ORIGINAL ARTICLES

Immunohistochemical detection of human cytomegalovirus, Epstein-Barr virus and human papillomavirus in invasive breast carcinoma in Egyptian women: A tissue microarray study

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ABSTRACT

Background and aim: Breast cancer is the commonest malignant tumor and a common cause of cancer death in women all over the world. Some recent studies attributed breast cancer to viral infection. This study aimed to evaluate the expression of HCMV, EBV and HPV in invasive carcinoma of the breast among the Egyptian women by immunohistochemistry and whether there is a relationship between the prognostic factors of breast carcinoma and these viruses.

Patients and methods: This retrospective study included 107 selected cases of invasive breast carcinoma. Slides cut from tissue microarray prepared blocks were stained immunohistochemically for HCMV, EBV and HPV antigens. The association of such viruses with the clinicopathological features, tumor recurrence and patient death was evaluated statistically.

Result: HCMV, EBV and HPV were present in 43.9%, 10.3% and 24.3% of cases respectively. HCMV was associated significantly with the tumor grade, mitotic count (P = .01), IDC, ER, PR, Her2/neu and molecular subtype (P = .032, .002, .02, .005, .003) respectively. EBV was associated with the tumor size, stage and histological type (P = . 025, .005, .009) respectively. HPV wasn't associated with any of the clinicopathological characteristics. None of these viruses was associated with the tumor recurrence or patient death.

Conclusion: HCMV and EBV might be contributing factors for the development and behavioural alteration of breast carcinoma, representing potential tools for the detection of specific therapies for this cancer. Further studies on a larger number of cases using other techniques such as CISH for specific typing of the viruses especially HPV can add more information.

Key Words: Breast cancer, Cytomegalovirus, Epstein-Barr virus, Human papillomavirus, Immunohistochemistry

1. INTRODUCTION

Breast cancer is the commonest cancer and a common cause of cancer death in women all over the world accounting for 22.9% and 13.7% respectively. In Egypt, it accounted for 37.7% of women cancer and 29.1% of cancer mortality in 2008.[1] Some risk factors have been detected such as the patient's age, family history and prolonged exposure to estrogen hormone. Sometimes an evident risk factor may be absent in 50%-80% of patients.[2] So recent researches have been performed to detect further risk factors that can be associated with this cancer.

Some studies suggested a causal association between breast

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cancer and viral infection like Epstein-Barr virus (EBV), mouse mammary tumor virus (MMTV), human papillomavirus (HPV) and human cytomegalovirus (HCMV).[3–6]

HCMV is one of the β-herpesvirus family with infection in 70%-90% of the world's population. It is reactivated periodically after latent infection in the host.[7] The nucleic acids and proteins of HCMV have been found in many cancers such as colon, prostate, breast cancers, glioblastoma, medulloblasoma, mucoepidermoid carcinoma of the salivary glands and rhabdomyosarcoma.[8–10] One study showed that HCMV was detected by immunohistochemical analysis in the normal epithelial cells of the breast tissue and the malignant epithelial cells of breast carcinoma but it was higher in the later.[6] On the other hand, a recent study didn't find HCMV in breast cancer tissue.[11]

EBV is also a member of human herpes virus family and is found in about 90%-95% of populations mostly in children and early adolescents with different manifestations.[12] It is also found in neoplastic diseases such as Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's lymphoma and gastric carcinoma.[13] It was considered by the International Agency for Research on Cancer (IARC) as one of group 1 carcinogens.[14] So, it has been investigated for its role in the development of breast carcinoma, where Labrecque *et al.* detected EBV in the epithelial cells of breast carcinoma.[15] However, the study carried out by Deshpande *et al.* showed that EBV was lacking in breast carcinoma.[16]

HPV is a DNA virus which is often associated with cervical cancer in women especially the high risk types 16 and 18. It was also found in anogenital and oral carcinomas and classified as an oncovirus by IARC.[14] After integration of the virus into the host cell genome the viral proteins such as E6 and E7 are expressed and inactivate the tumor supressor proteins P53 and Rb.[17] The detection of HPV in breast carcinoma showed contradictory results ranged from 0 to 86% in the different studies.[18]

