

Polymerase chain reaction for detection of Kaposi's sarcoma virus in breast tumors

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ABSTRACT

Human Herpes Virus 8 (HHV8) causes Kaposi's sarcoma, a common cancer among AIDS patients. It is proposed that this virus plays a role in formation of some blood vessel and lymphoma tumors. This study aimed to investigate the frequency of KSHV among benign and malignant breast tumors. A total of 24 carcinomas and 24 fibroadenomas paraffin embedded tumoral tissue samples were obtained from the pathology sections of Toos and Firoozgar hospitals in Tehran, Iran. All samples had been collected from patients during a period of time effective from June 2013 to February 2014. DNA was extracted from all samples and their infection with HHV8 was examined by PCR technique. The results obtained by this study showed that 6 out of 24 carcinoma samples (25%) were infected by KSHV, while the number of fibroadenoma samples infected by this virus was 9 (37.5%). The frequency of HHV8 infection was different among malignant and benign tumors. Based on the results obtained by this study, KSHV was observed in both benign and malignant tumors. Since KSHV was observed in both types of tumors therefore, it may not have significant relationship with tumor type. The role of KSHV in vessel tumors has been proved by many studies during the past decade. Some other studies have also defined a role for this virus in formation of breast fibroadenoma tumor. Therefore, further studies are needed to define the influence of this virus in breast tumors formation.

Keywords: Breast cancer; Carcinoma; Fibroadenoma; KSHV; PCR.

INTRODUCTION

Breast cancer is the uncontrolled growth of abnormal cells, which is created in various regions of the breast. This cancer may develop in different tissues such as ducts, which transfer breast milk, production tissue of the milk, and in unglandular tissue. Breast cancer is the most prevalent cancer in women [1]. Every year, a large number of women are afflicted by breast cancer, of which the disease is fatal for a number of sufferers [2]. According to the data presented by the US National Institute of Cancer, one out of every 8 women will be inflicted with breast cancer [3]. Although genetic factors such as mutation in the BRCA1 and BRCA 2 genes play an important role in occurrence of breast cancer [4-5], however, other susceptible factors cannot be neglected. For instance, the risk of suffering from breast cancer increases with age [6-7]. Almost, three quarters of the cases of affliction occur in women who are over 50 years old.

Except for age, other risk factors include family history of breast cancer, puberty before the age of 13, menopause after the age of 51, women who have never become pregnant and those who have had the first pregnancy after 30, obesity particularly after menopause, poor diet [8], alcohol consumption, and viral infection [5]. The studies carried out in two recent decades have provided evidences for the role of viruses in the occurrence of breast cancer [9-14]. Kaposi's sarcoma-associated herpes virus (KSHV) belongs to the gamma herpesvirus family and is the causative agent of various lymphoproliferative diseases in humans [15]. It was later shown that this virus causes Kaposi's sarcoma, which is a common cancer among AIDS patients. It is proposed that KSHV plays a role in formation of some blood vessel and lymphoma tumors. Attributes of this virus have a double strand DNA and icosahedral symmetry [16]. The nucleocapsid is surrounded

by a covering being of the lipid genus type [17]. Amplification of a small fragment of KSHV genome is the most common technique for detection of this virus in clinical samples [18]. This study aimed to investigate the frequency of KSHV among females with benign and malignant breast tumors.

MATERIALS AND METHODS

Demographic data of patients

The number of patients who suffered from carcinoma based on age groups included 5 patients (21%) below 35 years, 12 patients (50%) at 35-55 years, and 7 patients (29%) over 55 years old. The average tumor size in 6 patients (25%) was smaller than 2 cm, while in 18 patients (75%) the average tumor size was larger than 2 cm.

Regarding the lymphatic glands involvement, 18 patients (75%) showed involvement, while the rest 6 patients (25%) had no involvement. Among the studied carcinoma samples, the number of patients with ductal, lobular, and mucinous carcinomas were 22 (92%), 1 (4%) and 1 (4%), respectively. Four patients (16.6%) suffered from stage I malignant tumor, 9 (37.5%) had reached stage II, and 11 (45.9%) were at stage III (Table 1).

As to demographic data of patients with fibroadenoma, only ages of the individuals were available, of which 17 (71%) were under 35 years old and 7 (29%) between 35-55 years old.

Sample collection

Paraffin embedded samples of the breast carcinoma (N=24) and fibroadenoma (N=24) were collected from the pathology departments of Toos and Firozgar hospitals located in Tehran, Iran. The samples had been stored during a period of time effective from June 2013 until February 2014. The carcinogenicity of the samples was diagnosed based on the Richardson classification system by an experienced pathologist.

