

The presence of HPV DNA in breast cancer

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Abstract

Introduction and objective. For many years the incidence of breast cancer remained at a high level. There are many risk factors which increase the probability of occurrence of the development of neoplastic transformation. Human papillomavirus has oncogenic properties and many studies have demonstrated its role in malignant transformation of cervical epithelial. These properties seems to be of great importance also in the formation of cancers in different organs.

Objective. This study aims to assess the prevalence of HPV genetic material in women with breast cancer, and compare the prevalence of HPV DNA with a control group of women with non-malignant changes in the breast.

Material and methods. The biological material to be examined, obtained from 60 patients with breast cancer treated in Centrum Onkologii Ziemi Lubelskiej in Lublin, was fixed in paraffin blocks. The control group consisted of 30 patients with suspected tumour growth; however, the histopathological examination ruled out breast cancer cells. HPV DNA isolation was performed using a DNA Mini-Kit (Qiagen). Amplification and genotyping was carried out using a set of INNO-LIPA HPV Genotyping (Innogenetics) according to the manufacturer's instructions.

Results. The genetic material of the virus was detected in 8 women with breast cancer. DNA of HPV was not found in samples from the control group. HPV 16 occurred in 50%.

Conclusions. HPV genetic material is present in breast cancer cells. HPV is absent in women with benign breast changes. A positive correlation was found between the presence of the HPV genome and the age of the women: the virus is more common in women aged > 60 years, also noted was the presence of HPV associated with the type of histopathological diagnosis. HPV is significantly more frequent in invasive lobular cancer.

Key words

breast neoplasms, carcinogens, cell transformation, HPV DNA tests, oncogenic viruses, papillomaviridae

INTRODUCTION

Breast cancer is the most common cancer occurring in women in Poland. For many years the incidence and mortality rates of this diagnosis has been very high, although the implementation of a screening programme and progress in the oncology treatment has resulted in a reduction. According to the National Cancer Register, breast cancer has been diagnosed 15,784 times in 2010, and 5,226 women died. The problem predominantly affects women aged between 50 – 64 years of age, but the disease (however, in a small percentage) also occurs in the age group 20 – 24 years [1]. According to a report 'Prediction of cancer incidence and mortality in Poland up to the year 2025', it is estimated that the increase in incidence of women in the age group 50 – 69 years will continue to grow [2]. Because of this high incidence of the disease is extremely important to know the risk factors and possibility to reduce them. Knowledge of these risks can result in the application of preventive measures leading to a decrease in mortality; for example, current attempts to use tamoxifen and raloxifene. These drugs are used in chemoprevention in women to reduce the risk of breast cancer in groups with a higher risk of developing the disease. However, their use is subject to the occurrence of a number of side-effects, and presently the medical world is discussing their routine use

[3, 4]. The known risk factors for breast cancer include age, family history, geographic, socio-economic and hormonal factors, nutrition, physical activity, alcohol consumption, benign lesions in the breast, illness in the past on other types of cancer and the effects of ionizing radiation. Exposure to even several risk factors does not mean breast cancer will develop, but the coexistence of a number of factors increases the possibility of malignant transformation in the breast. Determination of the outcome takes into account principally the characteristics of TNM disease, as well as age, menopausal status, histological type, grade of cancer, level of expression of estrogen receptors (ER) and progesterone receptors (PgR). Early detection (prior to metastases) of a tumour is the most important prognostic factor, and the degree of expression of hormone receptors is the most important predictive factor [5, 6]. For microscopic evaluation of breast cancer, the WHO's histological classification is used. The most common type of breast cancer is ductal carcinoma, which begins in the cells of the ducts. Cancer that begins in the lobes or lobules is called lobular carcinoma and is more often found in both breasts than are other types of breast cancer. Inflammatory breast cancer is an uncommon type of cancer of the breast in which the breast is warm, red, and swollen.