There are many studies that investigated HCMV, EBV and HPV in breast carcinoma with PCR that can't differentiate the viruses in tumor cells from non-epithelial cells. So, immunohistochemical analysis can localize the viral proteins either in the malignant epithelial cells and non-epithelial cells giving accurate results.[19–21]

To our knowledge, there are no studies that investigated the expression of HPV and HCMV in breast carcinoma among the Egyptian women. Regarding EBV, only 2 studies were performed and evaluated the expression of EBV in breast carcinoma in Egypt. The first was performed by Fawzy *et al.* who evaluated EBNA1 by PCR.[22] The second was carried out by Zekri *et al.* who investigated the expression of CD21 and LMP1 antigens by immunohistochemistry, insitu hybridization and PCR.[23] So, we aimed to detect HCMV, EBV and HPV in carcinoma of the breast by immunohistochemistry (IHC) and whether there is a relationship between such viruses and breast carcinoma's prognostic factors and outcome.

2. PATIENTS AND METHODS

2.1 Data retrieval

This retrospective study included one hundred and seven selected cases of invasive breast carcinoma that have been obtained from the Oncology Center, Faculty of Medicine, Mansoura University (OCMU), Egypt between January 2010 to December 2012. The tumors were resected by modified radical mastectomy operation. All patients received postoperative therapy; hormonal, chemo or radiotherapy. Clinicopathological and postoperative follow up data were obtained from oncology center database until August 2015. Follow up period ranged from 32-68 months with a median follow up of 37 ± 20.51 months.

The haematoxylin and eosin (H&E) stained slides (cut from formalin fixed paraffin wax embedded specimens) were got back from the annals of the pathology lab in the OCMU then reviewed. Tumors were diagnosed according to WHO classification 2012 and were graded according to Nottingham grading system.[24,25]

2.2 Tissue microarray construction

The selected H&E stained slides were used as a guide for selection of the regions from which samples from the paraffin blocks were obtained. Tissue microarray (TMA) was assembled manually using a mechanical pencil tip.[26,27] Cores from the surrounding normal breast tissue were also taken as an internal control.

2.3 Immunohistochemical staining

The constructed TMA blocks were re-cut into 3-4 μm sections on slides of the coated type. After that the sections were deparaffinized followed by rehydration using alcohol of descending grades into water. Citrate buffer (at a different pH according to the type of the primary antibody) and heating in a microwave for 10 minutes were used for antigen retrieval. This was followed by incubation of the sections in 3% H_2O_2 blocking medium for 5 minutes then washing with distilled water and incubation with the following primary mouse monoclonal antibodies at the ordinary temperature for one hour: oestrogen receptors (ER) (1D5; 1:50, pH = 7.3 Dako, San Jose, USA), progesterone receptors (PR) (PR 636; 1:50, pH = 7.3, Dako, San Jose, USA), Her2/neu (CB11;

1:50, pH = 7.3 Novocastra, Newcastle, U.K), CMV late antigen (1.B.225; 1:200, pH = 6, Abcam, San Francisco-USA), EBNA1(E1-2.5; 1:1000, pH = 7.6, Abcam, San Francisco, USA) and HPV(K1H8; ready to use, pH = 6, Thermo Scientific, Fermont, CA, USA). The mouse DAB/peroxidase REAL^{TM} EnVision^{TM} method (K5007, Dako, Glostrup, Denmark) was carried out for immunostaining with reference to the producer orders. The internal positive control was normal breast duct epithelia for ER and PR. Positive external controls were ER, PR and Her2/neu positive breast carcinomas for ER, PR and Her2/neu respectively. The positive external controls for HCMV, EBV and HPV were colonic carcinoma, nasopharyngeal carcinoma and cervical carcinoma respectively. Negative controls were assessed by replacing the primary antibody by PBS.

