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Cent. Eur. J. Med. • 6(4) • 2011 • 456-462
DOI: 10.2478/s11536-011-0042-0

Central European Journal of **Medicine**

High-risk hpv infection in sexually active and non active young women

Research Article

Zana Bumbuliene[1*], Jonas Alisauskas[2], Agne Sepetiene[2]

[1] *Vilnius University, Faculty of Medicine, Clinic of Obstetrics and Gynecology, LT- 10207 Vilnius, Lithuania*

[2] *Vilnius University Hospital Santariskiu Klinikos, LT-08662 Vilnius, Lithuania*

Received 22 January 2011; Accepted 26 April 2011

Abstract: To determine the prevalence of high-risk HPV infection before first vaginal intercourse and after initiation of sexual activity in 14-22 years old girls, to find which HPV types occur most commonly. During the gynecologic exam cervical swabs were collected. Specimens were tested using Hybrid Capture 2 DNA test. In positive samples digene HPV genotyping test was performed. Subjects included 264 women, of whom 169 (64.0%) reported previous sexual activity and 95 (36.0%) had not yet had vaginal intercourse. There were 43 cases (16.3%) of high risk HPV, with 39 cases in those reporting sexual activity and 4 in virgins. Single strain HPV infection was detected in 62.8%, two strains in 23.2%, and three strains in 14.0% of subjects. HPV-16 was the most common type (20.9%), next more prevalent types were 18, 56, 31, 33 and 59. HPV-16 or HPV-18 were detected in 39.5% of the subjects. Not a single case was identified containing both types. The prevalence of high-risk HPV is common in sexually active young Lithuanian women. HPV were found in a small percentage of girls who had never had vaginal intercourse. The prevalence of types 16 and 18 among Lithuanian women was higher than in international data.

Keywords: *High-risk HPV prevalence • Virgins • Young women*

1. Introduction

Human Papillomavirus is one of the most widespread viral sexually transmitted infection (STI). HPV infects epithelial tissue of the skin and genital mucosae. Approximately 30 different types of HPV target the anogenital tract.

In 2005 the International Agency for Research on Cancer (IARC; Lyon, France) reassessed the carcinogenicity of Human Papillomaviruses. HPV strains was classified by their oncogenic risk into: high-risk (15 types), possible high-risk (3 types), low-risk (12 types), and not specified risk (3 types). The group of cervical carcinogens was expanded to include the following 15 high-risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82. HPV types 26, 53, 66 were included in the group of possible high-risk types [1].

Persistent infection with oncogenic HPV is a precondition of cervical cancer. Approximately 50% of cervical cancers worldwide are associated with HPV type 16 and nearly 20% with type 18. The next most common oncogenic HPV types are 45, 31, 33, 35, 52 and 58 [2,3].

HPV is transmitted by skin-to-skin contact. HPV requires access to basal cells through micro lesions in the epithelium that are often produced during sexual activity. HPV infection has been detected in women who have reported never having sexual intercourse with men [4]. Several studies have shown that HPV can be transmitted during non-intercourse foreplay through the use of fingers or sex toys [5-7].

Adolescent girls are at especially high risk for STIs for both social and biologic reasons. Socially, female adolescents often lack social power and skills to refuse sexual activity or demand safer sex. Early sexual debut, multiple partners, and no contraception use are common high risk behaviours in young people.

* *E-mail: zana.bumbuliene@yahoo.com*

Biological reasons: during puberty columnar epithelium gradually transforms into squamous: large areas of transitional squamous, glandular and metaplastic cells are formed, all of which support HPV replication [5].

In 2008, Smith JS et al. summarized worldwide data on genital HPV-DNA prevalence in women. Meta-analysis show that the peak of high prevalence of HPV is in younger women less than 25 years of age [8].

In Lithuania cervical cancer is one of the most common cancers. Only some studies examined the prevalence of HPV in Lithuanian women. We didn't know the prevalence of high-risk HPV infection in Lithuanian adolescents. Is it possible to have high-risk HPV before the first penetrative intercourse?

