

**INVESTIGATION OF THE ROLE OF HUMAN
PAPILLOMA VIRUS IN CERVICAL CARCINOMA IN
SOUTH-EASTERN HUNGARY**

**EPIDEMIOLOGICAL AND COST-EFFECTIVENESS
STUDIES**

PhD Thesis

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ABBREVIATIONS

ASCUS	Atypical squamous cells of undetermined significance
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia (CIN I, II, III, ca. in situ)
DNA	Deoxyribonucleic acid
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HSIL	High-grade squamous intraepithelial lesions
HUF	Hungarian forint
ICER	Incremental cost-effectiveness ratio
LEY	Life expectancy years
LSIL	Low-grade squamous intraepithelial lesions (CIN I)
OR	Odds ratio
PCR	Polimerase chain reaction
QALY	Quality-adjusted life years
RNA	Ribonucleic acid
STD	Sexually transmitted diseases

PUBLICATIONS OF THE AUTHOR

This thesis based on these articles:

1. **Kalmár L**, Szöllősi D, Thurzó L, Deák J, Nyári T, Kovács L and Pál A. High incidence of human papillomavirus infection in cervical carcinoma patients in South Hungary. *Acta Biologica Szegediens*, 2005: 49:15-17.
2. **Kalmár L.**, Szöllősi D., Bohus K., Thurzó L., Pál A. A humán papilloma vírusos fertőzés és a méhnyakrák közötti kapcsolat vizsgálata Dél-Magyarországon *Magyar Nőorvosok Lapja* 2006;69 (6): 563-565.
3. Nyári T, **Kalmár L**, Nyári Cs, Parker L, Kovács L and Thurzó L. Human papillomavirus infection and cervical intraepithelial neoplasia in a cohort of low-risk women. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2006;126:246-249.
4. Nyári T.A., **Kalmár L.**, Deák J., Szöllősi J., Farkas I. ,Kovács L. Prevalence and risk factors of Human Papilloma Virus infection in asymptomatic women in southeastern Hungary. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2004;115: 99-100.

SUMMARY

OBJECTIVES: In this thesis 1. determination of the prevalence of genital HPV infection in asymptomatic women in south-eastern Hungary; 2. examination of the role of HPV infection in the initiation of the development of low-grade squamous intraepithelial lesions; 3. determination of the incidence of HPV infection in cervical carcinoma patients in order to facilitate the prediction of the possibility of development of cervical cancer; and 4. a cost-effectiveness analysis of the screening of women for HPV infection questions were investigated:

METHODS: A cross-sectional study was carried out to determine the risk factors and prevalence of genital HPV infection. The role of HPV infection in the initiation of the development of low-grade squamous intraepithelial lesions were investigated by a prospective cohort study. A case-control study was applied to determine the incidence of HPV infection in cervical carcinoma patients and a cost-effectiveness analysis was used to estimate costs of HPV vaccination in south-eastern Hungary.

MAIN FINDINGS: In the cross-section study, a total of 397 women were examined with a mean of age of 35.5 years (SD 9.7). The overall rate of HPV infection was 23%. In the cohort study, the overall incidence rate of HPV infection was 0.12 cases/100 woman-months. The average duration of new LSIL was 20.1 months and 55.3 months in the HPV-positive and negative groups, respectively, these data being was statistically different ($p=0.001$). In the case-control study, the overall incidence of HPV infection in the cancer, non-negative cytology and normal cytology groups was 74%, 55% and 4%, respectively ($p=0.001$). In the cost-effectiveness study, the total direct costs per patient in the baseline and vaccinated cohorts were 111 251 and 191 541 HUF, respectively. The incremental cost-effectiveness ratio was 792 533.

CONCLUSIONS: The complex gynaecological examination is and will be suggested the most cost-effective method for preventing cervical cancer.

INTRODUCTION

Research into the human papilloma virus (HPV) has a long history. The association between HPV and many illnesses, including sexually transmitted diseases (STD) was known in ancient times. Dominico Antonio Rigoni-Stern, the health officer of Verona, used advanced statistical methods to analyse the causes of death in the city in the period 1760-1839, and in 1842 lectured on his results at the IV Congress of Italian Scientists. In the same year, he published his observations under the title “Fatti statistici relative alle malattie cancerose” in a paper in the pathological journal [94]. He pointed out that nuns contracted cervical cancer more rarely than married women, and that cervical cancer caused death mostly among prostitutes. He concluded that cervical cancer was caused by infection through sexual contact.

In 1907, the virus origin of warts was proved by autoinoculation [26]. In 1932 Richard Shope injected rabbits with the vaccine of a cotton-tail rabbit tumour-extract. This led to deformations in the animals, which was named papillomas. These were little horns on the head of the rabbits, caused by the highly contagious papilloma virus.

Professors József Baló and Béla Korpássy, famous pathologists at the University of Szeged published a monography in German [8], which has been cited in recent years by HPV researchers (e.g. Castaneda Iniguez MS, PhD thesis [24]).

In 1974, Harold zur Hausen (the Nobel prize winner 2009) et al. attempted to detect virus-specific DNA in human tumours using nucleic acid hybridizations with complementary RNA of human wart virus [126]. The gynecologist Valerie Beral replied with an article and raised a question in the Lancet “Cancer of cervix: a sexually transmitted infection?” [10]. In 1975 zur Hausen et al. demonstrated two HPVs, which were found in 70% of cervical carcinoma biopsy samples [125] and confirmed the positive answer to the above question. This is now known as one of the most frequent STD.

The family Papovaviridae contains 2 genera: Polyomavirus and Papillomavirus, which differ substantially in genomic organization and biological properties, although the morphology of the virions is similar.

In susceptible hosts, polyomaviruses frequently cause inapparent infections which may reactivate under immunosuppression and then result in overt disease. Representatives of this human-infecting virus group are the BK and JC viruses. Both are widespread in virtually all human populations; no recognizable disease is associated with the primary infection.

As regards the taxonomy the HPVs are categorized into the Alpha-, Beta- and Gammapapilloma virus genera. Other genera comprise the papillomaviruses of animals.

In 2005, a publication in Viral Taxonomy reported that more than 118 types of HPV had been sequenced and separated into 16 genera [34].

The HPVs are relatively small, DNA viruses without an envelope; the diameter is 55 nm. They have icosahedral symmetry, and 72 capsomers and a DNA which contains 6800-8400 bases pairs. The virus genome codes 8-10 proteins in which there are structured (L1 and L2) and non-structured (E1; E2; E4; E5; E6 and E7) proteins (Fig. 1). They are ether – resistant, and can tolerate 50 °C for 1 hour. They can not be detected in tissue cultures but only in keratinocyte cultures and xenographs [34].

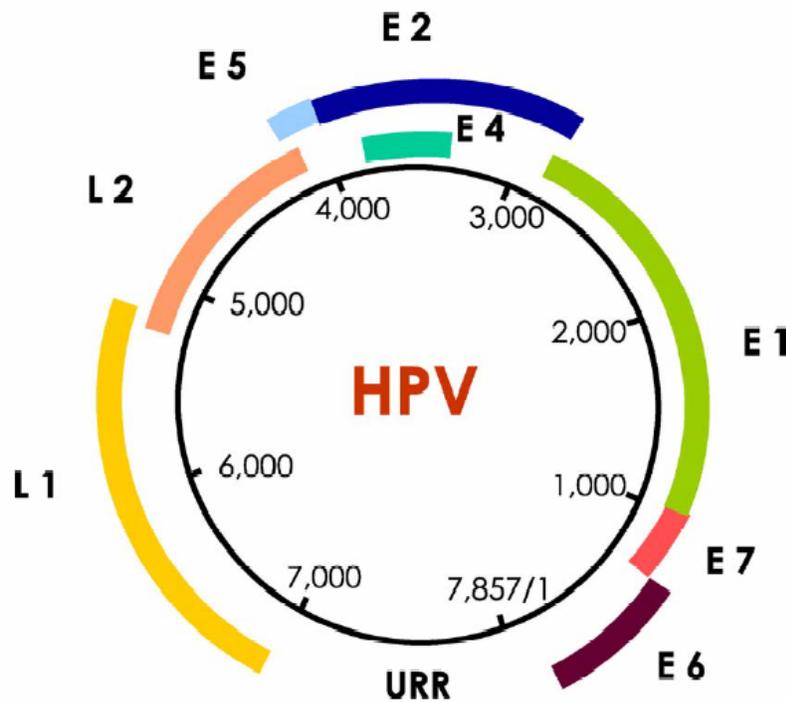


Fig. 1. Schematic presentation of the HPV genome showing the arrangement of the early E or nonstructural genes, the capsid genes (L1 and L2) and the upstream regulatory region.