Table 1. Clinicopathological and immunohistochemical features of the studied breast carcinoma cases (No. and %)

		NO.	Percentage
Tumor grade	G1	38	35.5%
	G2	45	42.1%
	G3	24	22.4%
Mitotic count	M1	48	44.9%
	M2	54	50.5%
	M3	5	4.7%
Tumor size	< 2 cm	5	4.7%
	> 2 cm	102	95.3%
Lymph node metastasis	N	26	24.3%
	P	81	75.7%
Tumor stage	Stage I	2	1.9%
	Stage II	48	44.9%
	Stage III	57	53.3%
Live or dead	Live	79	79.0%
	Dead	21	21.0%
Metastasis or recurrence	N	76	71.0%
	P	31	29.0%
Histological type	IDC	101	94.4%
	ILC	5	4.7%
	Mucinous	1	0.9%
ER	N	50	46.7%
	P	57	53.3%
PR	N	48	44.9%
	P	59	55.1%
Her2/neu	N	86	80.4%
	P	21	19.6%
HCMV	N	60	56.1%
	P	47	43.9%
EBV	N	96	89.7%
	P	11	10.3%
HPV	N	81	75%
	P	26	24.3%
Molecular subtype	Her2/neu	15	14.0%
	Lumial A	44	41.1%
	Luminal B	23	21.5%
	Triple negative	25	23.4%

Note. NO.: number of cases; M1: <11 mitotic figure /10 high power fields; M2: 11-22 mitotic figure/10 high power field; M3: >22 mitotic figure/10 high power fields; N: negative cases; P: positive cases.

2.4 Evaluation of immunohistochemistry

Tumors are considered positive for ER and PR when frank staining of the nuclei is $\geq 1\%$ of the tumor cells according to ASCO/CAP guidelines.[28] Her2/neu was scored according to the pattern of the membranous staining and percentage of stained tumor cells into: 0, no or weak incomplete staining in less than 10% of the tumor cells; 1) weak incomplete staining in more than 10% of the tumor cells; 2) weak or moderate complete staining in more than 10% of the tumor cells; 3) strong and complete staining in more than 10% of

the tumor cells. Only score 3 is taken into account as positive staining.[29] Different molecular subtypes were assessed after evaluation of Her2/neu, estrogen and progesterone receptors.[30] Immunostaining for antiviral antibodies was considered positive if > 1% of neoplastic cells displayed distinct brown cytoplasmic and or perinuclear staining for HCMV and nuclear staining for EBNA1 and HPV.

2.5 Statistical methods

Data analysis was done using the computer program SPSS (Statistical package for social science) version 17.0. Quantitative statistics were calculated in the form of a mean ± *SD* for parametric data and as a median ± *SD* for non parametric data. Qualitative statistics were calculated in the form of a frequency (NO and %). Chi-square and Fisher's exact probability tests were used for inter-group comparison of categorical data to detect the association between the virus expression and the different clinicopathological parameters. A *P*-value of < .05 was considered to be statistically significant.

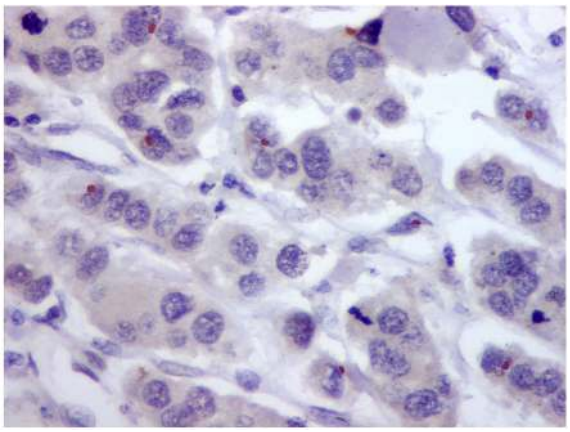

Figure 1. Immunohistochemical staining for HCMV in grade III infiltrating duct carcinoma of the breast showing brown perinuclear cytoplasmic inclusions × 400

Table 2. Association of HCMV expression with the clinicopathological characteristics of breast carcinoma

