Pelvic inflammatory disease is a risk factor for cervical cancer

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Summary

The acquisition of human papillomavirus (HPV), the most important etiological agent of cervical cancer, does not cause clinical complaints. Although HPV spreads together with agents causing pelvic inflammatory disease (PID) with complaints forcing the patient to seek medical advice, PID has not yet been evaluated as a predictor of cervical cancer. The present study aimed to determine the relationship between PID and HPV in order to evaluate the possible risk factor role of PID for cervical cancer. Two groups of patients were studied: (i) 2,215 women with PID; (ii) 4,217 women participating in a cervical cancer screening programme who were found to have cytological atypia, mucopurulent cervicitis or other colposcopically detected disorders but were free of symptoms of PID. The presence of HPV and other STD agents in cervical smears was detected with polymerase-chain reaction. HPV prevalence was 33.74% in patients with PID and 26.40% in the group of women without PID ($p < 0.001$). This suggests that patients suffering from PID apparently have a higher risk of cervical cancer.

Key words: Human papillomaviruses; Pelvic inflammatory disease; Cervical cancer; Polymerase chain reaction.

Introduction

The prevalence of infectious diseases in Hungary is fairly low. The mortality rates are close to the average level detected in the countries of the European Union (EU) [1]. The approaches, which were successful in achieving the favourable epidemiological status in the past, have become more and more insufficient due to the recent increasing trend of international migration, the changing sexual behaviour and many other social and economic factors. To maintain the effectiveness of control, the applied methods should be improved, especially in cases of sexually transmitted disease (STD), since the incidence of STDs has been gradually increasing during the last two decades in Hungary. Urgent interventions in prevention, detection and treatment of STDs are required.

The existing public health strategy of the European Parliament and the Council of the EU focuses on eight specific programmes including STDs and other communicable diseases, demonstrating clearly that the current operating systems must be adopted to the new challenges [2]. Hungary, a Central-Eastern European country, might soon join the EU so the present and future public health situation and problems in Hungary are expected to have an increasing significance for those of the EU. The need for continuous improvement is also emphasised by the fact that the incidence of STDs in some immediate eastern neighbour-countries to Hungary has been continuously increasing in recent years.

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The development of primary prevention for STDs (limitation of spreading of infectious agents) can be achieved by programmes to rationalise the sexual behaviour. The effectiveness of these interventions is highly dependent on the adaptation of the basic methods to the special needs of high risk groups. To optimise the methods, detailed data on occurrence of infective agents are obviously required [3]. Unfortunately, these data are scarcely available in Hungary.

One of the sexually transmitted agents, human papillomavirus (HPV) is the most important etiological agent of cervical cancer [4-6]. The standardised death rate for cervix cancer in Hungary is unacceptably higher than that in the EU, indicating a need to improve the effectiveness of the existing screening programmes. The prevalence of HPV infection and the risk distribution of cervical cancer in different subpopulations are not known. The lack of these epidemiological data hampers the improvement of screening programme organisation, since the specific needs of the subgroup can not be considered. Another consequence of this problem is that the women can not be informed about their specific risk and this is partly responsible for their low-level motivation to participate in screening [7].

Acquired HPV does not cause complaints, thus the hidden nature of the development of cancer prevents a visit to the gynaecologist in proper time. On the other hand, HPV spreads together with STD agents that can cause symptoms urging a visit to outpatient services. Pelvic inflammatory disease (PID) can cause such symptoms. Because HPV is not a causative agent of this disease, the routine microbiological diagnosis does not

investigate its presence. In spite of the well established importance of mixed infections in eliciting PID [8] and the well known association between HPV and the spread of other sexually transmitted agents, PID has not yet been evaluated as a predictor of cervical cancer [9-13]. The present study aimed to determine the relationship between PID and HPV infection in order to evaluate the possible risk factor role of PID for cervical cancer and the possible application of PID in defining high-risk groups for cancer screening.