Papillomaviruses are characteristically epitheliotropic and cause proliferative lesions in infected epidermal or mucosal epithelia. They are commonly designated wart viruses, although many members of the group induce only discrete lesions that differ histologically from common warts. Certain types may cause benign and certain types may cause malignant tumours. Non-melanoma skin cancers were also shown to contain novel human papillomavirus (HPV) genotypes [12].

The HPV transmission happens with tight contact, which can be genital-genital, manual-genital, oral-genital and anal-genital. The viruses get into the body by microlesions. It causes skin and mucous membrane diseases in a benign or malignant way. On the outer genitals mostly benign diseases are caused by the HPV 6 and 11 types. 70% of the cervix, anus, vulva and penis carcinomas are caused by the HPV 16 and 18 types [37, 38]. Most of these malignant diseases can be showed the DNA of HPV integration in human genom. More than 40 of these types can infect the genital tract through sexually contact. Thus, the HPV infection is a sexually transmitted disease.

The relationship of members of this virus group to these widely distributed cancers has evoked intense interest in their molecular and biological properties, in their interaction with the infected cell and in host defence mechanisms engaged in the control of viral carcinogenesis.

This thesis deals primarily with the relationship between human pathogenic papillomavirus and cervical carcinoma.

Papillomaviruses infect epidermal cells still capable of proliferating, commonly basal layer cells, via microlesions or at sites where such cells are naturally exposed to the surface. This occurs regularly at the junctions of different epithelia, and seems to account for the preferential localization of cervical intraepithelial neoplasias (CINs) at the transformation zone, where approximately 90% of all cervical lesions develop. The large transformation zone in young women seems particularly vulnerable to these infections [20, 22].

The mechanism of HPV infection developing

The initial infection provides a growth stimulus for the infected cells, leading to their lateral expansion [124] and in most instances to a delay in differentiation, outlined schematically in Fig. 2.

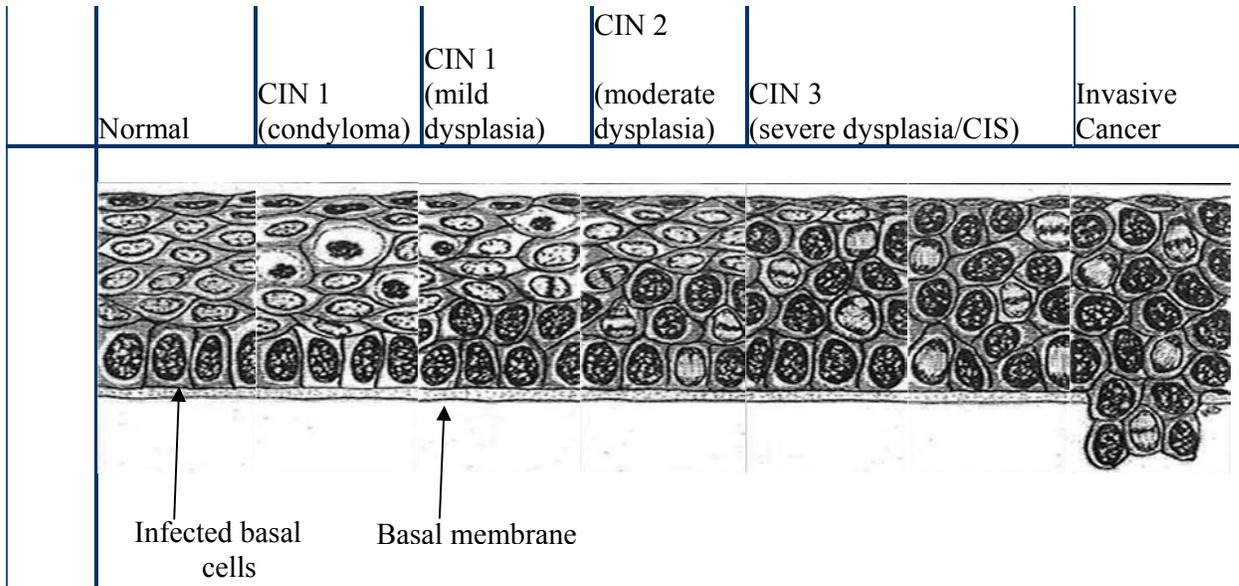


Fig. 2. Schematic representation of early events in HPV infection of keratinocytes

During the proliferative phase of the infected cells, viral gene expression is restricted. In HPV-16 and 18 infections, at most a low level of E6/E7 gene expression is detectable. Upon initiation of differentiation, however, this situation changes: early as well as late viral genes become abundantly expressed, viral capsid synthesis takes place within the stratum spinosum and stratum granulosum and virions can be visualized by electron microscopy in cell nuclei within the differentiating layers. Particle release occurs during desquamation from the surface of these lesions (Fig. 3.).

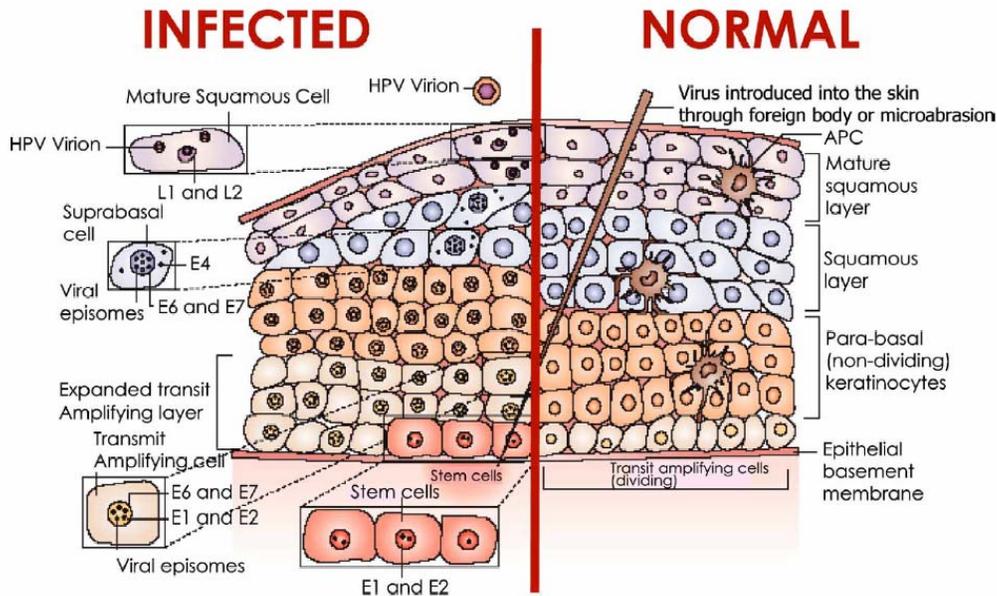


Fig. 3. The location in the squamous epithelium of the main stages of the papilloma virus life cycle.

Clinical aspects of HPV infections

Major manifestations of HPV infections are visible on the skin, the anogenital mucosa and the orolaryngeal mucosa. Skin warts can be classified according to appearance and histological criteria. Common warts, most frequently found on the soles of the feet, on hands and around knuckles, commonly contain HPV types 1, 2, 4, 7, 27 and 29. Flat warts (verruca plana) usually contain HPV type 3 or 10 [37].

Virus types were classified into two categories: high-risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), and low-risk (6, 11, 42, 43 and 44) types. The role of other HPV types in genital anomalies are not known.

Many cutaneous types have been described in Epidermodysplasia verruciformis (EV) (reviewed by Jablonska and Majewski in 1994)[71]. This condition is found world-wide, although at very low prevalence. Many of these papillomatous lesions contain more than one type of HPV. The EV-associated papilloma viruses form a separate subgroup of HPV. The same types of infections are also seen in organ allograft recipients and in some patients suffering from infections with the human immunodeficiency virus [17, 31, 103, 114]. It seems that, whereas infections with these HPV types must be very common in every population, the development of macroscopically visible lesions is well controlled by the immune systems of healthy individuals. Only under conditions of prolonged and severe immunosuppression do specific proliferative changes become apparent.

