Original Article

Association of Epstein-Barr virus with invasive breast carcinoma and its impact on well-known clinicopathologic parameters in Iranian women

Fereshteh Mohammadizadeh, Mojtaba Zarean[1], Maryam Abbasi[2]

Department of Pathology, [1]School of Medicine, [2]School of Nursery and Midwifery, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: The association between Epstein-Barr virus (EBV) and breast carcinoma in Iranian women is uncertain. We examined EBV latent membrane protein-1 (LMP-1) antigen expression in breast carcinoma and its relationship with clinicopathologic parameters among a population of Iranian patients.

Materials and Methods: This study was performed on formalin fixed and paraffin embedded tissue specimens with a diagnosis of invasive breast carcinoma archived at one university hospital in Isfahan city, Iran. We used immunohistochemistry to detect LMP-1 of EBV in carcinoma and its adjacent normal tissue. The frequency of LMP-1 expression in breast carcinoma and its relationship with age, tumor size, tumor type, tumor grade and lymph node status were then determined.

Results: A total of 80 cases were evaluated including 77 (96.3%) ductal, 1 (1.3%) lobular, 1 (1.3%) medullary and 1 (1.3%) mucinous carcinoma. LMP-1 expression was seen in 6 cases (7.5%) of breast carcinoma whereas normal breast tissue adjacent to carcinoma was negative for LMP-1 in all of the cases. A statistically significant association was seen between EBV and invasive breast carcinoma ($P = 0.03$). No significant relationship was observed between LMP-1 expression on one hand and age, tumor size, tumor type, tumor grade and lymph node status on the other.

Conclusion: EBV may play an etiological role in some of the cases of breast carcinoma in Iranian women. EBV expression does not seem to have a significant impact on the major clinicopathologic prognostic determinants of breast carcinoma.

Key Words: Breast carcinoma, Epstein-Barr virus, latent membrane protein-1 antigen

Address for correspondence:
Dr. Fereshteh Mohammadizadeh, Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: mohammadizadeh@med.mui.ac.ir

Received: 28.05.2013, **Accepted:** 16.09.2013

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.135158

INTRODUCTION

Breast carcinoma is most common cause of cancer related death among woman world-wide.[1] According to official statistics from cancer registry, breast cancer is the most common type of cancer among Iranian women.[2]

Although there are well-known risk factors associated

How to cite this article: Mohammadizadeh F, Zarean M, Abbasi M. Association of Epstein-Barr virus with invasive breast carcinoma and its impact on well-known clinicopathologic parameters in Iranian women. Adv Biomed Res 2014;3:141.

with breast carcinoma including early age of menarche, late age of menopause, nulliparity and positive family history of breast cancer, these cannot explain many cases of breast cancer.[3]

In recent years, certain viruses have been the focus of attention as potential risk factors for the development of breast cancer. Among viruses, *Cytomegalovirus* and Epstein-Barr virus (EBV) have been the subject of several studies in various parts of the world. The hypothesis of the presence of an association between EBV and breast cancer originates from some noteworthy observations. Incidence of breast cancer is high among men in Mediterranean countries, where EBV infection is endemic. Moreover, some EBV associated lymphomas have been reported in breast and there are striking morphological similarities between medullary carcinoma of breast and the well-known EBV associated nasopharyngeal carcinoma.[3,4] Reports of concurrent nasopharyngeal carcinoma and breast carcinoma further strengthen this hypothesis.[5,6]

EBV is a gamma herpes virus. Infection typically occurs early in life and may show a lifelong persistence, usually without serious health consequences. The association between EBV infection and certain malignancies such as Burkitt lymphoma and nasopharyngeal carcinoma is well-documented.[7] Despite several studies concerned with the issue of EBV association with breast cancer, the findings are controversial. In a recent study published in 2012, thirty investigations regarding the association of EBV and breast cancer have been reviewed with special attention to the methodology of these studies, sample size and proportion of specimens reported as positive for EBV. This review shows that the published data do not justify a conclusion that EBV is etiologically associated with breast cancer.[8]

According to controversial results, our purpose was to assess the presence of EBV in breast carcinoma from a series of Iranian patients. To the best of our knowledge, only one study regarding the etiologic role of EBV in breast carcinoma among Iranian women has been published in English literature.[9]

MATERIALS AND METHODS

This study was conducted on the archived formalin fixed and paraffin embedded tissue specimens in the pathology laboratory of Alzahra Hospital in Isfahan (Iran) with a diagnosis of invasive breast carcinoma between 2006 and 2010. The specimens had normal breast tissue adjacent to breast carcinoma. The cases of invasive ductal carcinoma were graded according to Bloom and Richardson grading system. The paraffin embedded tissue blocks were sectioned in 4 mm cuts and stained with immunohistochemistry (IHC) method for latent membrane protein-1 (LMP-1) antigen of EBV. The LMP-1 antibody used was monoclonal mouse antibody, isotype immunoglobulin G (IgG1), kappa, clone CS.1-4 (Dako Company, Denmark).

