Association between Epstein-Barr Virus (EBV) and Breast Cancer

Zhila Torabizadeh 1, Alireza Nadji 2, Farshad Naghshvar 1, Anahita Nosrati 1*, Mohsen Parsa 1

1Department of Pathology, Imam khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran.
2Virology research center, Masih Daneshvari hospital, Shahid Beheshti University of Medical Sciences, Sari, Iran.

Received: 4 Jul 2014
Revised: 1 Sep 2014
Accepted: 20 Sep 2014

Corresponding Authors:
Anahita Nosrati
Department of Pathology, Imam khomeini Hospital, Mazandaran University of Medical Sciences
E-mail: Anahita785@gmail.com

Abstract
Background: Breast cancer is the most common malignancy in females worldwide. Several etiological factors including environmental factors have been recognized for breast cancer. Epstein Barr virus as a viral etiological factor has been proposed. So far, several studies have investigated the relationship between development of breast cancer and Epstein virus, but few have been done in Iran. The aim of this study was to determine whether there was an association between EBV infection and female breast cancer in Iran.

Materials and Methods: We analyzed paraffin embedded breast tissue specimens by polymerase chain reaction (PCR) including breast cancer specimens (as case group) and breast fibroadenoma specimens (as control group). PCR was performed to amplify specific sequences of EBV.

Results: From 130 cases of breast samples, 67 cases of breast cancer tissues and 41 cases of breast fibroadenoma tissues had adequate quality and quantity of DNA to detect EBV. PCR for EBV was positive in 4 invasive ductal carcinoma specimens (7.3%) and only one of the fibroadenoma specimens (2.4%). No significant association was found between EBV infection and invasive ductal carcinoma (p> 0.05). Also, patient’s age and histological grade of IDC were not correlated with EBV infection (p>0.05).

Conclusion: We observed no etiologic association between EBV infection and invasive ductal carcinoma of female breast in our regions; however, further studies are required to elucidate this association.

Keywords: Breast cancer; Fibroadenoma; Epstein-Barr virus; Polymerase chain reaction

Please cite this article as: Torabizadeh Zh, Nadji A, Naghshvar F, Nosrati A, Parsa M. Association between Epstein-Barr Virus (EBV) and Breast Cancer. Res Mol Med. 2014; 2 (4): 24-29

Introduction
Breast cancer is the second reason of mortality in the world (1, 2) and the occurrence has increased by 2-fold over the past 30 years (2). The prevalence of breast cancer is 23% among all cancers in the world (3), and its mortality rate is about 16% (1), so it is the most common and fatal cancer in women (2,4). Risk factors of breast cancer are age, family history, menarche, delayed menopause, first pregnancy after 25 years of age, nulliparity, long-term consumption of exogenous estrogens, and obesity after menopause, and encountering ionizing ray (5). Epstein –Barr virus (EBV) has also been found to be as an etiological reason for breast cancer (5). This virus was first recognized by two researchers, Barr & Epstein in 1964 (6). It belongs to Herpesviridae family with subfamily of Gamma herpesviridae that is called Human herpes virus-4 (HHV-4) too. This virus had linear double strands DNA (7). The most important way of transferring EBV is through direct oral contact and saliva, while contamination through transfusion, transplantation and placenta is also possible (6, 8). Rate of involving this virus is about 100% in developing countries (9, 10, 11). Persistent lifetime infection stems from involvement of memory B-cells which are the main source of virus and amount of infection is between 1 to 50 infected cells per 10^6 B cells in healthy seropositive people (11). This virus causes some diseases such as...
infectious mononucleosis (IM), Burkett’s lymphoma, nasopharyngeal carcinoma (NPC), oral hairy leukoplakia, multiple sclerosis (MS), dendritic follicular cells malignancy (due to CD 21), Hodgkin’s lymphoma, Non-Hodgkin’s lymphoma, and gastric cancer (9,12,18,19). Some researchers indicated that EBV could change epithelial cells, and move toward malignancy (20-22). It is proved that EBV infection has no effect on e-myc and Bcl-2 -anti-apoptotic molecules-overexpression (23). It is shown that it can suppress Bax molecule expression, which plays a role in cellular apoptosis; thus, that could halter apoptosis in gastric cancer (24). This virus causes B cells growth and proliferation in Burkett’s lymphoma which is mediated by stimulation of IL10 production (25). So far, the association between EBV infection and breast cancer has not been expressed, and there are many controversities in this regard. Some studies implied an association between EBV and type of breast cancer (26, 27). On the other hand, others found no evidence of EBV infection (28-34). To the best of our knowledge, there are only a few studies about EBV in breast cancer in the Middle Eastern countries and few are from Iran as well. Therefore, in this study, we intended to investigate the presence of EBV in breast cancer specimens using polymerase chain reaction (Nested-PCR).