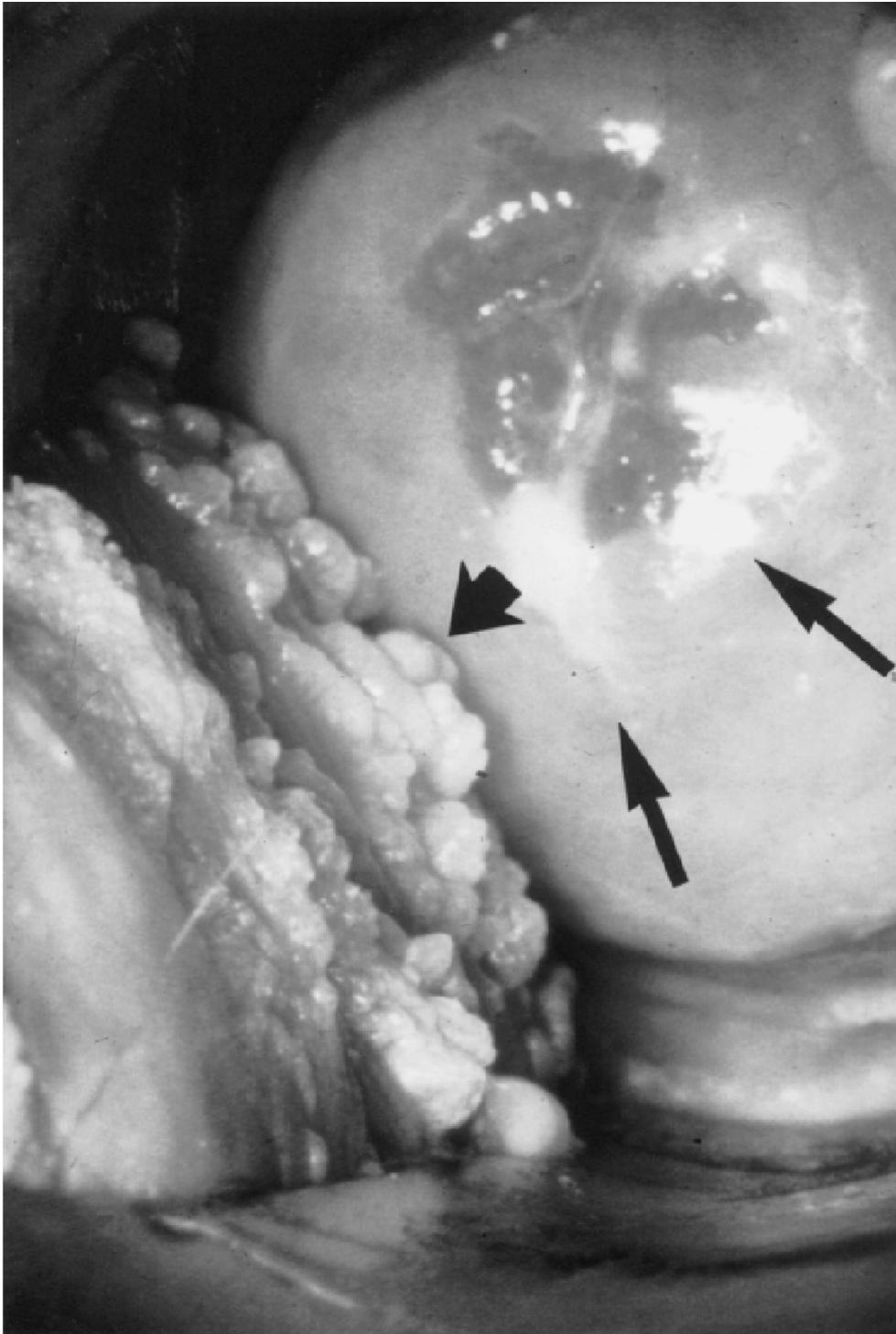


Fig. 4. Cervical intraepithelial lesions (thin arrow) and vaginal condylomata acuminatum (thick arrow).

In the anogenital tract some papillomavirus infections cause genital warts (condylomata acuminata) and others intraepithelial neoplasias (Fig. 4). Genital warts typically contain HPV-6 in circa 60% of all lesions or HPV-11 in around 30% [74, 117]. They develop into

cauliflower-like tumours. Histologically they are characterized by acanthosis, papillomatosis and, within the superficial layers, by an extensive koilocytosis. The same virus types are also found in inverse frequency in tumours at an entirely different location, viz. laryngeal papillomas. Here HPV-11 prevails, being present in approximately 60% of these lesions, whereas HPV-6 occurs in approximately 30%.

A rare anogenital form of condyloma acuminatum is represented by the Buschke-Löwenstein tumours. These are giant condylomata acuminata which grow invasively but metastasize only rarely. They can also be considered as a special variant of a verrucous carcinoma. All the tumours tested thus far contain either HPV-6 or HPV-11 DNA [91].

The second major clinical manifestation of anogenital HPV infections are intraepithelial neoplasias of the cervix or other anogenital sites, often diagnosed as Bowen's disease or as bowenoid papulosis [63]. CINs contain a spectrum of more than 30 different HPV types [72], some being found predominantly in low grade lesions, others more frequently in high grade intraepithelial neoplasias [18]. The most frequent type observed in the latter is HPV-16, found in the majority of studies in more than 50% of these lesions[52], followed by HPV-18, and in southeast Asia by HPV-58 [118]. Infections with these virus types, which are also regularly found in anogenital cancer biopsies, are considered as high risk for malignant conversion [54]. Consequently these types are considered as high risk viruses, by contrast with virus types commonly found in genital warts [39]. The latter rarely become malignant, the causal viruses thus being designated low risk HPV. A functional differentiation between these virus types has become possible: high risk viruses rapidly induce chromosomal instability and an aneuploid karyotype, whereas low risk viruses fail to do so [124].

In the oral cavity typical condylomata, verrucous hyperplasias and other types of papillomatous lesions have often been recorded. Whereas condylomatous lesions usually contain HPV-11 or HPV-6, in other papillomatous proliferations HPV-13 or HPV-32 are most common [48]. Papillomatous lesions in HIV-infected patients often contain HPV-7 DNA, otherwise mainly found in butchers' warts [117].

Laryngeal papillomatosis seems regularly to result from anogenital HPV infections, mainly by HPV-11 and HPV-6. The incidence rate of these lesions is low; they are acquired during early childhood, probably perinatally from mothers suffering from condyloma acuminatum or as early postnatal infections. They usually persist for long periods and recur regularly after surgical ablation [43]. Szentirmay et al. 2005. investigated the molecular biology and clinicopathological correlation of HPV in head and neck cancer [106]. This study has shown that the prevalence of HPV positivity and the prevalence of multiple HPV genotypes in the

same patient are presumably higher than assumed. The presence of HPV infection may be related to an increased risk for the development of a tumour disease. An increased radiocurability of HPV positive head and neck squamous cell carcinoma has also been demonstrated.

Both experimental and epidemiological data support a causal role of high risk HPV types in the development of cervical cancer: specific viral genes (E6/E7) are transcribed in HPV-positive cancer biopsies [30, 64, 66, 108]. In approximately two-thirds of the biopsies viral DNA is integrated into the host cell genome, resulting in a prolonged lifespan of E6/E7 oncoproteins [57]. E6/E7 genes immortalize human keratinocytes on transfection, permitting their unlimited growth in tissue culture. Their expression is apparently necessary for the initiation of permanent growth, for the maintenance of the immortalized cells and also for generating the malignant phenotype of cervical carcinoma cells. A switch-off of E6/E7 gene activity results in cessation of growth and an inability to cause tumours. Besides their property of directly stimulating cell proliferation, probably by activating cyclin E and cyclin A, E6 and E7 proteins interfere with cellular factors, p53 and pRB, negatively regulating the mitotic cycle. The genes for both proteins have been defined as immunosuppressor genes. The p53 is efficiently degraded by E6 and pRB is bound by the E7 protein, releasing it from a complex with the transcription factor E2F.

