RESEARCH ARTICLE

Almost Complete Lack of Human Cytomegalovirus and Human papillomaviruses Genome in Benign and Malignant Breast Lesions in Shiraz, Southwest of Iran

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Abstract

Breast cancer ranks as the most common cancer among women worldwide. There have been controversial reports regarding contributions of human papillomaviruses (HPVs) and human cytomegalovirus (HCMV) to its development. The aim of this study was to determine the frequency of HPV and HCMV positivity in benign and malignant breast tumors. **Materials and Methods:** Formalin fixed paraffin-embedded tissue specimens of 150 breast cancers (invasive ductal and lobular carcinomas) and 150 non-malignant breast lesions (fibroadenomas, fibrocystic disease and adenosis) were examined. All samples were first deparafinized then subjected to commercial DNA extraction. The β-globin gene fragment was amplified using polymerase chain reaction (PCR) to confirm the quality of extracted DNA. The presence of HPV and HCMV genomic DNA was determined using PCR and Real time PCR techniques, respectively. **Results:** The mean ages of the test and control groups were 35.2 and 45 years, respectively. For HCMV, none of the malignant lesions were positive and only 2 of the 150 benign samples demonstrated presence of the virus. No HPV genomic DNA was found in either malignant or benign cases. **Conclusion:** The results of this study indicated no relationship between HCMV or HPV infection with breast cancer development. Whether investigations in larger populations with longer follow-up might demonstrate any role remains unclear.

Keywords: Breast cancer- HPV- HCMV- benign breast tumors- malignant breast tumors

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Introduction

Breast cancer is generally the second most common cancer and life threatening in the women world wild. According to WHO reports, nearly 1,676,600 new breast cancer cases and 521,900 relevant deaths have been recorded in 2012, worldwide. Moreover, in 2012, in the developing countries, an estimated 882.900 new cases and 324.300 deaths are reported (Torre et al., 2012). A number of endogenous and environmental risk factors, such as: age, smoking, stress, anxiety, exposure to estrogen, familial history of breast cancer, obesity, alcohol consumption ,estrogen level, gene mutations, and epigenetic alterations in BRCA-1(Breast cancer-1) or BRCA-2 (Breast cancer-2), have been demonstrated to be attributed to/linked with breast cancer development (Hulka et al.,2008; Aceto et al., 2010). According to the previous studies, viral infections counted as the major risk factor for about 20% of all cancers (Mahmoudvand et al., 2015; Sarvari et al., 2014; Alibek et al., 2013). Recent evidences suggests a possible link between viruses such as Epstein–Barr virus (EBV), mouse mammary tumor virus (MMTV), human papillomavirus (HPV) and human cytomegalovirus (HCMV) with breast cancer (Alibek et al., 2013).

HCMV belongs to the family of Herpesviridae and infects a major part of human population with a lifelong persistence in about 70-90% of people worldwide. Infection with this virus is usually asymptomatic or may cause mild discomforts. HCMV replicates in a spectrum of human cells (especially endothelial cells and macrophages) persistently reason behind the viral latency establishment (Rahbar et al., 2015).

Regarding the possible HCMV carcinogenesis, some mechanisms supposed to be involved. It has been reported that immediate early protein 86(IE86) inhibits the blocking functions of pRb (Harkins et al., 2010). As a result, E2F, an important transcriptional factor, is released and enhance the expression of several S-phase genes as well as cell cycle progression. It also interacts and inhibits p53, resulting cell survival and increasing the rate of mutation (Song et al.,2005). Moreover,

