

Risk factors for cervical cancer among young women

Research Article

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Abstract: Background: The aim of this study was to assess the risk factors for cervical cancer and the occurrence of particular types of HPV among young women in the Polish population. Material and methods: Ninety-two women aged from 15 to 23 years old were involved in the study. Women were subjected to gynecological examination after completing the survey for the presence of risk factors for cervical cancer. Swabs for cytology, vaginal biocenosis evaluation and HPV DNA determination were collected. Results: 56.52% of population were infected with HPV, of which 40.4% by the oncogenic types. 22.8% of women were infected by more than one type of virus. The most common HPV type is 51, 16 and 31, as well as 52, 53 and 18. An isolated risk factor for HPV infection and thus cervical cancer in young women, statistically significant, was a high number of sexual partners. The other identified risk factors were: age of sexual initiation (16 years or less), ASCUS or LSIL cervical cytology, or bacterial infection present in the reproductive tract. Conclusions: The most important risk factors for cervical cancer among young Polish women are HPV infection, early sexual initiation and a large number of sexual partners.

Keywords: *Cervical cancer • HPV • Age • Sexual partner • Risk factor*

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1. Introduction

The etiology of cervical cancer is complex, although for several years, it has been a long-established view that among many factors that increase the risk of developing cervical cancer, specifically one of them is considered necessary, though insufficient. Epidemiological data and clinical studies provide evidence that the above mentioned factor is a cancer-causing HPV type leading to chronic infection of the cervix. The relationship between HPV and cervical cancer, meets all the criteria of the International Agency for Research on Cancer (IARC), which must be complied with to recognize the relationship between the factor and a cause of cancer [1,2]. Statistically, the most significant risk factors responsible for the occurrence of human papilloma virus

infection are very similar to the risk factors for cervical cancer. These include: age, early sexual initiation, High Sexual Activity, multiple sexual partners, history of abnormal Pap smears, venereal diseases and inflammatory changes within the vagina or vulva. Others, of less importance, are: smoking, use of oral contraceptives and number of previous pregnancies [2-4]. HPV infects the cells of the base layer of squamous epithelium of the cervix. The condition for the virus to reach this layer is its unveiling. Exposure of this layer occurs in the epithelial microtraumas due to sexual intercourse or an infection caused by various microorganisms. Peak prevalence of infections with human papilloma virus is between 20 and 25 years of age. Positive test results are obtained in about 40% of women in this age group in the general population. In these patients cancer is rarely observed

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[5]. The age at which cervical cancer occurs the most frequently is similar worldwide. The greatest morbidity affects women aged 45–54 years [6]. Epidemiological studies of cervical cancer's risk factors underline the important role of sexual activity, both women and men in the development of changes in the cervix [6–8]. The risk of HPV infection increases along increasing number of sexual partners. It is believed that the risk of developing cervical cancer is also higher if 1st sexual intercourse took place before reaching age of 16 years. Early initiation of sex life means that the most observed form of HPV infection is a clinical form [6]. The scientists are divided regarding the impact of cigarette smoking on the possible development of the human papilloma virus infection, and thus the development of cervical cancer [9,10]. Nevertheless, in the mucosa of female smokers, nicotine, and other substances with mutagenic properties were detected. There is a theory linking the presence of the above relationship with the transition state of local immunosuppression. The substances are thought to facilitate the primary infection and promote cell changes caused by the presence of HPV [3]. Many reports indicate the existence of the relationship between the development of human papilloma virus infection and low financial status. It is associated with a deficiency of certain micronutrients in the diet. Secondly, low socioeconomic status is also often related to poor living conditions and poor personal hygiene [3]. In addition, there is evidence that long-term, irregular use of hormonal contraception promotes the development of HPV infection. A significant increase in infection has been observed among women with endocrine disorders, pregnant and using oral hormonal contraception for multiple years [11,12].

