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European Journal of Cancer 37 (2001) 246–250

European Journal of Cancer

www.ejconline.com

The role of human papillomavirus in cervical adenocarcinoma carcinogenesis

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Received 2 May 2000; received in revised form 25 August 2000; accepted 4 October 2000

Abstract

Human papillomavirus (HPV) is considered the single most important co-factor in the development of cervical squamous cell carcinomas. Adenocarcinomas of the cervix are also related to HPV, but the correlation is reported to be less pronounced. In the present study, 131 cervical adenocarcinomas were identified through the Swedish Cancer Registry, examined morphologically and then analysed with sensitive polymerase chain reaction (PCR)-based HPV methods for a study of age-related prevalence of HPV. HPV was identified in 64% of the tumours after PCR amplification of the HPV *L1* gene only and in 71% following PCR amplification of both the *L1* and *E6* genes of HPV. HPV 18 was the most prevalent (52%), followed by HPV 16 (33%) and other types of HPV (15%). The prevalence of HPV was shown to be age-dependent. In women younger than 40 years, HPV was present in 89%, whereas in women 60 years and older, HPV was observed in only 43%. The difference was statistically significant, $P < 0.005$. The HPV-positive adenocarcinomas were represented by an age distribution similar to that of cervical squamous carcinomas with a maximum age, in the 40–49 year old group, whereas the frequency of HPV-negative adenocarcinomas increased with age, typical of most carcinomas occurring in elderly women.

Keywords: Cervix; Adenocarcinomas; HPV; PCR; Age related

1. Introduction

Most carcinomas of the cervix are of the squamous cell type, whereas adenocarcinomas are relatively few. The incidence of squamous carcinoma in Sweden has decreased constantly during the last three decades as a result of a combination of organised and sporadic cytological screening. In some age cohorts, the reduction of the incidence has been up to 70–75%. However, the incidence of cervical adenocarcinomas has doubled during the same study period. Consequently, cytological screening does not appear to be successful in reducing the incidence of adenocarcinomas [1].

In the cervical squamous carcinomas, human papillomavirus (HPV) is the dominating aetiological agent [2]. At present, almost 100% of the squamous cell tumours are considered to harbour oncogenic types of HPV [3]. For this reason, HPV testing has been considered an adjunct or an alternative to organised cytological screening in preventing the development of cervical carcinoma. Epidemiological studies indicate that HPV testing has a higher sensitivity and the same specificity as cytological screening for the detection of cervical intraepithelial neoplasia (CIN) [4].

Since there is an increase in the incidence of cervical adenocarcinomas despite organised cytological screening [1], it seems important to acquire further knowledge of the presence of HPV in these tumours. Furthermore, if HPV is also the dominating co-factor in the development of adenocarcinomas, HPV tests could have a

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0959-8049/01/$ - see front matter
PII: S0959-8049(00)00376-2

considerably larger impact on the prevention of adenocarcinomas than organised screening.

In the present study, blocks of 131 cervical adenocarcinomas were identified, through the Swedish Cancer Registry. The tumours were examined morphologically and the presence of HPV was analysed by sensitive polymerase chain reaction (PCR) techniques. An age-related variation in the prevalence of HPV was observed.

2. Patients and methods

2.1. Tumour material

Since 1959 all diagnoses of malignant tumours in Sweden have been collected in a national database (Cancer Registry of the National Board of Health and Welfare), where each specimen is identified by an individual topographical and morphological code [5]. One hundred and eighty adenocarcinomas of the cervix uteri, obtained between 1986 and 1996, were collected from six different departments of Pathology in the central area of Sweden. The tumour tissues had been formalin-fixed and paraffin-embedded. Serial sections from each block were cut. The first was stained with haematoxylin–eosin and evaluated histopathologically, whereas the rest were used for DNA preparation and subsequent HPV analysis. From the light microscopical examination and the women's medical records, 23 tumours were determined to be of non-cervical origin or contain too sparse tumour material for further analyses, 14 tumours were classified as squamous carcinomas and nine as adeno-squamous carcinomas.

2.2. DNA analysis

DNA was extracted from two 10 μm-thick sections of the formalin-fixed gynaecological specimens as described by Lungo and colleagues 1992 [6], but the incubation time was prolonged to 4 h. The section knife was replaced after each specimen block and an empty paraffin block was used alternatively to check for contamination. Distilled water was used as a negative control. To exclude false-negative results, all specimens were tested with the *β-globin* primers PC04 and GH20, resulting in a 268-base pair amplimer [7]. Three tumours were *β-globin*-negative and were not used for further HPV analysis (Table 1).

