



Original Research Article

Investigation the role of human cytomegalovirus in the invasive ductal breast carcinoma using real time PCR

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ABSTRACT

Keywords

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In Iraq, breast cancer is the commonest type of female malignancy. Increasing evidence in the last 10 years suggests that HCMV is associated with several human malignancies including breast cancer. This study aimed to investigate the DNA of HCMV in invasive ductal breast carcinoma by real time PCR. Fifty sample of cancer mass and non-neoplastic safe margin tissues of breast cancer collected. Each tumor mass and safe margin tissue divided in to 2 sections, one for DNA extraction then real time PCR while the other section processed for paraffin block and stained by eosin – hematoxylin stain. Thirty-eight samples (76%) of 50 samples was diagnosed as invasive ductal carcinoma (IDC). The result showed just nine (23.7%) sample was positive with mean of copy number equal to 3.772×10^3 copy number/ml and mean of threshold cycle (CT) equal to 36.8 from 50 cycle reaction. Many studies including our observation indicated to the association of HCMV with breast cancer but the role of HCMV in the pathogenesis of breast cancer is unclear.

Introduction

Breast cancer is the most frequently diagnosed cancer in women worldwide with an estimated 1.4 million new cases in 2008. (Institute for Health Metrics and Evaluation, 2011). In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the 2008 Iraqi Cancer Registry (Iraqi Cancer Board, 2007). This shows that the breast is the leading cancer site among the Iraqi population in general, surpassing even bronchogenic cancer (Alwan, 2010).

As in all cancers, the cause of breast cancer remains unknown. Research into its etiology has focused primarily on reproductive and other factors affecting circulating sex hormones as well as on genetic susceptibility. Hormones, as identified risk factors thought to explain only about half of all breast cancer incidences. Researchers are motivated to consider other routes of disease pathogenesis (Alwan, 2010).

The International Agency for Research on Cancer (IARC) reports that biological carcinogens cause 18-20% of cancers,

suggesting the tremendous potential of controlling microbe-related processes for cancer prevention (Parkin, 2006).

Human herpesviruses known for its oncogenic potential. HCMV and EBV of the Herpesviridae family have been implicated as a cause of breast cancer. There was significance of EBV expression in breast tissue of young patients with breast cancer (Hanna et al., 2011) and this finding confirmed by polymerase chain reaction (PCR)-based studies, positive correlation was shown (Amarante and Watanabe, 2009), and the presence of EBV DNA was associated with more severe forms of breast cancer (Mazouni et al., 2011).

Human Cytomegalovirus had been shown to be involved in many cancers including malignant glioma, and prostate, skin, and colorectal cancers (Alibek et al., 2013).

Increasing evidence in the last 10 years suggests that HCMV is associated with several human malignancies, and that HCMV gene products can modulate oncogenic properties of cells *in vitro* (Harkins et al., 2010). Since persistent of HCMV infection of breast epithelium could, in theory, promote malignant transformation of infected breast epithelium, that sought to determine the HCMV gene products in normal and neoplastic breast (Cox et al., 2010).

There might be several mechanisms of how CMV can cause breast cancer initiation and progression. Firstly, it was shown that HCMV gene products affect cell cycle regulation, inhibit apoptosis, activate angiogenesis and metastatic phenotype, and cause increased mutation rate, thereby overlapping with all established hallmarks of cancer cells (Dziurzynski et al., 2011).

Secondly, HCMV exhibits immunosuppressive properties, leading to escape of tumor cells from immune surveillance mechanisms (Zhu et al. 1995). HCMV has evolved multiple strategies for immune evasion resulting in persistent viral infection in the host (Cinatl et al., 1998). Several HCMV proteins, including those expressed with IE genes, block the host cell MHC class I antigen expression, which is essential for activation of CD8+ T-lymphocyte anti-tumor cytotoxicity. HCMV UL83 protein (pp65) blocks antigen presentation of HCMV epitopes to CD8+ T-cells, and expression of HCMV UL18, a MHC class I homologue, disrupts “natural killer” (NK) cell recognition of HCMV-infected cells (Farrell et al., 1997).

Thirdly, specific actions of virus-encoded interleukins (IL) could be implicated. IL-10 was shown to be differentially expressed in breast tumor cells and infiltrating lymphocytes. Elevated serum level of IL-10 observed in breast cancer patients. The fact that HCMV expresses a viral analogue of human IL-10 may lead to the conclusion that this could be one of the mechanisms of breast cancer promotion by the virus (Hamidullah et al., 2012).

Materials and Methods

The study prospectively designed. Fifty sample of cancer mass and safe margin tissues of breast cancer collected under supervision of surgeons from Al-Kadhimiya Teaching Hospital and Dijlah Private Hospital during February to July 2013.

Each tumor mass and safe margin tissue divided in to 2 sections, one preserve in normal saline in the freezing for DNA extraction while the other section preserve in neutral buffered formalin 10% for processing of paraffin block for eosin – hematoxylin staining.

DNA had be extracted from frozen mass and safe margin of breast cancer tissues according to the kit manufacture's manual of QIAamp DNA Mini kit from Qiagen – USA. Bosphore CMV quantification real time PCR v1 kit (Turkey)used to detect a region of DNA polymerase of HCMV in extracted DNA by Stratagene MX3005p instrument. This protocol performed according to the manufacture's manual including all preparations and thermal profile. The thermal protocol composed of an initial denaturation for activation the HotStar Taq DNA polymerase, a two-step amplification cycle and a terminal hold. The real time data collected at the second step of the amplification cycle.