The degradation of p53 is apparently the main contributor to the induction of chromosomal instability and aneuploidy, regularly observed as a consequence of high risk HPV infection [119]. Modifications in host cell DNA, apparently within genes regulating signalling pathways interfering with viral oncogene expression and transcription, eventually result in the dysregulation of viral oncogene activity and progression to a malignant phenotype. The mutagenic properties of oncoproteins of these papillomaviruses are not shared by low risk HPV, which probably explains their low carcinogenic potential [112].

The reported experimental data are supported by a rapidly increasing number of epidemiological case-control and cohort studies, pointing to high risk HPV as the main or even sole risk factor for the development of cancer of the cervix and its precursors [100]. Thus cervical carcinoma, as one of the most frequent malignancy in women world-wide, represents a major human cancer whose viral aetiology can be considered as proven [55, 87]. High risk HPVs are also demonstrable in cancers of the vulva and penis, and in anal and vaginal cancers. Vulval intraepithelial neoplasias and vulval cancers contain these viruses in 40-60% of all biopsies tested; similar data were recorded for vaginal cancers. A review of the available literature indicates that the overall prevalence of HPV in penile cancers is 54% [38].

A prevalence greater than 60% is recorded for anal and perianal cancers. It remains to be seen whether the tumours thus far negative for HPV DNA contain different types or have been caused by other factors.

Epidemiology of HPV infections

Most epidemiological studies have been concerned with genital HPV infections; much less is known about non-genital HPV infections. Genital HPVs are transmitted primarily through sexual contacts. In sexually inexperienced women, HPV DNA or antibodies to anogenital HPV types are only rarely detected [41, 97]. In addition, there is a strong positive trend between increasing numbers of sexual partners and the prevalence of genital HPV infections. Isolated reports of periungual infections with HPV-16 and of anogenital warts in children without evidence of sexual abuse may point to occasional manual transmissions from one site to the other [42, 46].

Transmission of non-genital HPV types occurs more efficiently on macerated or abraded epithelial surfaces. Concrete surfaces, such as the surrounds of swimming pools, seem to favour easy transmission of plantar warts. HPV-7-positive common warts, often found on the fingers of butchers and of fish and poultry handlers, may arise from accidental wounds from sharp implements.

The few available data on the incidence of genital warts indicate a dramatic rise between the 1950s and the late 1970s. In a study performed in Rochester, Minnesota, the age- and gender-adjusted incidence rose within this period from 13 per 100,000 to 105 per 100,000 [25]. Also within this period the rates of other sexually transmitted diseases (STDs) increased dramatically within Europe and the USA [67]. Similar data have been obtained in Sweden for carcinomata in situ of the cervix, where the rate per 100,000 increased from <10 in 1958 to approximately 100 in 1988 [1, 92].

Geographical differences in the prevalence of HPV infections are not readily apparent, although HPV-58 infections seem to occur with relatively high frequency in southeast Asia [72] the occurrence rate in Hungary 14,1%, and HPV-18 and 45 infections have been recorded more consistently in Africa and Indonesia than in Europe and the USA [15, 105].

A number of cohort studies have assessed the risk of genital cancer-linked HPV infections progressing to cervical intraepithelial neoplasia (CIN) and subsequently to invasive cancer (reviewed by Schiffman and Castle) [101]. In these, and even more in case-control studies, the odds ratios of the relative risks were mostly >10[47]. Well designed studies using reliable HPV test methods yielded the highest odds ratios. From the epidemiological data alone it can

be concluded that, world-wide, more than 90% of cervical cancers may be attributable to HPV infections.

HPV infection in Hungary

The nationwide research of HPV infections in Hungary started at the end of 1980s. In medical university research centers, and different HPV diagnostic methods have been carried out; the results were documented and published. (Medical University of Debrecen, Department of Microbiology, Department of Obstetrics and Gynaecology; Medical University of Szeged Department of Microbiology, Department of Obstetrics and Gynaecology). In Hungary, adult women or girls after two years became sexually active are recommended to be screened every year to detect and treat precancerous cervical lesions before the development of cervical cancer. However, despite important benefits from the cervical cancer screening programme, cervical cancers still occur and women continue to die from this disease.

A multicentre epidemiological survey was carried out in 1997 in order to determine the prevalence and some of the risk factors for persistent cervical HPV infection in asymptomatic women in Hungary. The observed overall average HPV infection rate was found to be 17.5% varying between 12.5% and 27.5%[35]; the prevalence 27.5% was detected in the Szeged region. (5, 33, 65).

Hybride capture system was used in the region of Debrecen and the presence of HPV infection proved to be 28,9% of the cases. 3,1% of the patients had acquired low risk, and 23,6% high risk virus type, however 2,1% of the women were infected both. (49, 98,)

Therapy of HPV infections

There is as yet no specific antiviral therapy for HPV infections. Surgical removal or ablation of the lesions by various local treatments (laser, electrodiathermy, cryotherapy etc.) are still the most reliable methods for treating papillomas and intraepithelial neoplasias.

Systemic and intralesional administration of interferon has been attempted in laryngeal papillomatosis and in recurrent condylomata acuminata. The evidence for success is so far not fully convincing. Retinoic acid may have some therapeutic potential, since this compound has been shown to suppress HPV transcriptional activity [9].

HPV vaccine

The prospect of HPV vaccine development offers hope for prevention of cervical, anogenital and some cancers of the head and neck region. Most HPV related diseases may be attributable to four HPV types: 6, 11, 16 and 18 [51]. High risk HPV types 16 and 18 account for approximately 75% of all cervical cancers and 55% of CIN 2 and CIN 3. [27, 28] It is also estimated that HPV types 16 and 18 may be responsible for approximately 35% of CIN 1 [56, 79, 84]. Recently bivalent (targets HPV types 16 and 18) and quadrivalent prophylactic recombinant HPV vaccines were developed [44,111]. A quadrivalent prophylactic recombinant HPV vaccine which targets HPV types 6, 11, 16 and 18 has recently been approved, designed for the prevention of cervical cancer, CIN, genital warts and other HPV related diseases. This vaccine was shown to be highly effective in phase III clinical trials. [83, 85, 113]

The aims of the thesis

A large number of epidemiologic, clinicopathologic and molecular studies have subsequently linked the presence of specific types of HPV to the development of anogenital cancers. It is now accepted that HPVs play a casual role in the pathogenesis of cervical cancers and their precursor alterations.

1. Study was carried out to establish the prevalence and risk factors (age, family status, smoking history, number of birth; cervical cytology) of genital HPV infection in 397 asymptomatic women in south-eastern Hungary.
2. The objective of this scientific question was a prospective examination including 504 sexually active women of the incidence of HPV infection in a low-risk female population who were HPV-negative at enrolment, and of the role of HPV infection in the initiation of LSIL development.
3. Determination of HPV infection using cervical cytology and hybrid capture assay, in order to facilitate the prediction of the possibility of development of cervical cancer in high-risk HPV infected 347 patients in south-eastern Hungary.
4. A cost-effectiveness analysis of the screening of women for HPV infection was performed.

MATERIALS AND METHODS

Diagnostic methods

HPV cannot be cultured reliably in a laboratory setting; therefore, HPV diagnostics rely on molecular technologies that detect HPV DNA in cervical/vaginal samples [35].

Molecular techniques can be broadly divided into those technologies that are not amplified, such as nucleic acid probe tests, and those that utilize amplification, such as polymerase chain reaction (PCR). Amplification techniques can be further divided into three separate categories: (1) target amplification, in which the assay amplifies the target nucleic acids (for example, PCR); (2) signal amplification, in which the signal generated from each probe is increased by a compound-probe or branched-probe technology; and (3) probe amplification, in which the probe molecule itself is amplified. To date, target and signal amplification techniques, in addition to non-amplified techniques, have been applied to the detection of HPV [61].