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the product of UL44 gene has been shown to inhibit transcriptional activity of p53 (Kwonet al., 2012). Hence, studies show that IE86 exhibits immunosuppressive and angiogenesis properties, which are two most keys factors in tumor growth and cancer survival (Harkins et al., 2010; Kwon et al., 2012; Dziurzynski et al., 2012; Johnsen et al., 2012). HCMV has the ability to infect the cells including, fibroblasts and endothelial cells which make up the tumor architecture/microenvironment. The aforesaid mechanisms raise the possibility of HCMV involvement with certain human cancers as a viral oncomodulator. Indeed, HCMV doesn't exert a direct role in tumor inducing but instead trigger tumor progression by regulation of cells cycle and programming either on tumor enhancing) immune induction (El-Shinawi et al., 2013). Therefore, the role of HCMV as an 'oncomodulator' is changing the tumor milieu in addition to initiation or progression of tumor (Richardson et al., 2015). HCMV genome, transcripts and proteins has also been detected in several types of cancers including: colorectal, prostate, breast, glioblastomas, medulloblastoma, mucoepidermoid cancer of the salivary gland, and rhabdomyosarcomas (El-Shinawi et al., 2013). In Iran, the presence of HCMV DNA genome has been detected in several type of cancers including colorectal (Tafvizi et al., 2013), Oral Squamous Cell Carcinoma (Delavarian et al., 2010) and Gastric Cancers (Leila et al., 2016). The study considering the contribution of HCMV in breast cancer development in the Middle-East is limited, so the investigation is demanding (Radley et al., 2016). Human papillomaviruses as a definite oncogenic virus is a small DNA virus belongs to the papillomaviridae family which infects the basal layer of epidermis (Mahmoudvand et al., 2015). The mechanism of carcinogenicity of papillomaviruses depends on the viral integration into the host DNA and expression of the oncoproteins E6 and E7 that inactivate the major tumor suppressor proteins P53 and Rb, respectively (Antonsson et al., 2011). The definite involvement of high-risk HPV strains such as type 16 and 18 with cervical, anogenital, and oral cancer is well-known (Radleyet al., 2016).

Although, HPV genome fragment has been detected in several other cancers including: esophageal squamous cell carcinoma, lung, prostate, ovarian, skin, colorectal, and urinary tract cancers, but its association with these cancers is controversial and has been remained to be proved (Mahmoudvand et al., 2015). Some reports have found association between breast cancer and presence of HPV DNA in invasive breast carcinoma samples (Glenn et al., 2012; Antonsson et al., 2011; De Villiers et al., 2004; Gumus et al., 2006). In contrast, some efforts failed to detect the presence of HPV DNA in samples of breast carcinoma (Silva et al., 2011; Mou et al., 2011; Lindel et al., 2007). In Iran, HPV genome fragments have been detected in lung, prostate, ovarian, skin, colorectal, bladder, as well as urinary tract cancers, and esophageal squamous cell carcinoma but not in breast cancer (Mahmoudvand et al., 2015; Jalilvand et al., 2014; Haghshenas et al., 2016).

Therefore, the aim of this study was to determine the frequency and evaluate the possible association of HCMV and HPV infections with benign and malignant breast tumors.

Materials and Methods

Subjects

A total of 300 paraffin-embedded biopsy breast specimens were gathered from biopsy bank of Faghihi hospital and Motaharri clinic, teaching centers affiliated with Shiraz University of Medical Sciences, according to the pathology report (Figure 1). The patients were admitted and surgery from January 2011 to December 2015. Specimens were included 150 subjects with invasive ductal/lobular carcinoma and 150 patients with benign breast tissues. The study was approved by the Ethics Committee of the University and informed consent was obtained before sample collection.

DNA extraction and qualification

DNA extraction was performed as previously describe (Mahmoudvand et al., 2017). Briefly, 10 sections (with the width of 10 μm) of the paraffin-embedded block were cut and put into a 1.5 mL Eppendorf tube followed by deparaffinization using 1200 μl of xylene. After incubation, the tubes underwent centrifugation and the supernatant was removed. Then, absolute ethanol was added, incubated at room temperature, underwent centrifugation step and the supernatant was removed. Both steps were repeated once more. The DNA was then extracted using a QIAamp DNA minikit (Qiagen, Dusseldorf, Germany) according to the manufacturer's instruction. The extracted DNA was stored at -20°C until use.

All extracted DNA samples were initially subjected to PCR with consensus primers PCO3/PCO4 (β-globin specific) for sample qualification/to confirm the quality of the extracted DNA. Negative samples were excluded from the study. For this purpose, the PCR reaction was performed on qualified samples in a total volume of 25μL, containing: 1mM MgCl2, 200 μM deoxyribonucleotide triphosphates solution (dNTPs), 1U Taq DNA polymerase (CinnaGene, Iran) and 1μM each specific primers (Table 1).