The objectives of our study were to determine the prevalence of high-risk HPV infection in sexually active 14-22 years old Lithuanian females, to assign the possibility of high-risk HPV infection in adolescents before the first vaginal intercourse, and to find which high-risk HPV types are frequent in the Lithuanian young women population, whether single or multiple infections predominate, as well as the prevalence of types 16 and 18.

2. Material and Methods

All girls aged 14-22 years attending Vilnius city outpatient clinics (Santariskiu and Seskines) for gynaecologic consultation or counselling were asked to take part in the prospective study. 264 of the women agreed. Inclusion criteria: asymptomatic sexually active women and virgins aged 14-22 years, whose visitation was related to gynecological pathology (e.g. menstrual disorders) or for prophylactic consultation, counseling. Exclusion criteria: girls with symptoms of infection or inflammation and women older than 23 years. Virginal status was ascertained in two ways: by self-report and hymen evaluation, assessed during gynecological exam.

Approval and informed consent were obtained from the patients before their participation. If a teenager was younger than 18, a signed agreement of one of her parents was compulsory, usually it was the mother who had came together.

During the visit a gynaecological examination was performed, cervical swabs for high risk HPV infection were collected. Gynaecological exam was done according to standard ACOG guidelines in the sexually active girls group. During the examination, materials for HPV consideration were gathered by a special brush from the cervix. For teenagers who self reported as virgins, the Huffman speculum was used. The cervix was not always clearly seen due to hymenal anatomy in the younger patients or those who were not tampon users or sexually active. Therefore, swabs for HPV were not always gathered from the os of the cervix. Sometimes swabs for HPV were taken from the upper vaginal third. Special collectors, softer than usual brushes were used for gathering samples from those who reported as virgins.

2.1. HPV DNA detection and genotyping

Vials with the testing material for HPV detection came to the laboratory, where high-risk HPV was detected using the molecular hybridization method (digene hybrid capture 2 version).

During the hybridization reaction the detected papilloma virus DNA signal is amplified *in vitro*. The reaction mixture contains a circular RNA probe, which is complementary to the whole papilloma virus genome. The formed RNA:DNA hybrid is recognized by the specific antibodies. Secondary antibodies, conjugated with alkaline phosphatase, specifically react with RNA:DNA:antibody hybrids. After adding a substrate to the reaction a discernible chemiluminescence signal is measured. The detected HPV signal can be multiplied up to 3000 times. Using this methodics a qualitive detection of the 13 high risk HPV viruses (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) DNA can be made in the sample.

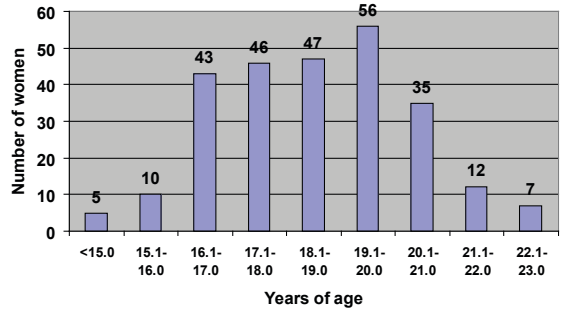
The clinical sensitivity of the molecular hybridization method is 5000 virus copies/reactions. The RNA probe corresponds to the whole papilloma virus genome and with genome deletions specifically detects the other part of the virus genome and hybridizates, in that case a possibility for a falsely negative result is reduced.

When high-risk HPV DNA was detected a further typing with digene HPV genotyping RH test was performed (Qiagen reagents) in order to detect which one of the high risk type a particular patient is infected with. This test provides reliable detection and differentiation of 18 individual HPV types, including 15 high-risk and 3 possibly high-risk types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82.

Identification of HPV genotypes is based on a reverse hybridization procedure. Denatured biotinylated amplicons, resulting from amplification of part of the L1 region, are hybridized with specific oligonucleotide probes, which are immobilized as parallel lines on membrane strips. After hybridization and stringent washing, HPV types are detected by a chromogenic reaction. Positive results show as a purple line on the test strip and can be easily interpreted visually.