Because there are many HPV types with differing oncogenic potential, diagnostic tests must not only detect HPV DNA, they also must determine the type(s) present in each specimen. The most widely used technique is the hybrid capture technology as described below. Hybrid capture technology, developed by the Digene Corporation, detects nucleic acid targets directly, using signal amplification to provide sensitivity comparable to target amplification methods. Digene has developed two products for the detection of HPV: the first-generation Hybrid Capture Tube test and the more recent Hybrid Capture II (HCAII) assay. Both assays detect “high-risk” HPV types. The Hybrid Capture Tube test detects the following high-risk types: 16, 18, 31, 33, 35, 45, 51, 52, and 56. However, four additional viral types were added to the high-risk category using second-generation HPV detection kit (HCA II) t: 39, 58, 59, and 68 [32]. The level of detection of the second-generation HCA II is rated at 5,000 viral copies per sample, or one picogram of HPV DNA per sample.

DNA target amplification is a laboratory-based procedure that duplicates DNA fragments from a target sequence of a gene, thus providing concentrated samples of a specific genetic sequence. Several types of DNA target amplification technologies exist; however, PCR is the most commonly employed in HPV detection [73]. PCR is a standard laboratory procedure, which can be adapted for the detection and typing of HPV.

PCR frequently is used as a diagnostic tool in epidemiologic investigations of HPV, but the associated costs and technology requirements often are inappropriate for large screening programs [96].

The sensitivity of HPV DNA testing of self-collected vaginal samples was 66.1 percent for detection of high-grade lesions and cancer; the false-positive rate was 17.1 percent. The sensitivity of HPV DNA testing of clinician-collected samples was 83.9 percent; the false-positive rate was 15.5 percent [122].

The cervical smears were classified by use of both Papanicolau and the Bethesda system for cytological diagnoses, since both of them were used for cytology evaluation during the period of our studies [7, 104].

Study population and data collection

Cross-sectional study

A cross-sectional survey was performed in the spring of 2000 at the Department of Obstetrics and Gynaecology of the University of Szeged. Cervical samples were collected for cytology and HPV testing from randomly selected fertile women seen at gynaecological outpatient clinics. Colposcopy and routine gynaecological examinations were performed in each case. The Papanicolau classification (Pap smear) was used for cytology evaluation. Sampling, sample transport and HPV DNA determination were carried out using HPV hybrid capture assay (DIGENE™ HPV). Sampling, sample transport and HPV DNA determination were performed according to the factory kit instructions.

The envisaged sample size was calculated by using Hsieh's formula [53]. In order to detect a 20% prevalence of infection at 5% significance level with a power of 90%, a sample size of 396 women was required. Data was extracted from the patient register about age, occupation, lifestyle, sexual practice and health status.

Cohort study

Between July 1998 and January 2002, a total of 504 sexually active women aged 20–60 years, non-smokers, and married or living with a constant partner, who presented for cervical cancer screening at an outpatient clinic – were invited to participate in a prospective study of cervical HPV infection. Only those women were eligible who did not have a history of preneoplastic or neoplastic lesions of the cervix or of conization or hysterectomy, who were willing to participate and who signed an informed consent form. At study entry, participants responded to a questionnaire on the risk factors for cervical cancer and underwent a pelvic examination for the collection of cervical cells for cytological testing and for the detection of HPV DNA. Additionally, colposcopy was carried out in each case as part of the gynaecological examination. Only samples from women with normal cytological results at enrolment were included in the analysis. Follow-up visits were scheduled 3-monthly. At each follow-up visit, a pelvic examination was performed, and cervical specimens were collected for cytological testing and HPV DNA detection. The cervical smears were classified by use of the Bethesda system for cytological diagnoses[21]. For the purpose of analysis, the following categories were used: normal; atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS), LSIL and HSIL or cancer [77].

HPV DNA determinations via HPV hybrid capture assay were carried out in accordance with the instructions of the manufacturer of the kit (DIGENE HPV hybrid capture 2). Virus types were classified into two categories: high-risk HPV, and low-risk types.

Case-control study

During the period between January 2002 and September 2003, a nested case-control study was performed to investigate the relationship between HPV infection and cervical carcinoma at the Department of Obstetrics and Gynaecology of the University of Szeged. Cervical samples were collected for cytology and HPV testing from women attending the gynaecological outpatient clinic. Colposcopic and routine gynaecological examinations were performed in each case. Both the Papanicolaou (Pap) and Bethesda classifications were used for cytology evaluation [2]. Sampling, sample transport and HPV DNA determination via HPV hybrid capture assay were carried out in accordance with the instructions of the manufacturer of the kit (DIGENE HPV hybrid capture 2).

Data concerning age, occupation, lifestyle and health status were extracted from the patient register system.

Statistical analysis

Cross-sectional study

Analyses were carried out using the STATA software package. Fisher-exact tests and Student's t-tests were performed. To obtain an overview of the risk, a multiple logistic regression analysis with dichotomous responses was performed, the outcome being defined by a positive or negative HPV result. Standard errors were estimated using the Huber-White procedure[121]. The goodness of fit of the models was checked using the Hosmer-Lemeshow method.

Cohort study

Person-time methods were used to calculate the HPV incidence density. Monthly unit of time was used to obtain more precise estimation. When interval-censored principles were used, a new infection was assumed to occur at the midpoint between the last negative and the first positive test result. Women continued to make follow-up visits until they developed a new infection or until the final visit, if they consistently gave negative test results. The age-specific

incidence of HPV infections was calculated as the number of new cases of infection per woman-year observed at 5-year demographical age intervals.

The risk of postenrolment SIL in relation to the HPV infection status during the study period was modelled by Cox proportional hazards regression. The time to event was measured from enrolment to the first instance of a lesion event or to the last recorded visit date for censored subjects.

The cumulative risk of acquiring a new HPV infection was estimated by use of the Kaplan-Meier method, under the assumption that infections occurred at the midpoint between the last negative and the first positive test result [121]. The time to infection was measured from the date of the study entry until the date of a new infection, with censoring at the visit with the last negative result. The Berslow and Day test was used for statistical comparison

Case-control study

Statistical analyses were carried out with the STATA software package. The statistical methods used were the chi-square test and analysis of variance. To obtain an overview of the risk, logistic regression analysis was performed. A probability level of $p < 0.05$ was considered statistically significant.

Cost-effectiveness study

The Markov model was utilized in decision analysis in an attempt to provide a comparatively accurate representation of this complex process [60, 76]. Markov models are used to reflect the complexity of transitions and to incorporate this complexity into the analysis. One-year cycle lengths were applied in order to calculate life expectancy; the MS-EXCEL program was applied.

Structure of the model

A previously published and validated US Markov model for the natural history of HPV infection and cervical carcinogenesis and for an estimation of the economic consequences of HPV-related diseases was adopted in view of the lack of national data, however, the epidemiology of HPV was assumed to be similar in developed countries [62, 69, 107].

Assumptions of the model

To produce a model with a manageable number of possible outcomes, some simplifying assumptions were necessary. The following list outlines the main underlying assumptions of the model and the rationale for making them.

The model assumes that all cases of cervical cancer begin with HPV infection. Estimates of the age-specific incidence of HPV infection were derived from published cohort studies (Table 1).

Table 1. The age-specific incidence of HPV infection [76, 78]

Age (years)	HPV incidence
13	0.030
14	0.050
15	0.100
16	0.100
17	0.120
18	0.150
19	0.170
20	0.150
21	0.120
22	0.100
23	0.100
24-29	0.050
30-49	0.010
50+	0.005

For the model, the HPV-infected state was defined as the presence of detectable HPV DNA with normal cervical cytology.

The cytology classification was as follows (Fig. 5): cytologic evidence of HPV infection and CIN I = low-grade SIL; CIN II, CIN III, and carcinoma in situ = high-grade SIL [45, 102]. Published age-specific regression rates of HPV and progression rates of 0.06667 and 0.1 for low-grade SIL and high-grade SIL, respectively, were also included in the model.