		HCMV				*P* value
		N		P		
		NO.	Percentage	NO.	Percentage	
Tumor grade	G1	28	46.7%	10	21.3%	.01
	G2	23	38.3%	22	46.8%	
	G3	9	15.0%	15	31.9%	
Mitotic count	M1	34	56.7%	14	29.8%	.01
	M2	25	41.7%	29	61.7%	
	M3	1	1.7%	4	8.5%	
Tumor size	< 2 cm	4	6.7%	1	2.1%	.27
	> 2 cm	56	93.3%	46	97.9%	
Lymph node metastasis	N	13	21.7%	13	27.7%	.47
	P	47	78.3%	34	72.3%	
Tumor stage	Stage I	1	1.7%	1	2.1%	.98
	Stage II	27	45.0%	21	44.7%	
	Stage III	32	53.3%	25	53.2%	
Histological type	IDC	54	90.0%	47	100%	.032*
	ILC	5	8.3%	0	0	----
	Mucinous	1	1.7%	0	0	----

Note. *Significance between the negative and positive cases of IDC.

3. RESULTS

This retrospective study was carried out on 107 patients with invasive breast carcinoma. These cases included one hundred and one (94.4%) cases with invasive ductal carcinoma (IDC) not otherwise specified (NOS), five (4.7%) cases with invasive lobular carcinoma (ILC) and one case with mucinous carcinoma (0.9%). The mean age of the patients was 54.6 ± 12 years with an age ranging from 31 to 88 years old. The other patients' clinicopathological features are represented in Table 1.

3.1 The relationship of HCMV expression with the clinicopathological and immunohistochemical characteristics of breast carcinoma

HCMV was present in 47 (43.9%) cases (see Figure 1). In positive cases, HCMV expression was limited to the tumor cells and absent in non tumor tissue and inflammatory cells. The mean age of HCMV positive cases was 54.57 years *vs.* 54.83 years in HCMV negative cases. There was no

statistically significant association between age and HCMV expression (P = .7). Table 2 shows that 21.3%, 46.8% and 31.9% of HCMV positive cases were grades I, II and III respectively with a statistically significant association between HCMV expression and the tumor grade (P = .01). There was also a statistically significant association between the mitotic count (M) and HCMV infection where 29.8%, 61.7% and 8.5% of HCMV positive cases were M1, M2 and M3 respectively (P = .01). In addition, there was a statistically significant association between HCMV expression and the IDC category (P = .032). On the other hand, HCMV expression didn't show any statistically significant association with the tumor size, nodal metastasis and tumor stage with P values = .27, .47 and .08 respectively.

Table 3. Association of HCMV expression with the hormonal status, molecular subtype and patient outcome

		HCMV				*P* value
		N		P		
		NO.	Percentage	NO.	Percentage	
ER	N	20	33.3%	30	63.8%	.002
	P	40	66.7%	17	36.2%	
PR	N	21	35.0%	27	57.4%	.02
	P	39	65.0%	20	42.6%	
Her2/neu	N	54	90.0%	32	68.1%	.005
	P	6	10.0%	15	31.9%	
Molecular subtype	Her2/neu	2	3.3%	13	27.7%	.003
	Luminal A	30	50%	14	29.8%	
	Luminal B	14	23.3%	9	19.1%	
	Triple negative	14	23.3%	11	23.4%	
Live or dead	Live	45	78.9%	34	79.1%	.98
	Dead	12	21.1%	9	20.9%	
Recurrence or metastasis	N	44	73.3%	32	68.1%	.55
	P	16	26.7%	15	31.9%	

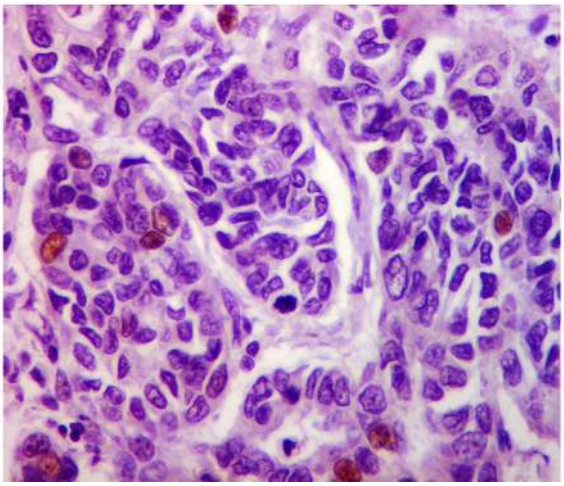

Figure 2. Immunohistochemical staining for EBV in grade II inf iltrating duct carcinoma of the breast showing brown nuclear staining × 400

