Evaluation frequency of Human Herpes Virus type 8 in Patients with Breast Cancer

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Abstract

Background

Breast cancer is one of the most common malignancies and the most common cause of death women around worldwide. Recently, viral etiology theory has been proposed on physiopathology of the breast cancer.

Method:

In a retrospective study, to study of non-familial agents and a viral factors breast cancer, the Real time PCR were used to detect Kaposi Herpes virus or Human herpes virus type8(HHV8) in 138 patients with breast cancer.

Result

Out of the 138 embedded paraffin breast cancer tissues, 17(12.3%) were positive for HHV8. Among the viral positive breast cancer samples, 8 samples (10.95%) were positive in intermediate grade and 10 samples (11.9%) were positive in 40–60 years old age group. There was no significant relation between grades of tumors and there was a significant relation between age and breast cancer.

Conclusion

Out of 17 positive samples 15 samples (88.23%) and 2 samples (11.77%) had more than 5 and less than 3 infected lymph node. In this study, there was a significant relation between infected lymph node and positive samples with HHV-8. Therefore, it is likely that the lymphocytes infected with HHV-8 virus may be the source of cancer cells and may also cause infected cells to spread to other organs, as well as the treatment of these patients is difficult.

Introduction

Breast cancer is one of the most common cancers in women[1], with more than 2.1 million people diagnosed with breast cancer annually[2]. Breast Cancer is the second most common cancer in women in the United States, with 268,600 new cases in the year. After lung cancer, breast cancer is the second deadliest cancer in the world and is responsible for 627,000 and 41760 deaths in the world and the United States respectively[2]. About 15% of women's cancer deaths are due to Breast cancer[1]. Until 2015, the incidence of breast cancer had an upward trend, but today the incidence of breast cancer has a steady trend[1]. However, the exact cause of breast cancer is unknown, and several factors such as lifestyle, smoking, obesity, aging, and infectious agents are considered as risk factors for breast cancer[2, 3]. According to the International Cancer Agency, infectious agents such as viruses are responsible for 15–

20% of human cancers[4]. Previous studies have shown that papillomavirus[5], Epstein-Barr virus (EBV) [2], mouse mammary tumor virus (MMTV)[6, 7], and bovine leukemia virus (BLV)[8] are associated with an increased risk of breast cancer. Moreover, several recent studies have suggested a possible relationship between Human Herpes Virus-8 (HHV-8) and breast cancer[9, 10].

The HHV-8 is the eighth human virus of the Herpesviridae and is also known as Kaposi's Sarcoma-associated Herpes Virus (KSHV)[11]. The HHV-8 virus causes Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease[12, 13]. Kaposi's sarcoma is more common among immunocompromised individuals such as AIDS patients[14]. Studies have shown that this virus is tumorigenic and is closely related to fibro adenoma tumors[9, 15]. However, the virus is not associated with cervical and oral tumors[16, 17]. A study by PAUL et al. Showed that the HHV-8 virus has the potential to invade and persist in the brain tissue[18]. Several studies have also shown that the HHV-8 virus has been detected in breast cancer patients. The study by Tsai showed that the prevalence of HHV-8 in breast cancer patients is higher than other viruses[9]. Also, the Amira S. Mohamed study showed, 28.8% blood samples of breast cancer cases were positive for HHV-8 DNA[10].

The HHV-8 virus has the ability to produce cytokine homologs such as interleukin-6 (IL-6). Studies have shown that an increase in IL-6 is associated with metastasis and the progression of breast cancer [19–21]. Increased IL-6 expression activates the virus lytic cycle and increases the expression of genes involved in pathogenesis, leading to the development of malignancy[22]. The ability to infect and proliferate in epithelial cells, the ability to produce interleukin homologs, and the detection of the hhv-8 virus genome in breast cancer tissue are factors that suggest that the hhv-8 virus may be associated with breast cancer[23].

We aimed to investigate the presence of HHV-8 genome in breast tissues in women with breast cancer.

Material And Method

Study population

A total of 138 formalin-fixed paraffin-embedded (FFPE) samples were collected from histologically confirmed cases of breast cancer diagnosed in the department of pathology of Kerman Hospital (Kerman, Iran) during the period between March 2016 and May 2018. Demographic information and cancer grade were also extracted from patients' records. This cross-sectional study was conducted according to the principles of the Declaration of Helsinki and received an ethics code from the Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran (IR.KMU.REC.1396.2137). Written informed consent was obtained from all participants enrolled in this study.

Sample collection and preparation

A thin 10- µm tissue section was obtained from the blocks, and deparaffinization was performed with xylene. The, a series of distilled water and graded ethanol solutions were used for rehydration, according

to previous studies.