The process of IHC was applied as follow:

- Placing the sections in 37°C oven for 48 h
- Rinsing in 100% xylol, graded ethanol (100%, 85% and 75%) and distilled water
- Rinsing in 10% phosphate buffered saline (PBS) solution
- Exposure to 10% H_2O_2 and methanol at a ratio of 1:9 for 30 min
- Rinsing in PBS
- Placing in citrate buffered solution (PH = 6.1) for 14 min at a microwave with power 800
- Rinsing in %100 PBS
- Adding blocking serum to the slides for 30 min and then drying
- Separately adding the specific LMP-1 antibody (1/200 dilution) for 30 min at room temperature
- Rinsing in PBS
- Adding broad spectrum antibody for 30 min
- Adding horseradish peroxidase-streptavidin for 30 min
- Adding diaminobenzidine (DAB) for 10 min
- Rinsing in 10% PBS
- Dehydration in graded alcohols (75%, 85%, 100%) and xylol
- Counterstaining with hematoxylin and mounting.

The slides were then evaluated by two pathologists using two-headed light microscope.

A well-documented EBV positive nasopharyngeal carcinoma was used as positive control. Nuclear staining with LMP-1 antibody irrespective of the intensity of staining and the percentage of the stained cells was considered as positive LMP-1 expression. Other data including age at the time of diagnosis, tumor size (greatest tumor dimension) and the status of axillary lymph node were obtained from the records available in the patients' documents.

The data were analyzed using the SPSS software (version 16, Chicago) for windows.

McNemar test was used to determine the association between EBV and breast carcinoma. The relationship between EBV and tumor grade was determined by

Mann-Whitney test. Independent *t*-test was used to determine the relationship between EBV and age, tumor size and lymph node status.

$P < 0.05$ was considered as statistically significant.

RESULTS

In this study, 80 paraffin blocks of breast carcinoma were evaluated. Cancer characteristics are presented in Table 1.

Six (7.5%) out of 80 carcinoma samples showed positive immunoreactivity for LMP-1 [Figure 1]. LMP-1 expression was not seen in any of the normal breast tissue samples adjacent to carcinoma. Mc Nemar test showed a statistically significant association between EBV and invasive breast carcinoma ($P = 0.03$).

Mann-Whitney test showed no statistically significant relationship between LMP-1 expression as the marker of EBV presence and tumor grade in invasive ductal carcinomas ($P = 0.847$) [Table 2].

Out of 80 samples, 77 were invasive ductal carcinoma. The remainder included one lobular carcinoma, one mucinous carcinoma and one medullary carcinoma. All the LMP-1 positive specimens belonged to invasive ductal carcinoma subtype. Since the numbers of carcinoma subtypes other than invasive ductal carcinoma were too small, study of relationship between LMP-1 expression as the marker of EBV presence and tumor type could not yield valid results [Table 3].

Independent *t*-test failed to show any statistically significant relationship between LMP-1 expression as the marker of EBV presence on one hand and age, tumor size and lymph node status on the other (P value of 0.833, 0.119 and 0.417 for age, tumor size and lymph node status, respectively) [Table 4].

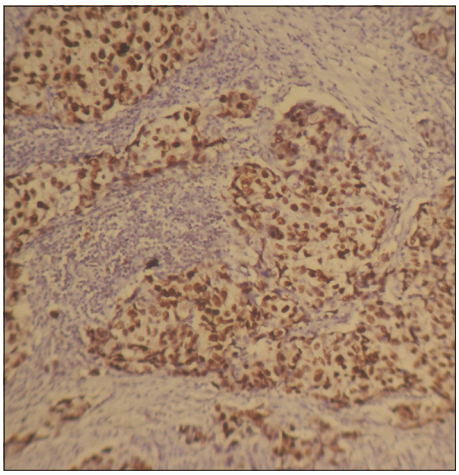

Figure 1: Positive immunoreactivity for latent membrane protein-1 antigen of Epstein-Barr virus in invasive ductal carcinoma (×10 objective)

