RESEARCH ARTICLE

Lack of Evidence for a Relationship between High Risk Human Papillomaviruses and Breast Cancer in Iranian Patients

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Abstract

Background: Whether there is any relationship between human papilloma virus (HPV) and breast carcinoma is not clear. Some previous studies have indicated a possible role in oncogenesis in the breast. In this study, we therefore analyzed the presence of HPV infection in breast tissues of Iranian women from Yazd city. Materials and Methods: In a cross-sectional study, formalin-fixed paraffin-embedded tissues from 87 patients with breast cancer and 84 cases with breast fibrocystic lesions (control group) were selected from a tissue archive. Grade of tumors and fibrocystic tissues were determined by two pathologists. The nested-PCR method was performed for detection of HPVs in samples. HPV genotypes were determined by sequencing and the phylogenetic tree depicted by MEGA software. Results: Of the 87 women with breast cancer, 22.9% (20 isolates) had positive results for HPV DNA. In the control group no HPV was detected. The HPV genotypes in positive samples were HPV-16 (35%) HPV-18 (15%), HPV-6 (45%) and HPV-11 (5%). The data did not approved a significant correlation between tissue pathology of breast cancer and the HPV genotype frequency. Conclusions: The data did not provide any evidence for a role of high risk HPV types in oncogenesis in the breast.

Keywords: Breast cancer - human papillomavirus - risk factor - Iran

Asian Pac J Cancer Prev, **17 (9)**, 4357-4361

Introduction

Breast cancer is one of the most common malignanciesy in women (Gradishar et al., 2015). The prevalence of breast carcinoma during previous years has increased in many countries (Molah Karim et al., 2015; Proctor et al., 2015). In 2012, the world health organization (WHO) was reported 521000 deaths caused by breast cancer (2014). This cancer includes 76% of common malignancies among Iranian women and death 1200 women per year in Iran (Tazhibi and Feizi, 2014). Gender, age, genetic factors, race, dense breast tissue and family history of disease are unchangeable risk factors for this cancer (Dossus and Benusiglio, 2015). Having children, hormone therapy after menopause, drinking alcohol and physical activity are changeable risk factors (Chlebowski and Anderson, 2015; Hvidtfeldt et al., 2015).

Also there are exist other effective unclear factors in incidence of breast cancer including diet and vitamin intake, Chemicals in the environment and tobacco smoke (Imtiaz and Siddiqui, 2014; Pimhanam et al., 2014). Genetic is responsible for ten percent of breast cancer cases, therefore environmental risk factors are also important (Marchina et al., 2010; Baker, 2015). During the past three decade, viral infections are considered as environmental causal factors (Alibek et al., 2013). Up today, Epstein bar virus (EBV) human herpes virus type 8 (HHV-8) and cytomegalovirus (CMV) are detected relating to breast carcinoma (Tsai et al., 2005; Yahia et al., 2014). Also genome of herpes simplex virus type 1 (HSV-1) has been detected in breast cancer tissues (Hsu et al., 2010).

The previous studies showed that human papilloma virus (HPV) is associated with 90% of anal cancers, 60% of oropharyngeal cancers, 65% of vaginal cancers, 35% of penile cancers, 50% of vulva cancers and almost all cases of cervical cancers (Zandberg et al., 2013). In general, it can be said that high-risk types of HPV are associated with approximately 5 percent of all cancers (de Martel et al., 2012). Because of immortalization human mammary normal cells in vitro by high risk types of HPV and detection of some types (16, 18, 31 and 33) in breast carcinoma tissues, the role of this virus in tumorigenesis of mammary cells is controversial hypothesis (Hsu et al.,

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2010). Since 1992, many studies have been carried out for detection of human papilloma virus in breast carcinoma tissues by polymerase chain reaction method (PCR), but the result of this research was inconsistent (Gumus et al., 2006; Lindel et al., 2007). For the first time, Relationship between HPV and breast cancer were evaluated by Di Lonardo et al. they were detected HPV-16 DNA in 29.4% of breast cancer tissues (Di Lonardo et al., 1992). Hennig et al. detected HPV-16 DNA in breast tissue of 46% of women with a history of high grade cervical intraepithelial neoplasia (CIN III) (Hennig et al., 1999b). Up to present, some researchers are detected HPV-18, -31,-33 and -58 DNA in breast carcinoma specimens (Fu et al., 2015). In contrast, in some studies, researchers have not detect HPV DNA associated with breast cancer (Kwong et al., 2013). Therefore, until the role of HPV DNA in pathogenesis of breast cancer is controversial. Further studies are needed for determination of the relationship between HPV infections and breast cancer. If the papilloma virus identified as a risk factor; therefore, preventive care and vaccination can decrease the cases of breast cancer. The aim of the present research is the study of prevalence all types of HPV (low-risk and high-risk) in breast cancer tissues and the evaluation of relationship between human papillomavirus and breast cancer in Yazd city patients of Iran.

