Original Article

Assessment of the Association between Human Papillomavirus Infection and Breast Carcinoma

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ABSTRACT

Background and Objectives: **Breast cancer is the most common malignancy in women throughout the world. There are controversial reports on the role of human papillomavirus (HPV) infection in breast carcinogenesis. The aim of this study was to assess the presence of HPV-DNA in invasive breast carcinoma to determine the association between HPV infection and breast carcinoma.**

Methods: **The study included formalin-fixed paraffin-embedded tissue samples of 100 cases with invasive ductal carcinoma of breast and 50 control tissues of mammoplasty specimens. HPV-DNA was purified and amplified through GP5+/GP6+ and MY09/MY11 primers.**

Results: **All tested carcinomas as well as normal tissues were negative for all types of HPV in PCR assay.**

Conclusion: **Our results do not support the association between HPV infection and breast carcinoma. Further studies involving larger number of cases are required to elucidate the role of HPV infection in breast carcinogenesis.**

Keywords: **Breast, Carcinoma, Human Papillomavirus (HPV)**

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Introduction

Breast cancer is the most common malignancy in women throughout the world. It was representing 22.9% of all new cancers in 2008 (an estimated 1.378 million new cases) and ranking second overall when both sexes are considered together (1). "Breast cancer affects Iranian women at least one decade younger than their counterparts in developed countries" (2). The mortality rate of breast cancer was 2.5 per 100,000 for female population of Iran (3).

Most breast cancers are carcinomas, which are malignant tumors arising from epithelial cells (4). In Iran infiltrative ductal carcinoma is the most common type of breast cancer and accounts for 77% of breast carcinomas (5). In many cases, the etiology of breast cancer remains unknown. Malignant transformation of breast represents a complex multistep process in which hormonal factors, genetic changes and environmental factors including dietary components, alcohol consumption, cigarette smoking, viruses and radiation may alter common cellular pathways, resulting an uncontrolled cell growth and malignancy (4, 6, 7).

Human papillomavirus (HPV) had been detected in several types of epithelial cancers such as cervix, vulva, anus, penis, oral cavity, larynx and esophagus (8-13). There are controversial reports on the role of HPV in breast cancer around the world. In the last two decades, some studies detected HPV-DNA in human breast cancer (14–21); however some authors had reported negative results regarding the association between HPV infection and breast carcinoma (22-24). Therefore, the possible role of HPV infection in breast carcinogenesis is still a great controversy.

Identification of HPV as a predisposing factor for breast cancer could be an important issue and have significant implications in public health. It is also helpful in identifying high risk groups and design investigations for new preventive and therapeutic strategies for patients with breast carcinoma. Furthermore, it is important to determine whether the HPV vaccines could have a role in reducing breast cancers caused by HPV viruses or not?

Considering the controversial reports on the association of HPV with breast carcinomas, the aim of this study was to assess the presence of HPV-DNA in invasive breast carcinoma to determine the association between HPV infection and breast carcinoma.

Materials and Methods

Study population

In this cross sectional study, formalin-fixed and paraffin-embedded tissue samples of 100 patients with invasive ductal carcinoma of breast (as study group) and 50 mammoplasty specimens (as control) from Tehran, Iran were provided for analysis. The study was approved by Pasteur Institute of Iran Ethics Committee.

Paraffin blocks were re-cut and prepared slides were stained with hematoxylin and eosin for histopathological review, confirming the diagnosis and marking areas for microdissection.

DNA extraction

Sections of 5–10 μm wide were prepared from each specimen, avoiding any cross-contamination between samples. Sections were subsequently deparaffinized by xylene and digested using digestion buffer containing proteinase K, followed by extensive extraction with phenol/chloroform. DNA quality was evaluated by PCR using primers PCO3/PCO4 that amplify a 110 bp product from the human β-globin gene. β-globin positive samples were subjected to nested PCR .

Nested PCR

Samples were screened for the presence of HPV using the nested PCR consisting of the MY09/11 primers (outer primers) and the GP5+/6+ primers (inner primers).

The MY09/MY11 primer set [MY9 (5' – CGT CCA/C AA/GA/G GGA A/TAC TGA TC - 3')] and [MY11 (5' - GCA/C CAG GGA/T CTA TAA C/TAA TGG – 3)], which amplify the L1 gene of HPV was capable of amplifying a wide spectrum of HPV types to produce a PCR product of 450 bp. Second round of PCR were done using GP5+/GP6+ primers. The GP5+/GP6+ primer set [GP5+ (5′- TTT GTT ACT GTG GTA GAT ACT AC-3′)] and [GP6+ (5′-AAA AAT AAA CTG TAA ATC ATA TTC-3′)] is a non-degenerate primer set that detects a wide range of HPV types using a lower annealing temperature during PCR and produces a PCR product of approximately 150 bp. The PCR was performed as previously explained by Aghakhani *et al.* (25). PCR products subjected to 1.5% gel electrophoresis to determine presence of HPV in specimens.

The Chi-square was used with the SPSS 16 Package program for statistical analysis (Chicago, IL, USA).

