome (9). Almost all cases of cervical cancer are caused by persistent infection with HPV HR types. HPV is the most common sexually transmitted disease in the world and approximately 80% of women and men will be infected with HPV at some point in their lives. Initial infection usually occurs during adolescence or early adulthood, with most women clearing the infection within 18 - 24 months. However, in 3 - 5% of women, HPV infection persists and they develop significant preinvasive disease, and <1% develop invasive cancer. HPV infection is also a cause of other malignant diseases, including cancer of the oropharynx, anus, penis, vulva, and genitalia (10).

Goal of this research is to examine the presence of risk factors in patients with developed cervical intraepithelial neoplasia.

Subjects and methods

The study included all female subjects treated at the Clinic for Gynecology and Obstetrics of the KCUS. The study is based on the analysis of prospectively collected relevant data obtained from 100 HPV genotyped female patients. In the experimental part of the study, the subjects were divided into two groups of fifty subjects each as follows:

- 1) **Group I** (n = 50): patients of reproductive age who were HPV genotyped, conized.
- 2) **Group II** (n = 50): patients of perimenopause and menopause who were HPV genotyped, conized.

As part of the comprehensive diagnostic workup, detection and verification of cervical dysplasia, four complementary methods play a role:

- 1) Pap smear.
- 2) Colposcopy with targeted biopsy.
- 3) Pathohistological analysis of the sample.
- 4) HPV genotyping of the virus, the results of which allow the separation of biological from morphological changes in the epithelium.

The database was compiled in Microsoft Office Excel 2013 and data from paper documentation were entered into it. After checking the integrity of the data, statistical analysis was performed in the IBM SPSS Statistics v.20.0 for Windows program. The data were presented in the form of tables and graphs, using classical methods of descriptive statistics, depending on the nature of the data and the scale of measurement.

2. Research Results

The largest number of respondents were born in the 1980s (n=9). The oldest respondent was 65 years old, while the youngest was 23 years old.

Table 1: Number of births of the entire sample

Number of Births					
	Frequency	Percent	Valid Percent	Cumulative Percent	
Valid	0	25	25.0	25.0	25.0
	1	29	29.0	29.0	54.0
	2	36	36.0	36.0	90.0
	3	10	10.0	10.0	100.0
	Total	100	100.0	100.0	

The highest percentage of respondents, 36%, had two births, followed by 29% one, 10% three births, and 25% had no children. The largest number of respondents from group I is without childbirth 40% (n=20), then with one childbirth 30% (n=15), with two childbirths 20% (n=10) and five of them with three childbirths 10%. In group II, which was to be expected, we have a larger number of multiparous women, the largest number of them with two births is 52% (n=26), then with one birth 28% (n=14), with three births is the same number as group I, five 10% of them, and there were also 10% of them without childbirth (n=5).

Table 2: Age of female respondents at first intercourse for the entire sample

Intercourse – Age					
	Frequency	Percent	Valid Percent	Cumulative Percent	
Valid	13	1	1.0	1.0	1.0
	15	2	2.0	2.0	3.0
	16	6	6.0	6.0	9.0
	17	15	15.0	15.0	24.0
	18	18	18.0	18.0	42.0
	19	10	10.0	10.0	52.0
	20	17	17.0	17.0	69.0
	21	9	9.0	9.0	78.0
	22	9	9.0	9.0	87.0
	23	2	2.0	2.0	89.0
	24	4	4.0	4.0	93.0
	25	2	2.0	2.0	95.0
	26	2	2.0	2.0	97.0
	27	1	1.0	1.0	98.0
	29	1	1.0	1.0	99.0
30	1	1.0	1.0	100.0	
Total	100	100.0	100.0		

Table 2 shows the age of the respondents at first intercourse for the entire sample. The largest number of respondents started sexual relations at the age of 18 (n=18), then at the age of 20 (n=17). The youngest respondent was 13 years old at the time of sexual intercourse, and the oldest was 30 years old. In relation to the groups, the largest number of respondents in group I entered into sexual relations at the age of 17 and 18, and in group II at the age of 20.