The aim of this study was to assess the prevalence of HPV infection among young women as well as risk factors for cervical cancer associated with HPV infection in the Polish population.

2. Material and methods

The study encompassed 92 women aged 15 to 23 years old, who were randomly admitted to the Outpatient Gynecologic Clinic and residents of the Youth Educational Center located at ul Kamienna 22 in Szczecin between 1 Jan 2010 and 31 Jan 2011. Patients were recruited on a voluntary basis, upon presentation of accurate information about the study and the purpose of research. They (or in case of underage, their legal guardians) signed a consent form to participate in the study. Each patient filled in a questionnaire consisting of 36 questions concerning the potential risk factors for cervical

cancer, obstetric and gynecological history, undergone treatment(s) and current health status. Afterwards all women were subjected to gynecological examination with the use of specula and a preliminary assessment of the macroscopic state of the cervix and vaginal biocenosis. All women underwent microbiological and cytological examination prior to colposcopy.

From each patient four swabs were taken: first, from the posterior fornix to assess the biocenosis of the vagina, then from the cervical canal to evaluate cytology, Chlamydia trachomatis infection and signs of HPV DNA. A sterile swab was taken to assess the biocenosis of the vagina. The specimen was applied directly on the glass slide and allowed to dry. Cervical swabs from the ecto and endocervix were collected with the use of a sterile brush Cervex–Brush (Rovers, Poland), smeared onto a slide and immediately fixated in 96% ethanol. The swab for Chlamydia trachomatis was taken with a sterile brush Vibra–Brush (Rovers, Poland), and applied to the crystal core for immunofluorescence. Investigation for Chlamydia trachomatis infection was carried out by using a sterile brush Vibra–Brush (Rovers, Poland). Swab was applied to a slide used for immunofluorescence. Sample after drying at room temperature was fixed with a methyl alcohol. Swab from the cervical canal in order to determine the HPV DNA was collected with a sterile brush Cervex–Brush Combi (Rovers, Poland) and fixed in liquid medium LBC. Cytological studies were carried out at the Department of Pathology, Faculty of Medicine Pomeranian Medical University. Samples were stained with hematoxylin and eosin and viewed under the optical microscope.

Microbiological tests were carried out in the Department of Microbiology and Immunology of Medicine Pomeranian Medical University. Direct preparations from the posterior vaginal vault were Gram stained, viewed under the optical microscope at a magnification of 1500x, and assessed for the presence of lactobacilli, other bacteria, fungi, epithelium and leukocytes. The presence of Chlamydia trachomatis elementary bodies in epithelial cells was tested by direct immunofluorescence method with the use of the Chlamydia trachomatis sets (MicoTrak Specimen Direct Test -Biotech). The sample was fixed with ethanol. Afterwards 30 ml of Chlamydia trachomatis reactive agent was applied onto the slide and incubated in a moist chamber at room temperature for 15 min. Then the specimen was washed in deionized water. A drop of Mounting Fluid was added when dry, a cover slip was applied, and evaluation under a fluorescence microscope at a magnification of 40x was performed. The result was considered positive if the number of elementary cells in the preparation was greater than or equal to nine. Marking of genotypes of