Table 1: Characteristics of the studied samples of invasive breast carcinoma

Cancer characteristics	Values (%)
Age (year)	51.3±12.4
Carcinoma type	
Ductal	77 (96.3)
Lobular	1 (1.3)
Medullary	1 (1.3)
Mucinous	1 (1.3)
Tumor size (cm)	3.91±1.99
Tumor grade in invasive ductal carcinomas	
I	5 (6.5)
II	40 (51.9)
III	32 (41.6)
Nodal involvement	
None	30 (37.5)
1-3	20 (25)
4-9	20 (25)
≥10	5 (4)
LMP-1 positivity in cancer tissue	6 (7.5)
LMP-1 positivity in normal tissue	0

Data are presented as mean±SD or number (%), LMP-1: Latent membrane protein-1, SD: Standard deviation

Table 2: Relationship between LMP-1 expression and tumor grade in invasive ductal carcinoma

Grade	Number	LMP-1 positive (%)
I	5	0
II	40	4 (10)
III	32	2 (6.2)
Total	77	6

LMP1: Latent membrane protein-1

Table 3: LMP-1 expression in various subtypes of invasive breast carcinoma

Carcinoma sub type	Number (%)	LMP-1 positive (%)
Ductal	77 (96.3)	6 (7.8)
Lobular	1 (1.3)	0
Medullary	1 (1.3)	0
Mucinous	1 (1.3)	0
Total	80	6

LMP-1: Latent membrane protein-1

Table 4: Relationship of LMP-1 expression with age, tumor size and lymph node status in invasive breast carcinoma

Variable	LMP-1 (Mean±SD)		*P* value
	Negative	Positive	
Age	51.4±12.7	50.3±9.7	0.833
Tumor size	4.03±2.02	2.7±1.05	0.119
Number of involved lymph nodes	3.3±5	1.7±1.5	0.417

LMP-1: Latent membrane protein-1, SD: Standard deviation

The relationship between LMP-1 expression and lymph node status was also determined by Mann-Whitney test. This test confirmed the lack of a statistically significant relationship between LMP-1 expression and lymph node status ($P = 0.49$).

DISCUSSION

Establishing an etiologic role for certain viruses in the development of breast carcinoma may have impacts on prevention and early detection of the breast cancer in infected and at risk populations in future.

In this study, we have investigated the expression of LMP-1 antigen of EBV in tumoral cells of invasive breast carcinoma and adjacent normal breast tissue using the IHC technique. We found LMP-1 expression in breast carcinoma cells in 7.5% of the studied samples, while none of the normal breast tissue samples adjacent to carcinoma showed LMP-1 expression. These results showed a statistically significant association between EBV and invasive breast carcinoma. Association between EBV and breast cancer has been reported in several studies from different countries. Mazouni *et al*. from France have reported the presence of EBV deoxyribonucleic acid (DNA) in 33.2% of the studied cases using the real-time quantitative polymerase chain reaction (PCR) technique.[10] Joshi *et al*. from India studied EBV nuclear antigen-1 (EBNA-1) in breast cancer tissue and benign breast diseases in rural Indian women. In both groups, they also measured anti-EBNA-1 IgG antibodies in sera of these patients. IHC for EBNA-1 was positive in 54.9% of breast cancer cases. No IHC positivity was detected in the samples from the control group with benign breast diseases. EBNA-1 IgG antibody levels were also significantly higher in breast cancer group compared to the control group.[3] Fawzy *et al*. from Egypt compared EBNA-1 expression by immunostaining and PCR in samples from breast carcinoma and fibrocystic disease. IHC positivity of EBNA-1 was found in 25% of breast cancer specimens. Of these, 80% were also positive for EBNA-1 DNA in PCR. Fibrocystic disease specimens were negative by both techniques.[11] Preciado *et al*. from Argentina used both IHC and PCR techniques and found EBNA-1 expression in 35% of breast carcinoma samples. None of the control samples with benign breast diseases were EBNA-1 positive.[12] In the study of Labrecque *et al*. from United Kingdom 21% of the breast carcinoma cases were positive for EBV DNA by PCR technique.[13] Glenn *et al*. from Australia identified EBV sequences by PCR technique in 68% of invasive breast cancer specimens.[14] Zekri *et al*. did a study on breast carcinoma specimens and normal breast tissue of Egyptian and Iraqi women. They used IHC, *in situ* hybridization and PCR techniques and detected EBV in 45% and 28% of Egyptian and Iraqi women with breast carcinoma, respectively. Control group was negative for EBV.[15] Hachana *et al*. used specific PCR assays and found EBV DNA in 27% of breast carcinoma cases in Tunisia.[16] Another study from Tunisia by Trabelsi *et al*. used IHC and *in situ* hybridization techniques and detected EBV virus in 16.6% of medullary carcinomas and 11% of high grade invasive ductal carcinomas with lymphoid stroma.[17] He *et al*. from China investigated serum immunoglobulin A (IgA) and IgG levels against EBV viral capsid antigen (VCA) and EBNA-1 in women with breast carcinoma and controls in a high incidence area of nasopharyngeal carcinoma. Their results showed a significant association between VCA IgA levels and increased risk of breast cancer. IgG variables were not related to breast carcinoma.[18] Huo *et al*. reviewed 24 studies and 1535 cases and found that 29.32% of the patients with breast cancer were infected with EBV. They reported the highest prevalence of EBV in Asia and the lowest prevalence of the virus in USA.[19]