Table 3: Number of partners of female respondents of both groups

Number of Partners					
	Frequency	Percent	Valid Percent	Cumulative Percent	
Valid	1	20	20.0	20.0	20.0
	2	25	25.0	25.0	45.0
	3	18	18.0	18.0	63.0
	4	13	13.0	13.0	76.0
	5	8	8.0	8.0	84.0
	6	6	6.0	6.0	90.0
	7	3	3.0	3.0	93.0
	8	2	2.0	2.0	95.0
	10	4	4.0	4.0	99.0
	20	1	1.0	1.0	100.0
Total	100	100.0	100.0		

Table 3 shows the number of partners of both groups of respondents. The largest number of respondents had two partners each, 25%, then one 20%, three 18%. The largest number of partners (n=20), was stated by one respondent. In group I, the largest number of respondents had two partners each, 26% (n=13), and in group II, the largest number of respondents also had two partners each, 24% (n=12).

Table 4: Type of contraception of the respondents included in this research

Type of Contraception	Frequency	Percent	Valid Percent
Pilula	3	3.0	3.0
Spirala	2	2.0	1.0
Total	100	100.0	100.0

A total of three respondents used contraceptive pills, of which two from group I and one from group II, and two used an intrauterine device as a form of contraception, i. e. spiral, one from each group.

Table 5: The percentage of cigarette consumption by the respondents included in this research

Cigarette Consumption					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	NE	48	48.0	48.0	48.0
	DA	52	52.0	52.0	100.0
	Total	100	100.0	100.0	

Out of a total of 100 female respondents in both investigated groups, 48% of them did not consume cigarettes, and 52%

did. In group I, 52% (n=26) of the respondents consumed cigarettes, and in group II, 52% also consumed cigarettes.

Table 6: Results of preoperative HPV genotyping for all subjects included in the research

HPV Genotyping Result					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	NEG	13	13.0	13.0	13.0
	POZ	87	87.0	87.0	100.0
	Total	100	100.0	100.0	

Table 6 shows the results of preoperative HPV genotyping. Of the 100 subjects included in this study, 13 were negative for human papillomavirus, and 87% were positive. In group I, 86% (n=43) were HPV positive, and in group II, 88% (n=44) were HPV positive.

Table 7: Pathohistological diagnosis of cervical biopsy by groups

Pathohistological Diagnosis of Biopsy		Frequency	Percent	Valid Percent	Cumulative Percent	
Group I	Valid	CIN I	5	10.0	10.0	10.0
		CIN I / II	3	6.0	6.0	16.0
		CIN II	14	28.0	28.0	44.0
		CIN II / III	3	6.0	6.0	50.0
		CIN III	20	38.0	38.0	88.0
		CIN III/ CIS	1	2.0	2.0	90.0
		CIS	5	10.0	10.0	100.0
		Total	50	100.0	100.0	
Group II	Valid	CIN I	5	10.0	10.0	10.0
		CIN I / II	2	4.0	4.0	14.0
		CIN II	10	20.0	20.0	34.0
		CIN II / III	4	8.0	8.0	42.0
		CIN II / H - SIL	1	2.0	2.0	44.0
		CIN III	20	40.0	40.0	84.0
		CIN III/ CIS	2	4.0	4.0	88.0
		CIS	6	12.0	12.0	100.0
Total	50	100.0	100.0			

The results of cervical biopsy in group I showed that the largest number of subjects, 38% (n=20), had a pathohistological diagnosis of CIN III, followed by 28% (n=14) of CIN II and 10% (n=5) of CIS. In group II, the largest number of subjects also had a pathohistological diagnosis of CIN III, 40% (n=20), CIN II 20% (n=10) and CIS 12% (n=6).

3. Discussion

The largest number of respondents in our study had first sexual intercourse at the age of 18 (n = 18), followed by 20 (n = 17). In relation to the groups, the largest number of respondents in group I had first sexual intercourse at the age of 17 and 18, and in group II at the age of 20. In group I, the largest number of respondents had two partners - 26% (n = 13), and in group II, the largest number of respondents also had two partners - 24% (n = 12). In the study by Fang et al., 57.6% of women had <10 lifetime sexual partners (11). In the study by Huic et al., less than 10% of respondents had more than four partners (12). Ferris et al. investigated HPV infection in adult women. 3, 817 women participated in the study, and factors associated with new HPV infections included younger age, younger age at first intercourse, women without a permanent partner, current tobacco use, and

a greater number of past and recent sexual partners (13), which correlates with the results of this study.