Statistical analysis: Statistical analysis estimated by used Fisher's exact test accomplished by SPSS software version 18 to compare the number cytomegalovirus – positive samples between invasive ductal breast carcinoma and safe margin and between the grade 1 and grade 2 of the invasive ductal breast carcinoma samples.

Ethical Approval

This research underwent to the terms of ethical considerations and in accordance with the form prepared for this purpose by the Iraqi Ministry of Health also got the approval of the research by the Committee of ethical standards in the Faculty of Medicine, Al-Nahrain University , one of the colleges affiliated to the Ministry of Higher Education and Scientific Research, Iraq.

Result and Discussion

Thirty-eight samples (76%) of 50 samples was diagnosed as invasive ductal carcinoma (IDC), 28 samples (73.7%) of 38 was estimated, as grade 2 while 10 samples

(26.3%) was grade 1. This diagnosis achieved by pathologist after hematoxylin and eosin staining.

Quantitative real time PCR performed to qualitative and quantitative detection of cytomegalovirus DNA in DNA samples that extracted from fresh tissue sample of invasive ductal carcinoma and safe margin. The result showed just (table 1) nine (23.7%) sample was positive with mean of copy number equal to 3.772×10^3 copy number/ml and mean of threshold cycle (CT) equal to 36.8 from 50 cycle reaction. This result showed there was significant differences ($P < 0.05$) between invasive ductal breast carcinoma samples and safe margin tissue (table 2). Regarding to association of the presence of CMV DNA and grade of differentiation of the invasive ductal breast carcinoma, the results showed there was no significant differences related the differentiation of the IDC.

Many studies indicated to the association of HCMV with breast cancer, but different results had be showed. This study gave significant result indicated to the presence of HCMV DNA in the invasive ductal breast carcinoma tissue regardless the grade of differentiation. This result confirmed by Harkins LE. et al., 2010 when used PCR and in situ hybridization to detect HCMV DNA in the breast ductal carcinoma in situ (DCIC) and invasive ductal carcinoma (IDC). In addition to that, Al-Alwany and Ali, 2013 used in situ hybridization to detect HCMV DNA in different grade of breast carcinoma and the results proved what came in this study. In contrast, Utera-Barillas et al., 2013 study show no association between HCMV infection and breast cancer progression when used real time PCR to detect HCMV DNA in the samples of breast cancer tissue.

Regarding control, in this study we used the safe margin tissue adjoining to the cancer mass tissue as a control to compare with samples of the invasive ductal breast carcinoma. The results showed absence of the HCMV DNA from the safe margin tissue when investigated by real time PCR and up to our best knowledge; this was the first study in Iraq used real time PCR to demonstrate the DNA of HCMV in invasive ductal breast carcinoma and safe margin tissue.

Other studies used normal breast tissue as control and the results were different, some studies showed absence of the HCMV DNA in the normal breast tissue while others showed 63% of HCMV DNA present in normal epithelial breast tissue (Harkins et al., 2010).

Recent investigations have linked viral infections such as EBV, MMTV, HPV and HCMV to breast cancer (Lee and Schmitt, 2003). Human cytomegalovirus had been shown to be involved in many cancers including malignant glioma, and prostate, skin, and colorectal cancers (Alibek et al., 2013). As mentioned above, HCMV had many mechanisms involved in the progression of the cancer. First evidence indicated to the association between HCMV

and breast cancer was the shedding of the virus with breast milk and presence of the epithelial cells in breast tissue, which consider the main tropism cell for HCMV (Harkins et al., 2010).

Many studies imply that inflammation is associated with cancer (Hanahan and Weinberg, 2011, Colotta et al., 2009). The inflammatory mediator COX-2 and its metabolites prostaglandins have been implicated as growth factors for tumor cells (Wang and Dubois, 2010). Many tumors, including breast cancer, are COX-2-positive, and high COX-2 expression levels are associated with poor clinical outcome (Soumaoro et al., 2004). Both selective and non-selective COX inhibitors decrease cancer incidence and show promise as anticancer agents (Thun et al., 2002). Interestingly, HCMV induces inflammation and at the same time utilizes sophisticated strategies to escape immune recognition (Michaelis et al., 2009, Powers et al., 2008). The HCMV protein US28 is a constitutively active chemokine receptor homologue that induces COX-2 expression and results in STAT3 phosphorylation, which increases the production of VEGF and IL-6 and consequently induce tumor formation in vivo (Maussang et al., 2009).

Table.1 The percentage of the presence of CMV DNA using real time PCR in the invasive ductal breast carcinoma (IDC) and non-neoplastic safe margin (SM) tissue samples

			Study groups		Total
			IDC	SM	
PCR	positive	Count	9	0	9
		%	23.7%	0.0%	13.2%
	negative	Count	29	30	59
		%	76.3%	100.0%	86.8%
Total		Count	38	30	68
		%	100.0%	100.0%	100.0%
P value			.003		
Relative Risk with (CI)			15.1 (0.91-249.47)		

Table.2 The presence of CMV DNA according to the grade of differentiation of invasive ductal breast carcinoma (IDC) tissue

	IDC grade 1	IDC grade 2
Positive	1 (2.6%)	8 (21%)
Negative	9 (23.7%)	20 (52.6%)
Total	10 (26.3%)	28 (73.6%)

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