Digene HPV genotyping RH test covers a bigger amount of HPV types in comparison with molecular

Figure 1. The age of enrolled subjects.



hybridization method: during typing HPV DNA high-risk types 73, 82 and possibly high-risk types 26, 53, 66 are present in addition.

2.2. Statistical methods

Analyzing data, means of the searching quantity parameters and standard deviations (SD), minimal and maximal values and the proportions of the quality features expressed in percents, were calculated. Comparing groups together, the significance of the differences between means of the respective parameters was evaluated by the Student's t-test, the difference was considered statistically significant when the bias likelihood was $p<0.05$. Comparing quality indicators the differences were evaluated using χ^2-test. The statistic data analysis was done using Microsoft Excel program.

The study protocol and informed consent forms were approved by the Lithuanian Bioethics Committee (2008.01.17; No3).

3. Results

The study population 264 young women from 14 to 22 years of age (mean age ± standard deviation: 18.6 ± 1.7 years), who were enrolled to the study during 2008. Most of them (87.0%) were 16-20 years old. (Figure 1).

Girls were divided into groups based on sexual activity. Group I consisted of 169 sexually active girls examined in Santariskiu and Seskines outpatient clinics (64.0% of subjects), mean age for sexually active participants was 19.3. Group II consisted of 95 self-reported virgins (36.0%) examined in Santariskiu outpatient clinic, mean age for girls started they had not had prior intercourse – 17.5 years. However in both groups were girls of various age, e.g. younger than 15 years or older than 21.

Prevalence of high-risk HPV types in asymptomatic sexually active and non active young females was 16.3%. High-risk HPV was detected in 39 of the 169 sexually active participants (23.1%) and in 4 cases (4.2%), among 95 non sexually active adolescents. The difference was statistically significant ($p<0,001$).

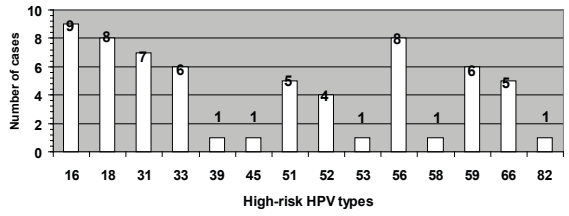
Among 43 HPV positive young women, one to three of 13 identifiable high-risk types were detected. The prevalence of single high-risk HPV infection was 27 cases (62.8%), double – 10 cases (23.2%) and in 6 cases (14.0%) triple infection.

In the entire study population 14 high-risk types from 18 possible were determined. HPV-16 was the most common type detected in 9 cases (20.9%) either as a single type or in combination with other types. Next more prevalent types were HPV-18 and HPV-56 (8/43 each), HPV-31 (7/43), HPV-33 and HPV-59 (6/43 each). (Figure 2) The vaccine HPV types 16 or 18 were

Table 1. Single high-risk HPV infection in sexually active and non active subjects.

High-risk HPV types	Sexually active (n=169) n	Non sexually active (n=95) n	Overall n=264) n
31	4	0	4
51	2	2	4
16	3	0	3
52	3	0	3
59	3	0	3
18	2	0	2
66	2	0	2
33	1	0	1
39	1	0	1
53	0	1	1
56	1	0	1
X-not identified	1	1	2
Total	23	4	27

Figure 2. Prevalence of high-risk HPV types according to a number of cases.



detected in 17 girls (39.5%).

The most prevalent high-risk types in single infection were HPV-31 and HPV-51. In virgins it was found only single high-risk type for each adolescent: in two cases it was estimated 51 type, in one case – 53 and for one patient – not identified type (Table 1).

The multiple high-risk HPV infection was found only in sexually active participants. The most prevalent high-risk types in multiple infections were HPV-56, followed by HPV-16 and HPV-18. Not a single case was identified when vaccine types 16 and 18 were detected together. When double high risk infections were detected, pairs of the virus types were not repeated. Only in one case of the triple infection high risk types 16, 33, 56 repeated. (Table 2).