2.3. HPV identification with the SHARP signal system

To study the presence of HPV DNA in the remaining 131 cervical adenocarcinoma specimens, MY09/MY11 primers were used for the PCR reaction on 5–10 μl of extracted tissue DNA essentially as optimised and previously described [8,9] (Table 1). Aliquots (10 μl) of each PCR product were resolved by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide. Cases with a visible band on the agarose gel were tested with the SHARP Signal System, allowing for identification of low- (6, 11, 42–44) and high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58) HPV types [10]. The hybrid capture detection was performed exactly according to the recommendations of the manufacturer.

2.4. Typing of high-risk HPV

A possibility of distinguishing HPV types 6, 11, 16, 18, 31, 33, 35,39, 45,51, 52, 56 and 58 by non-radioisotopic single-strand conformational polymorphism (SSCP) after PCR has been recently described [9]. All types demonstrated diverse band patterns, as initially previously tested by us on HPV plasmid DNA (kindly donated by E. de Villiers and H. zur Hausen, Deutsches Krebsforschungszentrum, Heidelberg, Germany; A.T. Lörincz, Digene Diagnostics Inc., Silver Springs, MD, USA and G. Orth, Institut Pasteur, Paris, France). PCR

Table 1
Primer pairs used for polymerase chain reaction (PCR) analysis

Primer pair	Sequence 5′-3′	Gene position	Length (bp)	Product size (bp)
β-globin/PCO4	CAACTTCATCCACGTTCACC	54–73	20	268
β-globin/GH20	GAAGAGCCAAGGACAGGTAC	195–176	20	
HPV/MY09	CGTCCMARRGGAWACTGATC[a]	*L1* (HPV6)	20	398
HPV/MY11[b]	GCMCAGGGWCATAAYAATGG[a]		20	
HPV/GP5+	TTTGTTACTGTGGTAGATACTAC	*L1*(HPV6)	23	141
HPV/GP6+	GAAAAATAAACTGTAAATCATATTC	*L1*(HPV6)	25	
HPV 16	CTAAAATTAGTGAGTATAGACATTA	*E6*	25	176
HPV 16	CCTTATATTATGGAATCTTTGC	*E6*	22	
HPV 18	ATGTTGCCTTAGGTCCATGCA	*E6*	21	570
HPV 18	ACCGAAAACGGTCGGGACC	*E6*	19	

HPV, Human papillomavirus.
[a] Degenerate code, M = A + C; R = A + G; W = A + T.
[b] Biotinylated at its 5′ end.

Table 2
Prevalence of human papillomavirus (HPV) in relation to age in cervical adenocarcinomas

Age (years)	HPV 18-positive	HPV16-positive	HPV positive (other types)	HPV negative (%)
⩽39	12/35	14/35	5/35	4/35 (11)
40–49	22/48	9/48	6/48	11/48 (23)
50–59	10/27	3/27	3/27	11/27 (41)
⩾60	4/21	5/21	0/21	12/21 (57)
Total	48/131 (37%)	31/131 (24%)	14/131 (11%)	38/131 (29)

SSCP detection sensitivity was achieved to the single-viral-copy level and was compatible with slot blot hybridisation. Briefly, semi-automated polyacrylamide gel electrophoresis (PAGE) was performed with the PhastSystem, using pre-cast homogenous 12.5% polyacrylamide gels and native buffer strips (Pharmacia LKB, Uppsala, Sweden). For SSCP, the denaturated GP5+/GP6+ PCR products [11] from the HPV *L1* gene were run under non-denaturing conditions and subsequently stained with 0.5% silver nitrate.

In this study, to optimise the HPV PCR analysis, all HPV-negative adenocarcinomas were subjected to a second HPV test using specific primers from the *E6* genes of HPV 16 and 18 in the PCR reaction [12] (Table 1). Thereafter, PCR products indicating infection with HPV 16 or 18, were identified on agarose gels.

2.5. Statistical analysis

The Chi-square test for trend was used for the statistical comparison of proportions [13]. A level of <0.05 was considered significant.

