

# HPV detection in cervical cancer patients in northern Poland

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**Abstract.** Human papillomaviruses (HPVs) are associated with various benign and malignant lesions including genital condyloma and anogenital cancer. Epidemiological data show that about 90% of all cervical cancer patients are HPV positive. The aim of our study was to determine the percentage of HPV infections in Polish population of examined women. To detect viral DNA, PCR method was used. To distinguish between different virus types, RFLP analysis was performed. Results obtained by PCR-RFLP method were verified by Hybrid Capture test. The presence of HPV DNA was detected in 53% of cervical cancer patients and in 2% of control group of healthy women. The agreement for HPV detection between PCR and Hybrid Capture methods was 81%. Our studies showed much lower incidence of HPV in Polish women with cervical cancer than among other populations as reported in world literature. HPV detection as well as determination of other factors involved in pathogenesis of cervical cancer is of great importance.

## Introduction

Human papillomaviruses (HPV) are small DNA viruses that infect differentiating keratinocytes of the stratified cutaneous and mucosal epidermis. They include about 30 types associated with the lesions in the anogenital tract. The anogenital associated-HPVs are typically grouped into 'low risk' HPVs (such as types 6 and 11) usually associated with benign warts and 'high risk' HPVs (such as types 16, 18) connected with moderate to severe dysplasias and invasive carcinomas (1-3). Recent studies have revealed that about 90% of cervical cancer carcinoma contain HPV DNA (4-7). There are even data suggesting that cervical cancer without HPV infection does not exist (8).

Cervical cancer takes third place (7.6%) after breast cancer (19%) and lung cancer (7.7%) in the classification for

the most frequently registered malignancy in Polish women. Moreover, mortality caused by cervical cancer in Poland is one of the highest among European countries, which testifies to the low effectiveness of diagnosis program. Statistical data show that cervical cancer is a severe problem in pathogenesis of uterine-genital system. Therefore, efficient prevention should be more accessible to allow early diagnosis of HPV infection.

The aim of our study was to determine the occurrence of HPV infection in population of women from the northern region of Poland by using simple and sensitive PCR-RFLP methods. We verified our results with Hybrid Capture II test.

## Materials and methods

*Clinical materials.* Swabs were obtained from 53 patients with cervical cancer, treated in the Oncology and Radiotherapy Clinic of the Medical University of Gdansk. The age range of the patients was from 28 to 82 (mean  $58 \pm 12.5$ ) years. Patients were in different clinical stages of squamous cell carcinoma of the cervix. Correlation between clinical stage and mean age of the studied population is shown in Table I.

Samples for the control group consisted of DNA extracted from 229 swabs and scrapes derived from healthy women undergoing routine gynaecological examination.

*PCR method.* To detect viral DNA, PCR method was used with L1 consensus primers (9). In PCR reaction the product of about 450 bp was amplified. To assess the adequacy of the DNA, a 268 bp fragment of the cellular  $\beta$ -globin gene of each sample was amplified using the specific primers PC 04 and GH 20 (9). HPV negative control DNA isolated from white blood cells was used. The reaction products were electrophoresed through a 3% agarose gel stained with ethidium bromide and viewed under UV light.

*Restriction fragment length polymorphism (RFLP).* To determine the viral type, RFLP analysis was performed. Specific enzymes were chosen on the basis of DNA sequences obtained from the GenBank (<http://www.ncbi.nlm.nih.gov>) and with the use of Clone Manager program (Table II).

Briefly, 10  $\mu$ l of the PCR product of each HPV DNA positive samples was digested using *RsaI* and restriction enzymes typical for each type. Digestion reaction was performed for 3 h in a final volume of 20  $\mu$ l. The products of digestion were analysed on 10% polyacrylamide gel stained with silver.

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Table I. Correlation between clinical stage and mean age of examined population.

Clinical stage	No. of cases	Mean age (years)
I	6	64.3
II	15	56.0
III	28	59.4
IV	4	40.3

Table II. Restriction enzymes used in the PCR-RFLP method.

HPV type	PCR product size	Restriction enzymes chosen for PCR product digestion (size of DNA restriction fragments in brackets)
6	448	<i>EaeI</i> (298, 150)
11	448	<i>HpaI</i> (397, 51)
16	451	<i>RsaI</i> (311, 70, 70) <i>EcoRI</i> (235, 216)
18	454	<i>RsaI</i> (136, 125, 85, 70, 38) <i>HpaII</i> (356, 98) <i>BstNI</i> (343, 111)
31	451	<i>RsaI</i> (381, 70) <i>StyI</i> (360, 91)
33	448	<i>RsaI</i> (236, 103, 70, 39) <i>ScaI</i> (378, 70)

*Hybrid Capture test.* PCR-RFLP results were verified by the commercially available Hybrid Capture II test according to the manufacturer's recommendation. Samples were examined in the Department of Virology, National Institute of Hygiene in Warsaw.

## Results

*Detection of HPV DNA in cervical cancer patients and healthy women.* Specimens from 53 patients with cervical carcinoma were tested by PCR using consensus primers MY09/MY11. The negative controls consisting of distilled water and HPV-negative DNA did not show amplification with PCR. HeLa cells containing integrated HPV 18 genome was used as the

Table IV. Agreement between Hybrid Capture and PCR methods.