Materials and Methods

Tissue sampling

For design a cross-sectional study, formalin-fixed paraffin-embedded tissues from 87 breast cancer patients and 84 cases with breast fibrocystic (control group) were selected from an archive of tissue of Shahid Sadoughi hospital laboratory in Yazd city. Grade of tumors and fibrocystic tissues were approved by two pathologists.

Tissue processing and DNA extraction

Tissue samples were cut by microtome (Accu-Cut® SRM™ 200, Sakura Finetek USA, Inc.) in 5 micron thickness. Then the slices of tissues collected in 1.5 ml micro tubes. One ml of xylene (Merck, Inc. Germany) added to each micro tube and mixture incubated at 55˚C (1 hour) for performing tissue deparaffinization. After centrifugation and removing of supernatant, this step was repeated with xylene. Tissues were washed by adding 1ml of ethanol 100% (Merck Inc. Germany), incubation at 37˚C and removing of supernatant (two time). Micro tubes were stored in an incubator for ethanol evaporation and drying of pellet. The tissue slices were digested using lysis buffer containing 250μg/ml proteinase K (Cinnaclone, Iran) for 1.5h at 60˚C followed by incubation for overnight at 37˚C. DNA extraction was carried out, from lysed tissues, using RIBO-prep extraction kit (AmpliSens, Russia). According to the manufacturer's instructions, 300μl of the warm lysis buffer were added to each 100μl of the sample and extraction were performed using standard protocol of kit.

HPV detection by PCR method

For detection of human papilloma virus in breast tissues, nested polymerase chain reaction (nested PCR) method was used followed by sequencing. We were used from 4 primers (MY09, MY11, GP5+ and GP6+) for detection of HPV genome and were used from Primers for β-globin gene as an internal control (summarized in Table1).

For any reaction with a final volume of 50μl, we were used 0.2μl of Taq polymerase (5u/μ, Thermo Scientific Inc., USA), MgCl2 (5 Mm, Thermo Scientific Inc., USA), 1μl of DNTPs (100mM, Thermo Scientific Inc., USA), 5μl of Buffer (10x, Thermo Scientific Inc., USA), 0.5μl of forward and reverse primers (10pmol, Metabion Inc., Germany), 10μl of Template and 31.5μl of DEPC water (Cinnaclone, Iran). The DNA of Hella cells and distilled water were used as PCR positive and negative controls, respectively. After amplification, PCR products were visualized on 1% agarose (Sigma-Aldrich, USA) gel by Gel Doc system (UVItec, United Kingdom).

Sequencing of HPV genome

Sequencing technique was carried out for confirmation of positive PCR results and for HPV typing. After purification of PCR product by Gene JET PCR Purification Kit (Thermo Scientific Inc., USA), the samples were sequenced by ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, USA). Data were analyzed by chromas software version 2.4.3 and types of HPV were determined using NCBI- BLAST web.

Phylogenetic Analysis

The sequences of the HPV-L1 gene were corrected by using the BioEdit software version 7.2.5.

Analyses for phylogenetic inference were performed using Maximum Composite Likelihood Method in MEGA software version 4.0.2.

Data analysis

Data were analyzed by using SPSS software version 18. Pearson Chi Fisher s Exact test was performed for evaluation of significant relationship.

Results

Samples

For this study, 87 breast cancer tissues were selected from archived paraffin-embedded blocks in the laboratory of Shahid Sadoughi hospital in Yazd city. The age of patient was between 22 and 77 years with overall average 47.7 ± 12.5 years. Two pathologists were determined the type of cancerous tissues that are summarized in Table 2. Also 84 approved noncancerous breast samples were selected as a control group.

Papillomavirus Genotypes in samples

Nested-PCR and sequencing methods were carried out for papillomavirus detection in selected tissue samples. HPV- DNA was detected in 22.9% of case samples. All control samples were negative for HPV genome. Only 4 types of human papillomavirus, including HPV-6, 11, 16 and 18 were detected. Data analysis for HPV Genotypes and tissue pathology grades has not shown any correlation

(p value: 0.427). This data summarized in Table 3.