Table 2. Multiple high-risk HPV infections in sexually active subjects.

	High-risk HPV types	Sexually active n
Double	16 / 33	1
	16 / 51	1
	16 / 56	1
	18 / 31	1
	18 / 33	1
	18 / 56	1
	18 / 59	1
	18 / 82	1
	52 / 66	1
	56 / 58	1
	Total	10
Triple	16 / 33 / 56	2
	16 / 56 / 66	1
	18 / 31 / 59	1
	31 / 59 / 66	1
	33 / 45 / 56	1
	Total	6

4. Discussion

Prevalence of high-risk HPV types in asymptomatic sexually active and non active Lithuanian females aged 14 to 22 years was 16.3%. It is difficult to compare our findings with the majority of investigations because they have been restricted to sexually active women or with small proportion of non sexually active girls.

In a South Korea study, 60% of the female students reported that they were virgins, the prevalence of HPV infection in sexually active and non-active students was 15.2%, high-risk types were predominant (69%) [9]. In our study the rate of high-risk HPV seems to be a little bigger, but less percent of participants (36%) were non sexually active. On the other side we used different methods for the collection of specimens.

Prevalence of high-risk HPV infection in asymptomatic sexually active Lithuanian females was 23.1%. Our results were comparable to the findings of the other Lithuanian studies: the number of infected teenagers who are sexually active met a general population-based number – 25.1% [10].

Z.Gudleviciene evaluated the prevalence of HPV in Lithuanian cervical cancer patients – 92.0% and in control women – 23.6%. This study included overall HPV types – high-risk and low-risk, females were older than 20 years [11].

In Shanghai, China high-risk HPV prevalence in sexually active study participants was 29.1%, the rate was highest 54.4% in women aged 20 or younger [12]. Tarkowski et al. detected HPV in 64% of 312 adolescent females, more than half of them had multiple HPV infection [13]. In 2010 Stamataki et al. detected prevalence of HPV DNA in Greek females aged 16 to 45 year, the highest prevalence 57.1% was found among women aged 16-20 years [14].

Students who were HPV negative, and reported never having sexual intercourse at enrollment, 32.3% acquired HPV within 12 months after initiating intercourse and more than 50% became HPV positive within four years [15]. HPV prevalence has been found to be highest among young females within the first few years after sexual debut. Moscicki et al. found prevalence rates of 54.5% for all genital HPV types and 29.1% for high-risk types in a cross-sectional study for adolescent females. Although HPV infection is common, studies suggest approximately 90% of infections clear within 2 years [5,16].

Across all geographical regions, data on HPV prevalence were generally limited to women over 18 years of age. Most of the studies evaluated overall (low and high risk together) HPV determination. Although prevalence of HPV in various geographical regions is different, in adolescents (10-19 years of age) and young adults (20-24 years of age) it is the most prevalent and varies from 20% to more than 60% [8,12,13,16-18].

Worldwide variations in HPV prevalence in young females appear to largely reflect differences in sexual behavior.

The pathways of HPV infection are sexual and non-intercourse (contact or perinatal). Generally HPV infection is transmitted by skin-to-skin contact during sexual vaginal intercourse. A number of studies, however, have suggested that non-sexually transmission may occur, HPV infection could be transmitted by finger-genital contact. Transmission between the hands and genital, as well as apparent self-inoculation events (primarily in men) were also observed [6,7,19]. The possibility of hand transmission is suggested by the reports of children with anogenital warts who were considered to have acquired their infection from close family members or by their own hand warts. But this study applies to the cases with warts caused by low-risk HPV infection [20,21].

Prevalence of high-risk HPV infection in non sexually active Lithuanian girls was 4.2%. Our results were similar to the findings of the above-mentioned South Korea study in virgins female students with overall HPV prevalence 4.7%, proportion of high-risk types 75%. HPV-16 was not identified in virgins, but HPV-18 was found in 2 cases. Multiple HPV infection was rare (12.5%) [9].