Hybrid Capture	PCR	No. of cases
Positive	Positive	18
Negative	Negative	16
Negative	Positive	6
Positive	Negative	2

positive control in PCR reaction. HPV DNA in examined samples was detected in 28 (53%) of 53 cervical cancer cases and in 4 (2%) of 229 healthy women (Table III).

We identified HPV type 16 as the most common type (89%) in the group of cervical cancer patients examined. Apart from type 16 we detected in single cases the following types: 33, 31 and a very rare type 58.

*Comparison of PCR-RFLP and Hybrid Capture methods.* Results from 42 of 53 examined cervical cancer patients were verified with Hybrid Capture method, as it is the only HPV DNA test approved by the US Food and Drug Administration (FDA). In 34 cases (81%) results showed agreement between the two methods (Table IV). Differences obtained by PCR and Hybrid Capture methods are not statistically significant ( $p=0.8$ ; McNemary test).

The Hybrid Capture assay is based on RNA:DNA hybridisation. It contains the RNA probe mixture for detection of low- and high-risk HPV types. However, the identification of the HPV virus type is not possible using this test.

## Discussion

Understanding the importance of HPV infection in the pathogenesis of cervical neoplasia has been accompanied by the increased number of epidemiological studies both in screening healthy women and patients with cervical cancer. HPV DNA detection requires development of a sensitive, specific and inexpensive method.

The aim of our studies was to determine the association between human papillomavirus infection and cervical neoplasia among the examined population of Polish women, using simple, sensitive and reliable PCR-RFLP method. The comparison of these two methods for HPV detection (PCR and Hybrid Capture) was also undertaken.

Table III. Prevalence of HPV DNA in cervical cancer patients and healthy women examined by PCR.

No. of cases tested	No. of negative cases (%)	No. of positive cases (%)						Total
		HPV 6	HPV 16	HPV 31	HPV 33	HPV 58	HPV X	
229 healthy women	225 (98)	1	1	-	-	-	2	4 (2)
53 cervical cancer patients	25 (47)	-	25	1	1	1	-	28 (53)

Table V. Comparison of HPV detection rate in cervical cancer patients from different regions of Poland.

No. of population examined	Frequency of HPV detection (%)	Detection methods	Geographical distribution of population examined	FIGO <sup>a</sup> classification	Clinical material	Refs.
75	46 (62)	Hybridization, Vira Pap DNA, HPV	Province of Cracow	0 ( <i>in situ</i> )	Smears	26
39	24 (61.5)	Hybridization <i>in situ</i>	Province of Cracow	I-III	Biopsies	27
10	9 (90)	PCR	Province of Lublin	Lack of data	Biopsies	28
93	62 (67)	PCR	Province of Gdansk	0-III	Smears	18
107	75 (70)	PCR	Province of Gdansk	0-III	Smears	29
24	23 (96)	PCR	Province of Poznan	Lack of data	Smears	30

<sup>a</sup>FIGO, International Federation of Gynaecology and Obstetrics.

Prevalence of HPV infection in healthy women vary significantly ranging from 3 to 33% depending on different risk factors for HPV infection, such as age of first sexual intercourse, number of sexual partners, ethnicity, race, and smoking (10-13; Zalewski J, *et al*, XIX Congress of Polish Oncol Soc., p120, 1998). The rate of HPV infection in the population of healthy women examined in our study was low (2%) but correlates with results reported by other authors.

Depending on the population examined and the method used, the percentage of oncogenic HPV type detection in cervical cancer patients can also vary significantly. A number of epidemiological studies indicate very high prevalence (about 90%) of HPV infection in cervical cancers (4,5,14). However, some other data show significantly lower HPV infection rate (about 60-70%) in patients with cervical cancer (15-22). HPV prevalence in our studies amounted to 53% of cervical cancers examined, which correlates with other low frequency results related to HPV infections in cervical cancer patients from Poland (17,18). Data concerning HPV infection in women with cervical cancer from different regions of Poland is grouped and presented in Table V. Such low percentage of HPV infection in Polish population may suggest differences in ethnic groups and involvement of other genetic mechanisms in cervical carcinogenesis.

We identified HPV type 16 as the most commonly present (89%) in the group of cervical cancer patients examined. Apart from type 16 we detected types: 33, 31 and a very rare type 58. Our results are consistent with previous reports which also indicated a high occurrence of HPV 16 (80-90%) in squamous cell carcinoma (23-25). The only exception is the Indonesian population in which the most frequent virus type is HPV 18 (6).

The comparison of the PCR-RFLP method and Hybrid Capture II test indicates 81% agreement but the difference in detection rate was not statistically significant. Although the Hybrid Capture test is the only FDA-approved assay, it could not distinguish samples with single or multiple infection and could not identify the HPV virus type. Moreover, using this test we are not able to estimate how much cross-hybridisation of HC probes with phylogenetically related new types could be anticipated. The potential advantage of PCR-RFLP

method (over Hybrid Capture test) is the lower performance cost compared to Hybrid Capture test.

Understanding the natural history of HPV-associated diseases requires sensitive and specific HPV detection and typing, so different methods should be considered for routine clinical use and large-scale epidemiological screening.

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