HPV molecular assay was performed with the use of Inno-Lipa HPV Genotyping Extra CE method (company Innogenetics). It allows the detection of 28 genotypes of HPV, including 15 recognized as high-risk (16^{HR}, 18^{HR}, 31^{HR}, 33^{HR}, 35^{HR}, 39^{HR}, 45^{HR}, 51^{HR}, 52^{HR}, 56^{HR}, 58^{HR}, 59^{HR}, 68^{HR}, 73^{HR}, 82^{HR}), 7 low-risk (6^{LR}, 11^{LR}, 40^{LR}, 43^{LR}, 44^{LR}, 54^{LR}, 70^{LR}), 3 moderate (26^{pHR}, 53^{pHR}, 66^{pHR}) and 3 ambiguously classified HPV genotypes (69*, 71*, 74*). Swabs from the cervical canal were fixed on the substrate LBC, stored at below -20 ° C for up to one month to complete the evaluation. Sets QIAmp DNA Mini Kit from QIAGEN was used for HPV DNA isolation, according to the manufacturer's protocol test. HPV L1 gene fragment was amplified by using specific primers SPF 10. HLA-DPB1 gene was used as a control in the process of isolation (multiplex PCR). Biotinylated amplicon was subjected to denaturation and hybridization with specific sequences for HPV plotted linearly on a nitrocellulose membrane. The next steps in the process of hybridization included flushing with rinsing solutions—a solution of streptavidin and alkaline phosphatase. In the presence of chromogen BCIP/NBT a linear system of bands was visualized, enabling the identification of individual virus genotypes. Tests were read using a table for typing (the INNO-LIPA HPV Genotyping Extra CE Interpretation Chart).

Statistical analysis was performed using STATISTICAL software v 6.1. In the analysis of the parameters expressed in nominal scale Chi square test was used. In order to assess the relationship between characteristics, Spearman correlation coefficient was calculated. The level of $p \leq 0.05$ was considered as a statistically significant difference. Odds ratios were determined for individual risk factors.

3. Results

HPV infection was found in 52 (56.52%) of 92 women tested. Oncogenic type infection was detected in 40.4%. In 22.8% of teenagers, infection was caused by more than one type of HPV. Most frequently reported type of virus was a type 51^{HR} (14% of respondents), 16^{HR} (13%) and 31^{HR} (10.87%). Type 52^{pHR} and 53^{pHR} was found in about 6% of respondents, while 18^{HR} and indeterminate type in more than 4% of young women (Table 1). Relevance HPV infection of the analyzed risk factors are shown in Table 2.

Nearly 10% (5 women) infected with HPV presented abnormalities in cytology in the form of ASCUS or LSIL, additionally in 3 patients (60%) 2 or more HPV types were detected. In HPV-negative women, all Pap tests were normal. The risk of changes in the form of ASCUS was almost twice as high in women infected with HPV and nearly fivefold in those infected by more than 2 types of HPV, in the case of a LSIL, respectively 2.7 and 9.5 times. The risk of HPV infection was almost twice as high in those women whose age at sexual initiation did not exceed 16 years, and quadrupled in the infected by more than 2 types of HPV, although it was not significant statistically. There was a significant fivefold increase in risk of HPV infection in patients, who in the survey indicated 5 or more partners. In these women the risk of infection by more than 2 types of HPV increases 9.5 times.

Infection of the vagina and cervix in women was determined on the basis of clinical symptoms, colposcopic examination, assessment of vaginal biocenosis, as well as evaluation of the samples screened towards

Table 1. The prevalence of HPV infection in the studied population (n = 92) with regard to particular types of virus.

HPV INFECTION										
TYPE	TOTAL	HPV 16 ^{HR}	HPV 18 ^{HR}	HPV 31 ^{HR}	HPV 33 ^{HR}	HPV 39 ^{HR}	HPV 45 ^{HR}	HPV 51 ^{HR}	HPV 52 ^{HR}	HPV 56 ^{HR}
N	52	12	4	10	4	6	1	13	6	3
%	56,52%	13,04%	4,35%	10,87%	4,35%	6,52%	1,09%	14,13%	6,52%	3,26%
TYPE	UNSPECIFIED	HPV 58 ^{HR}	HPV 68 ^{HR}	HPV 73 ^{HR}	HPV 6 ^{LR}	HPV 11 ^{LR}	HPV 54 ^{LR}	HPV 70 ^{LR}	HPV 53 ^{pHR}	HPV 66 ^{pHR}
N	4	1	1	1	3	1	1	3	5	1
%	4,35%	1,09%	1,09%	1,09%	3,26%	1,09%	1,09%	3,26%	5,43%	1,09%
LACK OF HPV INFECTION										
N	40									
%	43.48									

HR – High-Risk Human Papillomavirus

LR – Low-Risk Human Papillomavirus

pHR – possibly high-risk Human Papillomavirus

UNSPECIFIED – unknown genotype of undetermined significance in cervical cancer.