Table 3 shows that HCMV expression was negatively associated with ER, PR and Her2/neu expression with P values = .002, 0.02% and .005 respectively. A statistically significant association was also found between HCMV expression and molecular subtypes with P value .003. However, no statistically significant association was found between the expression of HCMV and patient death or tumor recurrence (P = .98 and .55) respectively.

3.2 The relationship of EBV expression with the clinicopathological and immunohistochemical characteristics of breast carcinoma

It was found that EBV was expressed in 11 (10.3%) cases (see Figure 2). The expression was found only in the malignant epithelial cells and absent in the surrounding normal breast tissue or lymphocytes infiltrating the tumor stroma. The mean age in EBV positive cases was 56.82 ± 14.62 years compared to 54.44 ± 11.74 years in EBV negative cases with no statistically significant association between the age and EBV expression (P = .5). As shown in Table 4, EBV expression shows a statistically significant association with a large tumor size and a higher tumor stage (P = .025 and .005) respectively. EBV expression also showed a statistically significant association with the histological type (P = .009), where 90.9% of EBV positive cases were of IDC type. On the other hand, there was no significant association with the histological grade, mitotic count or metastasis in lymph node (P = .15..4 and .08) respectively. From Table 5, the expression of EBV doesn't show any significant association with ER, PR, Her2/neu, molecular subtype, patient death or

tumor recurrence with *P* values = .9, .2, .08, .56, .8 and .36 respectively.

3.3 The relationship of HPV expression with the clinicopathological and immunohistochemical characteristics of breast carcinoma

HPV was found to be expressed in 26 (24.3%) cases (see Figure 3). The expression was limited to the tumor tissue but it was absent in the surrounding normal tissue. The mean age of HPV-positive cases was 56.77 years versus 54.01 years in HPV-negative cases with no statistically significant association ($P = .5$). Although grade 2 and grade 3 together were the highest among HPV-positive cases, there was no significant association between the tumor grade and HPV ($P = .8$). It was noticed that tumor size more than 2 cm accounted for 96.2% of HPV-positive cases but it wasn't significantly associated with the virus expression ($P = .8$). The same was found with the lymph node metastasis where cases with lymph node metastasis represented 69.2% of HPV-positive cases with no significant association ($P = .37$). Stage 2 and 3 accounted for 61.5% and 38.3% of HPV-positive cases respectively but with no significant association with the virus expression ($P = .12$). Regarding the association with ER, PR, Her2/ neu and the molecular subtypes, there was no significant association with HPV expression ($P = .7$, .76, .95 and .36) respectively. There was also no significant association between HPV expression and the tumor recurrence or patient death ($P = .44$ and .7) respectively.

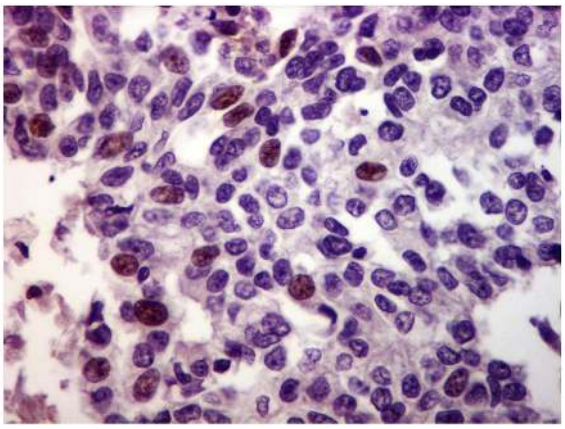

Figure 3. Immunohistochemical staining for HPV in grade II infiltrating duct carcinoma of the breast showing brown nuclear staining × 400