Material and Methods

In this retrospective case-control study, EBV genome of 79 paraffin embedded breast cancer tissue samples were used from pathology department of Bouali Sina and Imam Khomeini hospitals, Sari. The samples were 67 IDC cases (Invasive ductal carcinoma), 8 cases of ILC(Invasive lobular carcinoma) 2 cases of MC(medullary carcinoma) and 1 case of DCIS(Ductal carcinoma in situ), 1 case of IDC- ILC, and 51 cases of paraffin embedded tissue samples of fibroadenoma as controls for Nested-PCR. All samples belonged to female patients. Primary characteristics such as age, tumor type, and tumor grade were obtained from patient’s records and two experienced pathologists reconfirmed breast cancer diagnosis. 5-7 micrometer cut sections were prepared from all paraffin blocks and frozen to -70 °C.

PCR procedure was briefly;
1. Deparaффinizing formalin-fixed breast cancer paraffin blocks
2. DNA extraction from breast cancer cell
3. Amplifying DNA
4. Agarose gel electrophoresis
1. Deparaффinizing stage;

At first all samples were placed in xylene for 10 minutes and again in new xylene solution for 10 minutes consequently. Then, they were put in ethanol 100%, 95%, and 70% for 5 minutes, respectively. Finally, they were placed in distilled water for 5 minutes (10).

2. DNA extraction (in Nested-PCR);
DNA was extracted by using extracting kit produced by Iran KIAGEN called purification kit. All DNA samples were stored in -20 °C in freezer until the time of PCR test. General primers sequences used in nested-PCR for diagnosis of EBV genome were outer primers such as EBVS1 (sense) d(CTACAACAAAA CTGGTGGAAC) and EBVA1 (antisense) d(AGAC AGGTGCTAAGGGAGT) and inner primers such as EBVS2 (sense) d(TGCTCTCAAAACCTAGGCCA) and EBVA2 (antisense) d(TGATTAGCTAAGGCATTCCCA).

| Table 1. Comparison of case and control group for age and number of infected samples with EBV. |
|-----------------|-----------------|-----------------|-----------------|
| Variable        | Breast cancer   | Fibroadenoma    | P-value         |
| Age± SD (year)  | 48.05±12.5      | 34.2±9.7        | <0.05           |
| EBV-infected sample NO. | 4(7.3%) | 1(2.4%) | >0.05 |

Constituents of 50 microliter of primary PCR-mixture were as below:
1st round PCR 5μ
10xPCR buffer 2μ
50mM MgCl 2 1μ
Fast start DNA polymerase (5u,μl) 0.5μl
Primer S1 (10 μM) 5μl
Primer A1 (10μM) 5μl
PCR water 27μl
DNA Template 5μl

Distilled water for injection and EBV-infected cells was used as negative and positive controls, respectively.

2nd round PCR
10xPCR buffer 5μl
50mM MgCl 2 2μl
dNTps (10μM each) 1μl
Fast start DNA polymerase (5u,μl) 0.5μl
Primer S2 (10 μM) 5μl
PrimerA2 (10μM) 5μl
PCR water 27μl
1st round PCR product 5μl

3 Amplification in PCR process using external and internal primers was arranged in 35 cycles with three programs as follow:
1- One cycle consist of: 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. Then amplification was
followed for 33 cycles as; 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. Finally PCR was down in one cycle as 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 7 min.

4- Agarose gel electrophoresis;
At first, agarose gel was covered by buffer (25 cc boric acid containing ethidium bromide in 500 cc distilled water), then, 10-15 μl of PCR solution and negative and positive controls were added to the gel. Electrophoresis was done for 15-20 minutes. Later on, the gel was taken out and the amplified bands were read by Gel Doc.