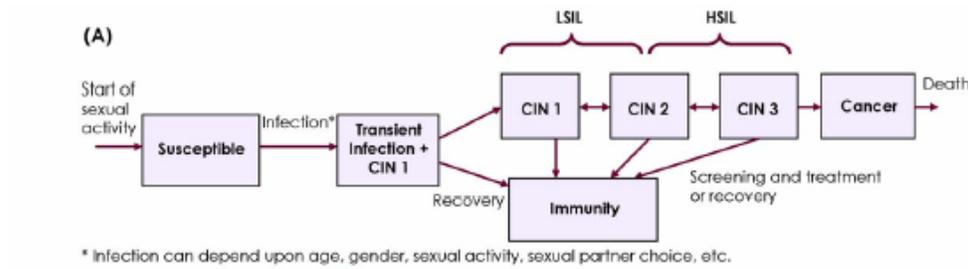


Fig. 5. Flow diagram representing a simple transmission model

The estimated number of 11-year-old girls in Csongrád County in 2007 was 2576 [36]. Thus, in the model, a hypothetical cohort of 2576 11-years-old girls were followed until the age of 75 years with different health states corresponding to the natural history of HPV infection (i.e. a healthy state, infected, LSIL, HSIL or cervical cancer). Movements between health states were based on yearly transition probabilities (Table 2)

Table 2. Transition probabilities used in the Markov model [76]

Age-specific HPV infection regression rate	probabilities
15-24 years	0.4700
25-29 years	0.3300
30+ years	0.1000
Age-specific regression rate (LSIL -> healthy)	
15-34 years	0.1083
35+ years	0.0667
Age-specific progression rate (LSIL -> HSIL)	
15-34 years	0.0167
35+ years	0.0583
Progression rate (HSIL -> cervical carcinoma)	0.0400

Additionally, the age-specific risk of women dying from other causes each year was based on published data [36].

Costs

Costs were based on the local charges of the Hungarian Health Insurance Organisation. Only direct costs were calculated in Hungarian forints (HUF). The costs of inpatient and outpatient treatment were defined by scores. Thus, these were multiplied by an average exchange rate of 1.46. to obtain the costs in HUF in cases of outpatient treatment. The costs of inpatient care included the full costs of treatment and hospitalization. These costs were calculated by using the current basic amount 146,000 HUF. Costs are presented in Table 3.

Table 3. Basic diagnostic and treatment costs used in cost-effectiveness analysis.

	Cost/case (HUF)
HPV DNA test	6 900
Gynecological visit for screening	3 577
Pap smear	
Colposcopy	
Treatment costs	
Conization	34 568
Transabdominal hysterectomy (TAH)	144 400
Radical hysterectomy (Wertheim's operation or Piver 3)	580 000
Irradiation	100 000
HPV screening (cytology screening and HPV DNA test)	10 477
LSIL (30% local treatment cost + 70% HPV screening)	7 784
HSIL (cytology screening and conization)	38 145
Cervical cancer (weighted average of costs of TAH + Piver 3)	659 420

The treatment costs were defined as shown in Table 3. The HPV DNA test was carried out on the basis of the cytological test results. The HSIL treatment costs were calculated as the weighted average costs of the gynaecological visit with the HPV DNA test and the cost of conization.

Vaccine parameters and vaccination programme

The results of recent clinical trials indicated that the quadrivalent HPV vaccine was 70% effective in preventing precancerous lesions, cervical cancers and genital warts due to HPV types 16 and 18. [14, 86].

On the basis of the proportions of precancerous lesions, invasive cancers and genital warts attributable to HPV 16 and 18 infections after vaccination, we modelled a reduction of approximately 33% for CIN 1, 52% for CIN 2/3 and 71% for cervical cancer [11]. The vaccine coverage was assumed to be 100% for all adolescent girls aged 11 years.

RESULTS

Cross-sectional study

A total of 397 women were examined with a mean of age of 35.5 years (SD 9.7). The overall rate of HPV infection was 23% (91/397). High risk HPV types were diagnosed in 15 cases (16% of all infections). Eleven of these (69%) were associated with a pathological cytology. Condyloma was found in 16 cases (4%) of which ten were associated with low risk HPV types and four were associated with high risk HPV subtypes. This difference was statistically significant (Fisher's $p=0.001$). Certain potential risk factors for HPV infection were examined. Young age (less than or equal to 24 years) was the factor most significantly associated with exposure to HPV infection ($\chi^2=8.35$; $p<0.001$). The results of the univariate analysis are summarised in Table 4. There was a statistically significant difference between the age at which HPV-infected patients (17.6 ± 0.19) and non-infected women (18.1 ± 0.14) became sexually active.

We found a higher proportion of HPV positive cases among smokers 28.9% (33/114) while among non-smokers it was only 20.5%(58/283); the difference is almost significant ($p=0.07$) (Table 4). Among unmarried women (49/181) also higher rate (27%) of HPV infection was observed comparing to married women 19% (42/216) ($p=0.069$). The rate of HPV infection is lower among women who had yet laboured (21%) compared with non-laboured women (29%) ($p=0.07$).

We also have found an inverse proportion between social situation, education and HPV prevalence. In case of a low level of education (8 or fewer grades) the proportion of HPV infected was slightly higher (26%), comparing to women with more education the number of HPV infected (22%), however, this difference was no significant ($p=0.23$) (Table 4).

In the multiple logistic regression analysis three variables found to be independent predictors of HPV infection (Table 5). Odds ratios (OR) for each were calculated; young age (less than or equal to 24 years) (OR=2.6; 95% CI: 1.44 - 4.82, $p = 0.002$), an abnormal Pap smear (OR=11.1 95%CI: 3.68 - 33.4, $p<0.001$) and having a condyloma (OR=4.9 95%CI: 1.72 - 14.1, $p = 0.003$). Smoking , family status and number of previous deliveries had no significant relationship to HPV infection in this multiple regression model. The Hosmer and Lemeshow statistic assessing goodness-of-fit had a value of 1.07 with 5 df ($p=0.783$). [81]

Table 4. Risk factors for exposure to genital human papillomavirus among 397 women. An odds ratios of 1.0 indicates the reference category.

Factor	No of cases	No. of HPV infected	Percentage of HPV infected	Odds ratio	95% confidence interval	Probability level of significance
Age						
≤ 24 year	62	23	37%	2.3	(1.29 - 4.13)	p=0.005 ^a
> 24 years	335	68	20%	1.0		
Family status						
Unmarried	181	49	27%	1.5	(0.96 - 2.46)	p=0.07 ^b
Married	216	42	19%	1.0		
Smoking history						
Yes	114	33	29%	1.6	(0.96 - 2.59)	p=0.07 ^b
No	283	58	21%	1.0		
Education						
elementary or less	108	28	29%	0.8	(0.47 - 1.32)	p=0.23 ^{NS}
grammar or higher	289	63	21%	1.0		
Number of births						
≥1	291	60	21%	0.6	(0.38 - 1.04)	p=0.07 ^b
0	106	31	29%	1.0		
Pap smear						
pathological	16	11	69%	8.3	(2.79 - 24.5)	p<0.001 ^a
normal	381	80	21%	1.0		
Condyloma						
Yes	16	9	56%	4.7	(1.69 - 12.96)	p=0.003 ^a
No	381	82	22%	1.0		

^a significant ^b borderline significant ^{NS} non-significant

Table 5. Results of multiple regression analysis for risk factors for exposure to genital human papillomavirus among 397 women. An odds ratios of 1.0 indicates the reference category. A p-value less than 0.05 was regarded as significance.

Factor	Odds ratio	95% confidence interval	Probability level of significance
Age	2.6	(1.44 - 4.82)	p=0.002
Pap smear	11.1	(3.68 - 33.4)	p<0.001
Condyloma	4.9	(1.72 - 14.1)	p=0.003
Smoking	1.4	(0.81 - 2.45)	p=0.22
Family status	1.27	(0.70 - 2.33)	p=0.42
Number of births	0.8	(0.39 - 1.48)	p=0.43

Cohort study

Of the 504 women who were invited to participate in the cohort study, 7 refused to do so. At the baseline, 27 women tested positive for HPV and 6 furnished abnormal cytological results, leaving 464 women who constituted the population of the study. At baseline, the median age was 31.1 years (interquartile range [IQR], 25.7-41.9 years). There were 17 cases of condyloma and 9 of them tested positive for HPV.

The total follow-up time was 16 923.4 person-months and the median duration of follow-up was 34.3 months (IQR, 25.7-45.6 months).