Frequency of HPV types in breast cancer tissues is shown in Table 3. This data did not show the significant correlation between tissue histopathology of breast cancer and the HPV genotype frequency (p value: 0.432).

Phylogenetic analysis for HPV genotypes

Phylogenetic analysis showed that HPV-6 sequences had same homology with Slovenia and Saudi Arabia. The sequences of HPV-11 isolates were more similar to American and Germany isolates. HPV-16 and HPV-18 types had homology with Slovenia and Indian, respectively (Figures 1-4).

Table 1. Specific Primers for HPV Detection in Breast Tissues

Primers name	Primer sequence	Amplicon size (bp)
HPV outer primers (L1)		
MY09-F	5'-GCM CAG GGW CAT AAY AAT GG-3'	450
MY11-R	5'-CGT CCM ARR GGA WAC TGA TC-3'	
HPV inner primers (L1)		
GP5+-F	5'-GTT ACT GTG GTA GAT ACT AC-3'	150
GP6+-R	5'- CTT ATA CTA AAT GTC AAA TAA AAA-3'	
β-globin gene primers		
PC03-F	5'-ACACAACTGTGTTCACTA GC-3'	110
PCO4-R	5'-CAACTTCATCCACGTTC ACC-3'	

Table 2. Histopathological Types of Cancerous Tissues in Case Samples

Histopathology	Frequency
Invasive Ductal Carcinoma (IDC)	66 (76%)
Invasive Lobular Carcinoma (ILC)	14 (16%)
Invasive Medullary Carcinoma (IMC)	7 (8%)
Total	87

Table 4. Results of HPV Frequencies in Different Grades of Breast Cancer

Pathology grade	HPV positive	HPV negative	Total	P value
I	8 (27.5)	21 (72.5)	29	
II	6 (27.2)	16 (72.8)	22	
III	6 (16.6)	30 (83.4)	36	
Total	20 (22.9)	67 (77.1)	87	0.427

Figure 1. Phylogenic Tree for Human Papilloma Virus Type 6

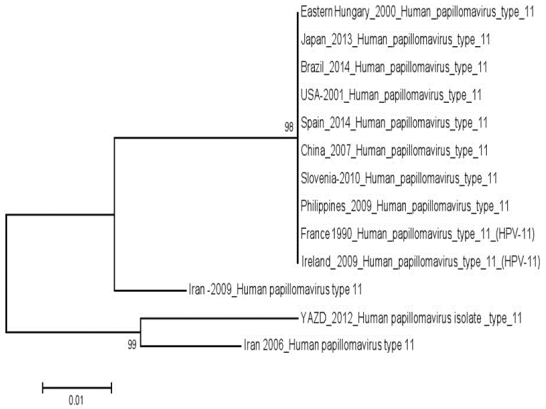

Figure 2. Phylogenic Tree for Human Papilloma Virus Type 11

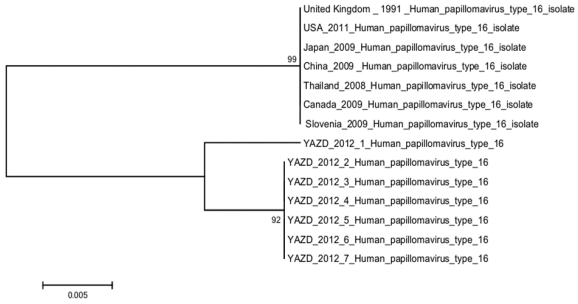

Figure 3. Phylogenic Tree for Human Papilloma Virus Type 16

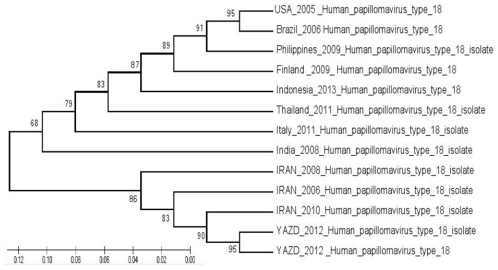

Figure 4. Phylogenic Tree for Human Papilloma Virus Type 18

Table 3. HPV Genotype Frequencies in Patients with Breast Cancer

Tissue Histopathology	HPV positive				HPV negative	Total	P Value
	HPV- 6	HPV- 18	HPV- 16	HPV- 11			
IDCa	5 (7.6%)	3 (4.5%)	4 (6%)	1 (1.5%)	53 (80.4%)	66	
ILCb	2 (14%)	0	3 (21%)	0	9 (65%)	14	
IMCc	2 (28%)	0	0	0	5 (72%)	7	
Total	9	3	7	1	67	87	0.432

a Invasive Ductal Carcinoma; b Invasive Lobular Carcinoma; c Invasive Medullary Carcinoma