Interpretation
No band has been seen in negative control group. Two bands have been seen in positive control group. EBV-related bands (267 to 325 bp) and bands related to human-DNA (723 bp) were identified. Statistical analysis: collected data were analyzed in SPSS V.16. To compare quantitative data t-student test was used and data Fisher exact test and χ² test were applied for qualitative data.

Results
In this retrospective case-control study, EBV genome was investigated in 79 samples of paraffin embedded breast cancer tissue including 67 cases (15.5%) of IDC, 8 cases (6.2%) of ILC, two cases (1.5%) of MC, 1 case (0.8%) of DCIS and 1 case (0.8%) of IDC – ILC and 51 sample of Fibroadenoma by Nested-PCR which all samples belonged to female patients.

The maximum and minimum ages in patients group were 22 and 75 years old, and in control group were 17 and 54 years, respectively. Statistical analysis between two age groups showed significant differences (P<0.05). From 67 samples of IDC, 24 samples (35.8%) were grade 1, 33 samples (49.3%) were grade 2 and 10 cases (14.9%) were grade 3 (Table 2).

The only breast fibroadenoma sample infected with EBV was found in a 69 year old woman. All four breast cancer samples containing EBV were IDC type. Three cases (75%) were grade 2, and one sample (25%) was grade 3, so there was not significant difference in statistical analysis (P>0.05). The mean ages of cancer samples infected with and without EBV were 54.75 +12.8 and 47.69+12.5, respectively, and there was no significant difference in statistical analysis (P>0.05).

Discussion
Breast cancer incidence rate has been reported differently in the world. The highest rate was in the Northern Africa and Western Europe with age standardized rates (ASR) 123.6 and 84.6 in one hundred thousand people, respectively (34). In our study, EBV genome was investigated in 79 samples of paraffin embedded breast cancer tissue including 67 cases (15.5%) of IDC, 6.2% ILC, 1.5% MC, 0.8% DCIS and 0.8% IDC – ILC and 51 sample of fibroadenoma by Nested-PCR, which all samples belonged to females. Modest incidence rate has been found in Mediterranean and Southern American countries (about 46.100000) and there are the lowest incidence rate in North Asia (25.100000) and central Asia (2.8.100000) too (36). The prevalence of breast cancer is low in Asia, however, its rate of mortality in some Asian countries is more than that of the Western countries (37). The possible causes are changes in behavior and life style (38). In Iran, breast cancer is the most common cancer among women.

Table 2. Characteristics of breast cancer samples infected with EBV compared to patient’s age.

| No | Type of cancer | Grade | Age (year) | Score |
|----|----------------|-------|------------|-------|
| 1  | IDC            | II    | 40         | 6     |
| 2  | IDC            | II    | 49         | 6     |
| 3  | IDC            | III   | 61         | 8     |
| 4  | IDC            | II    | 69         | 7     |

Table 2. Abundance of EBV-infected samples in suitable tissue samples for Nested-PCR in all types of available breast cancer samples.

| Type of breast cancer | IDC (N=45) | ILC (N=6) | IMC (N=2) | DCIS (N=1) | IDC-ILC (N=1) | P.V   |
|-----------------------|------------|-----------|-----------|------------|---------------|-------|
| Positive status       | 4(8.9%)    | -         | -         | -          | -             | >0.05 |
| Negative EBV-contaminated | 41(91.1%) | 6(100%)   | 2(100%)   | 1(100%)    | 1(100%)       |       |
It comprises 24.4% of all cancers (41) and the crude incidence rate was 17.81 and age-world-standardized incidence rate (ASR) of 23.65 per 100,000 in 2006 (40). For many years, the relation between EBV and cancer was limited to the presence of this virus in nasopharyngeal carcinoma and inflammatory cells (42). Although different factors are involved in breast cancer pathogenesis, its molecular fact has not been recognized yet (43). Trabelsi et al. considered presence of EBV in tumor cells and lack of them in non-tumor cells (natural cells) and in inflammatory cells for cause of breast cancer (44). Probably there is a relationship between infection with primary EBV in young adulthood and increased risk of breast cancer (45).