PCR program for β-globin was adjusted as follows: 10 min initial denaturation at 94°C, 35 cycles of denaturation at 94°C for 45 s, annealing at 44°C for 45 s, extension at 72°C for 1min, and one step of final extension at 72°C for 10 min.

HPV genome detection

HPV genome amplification was done on samples which were positive for β-globin gene. Briefly, using two separate set/pairs of HPV specific primers including: MY09/MY11 and GP5+/GP6+, L1 region of virus was targeted and amplified following two separate PCR tests (Table 1). PCR test for amplification of L1 region by MY09/MY11 primer pair was performed as previously described. Beside, amplification of L1 region using GP5+/GP6+ primer pair were also performed as follows: 5 min initial denaturation at 95°C, 50 cycles of denaturation at 95°C for 1 min, annealing at 48°C for 1 min and 45s, extension at 72°C for 3.5 min, and one step of final

extension at 72°C for 8 min. PCR products were then loaded into 2% agarose gel and visualized under UV light. In each run a positive sample that was confirmed as HPV positive included as described before (Mahmoudvand et al., 2015).

HCMV genome detection

Quantitative real time PCR was performed to qualitative and quantitative detection of HCMV genomic DNA on extracted DNA of malignant and benign breast tumor samples. Quantitative real time PCR was done using gensig real-time PCR kit (Primer Design Ltd TM, Advanced kit, UK) according to manufacturer's instruction. The reaction mix for PCR was adjusted in 10 µL total volume encompassing: 5µL Precision™ Master Mix, 0.5 µL primers and a probe targeting the glycoprotein B (gB) sequence and 2.5 µL of the template DNA.
The PCR program used for this reaction was included 1 cycle 95°C for 15 minutes, followed by 50 cycles of 95°C for 10 seconds and 60°C for 60 seconds using Step One Plus Real-Time thermocycler (Applied Biosystems-Grand Iland, NY, USA). This quantitative PCR assay was sensitive enough to detect as few as 100 copy of CMV genome per milliliter of biologic samples.

Statistical analysis

Statistical analysis was carried out using SPSS (Chicago, IL, USA) software version 23 for Microsoft Windows®. The results were processed statistically using the Chi square test and T-test.

Results

Demographic and pathological result

The mean age of participants in test and control groups was 35.2 ± 12.155 S.D and 45.0±9.465 S.D, respectively. The age of HCMV positive samples were 38 and 26, respectively. In the carcinoma group, 146 (97.3%) samples were ductal carcinoma, two (1.3%) samples were lobular carcinoma and two (1.3%) samples were invasive ductal and lobular carcinoma (IDC/ILC). Moreover the malignant tumors were diagnosed at stage I, stage II, and stageIII in 35 (23.3%), 67(44.7%), and 48 (32%) patients, respectively. Among the studied benign samples, 96 (64%) were diagnosed with fibroadenoma, 52 (34.7%) sample with fibrocystic and 2 (1.3%) samples were diagnosed as adenosis.

Real time PCR for HCMV

The result showed that just two sample of benign tumor were positive for HCMV genome. The number of genome detected in positive samples was 1,500 and 6,060 copy/ml, respectively. Interestingly, No HCMV genome positive sample was detected among malignant tumor samples.

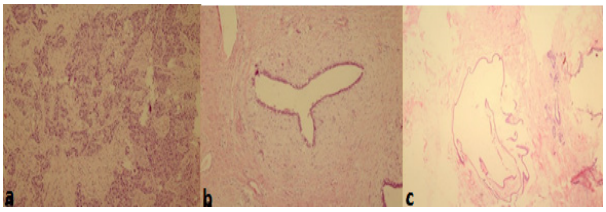

Figure 1. Photomicrograph of Human Breast Tumor Tissue. A) A photomicrograph of human breast cancer which is negative for the presence of HPV DNA. B) A photomicrograph of fibroadenoma which is negative for the presence of HPV DNA. C) A photomicrograph of fibrocytic which is negative for the presence of HPV DNA (H and E×250).