Variable prevalence of EBV association with breast carcinoma in these studies may be attributable to several factors including the sample size and methodology. PCR and *in situ* hybridization techniques are certainly more accurate compared with IHC technique. Moreover, proteins of EBV targeted for study have not been the same in all of these investigations. Another important factor seems to be the impact of the prevalence of EBV infection since the prevalence of the infection is different in various parts of the world.

Although most studies have suggested a possible association between EBV and breast carcinoma, there are studies that have failed to find such a relationship. Kadivar *et al*. from Iran studied EBV presence in breast carcinoma and benign breast diseases using IHC and PCR techniques. Studied EBV markers were negative in all breast cancer and control specimens.[9] Perrigoue *et al*. from USA used real-time quantitative PCR and reported lack of association between EBV and breast carcinoma.[20] According to the finding of the study of Baltzell *et al*. from USA who used *in situ* molecular methods of viral detection, EBV is unlikely to have a causative role in most types of breast carcinoma.[21]

We found no statistically significant relationship between LMP-1 expression on one hand and age,

tumor size, tumor grade and lymph node status on the other. Since tumor subtypes other than invasive ductal carcinoma were very small in number in our study, we could not obtain statistically valid results concerning the relationship between LMP-1 expression and tumor subtype in invasive breast carcinomas. The issue of relationship between EBV expression and clinicopathological prognostic determinants of breast carcinoma has been studied in some previous studies as well. Preciado *et al*. showed no significant relationship between EBV expression and worse clinicopathological determinants.[12] Labrecque *et al*. found no statistical association between EBV expression and tumor subtype.[13] Hachana *et al*. have reported the absence of any significant correlation between EBV expression and patient age, tumor grade, tumor size and lymph node status. However, they found a statistically significant relationship between EBV expression and estrogen receptor negativity.[16] Chu *et al*. also found that identification of EBV was not associated with tumor size, tumor grade and nodal status.[22]

On the other hand, some of the studies have reported the presence of significant association between EBV expression and some of the clinicopathological prognostic parameters of breast carcinoma. Mazouni *et al*. have found more aggressive features in breast cancers with EBV expression including more frequent estrogen receptor negativity and high histological grade.[10] According to results from the study of Fawzy *et al*., EBV-DNA positive breast cancers were associated with more than three lymph nodes involvement.[11] Glenn *et al*. reported that the presence of EBV in breast carcinoma is associated with young age of the patient at diagnosis.[14] Regarding tumor subtype, the strongest association might be present between lobular carcinoma and EBV infection.[19]

Since interleukin-10 and interferon-γ (INF-γ) play a critical role in the host responsiveness to EBV infection, their genetic variations may modify the association between EBV infection and the risk of breast carcinoma. Such a role has especially been suggested for genetic variations in INF-γ.[23]

Controversial results from the studies on the issue of EBV association with breast carcinoma emphasize on the need for further studies in this field to achieve more valid results. Serological studies to identify life-time EBV exposure in new cases of breast cancer and simultaneous study of EBV markers in breast carcinoma tissue of seropositive patients may further help us to clarify the complicated issue of association between EBV and breast cancer.

CONCLUSION

The results of this study suggest a potential causative role for EBV in some cases of Iranian women with invasive breast carcinoma. However, EBV expression does not seem to be correlated with the major clinicopathological prognostic determinants of the breast cancer.

ACKNOWLEDGMENTS

This paper is derived from a specialty thesis No. 390385 in Isfahan University of Medical Sciences. The university vice chancellery for research has approved and financially supplied the study. The authors wish to acknowledge the chancellery for their contribution to this work.