Oncogenic properties of human papillomavirus (HPV) have been confirmed in a number of test results, the best documented of which is the influence on the development of cervical cancer. In addition, it is responsible for the induction of cancer of the oral cavity, vagina, vulva, and lungs, among others [7, 8]. The presented article shows the correlation

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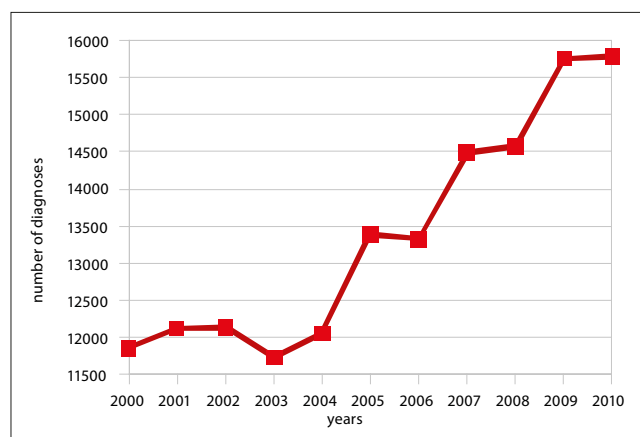


Figure 1. Incidence of breast cancer in Polish women.

between the prevalence of HPV infection in women with breast cancer. HPV belongs to family Papillomaviridae and is a non-enveloped virus with a diameter of 52–55nm. The genetic material of the virus is contained in a circular double-stranded DNA with a length of about 8,000 base pairs. It is organized in three regions: early (E), late (L) and regulatory (LCR – long control region). Division of the region encoding part of the early and late regions are apparent from the expression of the virus replication cycle. All the genes are clustered in the so-called open reading frame and are located on one strand of DNA that is transcriptionally active. The early region (which is nearly half the length of the genome) is responsible for transcription and replication of viral DNA. Proteins produced in this process are involved in the cellular transformation [9]. Especially, the proteins E6 and E7 working together play an essential role in the malignant transformation of HPV-mediated cell. They impair cell cycle control and cell maturation. The late region is responsible for encoding the structural proteins of the virion capsid. All types of viruses replicate in the nucleus of infected cells; however, those with low oncogenic potential do not connect with the host cell DNA, but replicate as extra-chromosomal episomes or plasmids. The formation of malignant tumours is associated with the ability to direct the integration of viral DNA into the host genome. Connection of the genome of HPV with cell-infected DNA leads to over-expression of oncogenic proteins E6 and E7. These oncoproteins are capable of inactivation of tumour suppressor gene production which leads to cell immortalization [10]. These properties are only potentially HPV highly oncogenic. Even if the infection does not lead directly to malignant transformation, the oncogenic properties of the protein may facilitate other carcinogenic factors that adversely affect the stability of the genetic material, leading to tumour formation [11].

Human papilloma viruses are a very large group of viruses that are classified in types and subtypes, based on the degree of compliance nucleotide sequences (DNA homology). The ability of single-stranded DNA to form a stable double-stranded DNA in certain physical and chemical conditions has led to the classification of different types of viruses. The order of detection decides on the granting of a sequence number. Nowadays, over 100 types of HPV are identified and classified. In general, viruses are classified into high potentially oncogenic types: 16, 18, 31, 33, 35, 39, 42, 44, 45, 51, 52, 56, 58, 59, 68, 73, 82, and low: 2, 3, 6, 7, 10, 11, 13, 32, 40, 43, 54, 57, 61, 70, 81. Human Papillomavirus infections can

occur through sexual contact, vertical, from mother-to-child during birth, and through infected medical instruments [12, 13]. Viruses have a special affinity for squamous epithelium covering the skin and mucous membranes, where the complete development cycle takes place (it can only occur in the differentiating epithelial cells). They infect cells and stimulates their growth by causing a different physiological form of the cells – papillary cells. Replication of the virus is very tightly linked to the differentiation of epidermal cells. Infectious progeny virus particles are detected only in the fully differentiated keratinocytes. The presence of virions in the superficial layers of the epidermis makes it possible to transfer germs by accidental contacts. Papillae may appear on the skin, mucous membranes of the mouth, respiratory tract, genital and urinary tracts [14, 15, 16].