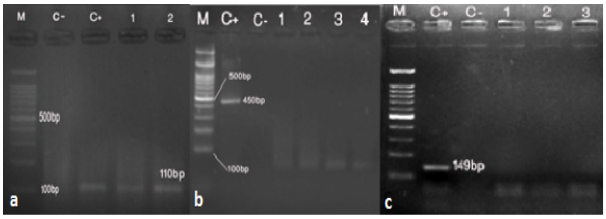

Figure 2. The Gel Electrophoresis Results of PCR Tests. A) Quality assessment by PCR analysis of DNA samples extracted from breast cancer tissue using β-globin primers indicated the presence of 110 bp bands in majority of samples. B) PCR analysis of DNA samples that were extracted from breast cancer tissue using HPV common primers (GP6+ GP5+) showed no positive test here, but a 450 bp band was demonstrative of positive control accuracy. C) PCR analysis of DNA samples extracted from breast cancer tissue using HPV common primers (MY09/ MY11).

PCR for HPV

In our study for more accuracy and sensitivity, two different set of PCR for detection of HPV genome fragment was employed. None of the sample was positive for the presence of HPV DNA in all malignant tumors and benign breast tissues.

Discussion

Breast cancer has the first rank among female cancers and remains as the second common cause of death as well, worldwide (Mousavi et al., 2007). Based on the report

Table 1. The Sequences and Other Characteristics of Primers Used in This Study, Enrollment in local colleges, 2005

Gene	Primers name	5' to 3' Sequence	Size, bp
β- globin	PCO3	5'ACACAACTGTGTTCACTAGC-3'	110
	PCO4	5'CAACTTCATCCACGTTCACC-3'	
HPV L1	MY09	5'- CGTCCMARRGGAWACTGATC-3'	450
	MY11	5'-GCMCAGGGWCATAAYAATGG-3'	
	GP6+	5′-GAAAAATAAACTGTAAATCATATTC- 3′	149
	GP5+	5′-TTTGTTACTGTGGTAGATACTAC- 3′	

published by the Iranian cancer registry, cancer is the third cause of mortality in this region. The incidence rate of breast cancer was 7.582 among Iranian's woman in 2009 and the age standardized incidence rate was 28.25 per 100,000 (Haghighat et al., 2016).

Although, the possible involvement of HPV and HCMV in breast cancer development has been considered, the data from Middle-East is limited. Even, from worldwide studies, here are controversial reports regarding the role of HPV and HCMV infections in introducing and/or developing of breast cancer (Rahbar, 2016). The present study investigated the presence of genomic DNA of HPVs and HCMV in cancerous and benign breast tissue to clarify their possible roles.

In our study, HPVs DNA did not detect in 150 invasive breast cancer tissues and 150 benign breast cancer tissues. This result was in parallel by several studies which were failed to detect HPV infection in breast carcinoma tissue. In Switzerland (Lindel et al., 2007), reported no evidence of HPV genome in invasive breast cancer tissues samples. Similarly, it was reported that none of 79 examined breast cancer samples of Brazilian patients were positive for HPV DNA (Silva et al., 2011). In this accordance, (De Cremoux et al., 2008) was also not detected HPV genomic DNA in 79 breast cancer samples of French patients. On the other hand, some studies found the HPV DNA in the invasive breast cancer tissues and benign breast tissue. In Egypt, Ahmed et al., (2016) reported that 26 of 107 (24.3%) invasive breast carcinoma tissue samples are infected with HPV as demonstrated by HPV protein detection through immunohistochistry. Tsai et al. (2005), found HPV DNA sequences in 8 of 62 (12.9%) invasive breast cancers tissue and 2 of 60 (% 3.3) benign breast tissues of Chinese women. In similar studies from Italy Di Lonardo et al., (1992) and Mou et al., (2011) and in China Damin et al., (2004) detected HPV genome among %10.6, %6.5 and %24.75 of tissue samples of breast carcinoma but none of all benign breast cancer tissues, respectively. In an interesting report from Germany, high rate of HPV genome has been demonstrated in the level of 86.2% and 68.9% for 29 tissue samples of breast carcinoma and 29 benign breast cancer tissues (de Villiers et al., 2004). In Iran, Sigaroodi et al., (2012) detected HPV DNA in tissues from 15 of 58 (25.9%) patients with breast cancer and 1 of 41 (2.4%) individuals with benign lesions.