Of the 464 women at risk, 20 presented with HPV infections (14 high-risk and 6 low-risk types) during the follow-up. Thus, the overall incidence rate of HPV infection was 0.12 cases/100 woman-months. The age-specific incidence of HPV infection according to 5-year intervals from ages 20-24 to 45-49 years and over 50 years were 0.23/100 woman-month, 0.13/100 woman-month, 0.04/100 woman-month, 0.11/100 woman-month, 0.07/100 woman-month, 0.05/100 woman-month and 0.11/100 woman-month, respectively. For any HPV, the highest incidence was observed in the age group 20-24 years old (Table 6). Thereafter, the incidence decreased harmonically with age.

Table 6. HPV the incidence according to age using person month time unit in the analysis.

Age groups (years)	Women (N)	HPV cases (N)	Person- months	Incidence (per 100 person – month)
20-24	104	8	3449.6	0.23
25-29	104	5	3688.3	0.13
30-34	77	2	4173.1	0.04
35-39	43	2	1676.1	0.11
40-44	56	1	1326.9	0.07
45-49	44	1	1772.3	0.05
50<	36	1	837.1	0.11
Total	464	20	16923.4	0.12

There were 19 incident reports of ASCUS, 21% of them involving HPV-positive cases. An LSIL event was diagnosed in 18 women. Among these women, 13 were HPV-positive (10 high-risk and 3 low-risk types). Fig. 6. illustrates the cumulative risk of LSIL for HPV-infected and non-infected women, respectively. The average duration of new LSIL was 20.1 months (95%CI: 13.9-26.3) and 55.3 months (95%CI: 45.7-64.9) in the HPV-positive and negative groups, respectively, these data being statistically different ($p < 0.001$ for Berslow-Day statistics). The cumulative incidence of LSIL for HPV-infected and non-infected women is displayed on Fig. 6.

With the use of Cox proportional hazard regression, we estimated the relative risk (RR) of a first instance of LSIL over the entire follow-up period among the 464 women free of lesions at study entry. The RR of LSIL was 90.0 (95%CI: 21.1–403.4) for women testing positive for HPV as compared with women testing negative for HPV.

No case of HSIL and CIN II+ occurred among the women in the study [82].

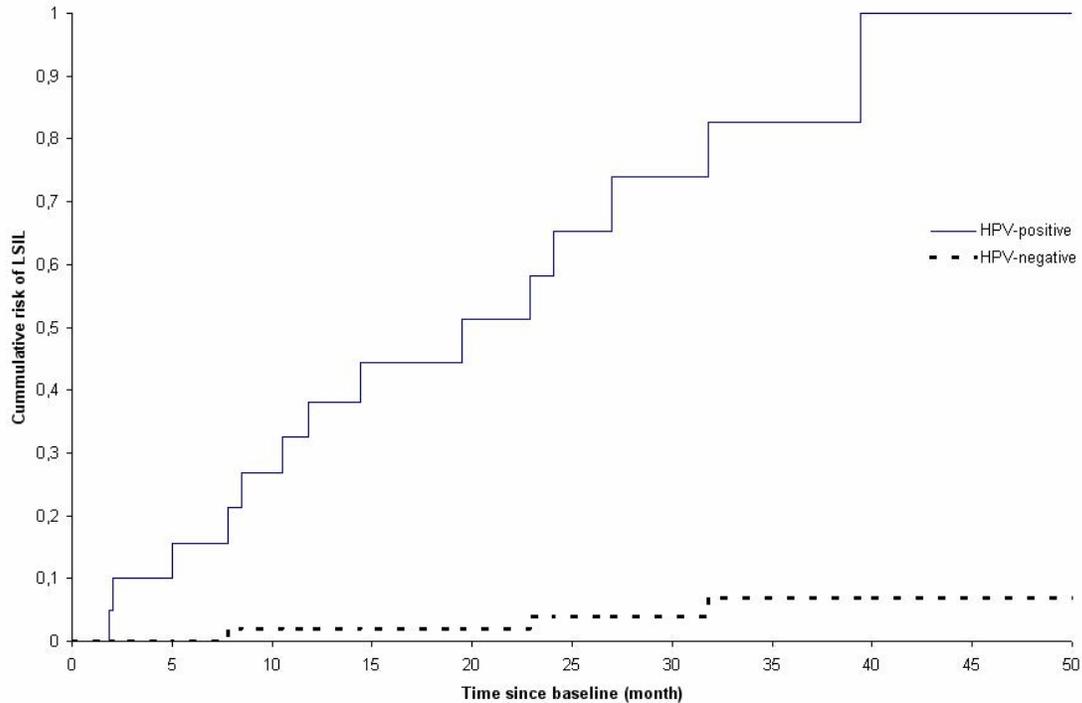


Fig. 6. The cumulative incidence of LSIL for HPV-infected and non-infected women.

Case-control study

A total of 347 women with a mean of age of 42.9 years (SD 9.5) were recruited into the case-control study: 178 of them gave normal Pap smear test (these women served as control group) and 169 women gave abnormal Pap smear tests (class III or higher), 39 of them were diagnosed with invasive carcinoma. This later group immediately underwent appropriate treatment. Thus, three groups (normal cytology, non-negative cytology and cancer groups) were used in the analysis.

The overall incidence of HPV infection in the cancer, non-negative cytology and normal cytology groups was 74% (29/39), 55% (72/130) and 4 % (7/178), respectively ($p < 0.001$). High-risk HPV subtypes were diagnosed in 86% (25/29 cases) of the HPV-infected cancer cases 16% (12/72) of the HPV-infected cases of those who gave abnormal Pap smear test. The distribution of the HPV subtypes is shown in Table 7 and the age-specific distribution of HPV infection in Table 8. There were 46 and 5 low-grade squamous intraepithelial lesions cases in the abnormal Pap smear group and the control group, respectively [58, 59].

Table 7. Distribution of HPV infections according to subgroups (negative, low-risk and high risk). The proportion of low-risk and high-risk types are significantly ($p < 0.001$) differs from the normal cytology cases in the abnormal Pap smear group and the cervical carcinoma groups.

Groups			Number of cases of HPV test results			
			Negative	Low-risk	High-risk	Total
Normal	Pap smear		171	4	3	178
Abnormal without carcinoma	Pap smear cervical		58	60	12	130
Cervical carcinoma			10	4	25	39

HPV infection significantly increased the risk of abnormal cytology (odds ratio (OR) 30.5 95% CI [13.3-70]) and the risk of cervical carcinoma (OR 68.8 95%CI [24.2-195.6]). Further, the OR for progression to cervical carcinoma when the HPV infection was associated with abnormal cytology was 2.16 (95%CI: [1.01-4.69]).

We did not find any significant difference in the incidence of HPV as a function of the living place or the previous obstetrical history of the women in our analysis.

Table 8. Age-specific distribution of HPV infection (n=347)

Age	Control Group				Abnormal cytology			
	Number of HPV-negative cases	Number of HPV-infected cases	Total	% of infected cases	Number of HPV-negative cases	Number of HPV-infected cases	Total	% of infected cases
20-29	0	0	0		10	30	40	75%
30-39	41	1	42	2%	10	21	31	68%
40-49	94	6	100	6%	25	15	40	38%
50-59	36	0	36	0%	9	6	15	40%
60-69	0	0	0		4	0	4	0%
Total	171	7	178	4 %	58	72	130	55%

Cost-effectiveness study

Our baseline model predicted 6 cases of cervical cancer, 180 cases of LSIL and 144 cases of HSIL and in a hypothetical cohort of girls aged 11 years followed until the age of 75 years (Table 9). HPV vaccination in the screening among the same cohort could prevent 81 cases of LSIL (45%), 64 cases of HSIL (44%) and 4 cases of cervical cancers (67%) (Tables 9 and 10). The numbers of life expectancy years (per patient) (LEY) in the baseline and vaccinated cohorts were 27.312 and 27.211, respectively. Thus, LEY increment was 0.101. The total direct costs per patient in the baseline and vaccinated cohorts were 111 251 and 191 541 HUF, respectively. The incremental cost-effectiveness ratio (ICER) was 792 533.