In this study using specific primer in 55 qualified samples of breast cancer tissue for Nested-PCR, 4 samples (7.3%) were found with EBV bands, while in 41 qualified samples of control group for Nested-PCR, one sample of fibroadenoma (2.4%) showed the EBV band. According to latter data, no statistical significant difference was noted. Thus, our area is a low risk EBV-infected region while other studies conducted in different part of the world showed different results. Our data were compatible with other low risk EBV-infected regions such as a study in the USA—prevalence rate of 7%) (46) and another in Germany (prevalence rate of 6.8%) (47). In the USA a prevalence rate of 2% has also been reported (48). EBV may exist during the early phase of malignancy in breast epithelial cells. But it will vanish through the trend of malignancy. On the other hand, presence of EBV in final step of tumor growth would induce severe oncogenic features such as invasion, angiogenesis and metastases (49, 50). These changes could cause more invasiveness of the subgroups of tumor cells (51). Due to the high prevalence of breast cancer, even a limited number of breast cancers having EBV are important (47). The data showed no relation between EBV, patient’s age and type of breast cancer.

Bonnet et al. also found similar results in lack of relation between EBV and tumor histology and other prognostic factors such as age at the time of diagnosis, tumor size and menopause, while in our study there was no association between tumor grade and presence of EBV. In addition, the ratio of positivity for EBV was significantly associated with higher grade cancer (52). The abundance of EBV-infected breast cancer in different places of the world is indicated in Table 4. Highest infection rate was reported in Japan (66%); however, just three cancer tissue samples were studied of which two had EBV-DNA (53). After Japan, Ukraine, France, some parts of the USA, and Taiwan also showed high prevalence rate (26, 36, 45, 52-54).

| Country        | Reference number | Percentage (%) |
|----------------|-----------------|----------------|
| Japan          | 54              | 66             |
| Ukraine        | 55              | 54             |
| France/Ukraine | 53              | 51             |
| France         | 26              | 46             |
| USA            | 36              | 45.8           |
| Taiwan         | 46              | 45.2           |
| USA            | 34              | 45             |
| USA            | 33              | 42             |
| Ukraine        | 56              | 40             |
| USA            | 57              | 36.36          |
| Egypt          | 56              | 25             |
| France         | 53              | 13.89          |
| Iran           | present study   | 7.3            |
| USA            | 47              | 7              |
| Germany        | 48              | 6.8            |
| USA            | 49              | 2              |
| Iran           | 58              | 0              |

Similarly, Fawzy et al. showed an association between EBV and some invasive breast cancers in Egyptian women and may play a role in their etiology (55).

**Conclusion**

Compared to other studies performed throughout the world we found north of Iran as a region with low-prevalence of EBV-infected breast cancer. This could be due to geographic differences, and genetic and environmental factors that affect the rate of this virus in different types of cancers especially breast cancer. Further studies in different regions and ethnicities in Iran are recommended to verify the role of EBV in breast cancer.

**Acknowledgments**

Authors would like to acknowledge all people in Mazandaran University of Medical Sciences, Boualimsina Hospital, in Sari, Masih Daneshvari and Imam Khomeini Hospitals in Tehran who kindly offered assistance in conducting this study. We also thank Dr Kamyar Khodadadi who helped us with primary editing and advanced searches.

**Funding/support**

This work was supported by a grant from the research council of Mazandaran University of Medical Sciences, Iran.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**Authors' Contributions**

TZh and NAR developed the original idea, the protocol, technical, and material support (as administrators).
NF contributed to the study by interpreting and analyzing the data. NA participated in the study by writing, revising and constructively supervising the whole manuscript for important intellectual content. PM drafted the manuscript, and provided the abstract.

