Prevalence of *Human Papilloma Virus* in group of Egyptian patients with breast cancer

Maha I. El Zaafarany[1], WafaaM. Badawy[2], Eman M. El Salakh[3]

[1]Oncology Centre, Mansoura University, Egypt.
[2]Student Hospital, Mansoura University, Egypt.
[3] Faculty of Medicine, Mansoura University, Egypt.

ABSTRACT

The etiology and the molecular mechanisms related to breast carcinogenesis remain poorly understood. Some reports have examined the role of *Human Papillomavirus* (HPV) in this disease. The purpose of this study was to determine the prevalence of HPV in breast cancer.Fifty four fresh frozen breast cancers samples were analyzed. Samples were tested for HPV by PCR, and products were sequenced. Findings were correlated with clinical and pathological characteristics.The HPV DNA prevalence in the breast cancer samples was 28 % (15/54). Sequence analysis in a subgroup of cases indicates the prevalence of high risk HPV16. In conclusion, the present study demonstrated for the presence of HPV in Egypt in a proportion of the malignant breast tissues. This finding suggests that HPV may have a biological significance in breast carcinogenesis.

Key Words: human papillomavirus, breast cancer, polymerase chain reaction (PCR).

INTRODUCTION:

Breast cancer is one of the main health problems in developed countries, and occupies a second place (15%) in incidence in the world, after the lung cancer (25% to 50%). (INCA 2011 and Estimativa, 2012). It is accounting for 22.9% of all female cancers. It occupies second place in frequency in female preceded only by cervical cancer .It is also the leading cause of cancer death in females accounting for 13.7% of their cancer-related mortality (Hortobagyi *et al*. , 2005).

In Egypt About 70% of the affected individuals will die from their disease, compared to 20-25% in Western countries. Late diagnosis and sub-optimal treatments are the most likely explanation for this gap (Corbex M *et al.*, 2009).

Worldwide, breast cancer is one of the main health problems and constitutes the highest number of deaths, involving 458,000 deaths per year, with the number of cases continuing to rise (JemalA *et al.*, 2007) . There are many risk factors associated with breast cancer development (age, familial history, personal history of breast cancer). Nevertheless, in 50–80% of cases, known risk factors have not been identified which has generated an interest in identifying new factors related to this cancer as virual infection. (Hortobagyi *el al.*, 2005).

The most studied viruses found to cause breast cancer in humans are mouse mammary tumor virus (MMTV), the Epstein-Barr virus (EBV) that occurring in up to 37% and 50% of breast carcinoma cases, respectively(Mant C.*et al.,* 2004). HPV is accepted as carcinogenic in human cervical, ano-genital, and head and neck cancers. Molecular and epidemiological studies have shown that a persistent infection with high-risk HPV is the most important risk factor for both cervical

cancer and its precursors (Chaturvedi *et al.*, 2002).

A number of studies have reported HPV DNA detection in extra-genital cancers, although the etiological involvement of HPV in those malignancies is still controversial (Jayaprakash *et al.,* 2011). It has been shown that HPV types 16 and 18 can immortalize normal breast epithelium. This raised the possibility that HPV might be involved in the pathogenesis of breast cancer(New York: Springer).

However, unlike cervical carcinoma, which is almost always associated with HPV, the causal role of HPV infection in the development of breast carcinoma remains controversial. The potential mechanism of transmission of HPV for the breast remains unknown, and opinions are divided between lymphatic and the blood spread. Although HPV transmission route is not yet determined, some types of HPV are found in both tumors cervical and breast (Widschwendter *et al.*,2004) (Yoshida K *et al.,*2011)

High risk HPV encodes aseries of proteins ,designated as early are (E1 - E7) or late (L1 and L2) . E6/E7 proteins inactivate two tumor suppressor proteins, p53 (inactivated by E6) and pRb (inactivated by E7). (Chaturvedi *et al.,* 2008) . The viral oncogenes E6 and E7 are thought to modify the cell cycle so as to retain the differentiating host keratinocyte in a state that is favourable to the amplification of viral genome replication and consequent late gene expression. (Rampias *et al.*, 2010).