Results

Formalin-fixed and paraffin-embedded tissue samples of 100 patients with invasive ductal carcinoma of breast and 50 mammoplasty specimens were analyzed for presence of HPV-DNA. The age of cases and controls ranged 20-80 and 21-50 years respectively. The tumor size was between 1.3 and 9 cm. 17.6%, 49.1% and 33.3% of cases had tumor grade 1, 2 and 3 respectively (Nottingham grading system) (26).

All tested carcinomas as well as normal tissues were negative for all types of HPV in PCR assay (Fig. 1).

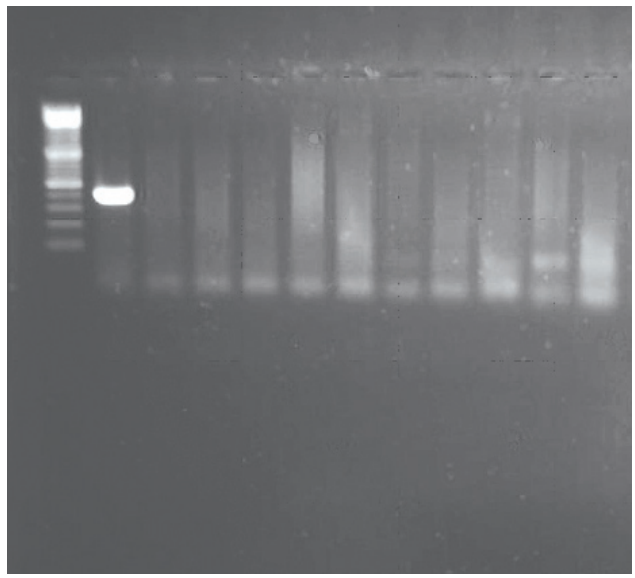

Fig.1: MY09/MY11 PCR amplicons of breast carcinoma samples with positive and negative controls. Lanes from left to right: DNA marker, positive control, negative control and samples which all are negative for HPV-DNA

Discussion

This study investigated the presence of HPV-DNA in invasive breast carcinoma to determine the relationship between HPV infection and breast carcinogenesis. We did not find HPV-DNA in breast cancer specimens regardless of patients' age or tumor grade and size.

Breast cancer is one of the most prevalent malignancies affecting women worldwide, with an annual incidence of about 1 million cases (27, 28). The development of combinative therapy for breast carcinomas including surgery, chemotherapy, radiology, biological and endocrine therapy, together with increasing public knowledge about early detection of breast cancer, has led to a good prognosis for many breast carcinomas. However, breast cancer is still the leading cause of cancer deaths in women (29, 30). The etiology of breast cancer remains poorly understood. Although many risk factors including family history, cigarette smoking, alcohol use and hormone levels are associated with breast cancer, however these factors cannot explain all cases of breast carcinomas (4, 6, 28).

Oncogenic papillomaviruses, especially HPV types 16 and 18, have been involved in malignant lesions of several sites, such as cervix, esophagus, prostate, bladder, and head and neck (8, 12, 13, 25). The possible participation of HPV in breast carcinogenesis has been proposed repeatedly in recent studies (14-24), but reports have been rather inconsistent.

Di Lonardo *et al.* (14) first demonstrated the relationship between HPV infection and breast cancer in 1992. After that, a growing number of investigations have detected HPV-DNA in breast cancer tissues, with the variable prevalence ranging from 0 to 86.2% (14-24). Several scholars (23, 24, 31, 32) did not find HPV- DNA in breast carcinomas. Low frequency of HPV infection (less than 16%) was reported by many authors (19, 33, 34) and high rates (between 20.9% and 86.2%) were reported by other investigators (14-17, 21).

Lindel *et al.* (23) showed no evidence of HPV infection in Swiss women with breast carcinomas. de Cremoux *et al.* (24) also analyzed invasive breast carcinoma tissues from French patients and no HPV infection were detected in any of breast cancer cases. Wrede *et al.* (32) investigated HPV infection in British women with breast cancer, but they also failed to detect any HPV infection. In a survey from North part of Iran, HPV-DNA was detected in 25.9% of breast cancer patients (35). de León *et al.* (36) reported the 29.4% rate of HPV-DNA positivity in breast cancers of Mexican women. Forty eifgt percent of Australian women with breast carcinoma were HPV positive in Kan *et al.* study (19). de Villiers detected HPV-DNA in 86.2% of breast carcinoma samples from German patients (18).

These discrepancies could be due to genetic, environmental and geographical variance in the study populations and differences in detection assays and tissue used for viral detection. Moreover, it seems that HPV load in breast tumors is much lower than its concentration in cervical cancer, which may cause harder detection of HPV in breast carcinomas despite amplification by PCR (37). In current study we did not find any association between HPV infection and breast carcinoma which is in agreement with other investigations which reported no HPV-DNA in breast cancers (23, 24, 32).

Other potential reason for these differences may be attributed to cross-contamination from other organs, which infected with HPV, during collection and processing of samples. In this study we avoided any cross-contamination between samples by sectioning the blocks to several small groups at different times, using new surgical blade for each sample, specially the first section of each specimen plus blade and gloves were discarded and new blade and gloves were used for main sectioning and using new filter tips during extraction and PCR.

Conclusion

Our results do not support the association between HPV infection and breast carcinoma. Further studies involving larger number of cases are required to elucidate the role of HPV infection in breast carcinogenesis.

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