Table 2. The relationship between human papilloma virus infection and the other studied risk factors for cervical cancer.

RISK FACTORS	HPV (+) n=52 n (%)	HPV (-) n=40 n (%)	OR	95% CI		p
AGE OF SEXUAL INITIATION ≤16YEARS	33 (63.46%)	19 (47.50%)	1.92	0.83	4.44	0.128
NUMBER OF SEXUAL PARTNERS ≥ 5	11 (21.15%)	2 (5.00%)	5.10	1.06	24.50	0.042
ORAL CONTRACEPTIVES	12 (23.08%)	26 (65.00%)	0.56	0.22	1.39	0.211
CONDOM USE	26 (50.00%)	24 (60.00%)	0.67	0.29	1.54	0.341
LOW SOCIOECONOMIC STATUS	2 (3.85%)	1 (2.50%)	0.43	0.18	1.47	0.071
SMOKING	26 (50.00%)	22 (55.00%)	0.82	0.36	1.87	0.634
BACTERIAL INFECTION	15 (28.85%)	7 (17.50%)	1.71	0.59	5.01	0.325
CANDIDIASIS	4 (7.69%)	3 (7.50%)	1.03	0.22	4.88	0.972
ASCUS	2 (3.85%)	0 (0%)	1.80	0.43	111.99	0.492
LSIL	3 (5.77%)	0 (0%)	2.70	0.64	149.46	0.243
COLPOSCOPIC CHANGES	11 (21.15%)	11 (27.50%)	0.71	0.27	1.85	0.479
CHLAMYDIA INFECTION	1 (1.92%)	0 (0.00%)	-	-	-	-
HPV INFECTION ≥ 2 TYPES (n=21)						
RISK FACTORS	n	%	OR	95% CI		p
AGE OF SEXUAL INITIATION ≤16YEARS	5	23.81%	4.15	0.46	37.02	0.203
NUMBER OF SEXUAL PARTNERS ≥ 5	2	9.52%	9.5	1.04	86.97%	0.046
ORAL CONTRACEPTIVES	1	4.76%	0.49	0.05	4.39	0.522
CONDOM USE	2	9.52%	0.40	0.07	2.28	0.299
LOW SOCIOECONOMIC STATUS	0	0.00%	0.90	0.05	16.78	0.585
SMOKING	2	9.52%	0.43	0.08	2.50	0.351
BACTERIAL INFECTION	2	9.52%	4.40	0.38	51.40	0.237
CANDIDIASIS	0	0.00%	0.90	0.04	8.08	0.467
ASCUS	1	4.76%	4.43	0.25	79.73	0.313
LSIL	2	9.52%	8.86	6.70	111.94	0.092
COLPOSCOPIC CHANGES	1	4.76%	0.62	0.07	5.60	0.670

Chlamydia trachomatis infection. The risk of HPV infection was almost twice as high in women with bacterial infection of the vagina and four times greater in women infected with more than two HPV types, although not statistically significant. There was no correlation between HPV infection and vaginal candidiasis. Chlamydia trachomatis infection was diagnosed only in one patient infected with HPV.

There was no correlation between HPV infection and low socioeconomic status, hormonal contraception, use of condoms as a means of preventing pregnancy and smoking.

