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Original Article

Prevalence of Human Herpes Virus-8 Infection Among Women with Breast Cancer

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Abstract

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

Materials and Methods: This cross-sectional study evaluated the presence of the Human Herpes Virus-8 genome in 138 formalin-fixed and paraffin-embedded (FFPE) breast cancer tissues, using real-time PCR. All samples were collected from the department of pathology of Bahonar Hospital (Kerman, Iran) and sent to the virology laboratory of Kerman University of Medical sciences with appropriate conditions.

Results: Out of the 138 FFPE breast cancer tissues, 17(12.31%) were positive for HHV8. Among the HHV-8 positive samples, 58.8% were in the 41-60 years old age group. Among HHV-8 positive cases, 47.05% were an intermediate grade and 82.05% have involvement of 7-9 lymph nodes. Also, there was a significant relationship between age and breast cancer.

Conclusion: 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.

Keywords: Human Herpesvirus 8, HHV-8, Breast Cancer

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).

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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 infection and its relationship with tumor grade and number of lymph nodes involved in women with breast tumor.

Methods

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 Bahonar Hospital (Kerman, Iran) during the period between March 2016 and May 2018. Nottingham modification of Bloom-Richardson system was used for breast cancer grading. Demographic information and cancer grade were also extracted from patients' records. Patients who did not have a sufficient sample size or whose file information was incomplete were also excluded from the study. 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-TGCAGCAGYTGTTGGTGTACCAC-AT-BHQ1), 30 pmol/ml of each following primers (5′-AGCCGAAAGGATTCCACCA-TT-3′) and (5′-TCCGTGTTGTCTACGTCC-AGA-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.

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.

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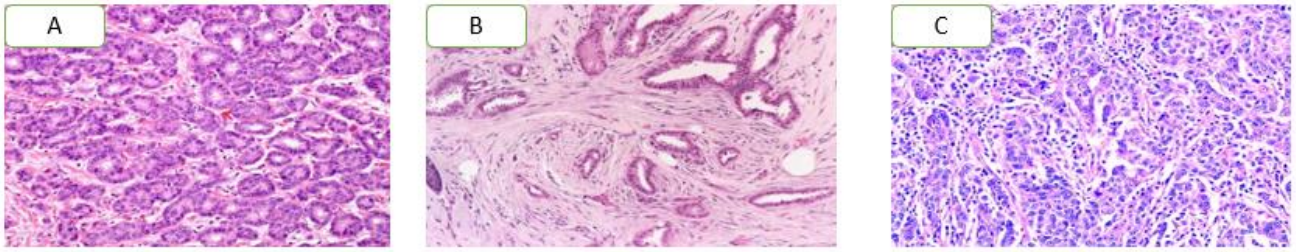

Fig. 1. Pathological feature of breast cancer Grades based on Nottingham modification of Bloom-Richardson system. A: Grade 1, B: Grade2, and C: Grade 3

Results

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. Based on Nottingham modification of Bloom-Richardson system, Out of 138 patients, 31 (22.5%) had high-grade tumor (Grade 3), 73 (52.9%) had intermediate (Grade 2), and 34 (24.6%) had low-grade tumor (Grade 1) (**Fig 1**). Tumor-infected lymph nodes were divided into four categories: <3, 3-6, 7-9, and >9 (**Table 1**).

Table 1: Demographic information of patients with breast cancer

	Variable	Frequency	Percent
Age group	20-40	33	23.91
	41-60	84	60.87
	61-80	21	15.21
	Total	**138**	**100**
Tumor grade	High Grade	31	22.5
	Intermediate	73	52.9
	Low Grade	34	24.6
	Total	**138**	**100.0**
Involved lymph node	<3	98	71.0
	3-6	26	18.8
	7-9	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 2**).

Table 2: 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 (23.5%) were in the age range of 20 to 40 years, 10 cases (58.8%) were in the age range of 41 to 60 years, and 3 cases (17.6%) 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 3**).

Table 3: Frequency of different age groups in positive and negative cases of HHV-8 in patients with breast cancer

Age group	HHV-8 positive	Total	P-value
20-40	4(23.5%)	33(12.12%)	0.046*
41-60	10(58.8%)	84(11.90%)	
61-80	3(17.6%)	21(14.28%)	
Total	17(100%)	138(12.31%)	

*: P-value < 0.05

The prevalence of viral infections in different tumor grades was also assessed, of which 17 (12.31%) were HHV-8 positive, 5 (29.41%) were high-grade, 8(47.05%) were intermediate, and 4 (23.52%) were low-grade. Statistical

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analysis showed that there was no significant relationship between the frequency of HHV-8 virus infection and tumor grade (P-value = 0.795) (**Table 4**)

Table 4: Frequency of tumor grades in positive and negative cases of HHV-8 in patients with breast cancer

Tumor grade	HHV-8 positive	Total	P-value
High grade	5(29.41%)	31(16.12%)	0.759
Intermediate	8(47.05%)	73(10.95%)	
Low Grade	4(23.52%)	34(11.76%)	
Total	17(100%)	138(12.31%)	

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 5**).

Table 5: Frequency of tumor-infected lymph nodes in positive and negative cases of HHV-8 in patients with breast cancer

Involved lymph nod	HHV-8 positive	Total	P-value
<3	1(5.88%)	85(1.17%)	0.016*
3-6	1(5.88%)	26(3.84%)	
7-9	14(82.35%)	23(60.86%)	
>9	1(5.88%)	4(25%)	
Total	17(100%)	138(12.31%)	

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 12.31%. 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 their 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 their 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).

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Conclusion

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.

Acknowledgment

None.

Conflict of interest

No conflict of interest is declared.

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