3. Results

3.1. Morphology

The 131 cervical adenocarcinomas, used in this study consisted of tumour cells growing in a tubular or papillary fashion or in a mixture of these features. Exophytic tumours were more often papillary, whereas in the infiltrating tumours, tubular and glandular structures predominated. Five tumours regarded as having a low differentiation status were growing in solid sheets, with only occasional glandular structures. A squamous differentiation was not present in any of the cases. Seventeen adenocarcinomas showed only superficial proliferation of the mucosa and obvious signs of infiltration into the surrounding stroma were not present. These tumours were regarded as *in situ* adenocarcinomas and the remaining 114 tumours as infiltrating cervical adenocarcinomas.

Table 3
Mean age (M±standard deviation (S.D.), in years) of women with HPV-positive and -negative cervical adenocarcinomas

	Cervical adenocarcinomas		
HPV test	Invasive	*In situ*	Total
HPV-positive	47±11	39±7	45±11
HPV-negative	55±15	51±10	54±15
Total	49±13	42±10	48±12

3.2. HPV analysis

With the PCR test using MY09/MY11 primers from the *L1* gene of HPV, 84/131 (64%) of the tumours were HPV-positive. After application of the second PCR test on the remaining HPV-negative cases with primers from the *E6* gene of HPV type 16s and 18, an additional nine tumours, five for the *E6* gene of HPV 18 and four for the *E6* gene of HPV 16, were HPV-positive, 93/131 (71%). In the HPV-positive adenocarcinomas, 48/93 (52%) were infected with HPV 18, 31/93 (33%) with HPV 16 and the remaining 14/93 (15%) with other or unknown types of HPV. In the *in situ* adenocarcinomas, 13/17 (76%) tumours contained HPV and in the invasive adenocarcinomas, 80/114 (70%). In all of the five poorly differentiated adenocarcinomas, HPV 18 was identified (Table 2).

3.3. Age distribution

The mean age of women with *in situ* adenocarcinomas was 42 years and in women with infiltration tumours, was 49 years. In women with HPV-positive tumours, the mean age was 45±11 years and in women with HPV-negative tumours, it was 54±15 years (Table 3).

The prevalence of HPV varied with age. In women younger than 40 years, HPV was identified in 89%, whereas in women 60 years and older, HPV was present in only 43%. This difference was statistically significant, $P<0.005$ (Fig. 1). The HPV-positive adenocarcinomas represented a distribution in age similar to that of cervical squamous carcinomas, with a maximum at ages 40–49 years, whereas the HPV-negative adenocarcinomas increased in number with age (Table 3).

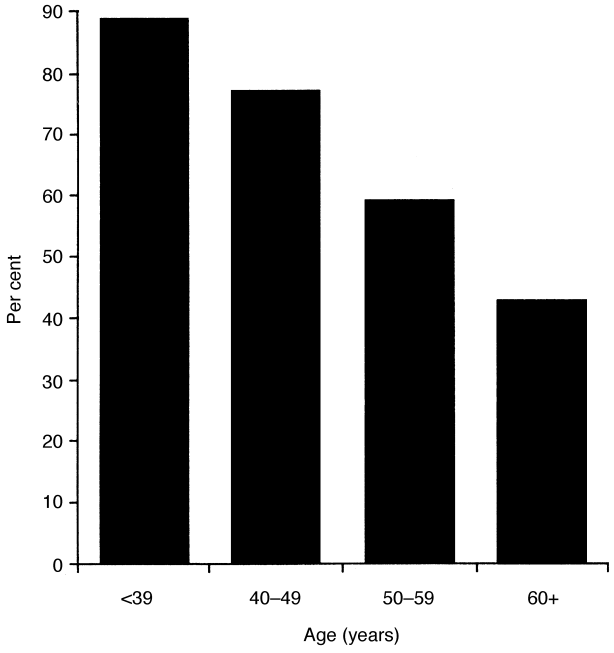

Fig.1. HPV-positive cervical adenocarcinomas in per cent in relationship to age at diagnosis. The difference in age distribution between HPV-positive and HPV-negative women was statistically significant ($P < 0.005$).

4. Discussion

The cervical adenocarcinomas had an overall prevalence of HPV of 71% in the present study. This is in agreement with previous reports from other countries [14,15]. However, stored formalin-fixed and paraffin-embedded tumours were used for the PCR analysis. If fresh frozen tumour tissues are examined, the prevalence of HPV may be higher, since alterations of the DNA sequence are caused by formalin fixation of stored specimens [16].