References
1. Fact Sheet of WHO Report. World Health Organization. The Top 10 Causes of Death in 2004.
2. Boyle P, Levin B. World cancer report 2008. IARC Press, International Agency for Research on Cancer, 2008.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics. CA Cancer J Clin. 2005; 55(2): 74-108. PMID: 15761078
4. Anderson BO, Jakesz R. Breast cancer issues in developing countries: an overview of the Breast Health Global Initiative. World J Surg. 2008; 32(12): 2578-85. PMID: 18283512
5. Robbins and Cotran. Pathologic basis of disease. 8th ed. Philadelphia: Saunders; 2011.
6. Henry JB. Clinical diagnosis and management by laboratory methods. 20th edition. New York: W.B. Saunders Co; 2001.
7. Wallach J. Interpretation of diagnostic tests. 7th edition. New York: Lippincott Williams & Wilkins; 2000.
8. Talaro KP, Talaro A. Foundations in microbiology. 4th edition. New York: McGraw-Hill; 2002.
9. Gandhi MK, Tellam JT, Khanna R. Epstein-Barr virus associated Hodgkin’s lymphoma. Br J Haematol. 2004; 125(3):267-81. PMID: 15086409
10. Meyer RM, Ambinder RF, Stroobants S. Hodgkin’s lymphoma: evolving concepts with implications for practice. Hematology Am Soc Hematol Educ Program. 2004: 184-202. PMID: 15561683
11. Yao QY, Rickinson AB, Epstein MA. A re-examination of the Epstein-Barr virus carrier state in healthy seropositive individuals. Int J Cancer.1985; 35(1):55-42. PMID: 2981780
12. Morshed K, Polz-Dacewicz M, Szymanski M, Ziaja M, Golabek W. Epstein-Barr virus antibodies in blood serum of patients with laryngeal cancer. Otolaryngol Pol. 2002; 56(1):45-8. PMID: 12053667
13. Pickard A, Chen CJ, Diehl SR, Liu MY, Cheng YJ, Hsu WL, Sun B, et al. Epstein-Barr virus seroreactivity among unaffected individuals within high-risk nasopharyngeal carcinoma families in Taiwan. Int J Cancer. 2004; 111(1):117-23. PMID: 15185352
14. Myhr KM, Riise T, Barrett-Conor E, Myrme H, and Vedeler C, Gronning M et al. Altered antibody pattern to Epstein - Barr virus but not to other herpes viruses in multiple sclerosis: A population based case-control study from Western Norway. J Neurol Neurosurg Psychiatry. 1998; 64:539-42. PMID: 9576551
15. Ascherio A, Munger KL, Lennette ET, Spiegelman D, Herman MA, Olek MJ et al. Epstein-Barr virus antibodies and risk of multiple sclerosis: A prospective study. JAMA. 2001; 286: 3083-8. PMID: 11754673
16. Sandlund JT, Gorban ZI, Berard CW, Sixbey J, Razzouk B, Talalayev AG et al. Large proportion of Epstein-Barr-virus-associated small noncleaved cell lymphomas among children with Non-Hodgkin’s lymphoma at a single institution in Moscow, Russia. Am J Clin Oncol. 1999; 22(5):523-5. PMID: 10521071
17. Vasaei MA, Ubaidaf MA, Khalidi HS, Almasri NM, Al-Abbadi M, Annab HZ. Association between Epstein-Barr virus and classic Hodgkin lymphoma in Jordan: a comparative study with Epstein-Barr virus-associated Hodgkin lymphoma in North American. Sotuth Med J. 2004; 97(3):273-7. PMID: 15043355
18. Lindhout E, Lakeman A, Mevissen ML, deGroot C. Functionally active Epstein-Barr virus-transformed follicular dendritic cell-like cell lines. J Exp Med. 1994; 179(4):1173-84. PMID: 8145036
19. Brooks L, Yao QY, Rickinson AB, Young LS. Epstein-Barr virus latent gene transcription in nasopharyngeal carcinoma cells: coexpression of EBNA1, LMP1 and LMP2 transcripts. J Virol. 1992; 66(5):2689-97. PMID: 1313894
20. Hummeli M, Anagnostopoulus I, Dallenbach F, Korbjuhn PH. EBV infection patterns in Hodgkin's disease and normal lymphoid tissue: expression and cellular location of EBV gene products. J Haematol. 1992; 82:689-4. PMID: 1336392
21. Hummeli M, Anagnostopulos I, korbjuhn P. Epstein- Barr virus in B- cell non- Hodgkin's lymphomas : unexpected infection patterns and different infection incidence in low- and high grade types. J Pathol.1995; 175: 263-71. PMID: 7745495
22. Fukayama M, Hino R, Uozaki H. Epstein-Barr virus and gastric carcinoma: virus-host interactions leading to carcinoma. Cancer Sci. 2008; 99(9):1726-33. PMID: 18616681
23. Lima MA, Ferreira MV, Barros MA, Pardini MI, Ferrari AC, Mota RM. Relationship between EBV infection and expression of cellular proteins c-Myc, Bcl-2, and Bax in gastric carcinomas. Diagn Mol Pathol. 2008; 17(2):82-9. PMID: 18382371
24. Ueda S, Maeda Y, Yamaguchi T, Hanamoto H, Hijikata Y, Tanaka M et al. Influence of Epstein-Barr virus infection in adult T-cell leukemia. Hematology. 2008; 13(3):154-62. PMID: 18702873
25. Samanta M, Ikwiri D, Takada K. Epstein-Barr virus-encoded small RNA induces IL-10 through RIG-I-mediated IRF-3 signaling. Oncogene. 2008; 27(30):4150-60. PMID: 18362887
26. Arbach H, Viglasky V, Lefeu F,Guinebretiere JM,Ramirez V, Bride N et al. Epstein–Barr virus (EBV) genome and expression in breast cancer tissue: Effect of EBV infection of breast cancer cells on resistance to paclitalex (Taxol). J Virol. 2006; 80:845-3. PMID: 16378986
27. Wong M, Pagano JS, Schiller JT, Tevethia SS,Raab-Traub N,Gruber J et al . New associations of human papillomavirus, Simian virus 40, and Epstein–Barr virus with human cancer. J Natl Cancer Inst 2002; 94:1832-36. PMID: 12488476
28. Gaffey MJ, Frierson HF Jr, Mills SE,Boyd JC, Zarbo RJ, Simpson JF et al. Medullary carcinoma of the breast. Identification of lymphocyte subpopulations and their significance. Mod Pathol. 1993; 6:721-8. PMID: 8302815
29. Lespagnard L, Cochaux P, Larsimont D,Degeyter M,Velu T, Heimann R et al. Absence of Epstein–Barr virus in medullary carcinoma of the breast as demonstrate by immunophenotyping, in situ hybridization and polymerase chain reaction. Am J Clin Pathol. 1995; 103:449- 52. PMID: 7726142
30. Glaser SL, Ambinder RF, DiGiuseppe JA, Horn-Ross PL,Hsu
Epstein-Barr Virus (EBV) in Breast Cancer