4. Discussion

The most important risk factors for cervical cancer in young women are the early initiation of sexual life, multiple sexual partners and infection with human papilloma virus, often simultaneously with many of its types

[13-17]. Confortini reports that almost 30% of women aged 18-24 years are infected with some type of human papilloma virus, while 19.3% are carriers of the oncogenic type [13]. Similar data are reported by Forhan after examining 838 teenagers aged from 14 to 19 years. Among those young women who began having sex, HPV infection was found in 29.5% [18]. In Papachristou studies, HPV infection was found in 49.1% of surveyed women, and 30.3% of them had an oncogenic type [19]. The greatest number of infected (68.4%) is given by Shikary, after examining 409 teenagers aged 13 to 26 years old. 59.5% of them had one or more types of high-risk HPV [20]. In the available literature, the authors indicate the percentage of infected patients varies from 19% to almost 70%. In each work, however, the number of women infected with high-risk type of HPV outweighs low-risk type [13,18-21]. In our study, the level of HPV-infected women is 56.52%. The percentage of carriers of oncogenic type of virus reaches 40.4% and 22.8% of teenagers are infected with more than one type of

HPV. These results are similar to those obtained by Papachristou and Menegazzi [19,22].

Similar results for the most common types of human papilloma virus detected in young women can be found in the literature worldwide. Lenselink reports that the type 16 (2.8% of respondents) was the most common type of virus, as well as the type 51 and 52 (2.5% of respondents), while 3.5% of cases had an undetermined type of infection [21]. In another work domination of types 16 and 6 in the studied population (ca.12%) was recorded, followed by types 51, 31, 66 and 53 (approximately 7-8%) [22]. Uusküla found type 16 (which is the most common type of HPV) infection in 6.4% and type 53 and 61 in approximately 4.5% of studied group respectively [23]. Confortini says that taking into account the high-risk type of virus among the surveyed women, the dominant type was 16 (8.53%), less frequently type 31 and 56 (2.44%), type 51 (2.08%) and 18 (1.88%), whereas among low-risk types type 6 and 11 were dominant (about 4%) [13]. Similarly, in our study population of young women, the dominant types were: 51 (14%), 16 (13%) and 31 (10.87%).

In our study, HPV infection has been associated with changes in cytology, although it was not statistically significant. HPV infection was found in all patients with ASCUS and LSIL abnormalities, whereas in 60% of cases women were infected with more than two types of HPV. In all HPV-negative women, Pap tests were normal. Similar results were obtained by other researchers. Boardman showed HPV infection in 80% of patients with ASCUS [24]. Wright showed the presence of HPV in 73.8% of women aged 25 years with cytological results: ASCUS ($p=0.002$) [25], while Allen in his study found HPV infection in 94.9% of patients with ASCUS [26]. González has shown that HPV infection five-fold increases the risk of intraepithelial neoplasia [27]. A similar association is shown in other studies [28,29]. The results show that changes in cytology in the form of ASCUS or LSIL are strongly associated with human papillomavirus infection.

Analysis of one of the best known risk factors affecting the development of various cancers (smoking) showed, that infection occurring in smokers is related with high-risk types of human papilloma virus [30]. Sarian et al provided data that infection caused by high-risk type of HPV occur statistically significantly more frequently in compulsive tobacco smokers (21.7%) compared to non-smokers (16.5%) or former smokers (13.5%) [31]. Silva, after examining nearly 70,000 women aged 14 to 79 years showed that the risk of human papillomavirus infection is twice as high in patients who smoke [32]. Similarly, the two fold increase in risk of HPV infection in the case of nicotine addiction has been reported by

Confortini among women aged 18 to 24 [13]. Also, other studies have linked smoking with HPV infection [33,34]. In our study, the relationship between cigarette smoking and HPV infection was not confirmed. Patients who reported themselves as non-smokers were infected with HPV slightly more frequent than smokers (50% vs 55%).

Literature data on HPV infection and the early age of sexual initiation is ambiguous. Some studies show that the very early age of sexual initiation is not an independent risk factor for cervical cancer [14,21] or the risk is slightly elevated (on the border of statistical significance [32]). Most of the work, however, significantly stresses the influence of early initiation of sexual activity with an HPV infection [13,15,17,35]. Our study confirms the relationship between the early start of sexual activity (under 16 years of age) and HPV infection. It was shown that the risk of HPV infection nearly doubles, and increases fourfold in women in whom two or more types of HPV were detected. Due to fact that this relationship is not statistically significant, it can be concluded that the age of starting sexual activity is not an isolated risk factor for HPV infection.