In sum, a number of studies acclaimed the association between HPV and breast cancer, while some others did not support these findings. This strong discrepancy may partly come from the differences in some factors including: genetic background, environmental effects, sample size, and life style (sexual behavior) of patients. Albeit, this kind of discrepancy may also be elucidated by some technical issue including: type of methods (PCR, immunohistochemistry or transcript analysis), type of sampling (fresh or fixed), sensitivity of detection approaches, probability of cross-contamination, as well as differences in the extraction procedures.

A simple look at the frequency of HCMV among malignant and benign beast tumor tissues reveled the very low frequency of HCMV, as only two samples of benign group were positive for HCMV DNA that was not significantly different between two groups. In agreement with this finding, Richardson et al., (2015) with founded no HCMV genomic DNA in none of 70 tumor but only in 2 of 70 (3%) of paired normal specimens. Hence, another study supporting no role for HCMV in breast cancer, Eghbali et al., (2012) reported that only 2 of 24 (8.3%) paraffin embedded breast carcinoma tissue and none of 24 sample of fibroadenoma tumors were positive for HCMV DNA. There are studies regarding high prevalence of HCMV but non-significant role among different groups. In this regard, the high rate of HCMV DNA in 12 of 12 (100%) breast cancer and 10 of 11 (91%) paired sentinel lymph node specimens has been reported Taher et al., (2013) in Swedish population. Also, some study only evaluated malignant breast cancer tissue in their study, therefore relying on the results of these study to prove the association of HCMV and barest cancer is questionable. In this regards, Antonsson et al., (2011) by using real-time PCR assays detected HCMV DNA in 5 of 54 (10%) breast cancer tissue. In addition, in Iran, karimi et al., (2016) reported HCMV DNA genomic fragment in 26 of 50 (24.75%) invasive breast ductal carcinoma. Also, In Egyptian women, it has been reported that 47 of 107 (43.9%) invasive breast carcinoma tissue sample infected with HCMV using immunohistochemistry staining method (Ahmed et al., 2016). In spite of our finding, several case-control studies have been shown a link between HCMV infections and breast cancer using RT-PCR. It has been reported that 3 of 32 (97%) samples of breast carcinomas and 17 of 27 (63%) normal epithelium were positive for HCMV DNA in Mexican subjects (Utera-Barillas et al., 2013). In England, Harkins et al., (2008) and his colleagues by using in situ hybridization have been showed that 31 of 32 (97%) ductal breast carcinoma samples and 17 of 27 (63%) normal epithelium were positive for HCMV DNA, emphasized on indicative role of HCMV in cancer development, In addition, they also detected HCMV DNA in 6 of 8 (75%) of breast cancer specimens and 1 of 4 (25%) breast reduction mammoplasty controls specimens using PCR method that partly was in agreement with previous their own study.

Like HPV, controversial results in the frequency of HCMV in breast cancer tissue may be from some differences in host factors including: race, genetic, and rate of infection. Beside other methodological issue like the differences in the sample size, method of DNA extraction, tissue handling and limitations of molecular analyses as well as type of tissue preparation (fresh and/ or paraffin embedded) are among impressive factors attributed to discrepancy. Variable findings may also be due to 'hit and run' oncogenic mechanisms, that means; virus triggers the oncogenesis process but it is vanished in further steps (Richardson et al., 2015).

In conclusion, our investigation has shown very low frequency of HCMV DNA in benign breast tumor tissue which was not supportive of the role of HCMV infection in progression of breast cncer. Moreover, our data don't suport the role of HPV infection in development of breast cncer. Regarding controversial issues, more

invesvetigation with a larger group from different part of the country may give us more useful data for conclusion.

Conflict of interest

All the authors declare no Conflict of interest.

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References

Aceto GM, Solano AR, Neuman MI, et al (2010). High-risk human papilloma virus infection, tumor pathophenotypes, and BRCA1/2 and TP53 status in juvenile breast cancer. *Breast Cancer Res Treat*, **122**, 671-83.