Patients and Methods

The study population consisted of two groups of women. The 2,215 patients with signs and symptoms of PID will be referred to as *Group 1*; they visited the Kenézy Hospital in Debrecen from 1999-2001. Diagnosis of PID was based on physical, clinical and laboratory findings. The clinical *minimum criteria* used to diagnose pelvic inflammatory disease were: lower abdominal tenderness, bilateral adnexal tenderness, cervical motion tenderness, no evidence of competing diagnosis, and negative pregnancy test. In order to avoid any unnecessary antibiotic treatment, in each case when this syndromic diagnosis seemed to be questionable, it was confirmed by additional or definitive criteria. *Additional criteria* were one or more of the following: fever (temperature at least 38.3°C), C-reactive protein (CRP) concentration > 20 mg/l, or erythrocyte sedimentation rate > 40 mm/h, leucocytosis (white blood cell count > $10x10^9$/l), abnormal cervical or vaginal discharge, evidence of *N. gonorrhoeae* or *C. trachomatis* in the endocervix, and purulent material from the peritoneal cavity by culdocentesis or laparoscopy. *Definitive criteria* for diagnosis of PID were: abdominal or transvaginal sonography showing thickened fluid-filled tubes with or without free pelvic fluid or tubo-ovarian complex, and laparoscopic abnormalities consistent with PID. Patients were excluded from the study who were pregnant, had received any antibiotic within 14 days, had undergone a bilateral tubal ligation, were younger than 15 years old, or were postmenopausal. Women with exacerbation of PID were not excluded.

Group 2 consisted of 4,217 women who presented at the outpatient services of the North-Eastern Region of the Great Hungarian Plain in 2000 as participants of a cervical cancer screening programme, and for whom microbiological investigation was indicated because of cytological atypia, mucopurulent cervicitis or colposcopically detected disorders. Patients with signs or symptoms of PID were excluded from this group.

Cervical samples were collected from 2,215 patients with PID (Group 1) and 4,217 women without PID (Group 2) with cytobrush and stabilised in transport solution. The samples were stored at -20°C until taken to the laboratory. DNA was extracted with the silica extraction method on a TECAN RSP-150 robotic system [14]. PCR was performed [15-17] to diagnose the presence of *Chlamydia trachomatis,* herpes simplex virus (HSV), HPV, *Mycoplasma genitalium, Neisseria gonorrhoeae,* and *Ureaplasma urealyticum.* PCR products were separated by agarose gel electrophoresis using 4% precast agarose gels containing 0.5 mg/ml ethidium bromide, visualised under UV on a transilluminator, and a Polaroid photo was taken for documentation. HPV-6 and HPV-11 were considered to belong to the low-risk group, the medium-risk group included HPV-31, HPV-33 and HPV-35, whereas HPV-16, HPV-18, HPV-45, HPV-52, HPV-56 and HPV-58 were considered to indicate high risk for cervical cancer.

Differences in infection prevalences between Group 1 and Group 2 were evaluated by the χ^2-test.

Results

In the cervical samples of our 2,215 patients with PID (Group 1) the most prevalent agent was HPV as it was detected in 33.74%. The frequency of detection was 22.40% for *Ureaplasma urealyticum*, 11.10% for *Chlamydia trachomatis*, 3.05% for *Mycoplasma genitalium*, 2.04% for *Neisseria gonorrhoeae* and 1.61% for HSV (Table 1).

In patients without signs or symptoms of PID (Group 2), similarly to Group 1, HPV was the most frequently detected agent, its frequency (26.40%) was significantly lower than in Group 1 ($p < 0.001$). The prevalence of *Ureaplasma urealyticum* was 18.69%. The occurrence of *Chlamydia trachomatís* showed a greater difference between the groups: it was 5.19% in women without PID, less than half of that observed among patients with PID. The prevalence of *Neisseria gonorrhoeae* was 1.32% in Group 2, as compared to 2.04% in Group 1. The prevalence of HSV was 0.92% and of *Mycoplasma genitalium* was 0.42% in Group 2. Although the difference observed for HSV was small, it was statistically significant ($p = 0.047$). The occurrence of all the other pathogens showed significant differences between Group 1 and Group 2, apart from *Neisseria gonorrhoeae* for which the difference was slightly above the limit of statistical significance ($p = 0.064$).

The age-distribution of women with cervical HPV-infection was found to be similar in both groups (patients with and without PID). The age distribution of patients with cervical HPV infection from both groups showed a peak of 32.2% at 20-24 years of age and almost 60% of the patients were between 20 and 29 years old (Figure 1).

Table 1. — *PCR detected prevalence of sexually transmitted pathogens in patients with and without pelvic inflammatory disease.*

	Patients with PID (n = 2,215)			Patients without PID (n = 4,217)			
Pathogen	No. of samples	No. of positive samples	Prevalence (%)	No. of samples	No. of positive samples	Prevalence (%)	p*
Human papillomavirus	2116	714	33.74	3977	1050	26.40	< 0.001
Ureaplasma urealyticum	1512	339	22.40	2483	464	18.69	0.004
Chlamydia trachomatis	1937	215	11.10	3834	199	5.19	< 0.001
Mycoplasma genitalium	1509	46	3.05	2645	11	0.42	< 0.001
Neisseria gonorrhoeae	1810	37	2.04	2431	32	1.32	0.064
Herpes simplex virus	492	24	1.61	2617	24	0.92	0.047

p*: χ^2 test for the differences between infection prevalences observed among women with and without PID.