The number of sexual partners in life clearly is associated with the risk of HPV infection. This confirms our study (OR=5.10, $p=0.042$), and many other published works on this subject [13,16,17,36-38]. Moscicki says that a large number of sexual partners is an independent risk factor for HPV infection and cervical cancer. He says that over 20% of patients who give a history of one partner are infected with human papilloma virus, while 69% of women having 10 or more partners are infected [7]. Confortini showed more than a tenfold increase in risk of HPV infection in patients reporting having 5 or more sexual partners [13]. Forhan reported already more than fourfold increased risk of HPV infection in the case of 3 partners [18]. Uusküla showed a similar association between the number of sexual partners and infection with human papilloma virus in a group of 845 women (almost threefold) [23]. Menéndez also highlights more than a threefold increased risk of HPV infection associated with many sexual partners [39]. Other studies also indicate an increased risk of infection from 2 to 8 times in case of multiple sexual partners [14,20,32]. An increased number of sexual partners is also connected with an increased risk of infection by more than one type of HPV. Soto-De Leon's research [15] and our own point to it (OR=9.5, $p=0.046$).

Our study demonstrated that the risk of HPV infection was almost twice as high in women with bacterial infection of the vagina, although statistically insignificant. Lippman showed a significant correlation between changes in normal vaginal biocenosis and HPV infection [40]. Confortini in turn indicates no statistically

significant association between infection and genital HPV infection [13].

Reviewing the world literature in terms of what kind of contraception is used, there are works that qualify the hormonal contraception to the group of the risk factors of HPV infection and cervical cancer and those that exclude this opinion. Porras indicates a nearly two fold increase in the risk of HPV infection in women using hormonal contraception, but observed no statistical relationship between condom use and infection with human papilloma virus [14]. Silva points to an increased risk of HPV infection at a similar level as Porras in women taking hormone contraceptives. He also recorded no statistically significant dependence in relation to the use of condoms and their protective action in relation to HPV [32]. Cotton, however, claims that the risk of infection with human papilloma virus is increasing among women using hormonal contraception [33]. Lensenlink undermines this opinion. His research indicates that use of hormonal contraception is not a risk factor for HPV infection. He also mentions the use of condoms, which are neither a protective nor a risk factor for HPV infection [21]. Confortini found no significant relationship between HPV infection and the use of hormonal contraception, but showed a protective role of condom use in women who use it every time, or in most cases [13]. Similar results were obtained by other authors exploring the relationship between HPV infection and the use of hormonal contraception [34,36,41,42]. In our study did

not show connection of HPV infection with the use of hormonal contraception and condoms.

Another factor taken into account that initiates the occurrence of cervical cancer is low socioeconomic status. Data was found in the literature both supporting and contradicting the hypothesis that low socioeconomic status is an important risk factor for HPV infection and HPV-dependent cancer. Flores says that in her studies, 57.4% of HPV-positive women in an interview reported low economic status, while 33% a high economic status and women indicated a protective effect of high material status in relation to low [43]. In an analysis of the association of socioeconomic status with HPV-dependent cancer, Benard reported nearly fifteen times greater risk of cervical cancer in women with low financial status compared to women with medium and high socioeconomic one [44]. Our study found no relationship between HPV infection and socioeconomic status.

In conclusion, it should be emphasized that only a large number of sexual partners was statistically significant as an isolated risk factor for HPV infection and thus cervical cancer in young women in the Polish population. Other risk factors were: age of sexual initiation 16 years or less, changes in cytology in form of ASCUS or LSIL, and bacterial infection in the reproductive tract. No correlation was found between HPV infection and low socioeconomic status, history of smoking, use of hormonal contraception and condoms.

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