Material for research on detection of HPV can be provided from swabs taken from the genitor-urinary tract, oral cavity and pharynx, fresh tissue obtained intraoperatively, as well as tissue preserved in paraffin blocks. Pap smears and histopathology provide only the possibility of an indirect confirmation of the presence of the virus on the basis of the changes caused in the cells and tissues (koilocytosis, dyskeratosis, dysplasia, neoplasia). Serological methods and laboratory animal breeding are not applicable, because there is no possibility of typing of the virus. The most commonly used technique is the detection of HPV in polymerase chain reaction (PCR), which allows multiple copies of the DNA fragments sought to be obtained quickly. This method is highly specific and sensitive [17, 18].

Objective. The aim of the presented study is to assess the contribution of HPV in the pathogenesis of breast cancer. This aim was achieved by isolating HPV DNA from tumour tissue from the investigated group, PCR reaction viral genome, and determination of HPV genotypes. Another aim was to determine the prevalence of the viral genetic material in women with breast cancer, divided into types of viruses, and comparing the results with patients in the control group. Furthermore, relationships were sought between the presence of viral DNA and histopathological diagnosis, staging and grading characteristics, the expression of receptors on the surface of cancer cells, as well as demographic characteristics, such as age of the patients, number of births, and time of first menstruation in women with evidence of the presence of HPV genetic material.

MATERIALS AND METHOD

The material consisted of 60 paraffin blocks containing specimens taken from patients with breast cancer who had been treated in the St Johns' Oncology Centre in Lublin (COZL). The analysed fragments of cancer tissue had a size of 5 x 10 microns. Isolation was performed using a set of DNA Mini-Kit (Qiagen) according to manufacturer's instructions. To determine the suitability for molecular research, DNA was obtained from histological preparations, and amplification reaction of human β -globin gene was performed as a reference gene. Primers with a length 22 (KM29) and 20 (PC04) base pairs were used. The size of the amplified fragment was 205 bp. The sequence of the set primer of PC04 was d(5'-CAA CTT CAT CCA CGT TCA CC-3') and for KM29 was d(5'-GGT TGG CCA ATC TAC TCC CAG G-3').

Polymerase Chain Reaction and amplification was carried out using a set of INNO-LIPA HPV Genotyping CE Amp (Innogenetics) according to manufacturer's instructions, product 65 bp; HPV DNA was amplified by the short PCR fragment SPF10 HPV primer set. The SPF10 primers amplify a 65bp fragment from the L1 region of the HPV. The presence of PCR products (65bp) was checked by electrophoresis. Genotyping performed with a set of INNO-LIPA Genotyping (Innogenetics) permitted the specific detection of 16 HPV genotypes: 6, 11, 16, 18, 31, 33, 40, 51, 53, 54, 58, 59, 66, 68, 70, (73). The set applied did not allow for differentiation between HPV 68 and HPV 73.

The studied group consisted of 60 women with histopathologically-confirmed diagnosis of breast cancer who were treated in the St. Johns' Oncology Centre in Lublin (COZL). The average age of patients was 56.85 ± 10.08 , age at menarche – 14.28 ± 1.47 . Among them, 7 women had not given birth to children, 7 women had one child, 25 had two children, and 21 had three or more children. According to histological material of patients of this group, ductal invasive carcinoma was diagnosed in 76.67% (n=46), lobular invasive carcinoma in 13.33% (n=8), other histological types of carcinoma – mucinous, intraductal and tubular carcinoma – were diagnosed in 10% (n=6).

Table 1. Characteristic of TNM and G in women with breast cancer

Stage	quantity	%
T1	15	25.00%
T2	37	61.67%
T3	4	6.67%
T4	4	6.67%
N0	38	63.33%
N1	18	30.00%
N2	4	6.67%
M0	59	98.33%
M1	1	1.67%
G1	5	8.33%
G2	33	55.00%
G3	22	36.67%

Table 2. Increased expression receptors in women with breast cancer

Increased expression	HER 2	PgR	ER
-	31	22	20
+	11	11	11
++	2	8	11
+++	16	19	18

In the control group of 30 women with suspected breast cancer, based on physical examination and diagnostic imaging, there were no tumour cells in the histopathological material. Fibrocystic breast changes were recognised by microscopic examination 16 times: 4 women had fibroadenoma, 3 had intraductal papillomas, and in 7, the analysed preparations established other histopathological diagnosis. The average age of patients in control group was 54 ± 9.28 , age at menarche – 14 ± 1.47 .