Several studies had included self-reported virgins, but in a low proportion. In 1994 Rylander et al. found HPV infection in 1.8% of virginal women, the samples were collected from cervical [22]. In 2002 Winer et al. study detection of HPV in genital samples from virginal women was 1.7% [15]. In 2007, epidemiological data from United States Survey showed that overall HPV infection was detected in 5.2% of females who reported never having had sex [17]. All these studies, as well as our, confirm possibility of high-risk HPV infection in virgins and support the existence of alternative routes of transmission. HPV is transmitted through genital skin to genital skin sexual contact and penetrative intercourse is not required.

In our study 62.8% young women were infected by single HPV infection and 37.2% by multiple HPV infection. Our results were similar with the Lithuanian Medical Research Centre “Sorpo” data. They analyzed 174 high-risk HPV positive samples and 33.3% of it had more than one type: 27% of women was infected by two types, 4.6% by three types, and the rest had 4 or 6 high-risk types [23].

In 2005 Brown et al. detected high-risk HPV infection in 38.6% of adolescent women, aged 14-17. The mean number of HPV types was 4.9 per HPV-positive subject.Multiple HPV types were detected in 81.6% of individuals. The most frequently detected high-risk types were HPV-52 (38%), HPV-16 (31%), and HPV-59 (23%). They hypothesize that maybe HPV-52 preferentially infects younger women [24].

In our study the proportion of multiple infections was lower but quite significant, HPV-52 was found only in 9.3% (4/43) of infected females. The most prevalent types were HPV-16, HPV-18 and HPV-56. Possible high-risk HPV-53 type was observed only as single infection and only for virgins. This type has not been found and in medical research center “Sorpo” laboratory, where sexually active women of various age were tested.

Nearly 100% of all cervical squamous cell carcinomas and more than 70% of adenocarcinomas are associated with papillomavirus DNA. HPV 16 type is the most frequent in cervical squamous cell carcinomas, high-risk 18 and 45 types in cervical adenocarcinoma [25]. According to our research vaccine HPV types 16 or 18 were detected in 39.5% of subjects, non of them was found in virgins, so the prevalence of vaccine HPV types among sexually active women are higher and reached 43.6%. According to Lithuanian „Sorpo“ study results the types 16 and 18 were found in 51.7% cases [23]. De Sanjose S. et al. reported that around 291 million women worldwide are carriers of HPV DNA, of whom 32% are infected with HPV16 or HPV18, or both [26].

Our study has some limitations. Noncoitally active females may have been sexually active in other ways. We asked them and turn our attention only to vaginal sexual intercourse.

As it is mentioned above during hybridization it is possible to find 13 high risk HPV types. The *Genotyping RH* test identifies one high risk type from 18. HPV types 26, 53, 66, 73 and 82 are absent in hybridization, they are determined during typing. HPV-53 and HPV-66 HPV determined during typing were found by one, although the circular RNA probe should not record those cases. This might be due to non specific circular RNA sond’s reaction with these HPV types.

Although typing covers a bigger amount of high risk types in comparison with molecular hybridization method, in two cases the HPV type found during hybridization was not determined during genotyping. It is thought to be connected with the inhibition of Taq polymerase during amplification, or HPV DNA deletions, involving L1 gene, which might occur during cell infection.

In conclusion, the prevalence of high-risk HPV infection is common in Lithuanian sexually active adolescent and young women. Our research confirm that human papillomavirus infection is predominantly acquired in adolescence. High-risk HPV in a small percentage is found in virgins and support the existence of alternative modes of HPV transmission. Approximately one-third of the enrolled females had multiple infection.

The prevalence of the most aggressive HPV types 16 and 18 among young Lithuanian women was higher than in international data, so expected benefits and the effect of vaccines in Lithuania are respectively higher and might help to reduce the risk of cervical cancer in our country.

Acknowledgments

We appreciate the support of the Lithuanian State Science and Studies Foundation in this study (contract with the Lithuanian Sciences and Study Foundation, No. T–95/08).

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