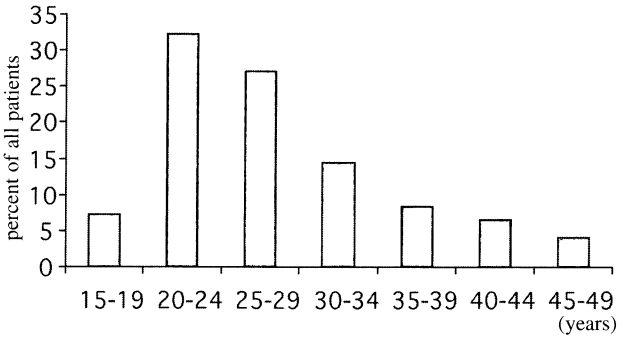

Figure 1. — Age distribution of all HPV-positive patients (100% = 1,764).

Medium- and high-risk HPV types were identified in 75.21% of HPV-positive patients if they suffered from PID, and in 66.86% if they did not suffer from this disease (Table 2); the difference was statistically significant ($p < 0.001$).

Table 2. — *Pathologic findings.*

Type of HPV	Women with PID		Women without PID		p*
	No. of cases	Prevalence (%)	No. of cases	Prevalence (%)	
Low-risk	177	24.79	348	33.14	< 0.001
Medium- and high-risk	537	75.21	702	66.86	< 0.001
Total	714	100.00	1050	100.00	

p*: χ^2 test for the differences of different HPV types between women with and without pelvic inflammatory disease.

Discussion

High-risk types of human papillomavirus (HPV) are known to be causative agents in the development of cervical intraepithelial neoplasia and, if the virus is not cleared, persistent high-risk HPV infection can lead to CIN 3 and finally cervical cancer [5]. HPV infection, however, usually does not cause symptoms forcing the patient to seek medical advice and, therefore, may remain undetected in the early stages of the disease. HPV is mostly sexually transmitted, and its spread may be associated with the transmission of other agents that can cause pelvic inflammatory disease (PID) with complaints serious enough to force the patient to visit the gynaecologist. It has been demonstrated that CIN occurs more frequently in women treated for PID [9]: our present study was aimed to detect a possible association of PID with cervical HPV infection.

In addition to HPV, the prevalence of *C. trachomatis* and four other sexually transmitted agents, listed in Table 1, were studied in patients with and without PID. These findings, together with the age-distribution of patients (Figure 1), corroborate the validity of patient selection.

In patients with PID the prevalence of cervical HPV infection was found to be significantly higher than in patients without PID. This finding was expected, since sexual behaviour is an epidemiological factor for both PID [18] and cervical HPV infection [19-21]. The prevalence of medium- and high-risk HPV types in women with PID was found to be significantly higher than in those who had no signs or symptoms of PID ($p < 0.001$).

Although we did not study the persistence of high-risk HPV types in our patients, it is reasonable to suppose that the duration of the persistence was similar in the two groups. Therefore, the probability of developing of CIN or cervical cancer may be higher in patients with PID than in those who do not show any signs or symptoms of pelvic inflammatory disease.

The prevalence of cervical *C. trachomatis* infection was also found to be significantly higher in women with PID. As the causal relationship between high-risk HPV types and cervical cancer has been firmly established, and cervical *C. trachomatis* infection may be considered as an independent risk factor for the development of invasive cervical squamous cell carcinoma [22-25], the observed significantly higher prevalence of these agents in patients with PID as compared to those without PID in this study suggests that women suffering from pelvic inflammatory disease may have a higher risk of cervical cancer. More investigation is also needed to confirm our findings.