Ahmed RA, Yussif SM (2016). Immunohistochemical detection of human cytomegalovirus, Epstein-Barr virus and human papillomavirus in invasive breast carcinoma in Egyptian women: A tissue microarray study. *J Solid Tumors*, **6**.

Alibek K, Kakpenova A, Mussabekova A, Sypabekova M, Karatayeva N(2013). Role of viruses in the development of breast cancer. *Infect Agent Cancer*, **8**, 32.

Antonsson A, Bialasiewicz S, Rockett RJ, et al (2012). Exploring the prevalence of ten polyomaviruses and two herpes viruses in breast cancer. *PLoS One*, **7**, e39842.

Antonsson A, Spurr TP, Chen AC, et al (2011). High prevalence of human papillomaviruses in fresh frozen breast cancer samples. *J Med Virol*, **83**, 2157-63.

Damin AP, Karam R, Zettler CG, Caleffi M, Alexandre CO (2004). Evidence for an association of human papillomavirus and breast carcinomas. *Breast Cancer Res Treat*, **84**, 131-7.

Delavarian Z, Pakfetrat A, Falaki F, Pazouki M, Pazouki N (2010). The role of viruses in oral squamous cell carcinoma in young patients in Khorasan (Northeast of Iran). *J Appl Sci*, **10**, 981-5.

de Cremoux P, Thioux M, Lebigot I, et al (2008). No evidence of human papillomavirus DNA sequences in invasive breast carcinoma. *Breast Cancer Res Treat*, **109**, 55-8.

Di Lonardo A, Venuti A, Marcante ML (1992). Human papillomavirus in breast cancer. Breast Cancer Res Treat, **21**, 95-100.

de Villiers E-M, Sandstrom RE, zur Hausen H, Buck CE (2004). Presence of papillomavirus sequences in condylomatous lesions of the mamillae and in invasive carcinoma of the breast. *Breast Cancer Res*, **7**, R1.

Dziurzynski K, Chang SM, Heimberger AB, et al (2012). Consensus on the role of human cytomegalovirus in glioblastoma. *Neuro Oncol*, **14**, 246-55

Eghbali M, Ghane M, Mirinargesi M (2012). Frequency of cytomegalovirus (CMV) in benign and malignant tumors. *Int J Mol Clin Microbiol*, **2**, 175-9.

El-Shinawi M, Mohamed HT, El-Ghonaimy EA, et al (2013). Human cytomegalovirus infection enhances NF-kappaB/p65 signaling in inflammatory breast cancer patients. *PLoS One*, **8**, e55755.

Glenn WK, Heng B, Delprado W, et al (2012). Epstein-Barr virus, human papillomavirus and mouse mammary tumour virus as multiple viruses in breast cancer. *PLoS One*, **7**, e48788.

Gumus M, Yumuk P, Salepci T, et al (2006). HPV DNA frequency and subset analysis in human breast cancer patients' normal and tumoral tissue samples. *J Exp Clin Cancer Res*, **25**, 515-21.

Haghighat S, Akbari ME, Yavari P, Javanbakht M, Ghaffari S (2016). Cost-effectiveness of three rounds of mammography breast cancer screening in Iranian women. *Iran J Cancer Prev*, **9**, e5443.

Haghshenas MR, Mousavi T, Moosazadeh M, Afshari M (2016). Human papillomavirus and breast cancer in Iran: a meta-analysis. *Iran J Basic Med Sci*,**19**, 231-7.

Harkins LE, Matlaf LA, Soroceanu L, et al (2010). Detection of human cytomegalovirus in normal and neoplastic breast epithelium. *Herpesviridae*, **1**, 1.

Hulka BS, Moorman PG (2008). Breast cancer: hormones and other risk factors. *Maturitas*, **38**, 103-13.

Jalilvand S, Shoja Z, Hamkar R (2014). Human papillomavirus burden in different cancers in Iran: a systematic assessment. *Asian Pac J Cancer Prev*, **15**, 7029-35.

Johnsen JI, Baryawno N, Söderberg-Nauclér C(2012). Is human cytomegalovirus a target in cancer therapy. *Oncotarget*, **2**, 1329-38.