After initial identification of HPV DNA with a PCR technique (MY09/MY11), recognising a sequence in the *L1* gene, a subsequent PCR HPV analysis of the HPV-negative adenocarcinomas was performed with primers, identifying a sequence in the *E6* gene of HPV types 16 and 18. Five additional HPV 18- and four HPV 16-positive adenocarcinomas were obtained by this procedure.

Since approximately 95% of the stored blocks of cervical squamous carcinomas were HPV-positive determined by a similar PCR technique as described above, it is presumed that a subset of cervical adenocarcinomas is unrelated to HPV [17,18].

However, as mentioned above there may have been some false-negatives due to the use of formalin that may have led to lower detection rates in the PCR. This probably explains why there is some variance with previous studies. One study, including 19 adenocarcinomas and 12 adenosquamous carcinomas found lower rates of HPV in these cancers compared with the rate in 85 squamous tumours. However, another study, including 24 adenocarcinomas and 17 adenosquamous carcinomas did not find lower rates of HPV in the tumours compared with squamous samples. A further hybridisation experiment following the second PCR for the *E6* gene of HPV16 and HPV18 may have detected a few false-negatives.

It is sometimes difficult to discriminate between uterine adenocarcinomas of endometrial and cervical origin. Both clinical and morphological methods were used to identify the cervical tumours, which were all growing in the cervical mucosa or infiltrating the cervical stroma tissue. Furthermore, all 23 adenocarcinomas, considered as non-cervical were HPV-negative. In some earlier reports, HPV was described in endometrial adenocarcinomas [21,22], whereas Hording and colleagues did not find any HPV in these carcinomas. They argued that HPV analysis is of value for differentiating between cervical and endometrial adenocarcinomas [23].

The prevalence of HPV in the adenocarcinomas was age-dependent. In women younger than 40 years, HPV was present in 89%, whereas in those 60 years and older, HPV was observed in only 43%. Tenti and colleagues reported a similar trend, with a mean age of HPV-positive women of 52 years and of HPV-negative women of 63 years [24].

Studies from population-based cancer registers reveal an increase in the incidence of cervical adenocarcinoma in young women [25,26]. In Norway, a 38% increase in the incidence of cervical adenocarcinomas was recorded in young women (24–34 years of age) during 1970–1984 compared with a 30% decrease in the incidence of squamous cell carcinomas [27]. Chilvers and colleagues showed in three English regional registers an increasing incidence of adenocarcinomas in women younger than 35 years [28].

It has been suggested that a risk factor for developing adenocarcinoma of the cervix uteri is the long-term use of oral contraceptives [14,29]. Pater and colleagues reported that transformation of the cervical epithelium was initiated by the combination of HPV 16 DNA and *ras* oncogene activation in the presence of either progesterone or the chemically related progestin, norgestrel, the pharmacologically active component of oral contraceptives [30].

In contrast to squamous carcinomas, adenocarcinomas are predominated by HPV 18. Thus, an apparent local trophism exists for the oncogenic HPV types of the genital tract. Kurman and colleagues [31] provided evidence for a more aggressive behaviour of tumours containing HPV 18, since HPV 18 was present in only 3% of CIN lesions compared with 22% of invasive carcinomas. All of the five poorly differentiated adenocarcinomas were HPV 18-positive. In accordance with previous

reports, an association between poorly differentiated tumours and HPV 18 infection is indicated [32,33].

Cytological screening has decreased the incidence of squamous carcinoma of the cervix. However, it has not been effective in decreasing the incidence of cervical adenocarcinoma [1]. Recent data indicate that a HPV test is more sensitive and equally specific as a cytological examination for the detection of women at risk of developing cervical cancer [4]. Since a large proportion of adenocarcinomas are HPV-positive, it is possible that HPV tests have a stronger impact on the prevention of adenocarcinomas than cytological screening, especially in young females.

Acknowledgements

We wish to thank Sonja Andersson and Margit Gustafsson for technical assistance. The photographic work of Frank Bittkowski is greatly appreciated. This study was supported by the Swedish Cancer Foundation, the Karolinska Institutet Foundation, Stockholm and the Medical Faculty of the Uppsala University, Sweden.

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