The aim of this study was to document the association of HPV and its types with breast carcinoma cases in Egypt. The study also aimed to identify the correlation of HPV and its types with known prognostic and predictive markers, namely age, histological grade, tumor size, lymph node metastasis.

MATERIALS AND METHODS

Study subjects:

The study was conducted on 54 fresh tissue biopsies of breast carcinoma, collected without any pre-selection criteria from the Oncology Center at Mansoura University which were typed according to the American Joint Committee on Cancer, included: 37 invasive ductal carcinomas, 12 invasive lobular, 5 patients having both a history of invasive cervical cancer and breast cancer as second primary cancer were selected for enrolment in a study of breast carcinomas for the presence of HPV. Clinicopathological information, including age, tumor histology, clinical stage, the expression of estrogen receptors were obtained from medical and pathological records.

Polymerase Chain Reaction (PCR) for HPV Detection:

DNA was extracted from tumor fresh biopsies using QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) following manufacturer's instructions.

The presence of HPV DNA sequences was verified by amplification with two sets of primers. A first round with degenerated primers MY09, 5′-CGT CCM ARR GGA WAC TGA TC-3′ and MY11, 5′-GCM CAG GGW CAT AAY AAT GG -3′, amplifying 450 bp long fragment in highly conserved region in L1 gene; and a second round with consensus primers GP5+, 5′ TTT GTT ACT GTG GTA GAT ACT AC- 3′ and GP6+, 5′-CTT ATA CTA AAT GTC AAA TAA AAA 3′ generate 140 to 150 bp fragment of the L1 region of the virus. Both systems of primers detect

a broad spectrum of oncogenic and non-oncogenic HPV types. *(HPV: human papillomavirus.* Primer sequence abbreviations: M = A or C; W = A or T; R = A or G; Y = C or T; I = inosine)

Reaction mixture (50 µl) for nested PCR consisted of 1×reaction buffer. PCRs were run using following profile: initial denaturation at 95°C for 5 min, 40 cycles of 95°C for 1 min, either 50°C (annealing for MY09/11 primers) or 40°C (GP5+/6+primers) for 1 min and 72°C for 1 min, final incubation by 72°C for 5 min. PCR were run on the cycler (Thermo scientific ARKTIK) thermal cycler Finland . Products of all PCRs were separated in a 2% agarose gel . Successfully amplified PCR products were purified with a gene jet PCR purification kit (Fermentous), sequenced using a Big Dye Terminator Sequencing kit (Applied Biosystems) , and run on an automated sequencer ABI Prism 310 (Genetic analyzer, USA) at an constant voltage of 11.3 kV for 20 minutes. Results were evaluated by BLAST program http://www.ncbi.nlm.nih.gov/BLAST.

RESULTS

Statistical analysis:

Statistical analysis was performed using Prism 5.0 statistical package (GraphPad Software, San Diego, USA, 2007). Categorical data was compared between groups by using Chi-square (χ^2) or Chi-square (χ^2) with Yates correction. Categorical variables were represented with frequency and related percentage values. All the P-values presented are two-sided. A p value of < 0.05 was considered statistically significant.

We examined 54 female breast cancer cases, aged from 25 to 64 years with a mean of 49. Patients' clinical characteristics and HPV association are shown. 15 out of 54 patients (27 %) were HPV positive for MY09/MY11 and GP5+/GP6+ amplification.11 were positive for HPV16(73.3%),4 were positive for HPV 18 (26.7%) There was no significant difference in the HPV detection rate between histological types of invasive carcinomas. The presence of HPV was not related to the expression of estrogen and progesterone receptors. (Table1).