Karimi M, Hosseini SZ, Nikkhoo B, Soleimani-Mohammadi F (2016). Relative frequency of cytomegalovirus (CMV) in tissue samples of women with breast cancer in Sanandaj, Iran. *Int J Bioassays*, **5**, 4907-11.

Kwon Y, Kim MN, Young Choi E, et al (2012). Inhibition of p53 transcriptional activity by human cytomegalovirus UL44. *Microbiol Immunol*, **56**, 324-31.

Leila Z, Arabzadeh SA, Afshar RM, Afshar AA, Mollaei HR(2016). Detection of Epstein-Barr virus and cytomegalovirus in gastric cancers in Kerman, Iran. *Asian Pac J Cancer Prev*, **17**, 2423.

Lindel K, Forster A, Altermatt HJ, Greiner R, Gruber G (2007). Breast cancer and human papillomavirus (HPV) infection: no evidence of a viral etiology in a group of Swiss women. *Breast J*, **16**, 172-7.

Mahmoudvand S, Safaei A, Erfani N, Sarvari J (2015). Presence of human papillomavirus DNA in colorectal cancer tissues in Shiraz, Southwest Iran. *Asian Pac J Cancer Prev*, **16**, 7883-7.

Mahmoodvand S, Zamani K, safaei A, et al (2017). No detection of Streptococcus g allolyticus and Helicobacter pylori in colorectal cancer tissue samples in Shiraz, Iran. *Iran J Cancer Prev*, **10**, e6337

Mousavi SM, Montazeri A, Mohagheghi MA, et al(2007). Breast cancer in Iran: an epidemiological review. *Breast J,* **13**, 383-91.

Mou X, Chen L, Liu F, et al (2011). Low prevalence of human papillomavirus (HPV) in Chinese patients with breast cancer. *J Int Med Res*, **39**, 1636-44.

Radley D, Saah A, Stanley M (2016). Persistent infection with human papillomavirus 16 or 18 is strongly linked with high-grade cervical disease. *Hum Vaccin Immunother*, **12**, 768-72.

Rahbar A (2015). Promotion of tumorigenesis and clinical implications of viral infection in breast cancer. *J Carcinog Mutagen*, **6**, 217.

Richardson AK, Currie MJ, Robinson BA, et al (2015). Cytomegalovirus and Epstein-Barr virus in breast cancer. *PLoS One*, **10**, e0118989.

Sarvari J, Mojtahedi Z, Kuramitsu Y, et al (2014). Comparative proteomics of sera from HCC patients with different origins. *Hepat Mon*, **14**, e13103.

Sigaroodi A, Nadji SA, Naghshvar F, et al (2012). Human papillomavirus is associated with breast cancer in the north part of Iran. *Sci World J*, 10.1100/2012/837191.

Silva Jr RG, Da Silva BB (2011). No evidence for an association of human papillomavirus and breast carcinoma. *Breast Cancer Res Treat*, **125**, 261-4.

Song YJ, Stinski MF (2005). Inhibition of cell division by

the human cytomegalovirus IE86 protein: role of the p53 pathway or cyclin-dependent kinase 1/cyclin B1. *J Virol*, **79**, 2597-603.

Tafvizi F, Fard ZT (2014). Detection of human cytomegalovirus in patients with colorectal cancer by nested-PCR. *Asian Pac J Cancer Prev*, **15**, 1453-7.

Taher C, de Boniface J, Mohammad AA, et al(2013). High prevalence of human cytomegalovirus proteins and nucleic acids in primary breast cancer and metastatic sentinel lymph nodes. *PLoS One*, **8**, e56795.

Torre LA, Bray F, Siegel RL, et al (2012). Global cancer statistics. *CA Cancer J Clin*, **65**, 87-108.

Tsai JH, Tsai CH, Cheng MH, et al (2005). Association of viral factors with non-familial breast cancer in Taiwan by comparison with non-cancerous, fibroadenoma, and thyroid tumor tissues. *J Med Virol*, **75**, 276-81.

Utrera-Barillas D, Valdez-Salazar H-A, Gómez-Rangel D, et al (2013). Is human cytomegalovirus associated with breast cancer progression. *Infect Agent Cancer*, **8**, 1.