Table 4. Association of EBV with the clinicopathological characteristics

		EBV				*P* value
		N		P		
		NO.	Percentage	NO.	Percentage	
Tumor grade	G1	35	36.5%	3	27.3%	.15
	G2	42	43.8%	3	27.3%	
	G3	19	19.8%	5	45.5%	
Mitotic count	M1	45	46.9%	3	27.3%	.4
	M2	47	49.0%	7	63.6%	
	M3	4	4.2%	1	9.1%	
Tumor size	< 2 cm	3	3.1%	2	18.2%	.025
	> 2 cm	93	96.9%	9	81.8%	
Lymph node metastasis	N	21	21.9%	5	45.5%	.08
	P	75	78.1%	6	54.5%	
Tumor stage	Stage I	2	2.1%	0	0.0%	.005
	Stage II	38	39.6%	10	90.9%	
	Stage III	56	58.3%	1	9.1%	
Histological type	IDC	91	94.8%	10	90.9%	.009
	ILC	5	5.2%	0	0	
	Mucinous carcinoma	0	0	1	9.1%	

4. DISCUSSION

This study was carried out to investigate the existence of HCMV, EBV and HPV in invasive carcinoma of the breast and their association with its prognostic factors by IHC which has been constantly debated over the past decade.

HCMV was detected in 43.9% of our cases. One study detected the virus in 7.4% of cases by PCR but they didn't find any association with the clinicopathological parametrs and patient survival.[19] The latter study explained the absence of correlation with prognostic parameters by the hit and run theory. On the other hand, the present study revealed a statistically significant negative association with ER, PR

and Her2/neu and the molecular subtype where the higher expression was among the Her2- positive and triple negative cases. It was also found to be associated significantly with the tumor grade and the mitotic count. Others found the virus proteins in 100% of cases and in the epithelial cells of metastasis in the sentinel lymph nodes suggesting its role in development of breast cancer and its metastasis.[31] It has been found that there is an association between HCMV and lower disease free and overall survival times.[5] However, we found that HCMV wasn't associated with tumor recurrence or patient death. The virus wasn't detected in non - tumor tissue or inflammatory cells in our study which is consistent with the results detected by Taher *et al.*[31] In addition, others didn't find the virus in the fibroadenoma tissue.[19]

Table 5. Association of EBV expression with the hormonal status, molecular subtype and patient outcome

		EBV				*P* value
		N		P		
		NO.	Percentage	NO.	Percentage	
ER	N	45	46.9%	5	45.5%	.9
	P	51	53.1%	6	54.5%	
PR	N	45	46.9%	3	27.3%	.2
	P	51	53.1%	8	72.7%	
Her2/neu	N	75	78.1%	11	100.0%	.08
	P	21	21.9%	0	0	
Molecular subtype	Her2/neu	15	15.6%	0	0	.56
	Luminal A	39	40.6%	5	45.5%	
	Luminal B	20	20.8%	3	27.3%	
	Triple negative	22	22.9%	3	27.3%	
Live or dead	Live	70	78.7%	9	81.8%	.8
	Dead	19	21.3%	2	18.2%	
Recurrence or metastasis	N	67	69.8%	9	81.8%	.36
	P	29	30.2%	2	18.2%	

EBV has been suggested to be associated with breast cancer indicated by detection of the virus in breast milk, presence of some EBV associated lymphomas in the breast and that breast cancer has epidemiological similarities to young adult Hodgkin's lymphoma.[20] Immunohistochemical study of EBNA1 revealed different results ranged from 0%, 4%, 25%, 37%, 42% and 51%.[20] Our study found EBV in 11 (10.3%) cases. One Egyptian study detected EBV in 25% of the breast carcinoma specimens stained for EBNA-1 in the Egyptian women but by PCR analysis.[22] The difference among the different studies can be attributed to the difference in the studied clones, the studied antigens (EBNA1, EBNA2 or LMP1), the genetic predisposition or due to difference in the geographical distribution. The expression of EBV has been found in 5%-30% of the tumor cells which is consistent with ours.[32] It has also been demonstrated that the virus expression ranged from 5%-50% by another study.[33] The different proportion of stained tumor cells can be attributed to that breast carcinomas have highly heterogenous genomic content and distribution. So, it is suggested that EBV might play a role in the development of breast cancer in association with other co-factors but not a primary etiological agents.[20]