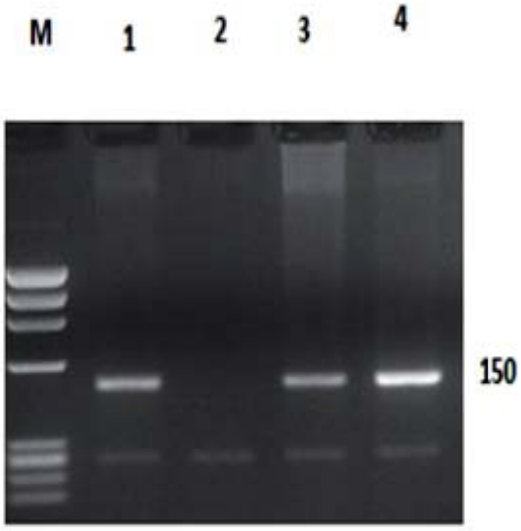
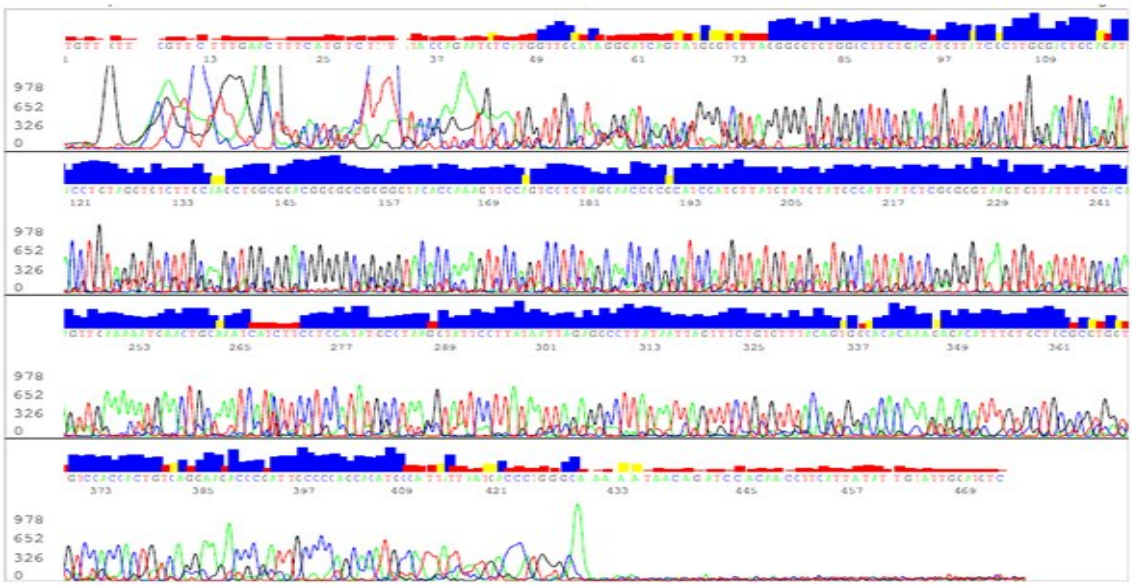


Figure 1. Detection of HPV infection from breast cancer biopsy in 2.0% agarosegel electroforetical analysis of nested PCR products. In left line – MW marker, lines marked 1 – 4are negative and positive samples, PCR product 150 bp.



Sequencing of product of nested PCR of HPV by genetic analyzer

Table 1. HPV status according to clinical parameters in breast carcinoma cases.

	All Cases N (%)	**HPV Positive N (%)**	**HPV Negative N (%)**	**p Value**
Number of Cases:	54 (100)	15 (27)	39 (73)	
Age Groups:				0.4991
< 40	8 (100)	1 (13)	7 (88)	
41-50	21 (100)	8 (38)	13 (62)	
51-60	22 (100)	5 (23)	17 (77)	
> 60	3 (100)	1 (33)	2 (67)	
Tumor Histology:				0.5478
Invasive Ductal:				
NOS:	26 (100)	6 (23)	20 (77)	
Mucinous:	9 (100)	2 (22)	7 (78)	
Medullary:	3 (100)	1 (33)	2 (67)	
Invasive Lobular:	11 (100)	3 (27)	8 (73)	
Both Cervical & Breast Cancer:	5 (100)	3 (60)	2 (40)	
Clinical Stage:				0.5951
Early:	10 (100)	3 (30)	7 (70)	
Advanced:	37 (100)	9 (24)	28 (76)	
Unclassified:	7 (100)	3 (43)	4 (57)	
Axillary LN Metastasis:				0.9371
Positive:	41 (100)	11 (27)	30 (73)	
Negative:	13 (100)	4 (31)	9 (69)	
Estrogen ReceptorExpression:				0.7533
Positive:	35 (100)	9 (26)	26 (74)	
Negative:	19 (100)	6 (32)	13 (68)	

HPV: human papillomavirus.