In this study, out of a total of 100 female respondents in both investigated groups, only 5% used contraception. A total of three respondents used contraceptive pills, of which two from group I and one from group II, and two used an intrauterine device as a form of contraception, i. e. spiral, one from each group. In the research by Huic et al., 30% of the respondents used contraception (12), which does not correlate with the results of our research.

The analysis of tobacco use revealed the following data: in group I, 52% of the subjects smoked cigarettes, and in group II, 52% also smoked cigarettes. In the study by Fang et al., 41.6% were current smokers (11), which is in line with the results of our study. In the study by Huic et al., the patients were divided into three age subgroups (<29, 30 - 39, and >40 years), and 50% of them smoked (12), which is in line with the results of our study. In the study by Huic et al., the highest percentage of individual HPV HR infections and HPV HR coinfections was recorded in women in the age groups 30 - 39 and <30 years. Smoking (p = 0.0330) and sexual behavior (p = 0.0180 for more than three sexual partners; p = 0.0238 for cases when the partner was involved in another sexual relationship) were associated with viral persistence, while

long - term contraception did not yield statistically significant results (12).

In this study, the largest number of subjects in group I had a cytological diagnosis of CIN II (26%), followed by CIN III (22%), the prevalence of CIN I and CIN II/III was 16%, and CIS was diagnosed in 8% of subjects. In group II, the highest percentage of subjects had a diagnosis of CIN II (32%) and CIN III (30%), as in the previous group. Then CIN I (14%), and CIS 6%. Out of a total of 100 subjects in both examined groups, 60% of them had a colposcopic diagnostic examination, 54% of group I (n = 27) and 66% of group II (n = 33). Out of 100 subjects included in this research, 13 of them were negative for human papillomavirus, and 87% were positive. In group I 86% (n=43) were HPV positive, and in group II 88% (n=44) were HPV positive. Bogani et al conducted a retrospective, multi - institutional study with the aim of evaluating the outcome of high - risk HPV positive and negative women affected by high - grade cervical dysplasia. Medical records of patients with high - grade cervical dysplasia, who underwent conization between 2010 and 2014, were collected. All included patients were followed for at least five years. A total of 2, 966 women with high - grade cervical dysplasia were examined. The study population included 1, 478 (85%) and 260 (15%) women with high - grade HPV - positive and HPV - negative cervical dysplasia, respectively. The prevalence of CIN II and CIN III among the HPV positive and negative cohorts was similar (p = 0.315). Patients with HPV - positive high - grade cervical dysplasia had a higher risk of 5 - year recurrence (after primary conization) than HPV - negative patients (p < 0.001), which is consistent with the results of our study. By multivariate analysis, HPV - negative women were at low risk of recurrence (p = 0.018). In comparing HPV - negative patients with HPV - positive patients, CIN III was associated with an eight - fold increased risk of recurrence (p < 0.001). The authors conclude that HPV - negative high - grade cervical dysplasia is not uncommon and accounts for 15% of population in this study. These patients have more favorable outcomes than those with documented HR HPV infection. They also believe that further prospective studies are needed to confirm their data (14).

4. Conclusion

The largest number of respondents in group I had a diagnosis of CIN II (26%), followed by CIN III (22%), the prevalence of CIN I and CIN II/III was 16%, and 8% of respondents had a diagnosis of CIS. HPV HR as the most significant risk factor was positive in 87% of respondents. A total of 52% of respondents consumed cigarettes. The largest number of respondents had sexual intercourse at the age of 18, and the largest number of respondents had two partners, 25% of them, then one 20%, three 18%. We conclude that risk factors are extremely present in patients with cervical intraepithelial neoplasia and that it is necessary to implement a public health campaign to raise awareness about the presence and prevention of risk factors.

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