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. Sadjadi A, Nouraie M, Ghorbani A, Alimohammadian M, Malekzadeh R. Epidemiology of breast cancer in the Islamic Republic of Iran: First results from a population-based cancer registry. East Mediterr Health J 2009;15:1426-31.
3. Joshi D, Quadri M, Gangane N, Joshi R, Gangane N. Association of Epstein Barr virus infection with breast cancer in rural Indian women. PLoS One 2009;4:e8180.
4. Mant C, Hodgson S, Hobday R, D'Arrigo C, Cason J. A viral aetiology for breast cancer: Time to re-examine the postulate. Intervirology 2004;47:2-13.
5. Pan X, Zhu X, Li QQ. Case report of concurrent primary malignancies of the breast and nasopharynx. Oncol Lett 2012;4:285-8.
6. Yilmaz K, Koptur B, Dede DS, Aksoy S, Altundag K. Nasopharyngeal carcinoma with synchronous breast cancer; possible role of Epstein-Barr virus infection in the carcinogenesis of both cancers. J BUON 2012;17:600.
7. Hippocrate A, Oussaief L, Joab I. Possible role of EBV in breast cancer and other unusually EBV-associated cancers. Cancer Lett 2011;305:144-9.
8. Joshi D, Buehring GC. Are viruses associated with human breast cancer? Scrutinizing the molecular evidence. Breast Cancer Res Treat 2012;135:1-15.
9. Kadivar M, Monabati A, Joulaee A, Hosseini N. Epstein-Barr virus and breast cancer: Lack of evidence for an association in Iranian women. Pathol Oncol Res 2011;17:489-92.
10. Mazouni C, Fina F, Romain S, Ouafik L, Bonnier P, Brandone JM, *et al*. Epstein-Barr virus as a marker of biological aggressiveness in breast cancer. Br J Cancer 2011;104:332-7.
11. Fawzy S, Sallam M, Awad NM. Detection of Epstein-Barr virus in breast carcinoma in Egyptian women. Clin Biochem 2008;41:486-92.
12. Preciado MV, Chabay PA, De Matteo EN, Gonzalez P, Grinstein S, Actis A, *et al*. Epstein-Barr virus in breast carcinoma in Argentina. Arch Pathol Lab Med 2005;129:377-81.
13. Labrecque LG, Barnes DM, Fentiman IS, Griffin BE. Epstein-Barr virus in epithelial cell tumors: A breast cancer study. Cancer Res 1995;55:39-45.
14. Glenn WK, Heng B, Delprado W, Iacopetta B, Whitaker NJ, Lawson JS. Epstein-Barr virus, human papillomavirus and mouse mammary tumour virus as multiple viruses in breast cancer. PLoS One 2012;7:e48788.
15. Zekri AR, Bahnassy AA, Mohamed WS, El-Kassem FA, El-Khalidi SJ, Hafez MM, *et al*. Epstein-Barr virus and breast cancer: Epidemiological and molecular study on Egyptian and Iraqi women. J Egypt Natl Canc Inst 2012;24:123-31.
16. Hachana M, Amara K, Ziadi S, Romdhane E, Gacem RB, Trimeche M. Investigation of Epstein-Barr virus in breast carcinomas in Tunisia. Pathol Res Pract 2011;207:695-700.

17. Trabelsi A, Rammeh S, Stita W, Mokni M, Mourou A, Korbi S. Detection of Epstein-Barr virus in breast cancers with lymphoid stroma. Ann Biol Clin (Paris) 2008;66:59-62.
18. He JR, Tang LY, Yu DD, Su FX, Song EW, Lin Y, *et al*. Epstein-Barr virus and breast cancer: Serological study in a high-incidence area of nasopharyngeal carcinoma. Cancer Lett 2011;309:128-36.
19. Huo Q, Zhang N, Yang Q. Epstein-Barr virus infection and sporadic breast cancer risk: A meta-analysis. PLoS One 2012;7:e31656.
20. Perrigoue JG, den Boon JA, Friedl A, Newton MA, Ahlquist P, Sugden B. Lack of association between EBV and breast carcinoma. Cancer Epidemiol Biomarkers Prev 2005;14:809-14.
21. Baltzell K, Buehring GC, Krishnamurthy S, Kuerer H, Shen HM, Sison JD. Epstein-Barr virus is seldom found in mammary epithelium of breast cancer tissue using *in situ* molecular methods. Breast Cancer Res Treat 2012;132:267-74.
22. Chu PG, Chang KL, Chen YY, Chen WG, Weiss LM. No significant association of Epstein-Barr virus infection with invasive breast carcinoma. Am J Pathol 2001;159:571-8.
23. He JR, Chen LJ, Su Y, Cen YL, Tang LY, Yu DD, *et al*. Joint effects of Epstein-Barr virus and polymorphisms in interleukin-10 and interferon-γ on breast cancer risk. J Infect Dis 2012;205:64-71.

Source of Support: Nil. **Conflict of Interest:** None declared.