DISCUSSION

The transmission route of HPV detected in breast carcinoma is yet unclear. Two independent studies suggested possible hematogenic and/or lymphatic transfer of the virus from one organ to another (Widschwendter *et al.*, 2004) Interestingly other study detected HPV-16 DNA in 46% of breast cancer occurring among women with a history of high-grade cervical intraepithelial neoplasia (CIN III). Their finding suggests that HPV-associated cervical neoplasia might be the original site of HPV infection from which the virus could be transported to the breast (Hennig EM *et al.,* 1999). In our study, there were five breast cancer cases with a history of cervical cancer but only 3 of them was HPV-positive in their breast tumor.

Results of this work 15 out of 54 patients (27 %) were HPV positive for MY09/MY11 and GP5+/GP6+ amplification. confirm and broaden earlier reports that also described a similar proportion of positive cases, such as the 23% HPV association with invasive ductal carcinoma, reported in Australia,(Heng *et al.,* 2009) the 21% described in Japan by(Khan *et al*., 2008) or the 25.9% HPV positive cases in Iran. (Sigaroodi *et al.,* 2012) In line with these,(Li *et al*.,2011) conducted a meta-analysis and revealed that 24.5% of the breast carcinoma cases were associated with HPV, of which 32.4% occurred in Asia and 12.9% in Europe. Increased HPV incidence in breast cancer was observed in Australia, were both Glenn *et al.* (2012) and Antonsson *et al*. (2011) described HPV DNA prevalence in the breast cancer samples of 50%. Conversely, only a 6.5% HPV association was described in China (Mou X *et al*., 2011).

Between 1992 and 2012 the worldwide systematic revision of a number of studies about HPV relation in breast cancer, showed that prevalence varies between 4% (3/67) (Mendizabul-Ruiz *et al.,* 2009) in Mexico to 86% (25/29) in the USA. (de Villiers EM *et al.,*2005).

Kenji Ohba *et al.* (2014) reported that HPV-positive breast cancer showed better Prognosis than virus-negative tumors as HPV-positive tumors showed significantly longer duration period for recurrence after breast cancer resection as compared to virus-negative tumors .

Results of this work show no significant difference in the HPV detection rate between histological types of invasive carcinomas. The presence of HPV was not related to the expression of estrogen and progesterone receptors .In coordination of our results that his report, clinical parameters such as tumor histology, axillary lymph node status or estrogen and progesterone receptors were not statistically associated with presence of HPV (de León *et al.*, 2009) (Aguayo *et al.*, 2011) However, (Kroupis *et al.*, 2006) described the only breast cancer series harboring high-risk HPV DNA sequences related to clinical parameters; in which those HPV+ cases displayed less estrogen-receptor and were proliferative. (Kroupis *et al.,* 2006)

In results of this work 11 were positive for HPV16(73.3%), 4 were positive for HPV 18 (26.7%).In coordination of our results Di Lonardo *et al*. were the first to report HPV 16 DNA using PCR in 29.4% of 40 breast cancer specimens. (Di Lonard *et al.,* 1992). Using PCR, HPV types 11, 16 and 18 have been reported with increasing frequency from women living in USA and Brazil, while HPV type 18

was present in the majority of Australian women (Kan CY *et al.,* 2005). Others reported that HPV 33 was seen in 34.1% of breast carcinoma cases in Japan (YU Y *et al.,*2000), Studies from the Middle East reveal that HPV types 18, 33 and 35 were present in cancerous and normal breast tissue in Turkish women. (Gumus *et al*., 2006) In Syria, a prevalence of HPV 16 (9%), HPV 33 (56%) and HPV 35 (37%) was observed. (Akil *et al*., 2008). In conclusion, The present study demonstrates the presence of HPV in Egypt in a significant proportion of the malignant breast tissues .As HPV vaccine are now available for HPV16,18 in a number of countries with good effectiveness to prevent cancer cervix and genital warts ,so studies to detect effectiveness of the vaccine as specific therapy in breast cancer .

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