RESULTS

The conducted analysis of the research results allowed the detection of HPV in samples from 8 patients with the breast cancer. In the control group, HPV DNA was not detected. Comparison of the prevalence of the virus in these two groups there a was statistically significant difference (Fisher's exact test $p = 0.033$). In the process of genotyping, HPV type 16 was identified in 50% of samples, HPV type 6 in 25% of samples, and in 25% of samples it was not possible to determine the type of virus.

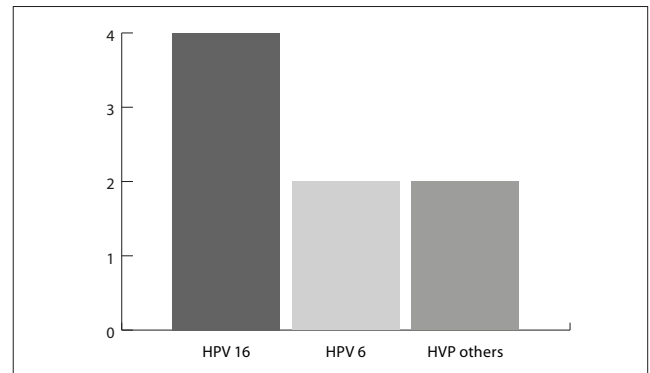


Figure 2. Types HPV identified in women

A positive correlation was found between the presence of the virus and cancer histological type. HPV was detected in 50% of patients with invasive lobular carcinoma and in 8.70% of patients with invasive ductal carcinoma. In contrast, no virus was detected in other types of cancer. The genetic material of the virus frequently found in invasive lobular cancer ($p = 0.006$). There was no statistically significant association between the prevalence of HPV in women and the staging and grading characteristics or the degree of expression of the receptors (HER2, PgR, ER).

Table 3. Presence of the virus and grading

Grading categories	G1	G2	G3	All
HPV -	4	28	20	52
HPV +	1	5	2	8
Together	5	33	22	60

Analysis of the demographic information of patients demonstrated a relationship between patient age and the presence of virus in patients. In patients > 60 years, HPV was detected in 35% of the material, in the age range 51–60 in 3.45% samples, whereas no patient aged <50 years no HPV DNA was found. Other demographic characteristics did not correlate with the presence of the virus.

DISCUSSION

Despite much research, the role of human papillomavirus in malignant transformation of mammary glands cells is still unclear. Available research results are inconclusive. There are many publications in which the presence of virus was excluded in women with breast cancer [19, 20], as well as those where the relationship has been demonstrated [21, 22]. The

large variability of results may be due to different tests used to detect the presence of the virus, the selection of appropriate primers, as well as the biological material taken to isolate the genetic material of the virus. The mechanism for inducing malignant transformation in cells has also been discussed. While the role of oncogenic proteins E6 and E7 is evidenced in malignant transformation of epithelial cells of the cervix, some researchers have cast doubt on this mechanism in the case of breast cancer [23]. Undoubtedly, further study is still necessary to confirm the correlation between infection with HPV and breast cancer. As the results of the presented study confirm, this gives great opportunities for the introduction of vaccination against certain types of virus (as is currently using in the case of cervical cancer). This would create the possibility of primary prevention in the fight against the 'epidemic' of breast cancer, both in Poland and worldwide.

CONCLUSIONS

The results suggest an effect of HPV on the development of breast cancer. In the presented study, HPV type 16 (in 50%) and type 6 (in 25%) were identified; other types were also found but their identification was not possible. HPV genome was detected in 13.33% of patients with breast cancer. This study did not demonstrate the presence of HPV in women with benign breast changes. Correlations were observed between the presence of HPV and the type of histopathological diagnosis, as well as a positive correlation between the presence of HPV and age of the patients.

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