References

[1] World Health Organization: Health for All Dataset, www.who.ch.
[2] Editorial commentary: "The EU's answer to future public-health challenges". *Lancet,* 359, *2211,* 2002.
[3] Cuzick J., Szarewski A., Terry G., Ho L., Hanby A., Maddox P. *et al.*: "Human papillomavirus testing in primary cervical screening". *Lancet,* 1995, *345,* 1533.
[4] Meijer C.J., Helmerhorst T.J., Rozendaal L., van der Linden J.C., Voorhorst F.J., Walboomers J.M.: "HPV typing and testing in gynaecological pathology: has the time come?". *Histopathology,* 1998, *33,* 83.
[5] Josefsson A.M., Magnusson P.K., Ylitalo N., Sorensen P., Qwarforth-Tubbin P., Andersen P.K.: "Viral load of human papiliomavirus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control study". *Lancet,* 2000, *355,* 2189.
[6] Meijer C.J., Rozendaal R., Walboomers J.M.: "Clinical role of HPV testing". In: "Global challenge of cervical cancer screening and prevention". J. Monsonego (ed.). *CME J. Gynecol. Oncol.,* 2000, *5,* 14, 26.
[7] Jacobs M.V., Walboomers J.M., Snijders P.J., Voorhorst F.J., Verheijen R.H., Fransen-Daalmeijer N., Meijer C.J.: "Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age related patterns for high-risk and low-risk types". *Int. J. Cancer,* 2000, *87,* 221.
[8] Report of the US Preventive Services Task Force: "Guide to Clinical Preventive Services". Baltimore, Williams and Wilkins, 1989.
[9] Chiarini F., Pisani S., Gallinelli C., Papi E., Seganti L., Degener A.M. *et al.*: "Simultaneous detection of HPV and other sexually transmitted agents in chronic urethritis". *Minerva Urol. Nephrol.,* 1998, *50,* 225.
[10] Giraud J., Coiffic J., Poulain P., Kerisit J.: "High prevalence of cervical intra-epithelial neoplasia in women treated for pelvic inflammatory disease". *Eur. J. Obstet. Gynecol. Reprod. Biol.,* 1998, *81,* 51.
[11] Briggs R.M., Holmes K.K., Kiviat N., Barker E., Eschenbach D.A., De Yong R.: "High prevalence of cervical dysplasia in STD clinic patients warrants routine cytologic screening". *Am. J. Public. Health.,* 1980, *70,* 1212.

[12] Becker T.M., Wheeler C.M., McGough N.S.: "Sexually transmitted diseases and other risk factors for cervical dysplasia among south-western hispanic and non-hispanic white women". *J. Am. Med. Assoc.,* 1994, *271,* 1181.
[13] Kamb M.L.: "Cervical cancer screening of women attending sexually transmitted disease clinics". *Clin. Infect. Dis.,* 1995, *20* (suppl. 1), S98.
[14] Boom R., Sol C.J., Salimans M.M., Jansen C.L., Wertheim-van Dillen P.M., van der Noordaa J.: "Rapid and simple method for purification of nucleic acids". *J. Clin. Microbiol.,* 1990, *28,* 495.
[15] Farrell D.J.: "Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using cppB nested PCR and 16S rRNA PCR". *J. Clin. Microbiol.,* 1999, *37,* 386.
[16] Catry M.A., Borrego M.J., Cardoso J., Ázevedo J., Santo I.: "Comparison of the Amplicor Chlamydia trachomatis test and cell culture for the detection of urogenital chlamydial infections". *Genitourin Med.,* 1995, *71,* 247.
[17] Blanchard A., Crabb D.M., Dybvig K., Duffy L.B., Cassell G.H.: "Rapid detection of tetM in *Mycoplasma hominis* and *Ureaplasma urealyticum* by PCR: tetM confers resistance to tetracycine but not necessarily to doxycycline". *FEMS Microbiol. Lett.*, 1992, *74,* 277.
[18] Grodstein F., Tothman K.J.: "Epidemiology of pelvic inflammatory disease". *Epidemiology,* 1994, *5,* 234.
[19] Cox J.T.: "Epidemiology of cervical intra-epithelial neoplasia: the role of human papillomavirus". *Clin. Obstet. Gynecol.*, 1995, *9,* 1.
[20] Bornstein J., Rahat M.A., Abramovici H.: "Etiology of cervical cancer: current concepts". *Obstet. Gynecol. Surv.,* 1995, *50,* 146.
[21] Duggan M.A.: "Human papillomaviruses in gynecological cancer". *Curr. Opin. Obstet. Gynecol.,* 1996, *8,* 56.
[22] Lehtinen M., Dillner J., Knekt P. *et al.*: "Serologically diagnosed infection with human papillomavirus type 16 and risk for subsequent development of cervical carcinoma". *Br. Med. J.*, 1996, *312,* 537.
[23] Dillner J., Lehtinen M., Bjørge T. *et al.*: "Prospective seroepidemiologic study of human papillomavirus infection as a risk factor for invasive cervical cancer". *J. Natl. Cancer Inst.*, 1997, *89,* 1293.
[24] Koskela P., Anttila T., Bjørge T. *et al.*: "*Chlamydia trachomatis* infection as a risk for invasive cervical carcinoma". *Int. J. Cancer,* 2000, *85,* 35.
[25] Anttila T., Saikku P., Koskela P. *et al.*: "Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma". *JAMA,* 2001, *285,* 47.

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