JL et al. Absence of Epstein-Barr virus EBER-1 transcripts in an epidemiologically diverse group of breast cancers. Int J Cancer. 1998; 75:555-8. PMID: 9466655

31. Chu JS, Chen CC, Chang KJ. In situ detection of Epstein-Barr virus in breast cancer. Cancer Lett. 1998; 124:53-57. PMID: 9500191

32. Kijjma Y, Yokota S, Takao S, Baba M, Natagoe S, Yoshinaka H. et al. Epstein-Barr virus involvement mainly restricted to lymphoepithelial type of gastric carcinoma among various epithelial neoplasms. J Med Virol. 2001; 64:513-8. PMID: 11468737

33. Deshpande CG, Badve S, Kidwai N, Longnecker R. Lack of expression of the Epstein-Barr virus (EBV) gene products, EBERs, EBNA1, LMP1 and LMP2A, in breast cancer cells. Lab Invest. 2002; 82:1193-9. PMID: 12218080

34. Perrigoue JG, den Boon JA, Fiordi A, Newton MA, Ahlquist P, Sugden B. Lack of association between EBV and breast carcinoma. Cancer Epidemiol Biomarkers Prev. 2005; 14:809-811. PMID: 15824148

35. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009; 59: 225-49. PMID: 19474385

36. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005; 55: 74-108. PMID: 15761078

37. Shibuya K, Mathers CD, Boschi-Pinto C, Lopez AD, Murray CJ. Global and regional estimates of cancer mortality and incidence by site: I. Results for the global burden of disease 2000. BMC Cancer. 2002; 2: 37. PMID: 12502432

38. World Cancer Report 2008. WHO. http://www.iarc.fr/en/publications/pdfonline/wcr/2008/index.php (2 January 2010, date last accessed).