DNA Extraction

The DNA extraction was performed by using viral nucleic acid extraction kit (North Korea), according to the manufactures instruction. Spectrophotometry Nano drop ND-1000 (thermo Fisher Scientific Inc. Waltham, MA) was used for evaluation of the extracted DNA. The extracted DNA was stored at -20°C until molecular tests were performed.

DNA amplification

For the detection of HHV-8-DNA, a Taq Man real-time PCR approach was applied. About 40 ng of extracted DNA was used as the template in a reaction including 10µl of 2x-PCR master mix (amplicon, Austria), 10 pmol/ml of probe (FAM-TGCAGCAGYTGTTGGTGTACCACAT-BHQ1), 30 pmol/ml of each following primers (5'-AGCCGAAAGGATTCCACCATT-3') and (5'-TCCGTGTTGTCTACGTCCAGA-3'), then brought to 20µl using sterile distilled water. The test was performed in 45 cycles, started by one cycle 10 min at 94°c, followed by 45 cycles of amplification, consisting 10 s at 94°c and 40 s at 60°c (**Table 1**).

Table 1: Temperature profiles used in this study

cycle	time	temperature	state
1	10 min	95°c	Holding
45cycles	10 sec	95°c	Denaturation
	40sec*	60°c	Annealing, Extension

Statistical analysis

All statistical analysis was performed using the IBM SPSS version 18 (SPSS Inc, USA). Data was reported as mean ± standard deviation. P-values less than 0.05 were considered to be statistically significant.

Result

Out of 138 FFPE samples collected from breast cancer patients, the prevalence of HHV-8 virus infection was evaluated. The age range of patients was from 28 to 79 years [mean ± standard deviation (48.47 ± 11.33)]. Among 138 patients, 33 was in the age range of 20–40 years (23.91%), 84 was in the age range of 41–60 (60.87%) and 21 was between 61 to 80 (15.21%) years. The highest frequency of patients is in the age range of 41 to 60 years (Table 2).

Table 2
Frequency of different age groups in patients with breast cancer

Age group (yrs.)	Frequency	Percent
20–40	33	33(23.91%)
40–60	84	84(60.87%)
60–80	21	21(15.21%)
Total	138	138(100%)

Out of 138 patients, 31(22.5%) had high-grade tumor, 73 (52.9%) had intermediate, and 34 (24.6%) had low-grade tumor (Table 3).

Table 3
Frequency of tumor grades in patients with breast cancer

Type of grade	Frequency	Percent
High Grade	31	22.5
Intermediate	73	52.9
Low Grade	34	24.6
Total	138	100.0

Tumor-infected lymph nodes were divided into four categories: <3, 3–6, 6–9, and > 9 (Table 4).

Table 4
frequency of lymph nodes infected with the tumor in patients with breast cancer

Mean of lymph node	Frequency	Percent
< 3	98	71.0
3–6	26	18.8
6–9	10	7.2
> 9	4	2.9
Total	138	100.0

Of the 138 samples, the HHV-8 genome was identified in 17 (12.32%) cases. And 121(87.7%) samples had no HHV-8 DNA. (Table 5)

Table 5
HHV-8 frequency among patient with Breast cancer

HHV8			
		Frequency	Percent
HHV-8	positive	17	12.3
	Negative	121	87.7
	Total	138	100.0

Of the genome frequency among different age groups, 4 cases (2.90%) were in the age range of 20 to 40 years, 10 cases (7.25%) were in the age range of 41 to 60 years, and 3 cases (2.17%) were in the age range of 61 to 80 years. Chi-square test showed that there was a significant relationship between different age groups and the prevalence of HHV-8 virus infection (P. Value = 0.046) (Table 6).

Table 6
Frequency of different age groups in positive and negative cases of HHV-8 in patients with breast cancer

Age group	HHV8		Total	P-value
	Positive	Negative		
20–40	4(2.9%)	29(21.01%)	33(23.91%)	0.046*
40–60	10(7.25%)	74(53.62%)	84(60.87%)	
60–80	3(2.17%)	18(13.04%)	21(15.21%)	
Total	17(12.32%)	121(87.67%)	138(100%)	

The prevalence of viral infections in different tumor grades was also assessed, of which 17 were HHV-8 positive, 5 were high-grade, 8 were intermediate, and 4 were low-grade. Statistical analysis showed that there was no significant relationship between the frequency of HHV-8 virus infection and tumor grade (P-value = 0.795) (Table 7)

Table 7
Frequency of tumor grades in positive and negative cases of HHV-8 in patients with breast cancer

Type of grade	HHV8		Total	P-value
	Positive	Negative		
High Grade	5(3.62%)	26(18.84%)	31(22.46%)	0.759
Intermediate	8(5.90%)	65(47.10%)	73(52.90%)	
Low Grade	4(2.90%)	30(21.47%)	34(24.37%)	
Total	17(12.49%)	121(87.41%)	138(100%)	