Table 9. Economic and cost effectiveness outcomes of baseline analysis

Baseline	No. of cases	Cost/case (HUF)	Total cost (HUF)
No of women	2576		
Total no. of visits	70 358	3 577	251 669 974
HPV–infected	2 895	10 477	30 333 217
LSIL	180	7 784	269 302
HSIL	144	38 145	514 618
Cervical cancer	6	659 420	3 794 791
Total			286 581 902

Table 10. Economic and cost–effectiveness outcomes associated with quadrivalent HPV vaccination

With vaccination	No. of cases	Cost/case(HUF)	Total cost (HUF)
Vaccination	2 576	82 500	212 520 000
No. of visits	70 097	10 477	250 736 479
HPV–infected	2 411	3 577	25 260 047
LSIL	99	7 784	766 862
HSIL	80	38 145	3 066 859
Cervical cancer	2	659 420	1 060 348
Total			493 410 594

Sensitivity analysis was carried out for the outcomes in the model to determine the changes in estimated values. The ICER was increased if the vaccine efficacy was decreased to less than 90%.

DISCUSSION

Cross-sectional study

In our study, demographic (young age, unmarried status and being nulliparous) and behavioural (smoking) factors were statistically significant predictors of HPV infection. Overall almost a quarter of women were HPV-infected, however, the age group under 24 years age group displayed a very high rate of infection (37%).

The relationship between clinical symptoms and signs, and the probability of HPV infection was also examined. There was a statistically significant difference $p < 0.001$ in the recalled Pap smear results - 12% in the HPV positive patients compared to 1.6% in the HPV negative patients. In addition, a significant association was found between exposure to HPV infection and genital condyloma. Our findings confirm that HPV infection is more frequent in young adults [50, 108, 120].

The aim of preventive strategies is to identify HPV infection in the asymptomatic phase. Cervical cytology is a reliable method for detecting the pathological lesions of epithelium and of dysplasia of the cervix. Cervical cytology could also detect the sign of HPV infection which could confirm using HPV-DNA test. Hence, regular gynaecological cervical screening may lead to significantly reduced rates in mortality and morbidity of cervical cancer. [110].

Cohort study

The results of this prospective study describe the incidence of HPV infection in a cohort of women of a broad age range and from a population at low risk of cervical cancer. The cohort comprised women 19–60 years old who were monitored on average for 3 years. We were able to estimate the age-specific incidence rates of the various HPV types and estimated the relative risk of a first instance of LSIL over the entire period of follow-up for women testing positive for HPV as compared with women testing negative for HPV.

The traditional cross-sectional epidemiological study design does not allow an understanding of the role of HPV and the pattern of changes in the history of cervical dysplasia, whereas screening for cervical lesions on multiple occasions during follow-up does [13, 30, 123]. The restriction to prevalence measures in studies produces similarly elevated risk associations for concurrent HPV infection and lesion development.

Our cohort study had several strengths, including the broad age range of the women enrolled, the very low proportion of those refusing to participate, the long follow-up period, and the use of sensitive and well-validated assays for the detection of HPV DNA. However, a limitation

of this study is the lack of information on the sexual behaviour of the sex partners of the women included in the cohort.

Approximately 80% of HPV infections is transient and asymptomatic [42, 47, 79]. These infections do not produce epithelial abnormalities. Only 20% of high-risk HPV infections cause morphologic changes in the epithelium of the cervix without intervention[51, 64]. In our study, the 90.0 of RR of LSIL revealed similar relative risk to data in the literature for women testing positive for HPV as compared with women testing negative for HPV[3, 75]. However, progression of premalignant lesions is preceded by clearance of HPV. Similar to other reports [70, 77, 80] we suggest that the cases who are HPV positive but have negative cytological test should be follow up more frequently. Nevertheless, the women who are HPV negative as well as cytologically negative and no inflammation might be screened at longer interval. Further, more information about the natural history of HPV infection should be provided by appropriate education which will certainly increase participation in cervical cancer screening programs.

Case – control study

This case – control study focused on the relationship between the incidence of HPV infection and that of cervical cancer. HPV infection associated with abnormal cytology correlated significantly with the development of cervical carcinoma.

In the 39 cancer cases, we found a rate of HPV infection of 74% (29 cases). 25 of these 29 cervical cancer patients were infected by high-risk HPV, thus the proportion of high-risk HPV among the HPV-infected cancer patients was very high (86%). The corresponding proportion among the HPV-infected non-cancer patients was only 16% (12/72). The odds ratio for the progression to cervical carcinoma when the HPV infection was associated with abnormal cytology was 2.16 which represents significantly increased risk to develop cervical carcinoma.

In the second half of the 1990s, HPV testing was generally applied for the clinical screening of women of fertile age in Hungary. However, with regard to the results of international studies on large numbers of patients, and from cost-benefit considerations this practice was later modified[99]. We currently perform HPV testing only when this is suggested by the results of cytological examinations carried out because of the possibility of HPV infection.

The regular clinical cytological screening of HPV-infected patients and the treatment of abnormal cytology by conization has effectively reduced the development of cervical cancer

[95, 109, 118]. One result of our study was a knowledge of the incidence of HPV infection in cervical cancer in Hungary, which had previously not been well documented.

Cost-effectiveness study

Cost-effectiveness analysis has an implicit goal the determination of whether a treatment or intervention is or is not cost effective. Cost effectiveness is measured as a ratio of cost to effectiveness. An incremental or marginal cost-effectiveness ratio is an estimate of the cost of using one treatment/intervention in preference to another: difference in cost divided by the difference in effectiveness. The life expectancy is originally defined by actuaries as the average future lifetime of a person which is the most appropriate measure of the effect of an intervention on survival. In many cases, decision analysis and utility analysis are used to estimate the effect of the intervention on quality adjusted life expectancy. [89]. In some studies utility analysis are used to estimate the effect of intervention on quality-adjusted life expectancy, which is a measure that combines life expectancy with expected quality of life in a single metric [4, 19].

This is the first study of the cost-effectiveness associated with quadrivalent HPV vaccination in Hungary. Since the Data TreeAge software developed for cost-effectiveness studies was not available for us, our analysis was carried out with the MS-EXCEL program. Thus, the model was simplified and utility analysis could not be carried out. However, the values of the life expectancy were close to those that would have been calculated with the Data TreeAge software and reported by Kulasingam and Myers [69].

The costs for HPV vaccination of girls aged 12 years have been analysed in several health economic model studies [93, 107]. The estimated cost per life-year saved varies, under the assumption that vaccinated girls will participate in cervical cancer screening programs. The relationship between cost and effect is influenced by several factors, among them, the price of the vaccine and the percentage of cancer cases that could be prevented by vaccination. All studies have assumed the latter to be 70% [68, 90, 116]. The percentage of cancer cases that could be prevented by vaccination against HPV 16 and 18 might be lower [115]. Hence, all of the model studies might have overestimated the effects of a general childhood vaccination. Thus, only the vaccination against oncogenic HPV types of whole population could reduce significantly the percentage of cervical cancer cases [16].

NEW STATEMENTS

1. The overall prevalence of HPV infection was 23%. Young age (under 24 years) and abnormal cervical cytology were significantly associated with exposure to HPV infection ($p=0.001$; $p=0.005$).
2. During the follow up, 20 women presented with HPV infection and in 18 developed LSIL of the 464 risk population. Among these women, 13 were HPV positive (10 high, and 3 low-risk types). The average duration of new LSIL was 20,1 months, (95% CI 13.9-26.3) and 55.3 months (95% CI. 45.7-64.9) in the HPV-positive and negative groups, respectively ($p=0.001$). The relative risk as 90.0 for a first instance of LSIL among women testing positive for HPV as compared with negative for HPV.
3. The incidence of HPV infection in south-eastern Hungary based on the clinical, cytological and virological examination, was in the cancer, positive cytology and normal cytology groups 74% (29/39) and 4% (7/178), respectively ($p=0.001$). When the HPV infection was associated with abnormal cytology the odds ratio for progression to cervical carcinoma was 2.16 which represents significantly increased risk to development cervical cancer.
4. The number of life expectancy years (per patients) (LEY) in the baseline and vaccinated cohorts were 27.312 and 27.211, respectively (LEY increment 0.101). The total direct costs per patient in the baseline and vaccinated cohorts were 111 251 and 191 541 HUF, respectively. The incremental cost-effectiveness ratio (ICER) was 792 533 HUF. The complex gynaecological examination is and will be suggested the most cost-effective method for preventing cervical cancer.

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