39. Sadjadi A, Nouraei M, Mohagheghi MA, Mousavi-Jarrahi A, Malezkadeh R, Parkin DM. Cancer occurrence in Iran in 2002, an international perspective. Asian Pac J Cancer Prev. 2005; 6(3): 359-63. PMID: 16236000

40. Mousavi SM, Gouya MM, Ramazani R, Davalou M, Hajisadeghi N, Sedighi Z. Cancer incidence and mortality in Iran. Ann Oncol. 2009; 20(3): 556-63. PMID: 19073863

41. Goya M. Iranian Annual Cancer Registration Report 2005/2006. Ministry of Health and Medical Education, Health Deputy, Center for Disease Control and Prevention (In Persian). Tehran, Iran, 2007.

42. Amarante M K and Watanabe M A E. The possible involvement of virus in breast cancer. J Cancer Res Clin Oncol 2009; 135(3): 329-37. PMID: 19009309

43. Dimmock NJ, Primrose SB. Carcinogenesis and tumor viruses. Introduction to modern virology. 4th edition. London: Blackwell Science, 1994.

44. Trabelsi A, Rammeh S, Sitia W, Mokni M, Mourou A, Korbi S. Detection of Epstein-Barr virus in breast cancers with lymphoid stroma. Ann Biol Clin (Paris). 2008; 66(1):59-62. PMID: 18227005

45. Yasui Y, Potter JD, Stanford JL, Rossing MA, Winget MD, Bronner M, Daling J. Breast cancer risk and "delayed "primary Epstein-Barr virus infection. Cancer Epidemiol Biomarkers Prev. 2001; 10(1):9-16. PMID: 11205495

46. Thorne LB, Ryan JL, Elmore SH, Glaser SL, Gulley ML. Real-time PCR measures Epstein-Barr Virus DNA in archival breast adenocarcinomas. Diagn Mol Pathol. 2005; 14(1):29-33. PMID: 15714061

47. Herrmann K, Niedobitek G. Lack of evidence for an association of Epstein–Barr virus infection with breast carcinoma. Breast Cancer Res. 2003; 5(1):R13-7. PMID: 12559053

48. McCall SA, Lichy JH, Bijwaard KE, Aguilera NS, Chu WS, Taubenberger JK. Epstein-Barr virus detection in ductal carcinoma of the breast. J Natl Cancer Inst. 2001; 93(2):148-50. PMID: 11208885

49. Murono S, Inoue H, Tanabe T, Joab I, Yoshizaki T, Furukawa M et al. Induction of cyclooxygenase-2 by Epstein-Barr virus latent membrane protein 1 is involved in vascular endothelial growth factor production in nasopharyngeal carcinoma cells. Proc Natl Acad Sci USA. 2001; 98(12):6905-10. PMID: 11381123

50. Wakisaka N, Murono S, Yoshizaki T, Murono S, Furukawa M, Pagano JS. Epstein-Barr virus latent membrane protein 1 induces and causes release of fibroblast growth factor-2. Cancer Res. 2002; 62(21):6337-44. PMID: 12414666

51. Wakisaka N, Pagano JS. Epstein–Barr virus induces invasion and metastasis factors. Anticancer Res. 2003; 23(3A):2133-8. PMID: 12894587

52. Bonnet M, Guinebretiere JM, Kremmer E, Grunewald V, Benhamou E, Contesso G, et al. Detection of Epstein-Barr virus in invasive breast cancers. J Natl Cancer Inst.1999; 91(16):1376-81. PMID: 10451442

53. Horiiuchi K, Mishima K, Ohsawa M, Aozasa K. Carcinoma of stomach and breast with lymphoid stroma: localization of Epstein–Barr virus. J Clin Pathol.1994; 47:538-40. PMID: 8063937

54. Luqmani YA, Shousha S. Presence of Epstein–Barr virus in breast carcinoma. Int J Oncol. 1995; 6(4):899-903. PMID: 21556618

55. Fawzy S, Sallam M, Awad NM. Detection of Epstein–Barr virus in breast carcinoma in Egyptian women. Clin Biochem.2008; 41(7-8):486-92. PMID: 18258188