The relationship between the number of lymph nodes involved in the tumor and viral infection was also examined. In 14 cases between 6 and 9 lymph nodes were involved and in 1 case more than 9 lymph nodes were involved. Statistical analysis showed that there was a significant relationship between the number of lymph nodes involved in the tumor and the frequency of HHV-8 virus infection (P-value = 0.016) (Table 8)

Table 8
Frequency of tumor-infected lymph nodes in positive and negative cases of HHV-8 in patients with breast cancer

Lymph nod	HHV8		Total	P-value
	Positive	Negative		
< 3	1(0.72%)	84(60.87%)	98(71.01%)	0.016*
3–6	1(0.72%)	25(18.12%)	26(18.84%)	
6–9	14(10.14%)	9(6.52%)	10(7.24%)	
> 9	1(0.72%)	3(2.17%)	4(2.89%)	
Total	17(12.32%)	121(87.68%)	138(100%)	

Discussion

Breast cancer is one of the most common and deadly diseases that has an unknown cause[24]. There are several factors that increase the risk of breast cancer, including infectious agents, especially viruses[25]. Various studies have shown that the prevalence of viral infections in breast cancer patients is higher compared to the control group. A study by Amira S. Mohamed and colleagues showed that the HHV-8 virus genome was found in the blood of 28.8% of breast cancer patients[10]. While our results showed that the frequency of HHV-8 virus genome in breast cancer tissue samples was 12.38%. In the present study, we did not find a significant relationship between tumor grade and the frequency of viral infection, while in the Amira study, the prevalence of viral infection also increased with increasing tumor grade.

Similar to Amira study, our results showed that there is a significant relationship between tumor-infected lymph nodes and the prevalence of viral infection, so that with the increase of cancer-infected lymph nodes, the prevalence of viral infection has also increased (P-value < 0.05). Therefore, it is likely that the lymphocytes infected with HHV-8 virus may be the source of cancer cells and may also cause infected cells to spread to other organs.

The first study on the possible role of HHV-8 in breast cancer was conducted in 2003 by Newton. The study found that HHV-8 was present in 55% of breast cancer samples. For the first time, Newton and colleagues reported that the HHV-8 could be associated with breast cancer[26]. Tsai et al. In 2005 examined the prevalence of viral infections in patients with breast cancer, the results of this study showed that 45.2% of the samples were HHV-8 positive[9]. In this study, the prevalence of HHV-8 viral infection was higher than the Human Papillomavirus (HPV) and EBV. In 2007, Liao et al. examined the prevalence of various viral infections in patients with breast cancer. The results showed that 87.50% of patients and 45.16% of controls were positive for the presence of the HHV-8 genome[27]. Chun-Ru Hsu and colleagues in 2010 conducted a study entitled Possible Factors of Viral DNA in Breast Cancer. In this study, it was found that HHV-8 is present in 43.8% of breast cancer samples[15].

The HHV-8 virus is associated with a variety of tumors, such as prostate tumors[28], endothelial cell tumors[29], and B cell lymphocytes[30]. Studies have also shown that the HHV-8 has the ability to immortalize and transform breast cells by inhibiting apoptosis, inducing cell proliferation, cell survival, increasing angiogenesis, and modulating the immune system. In addition, the virus can induce tumor genesis by producing interleukin homologs[10].

The results of this study showed that the prevalence of HHV-8 infection in patients with breast cancer is high and may be associated with an increased risk of breast cancer. In addition, it is likely that the lymphocytes infected with HHV-8 virus may be the source of cancer cells and may also cause infected cells to spread to other organs, as well as the treatment of these patients is difficult.

Abbreviations

Human Herpes virus-8 (HHV-8)

Kaposi's Sarcoma-associated Herpes Virus (KSHV)

Epstein-Barr virus (EBV)

Mouse mammary tumor virus (MMTV)

Bovine leukemia virus (BLV)

Human Papilloma virus (HPV)

Formalin-fixed paraffin-embedded (FFPE)

Interleukin-6 (IL-6)

Declarations

Acknowledgments

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None

Availability of data

All data used and analyzed are available from the corresponding author

Ethical approve

This cross-sectional study was conducted according to the principles of the Declaration of Helsinki and received an ethics code from the Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran (IR.KMU.REC.1396.2137). Written informed consent was obtained from all participants enrolled in this study

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no conflicts of interest.

Author contribution

E.F. and R.M.F. Conceived and designed the study. H.R.M, and S.A.A analyzed the data. E.F, S.H.M collecting the samples and perform laboratory tests, E.F and M.I wrote the paper. All authors read and approved the final manuscript

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