It was found that EBV is associated with aggressive tumors where Bonnet *et al.* found a significant association with high grade tumors, ER-negative tumors and presence of the virus in metastases of EBV positive primary tumors.[32] Our study, on the other hand didn't find any significant association with the tumor grade, lymph node metastasis, the hormonal status, the molecular subtype, tumor recurrence or patient death. However, there was a significant association with the tumor size (supporting the role of the virus in tumor proliferation), the tumor stage and the histological type where higher expression was found in infiltrating duct carcinoma than infiltrating lobular carcinoma. Others found that the higher expression of EBV was present in the medullary than the lobular carcinoma.[34]

EBV tends to infect young persons of both sexes suggesting a genetic predisposition or early life exposure. It also affects older ages if the immune system is diminished.[20] It has been reported that EBV positivity is higher in women less than 50 years than those older than 50 years which isn't consistent with ours.[35] The virus wasn't found in the surrounding normal tissue or the lymphocytes infiltrating the tumor stroma in our cases, which is consistent with other studies suggesting the role of EBV in breast carcinoma.[15,33,35]

HPV was present in 26 (24.3%) cases in our study. This agrees with the study carried out by Pereira *et al.* where HPV

was present in 26% of cases but was detected by PCR.[36] In a study carried out in Kuwait, HPV was found in 16.7% of cases by IHC and 35.4% by chromogenic insitu hybridization (CISH). They also didn't find a statistically significant association with the prognostic factors, which is consistent with our results.[37] One study stated that the prevalence of HPV varied from 4% in Mexican to 86% in American women.[38] This wide difference among literatures could be attributed to the methods of detection where most studies utilized PCR technique with the higher sensitivity but low specificity as compared to CISH and IHC.[37] The expression of HPV was found only in the tumor tissue and absent in the surrounding normal tissue in our study which agrees with the previous studies.[37,38] However, one Turkish study detected the virus in the normal tissue but at a lower level.[39] On the other hand, Eslamifar *et al.* didn't detect HPV in all tested carcinomas or the normal breast tissue.[40]

5. Conclusion

The present results demonstrated HCMV, EBV and HPV in a fraction of breast carcinomas in Egyptian women (OCMU) by IHC method, which is more accurate than the PCR technique in distinguishing the tumor cells from the nontumor tissue and the inflammatory cells. HCMV was the most common in our cases and it was associated with the tumor grade, mitotic count and the hormonal status. Although EBV was the least expressed one, was associated with tumor size, tumor stage and the histological type. The previous findings indicate that HCMV and EBV might be contributing factors for development and behavioural alteration of breast carcinoma supported by their restriction to the epithelial cells. In addition, these results represent potential tools for the development of specific therapies such as immunotherapeutic strategies based on EBV specific cytotoxic T cells which are recently being used for the treatment of Hodgkin's lymphoma and nasopharyngeal carcinoma positive for EBV. Although HPV was present in breast carcinomas, it wasn't associated with the clinicopathological characters of this carcinoma which requires further investigations.

6. Recommendations

Further studies on a larger number of cases obtained from the different oncology centers in Egypt are recommended to obtain more accurate results to be compared with the worldwide data. In addition, using other techniques such as CISH for specific typing of the viruses or using different methods such as PCR, CISH and IHC at the same time and compare all data can add more information. Understanding the association between these viruses and breast carcinoma is important to identify women at risk for this cancer and those who can benefit from the use of antiviral therapy as a prophylactic therapy or as a therapy for a residual disease. In future, it would be good to carry out the additional studies by monitoring breast cancer incidence amongst women vaccinated against these viruses.

Conflicts of Interest Disclosure

No potential conflicts of interest are disclosed.

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