Discussion

The relationship between viruses and breast cancer be considered in medical sciences in recent years (Gumus et al., 2006). Up today, the role of viruses in the etiology of this cancer has not been clear. The members of herpesviridae including HSV, EBV and HHV-8 are detected in breast tumors (Hsu et al., 2010). According to previous researche, it is accepted that human papilloma virus can infect the breast tissues (Zandberg et al., 2013). There are exist many studies that are evaluated the presence HPV genotypes in breast cancer lesions (Fu et al., 2015). The various studies were reported that the rate of HPV is in the range of 4 to 86 percent (Hsu et al., 2010).

In 2010, a meta-analysis was performed to estimate the prevalence of human papillomavirus and evaluate the association this virus with breast cancer. Data analyses showed that 24.49% of the breast cancer cases were associated with HPV. 12.91% of the cases were occurred in Europe and 32.42% in Asia. Data were not rule out the possibility of the association of HPV and breast cancer (Li et al., 2011). We detected the HPV in breast carcinoma lesions that is consistent with many previous publications, but data statistical analysis did not showan association between this virus and breast carcinoma.

In the past years, three studies were carried out in Iran for detection of HPV in patients with breast cancer. Tahmasebifard et al. cannot find any types of HPV in patient's tissue lesions (Tahmasebi fard, 2013) but sigaroodi et al. reported that HPV-6, -11, -16, -18, 23, 124 and -15 have been present at 25% of breast carcinoma samples (Sigaroodi et al., 2012). In our study, HPV-6, -11, -16 and -18 were detected in 22.9% of cases, but HPV-6 was more than other types while in sigaroodi study the HPV-16 and -18 were the most abundant (Sigaroodi et al., 2012). In both studies, most HPV types reported in invasive ductal carcinoma, but did not show a significant relationship between HPV types and IDC.

In the latest study conducted in Iran, Ahangar-Oskouee et al. were detected HPV in 33.8% of breast cancer specimens, but were not found the association between HPV higt risk types with this carcinoma (Ahangar-Oskouee et al., 2014). In this research, like our study, the HPV-6 was found the most frequent type.

In 2008, De cremoux et al. were not founded any HPVs in 50 breast cancer specimens (de Cremoux et al., 2008). We used same primers for PCR testing and achieved positive results. Our finding showed that 55% of breast cancer tissues had high risk HPV types. The same data were acquired by De Villiers et al. previously (35).

Henning et al. were detected only HPV-16 in 46% of breast carcinoma lesions in South Korea (Hennig et al., 1999a). Some researchers, including Widschwendter, Damin and Kan were showen the evidence for an association of HPV and breast carcinoma (Damin et al., 2004; Widschwendter et al., 2004; Kan et al., 2005) but we have not found a significant association between HPV types and breast cancer like De cremoux study (de Cremoux et al., 2008).

In 1993, Ong et al. were depicted the phylogenic tree for HPV types. They were showing that HPV-18 types of Asia are closely related to America and Europa (Ong et al., 1993). Phylogenic analysis of our HPV isolates supported this finding. Also the HPV-6 and HPV-16 isolates are close to Asian and European isolates. The HPV-11 isolates are more related to Iranian 2006 types, whereas 2009 isolates located close to European country isolates.

It is possible that various reported results of HPV prevalence in an aria be caused by differences in performance of diagnostic methods or sampling. HPV concentration in breast cancer lesions seems to be lower than its concentration in cervical samples, therefore, HPV detection in breast tumor specimen is more difficult (Ahangar-Oskouee et al., 2014).

In conclusion, The present study reveals the presence of the HPV genome in breast tumor tissues in women of Yazd city in Iran. We were not founded the potential evidence for the role of high risk HPV types in oncogenesis of breast tissue. Therefore, further research is needed to clear this hypothesis.

Acknowledgements

The authors acknowledge the cooperation of the staff of the Animal viral Disease Research and Diagnosis, Razi institute.

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