Deparaffinization of the samples

Paraffin embedded sample blocks were cut in 5 µ slides by sterile microtome blade (N35). The sample slides were kept into sterile containers until deparaffinization. To deparaffinize, the samples were left in a xylene solution (Merck Germany) for 30 minutes

followed by soaking in respectively 100%, 80%, 60%, and 40% ethanol (Merck Germany), each for 10 s. The tissue obtained was then transferred to sterile microtubes and stored at -20°C until DNA extraction.

DNA extraction

DNA extraction was carried out by kit according to the manufacturer instruction (Qiagene, Lot No: 11872534, Cat No: 51306). The purity of extracted DNAs was analyzed based on their absorbance at 260 and 280 nm wavelengths by biophotometer (Eppendorf, Germany).

Human beta-globin gene amplification

Human beta-globin gene was co-amplified with the target fragment, as an internal amplification control, using the following primers: b2-microglobulin-F: 5'-TCCAACATCAACATCTTGGT-3'; and b2-microglobulin-R: 5'-TCCCCAAATTCTAAGCAGA-3' [9].

Each reaction was performed in a total volume of 25 µl containing 14 µl of molecular biology-grade water (Sigma Aldrich Company LTD., USA), 2.5 µl of 10×PCR buffer (Promega, USA), 1 µl of 10 pmol of forward and reverse PCR primers, 1 µl of 10 mM dNTPs (Promega, USA), 0.5 µl of smart taq DNA polymerase (Promega, USA), 1 µl of 50 mM MgCl₂ (Promega, USA) and 5 µl of DNA template. The negative control tube contained the same PCR reagents as above but had 5 µl of water substituted for the DNA template.

PCR amplification conditions on thermocycler (Biorad-Germany) were as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 50 s, 54°C for 45 s and 72°C for 40 s, with a final extension at 72°C for 5 min.

HHV8 gene (ORF 26) amplification

Specific Primers produced by TAG Copenhagen (Denmark) were used to amplify the KSHV gene. The sequences of forward and reverse primers were 5'-CTCGAATCCAACGGATTTGA-3' and 5'-ATATGTGCGCCCCATAAATG-3', respectively [19]. Each reaction was performed as mentioned former for standard gene. PCR amplification conditions on thermocycler (Biorad-Germany) were as follows: 95°C for 5 min, followed by 35 cycles of 95°C for 50 s,

54.5°C for 50 s and 72°C for 50 s, with a final extension at 72°C for 5 min.

Electrophoresis

An aliquot of all PCR products was run on a 1.5% (w/v) agarose gels with a 100 bp DNA ladder (Fermntas-Russia) and electrophoresed at 75 V for 40 min. The bands were visualized using ethidium bromide staining and photographed after UV treatment by a transilluminator (UV doc, England).

Statistical analysis

Chi square test was used to determine whether there was any significant difference between the

frequency of KSHV in the carcinoma and fibroadenoma samples and its relationship with the breast cancer (SPSS software 19).

RESULTS

In order to identify the DNA of virus in the tissue samples, the PCR technique was used. The amplified fragments of human beta-globin gene and viral DNA were 122 bp and 233 bp, respectively. Some examples of beta globin gene and KSHV amplification products electrophoresed on 1.5% agarose gels are given in Figures 1 and 2.

Table 1. Distribution of KSHV by demographic variables in patients who suffered from carcinoma

Demographic variable	Status	No. Tested	No. KSHV+	No. KSHV-
Age (years)	< 35	5	0	5
	35-55	12	3	9
	> 55	7	3	4
tumor size	smaller than 2 cm	6	2	4
	larger than 2 cm	18	4	14
lymphatic glands involvement	yes	18	3	15
	No	6	3	3
Type of carcinoma	ductal	22	6	16
	lobular	1	0	1
	mucinous	1	0	1
Stage of tumor	I	4	1	3
	II	9	2	7
	III	11	3	8
Total		24	6	18

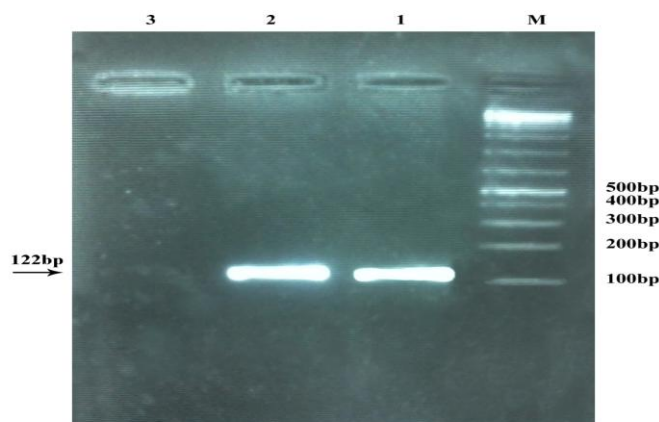


Figure 1. Beta globin gene amplification products electrophoresed on a 1.5% agarose gel. Lane M: 100 bp molecular marker; lane 1 and 2: Human beta-globin gene; lane 3: Negative control

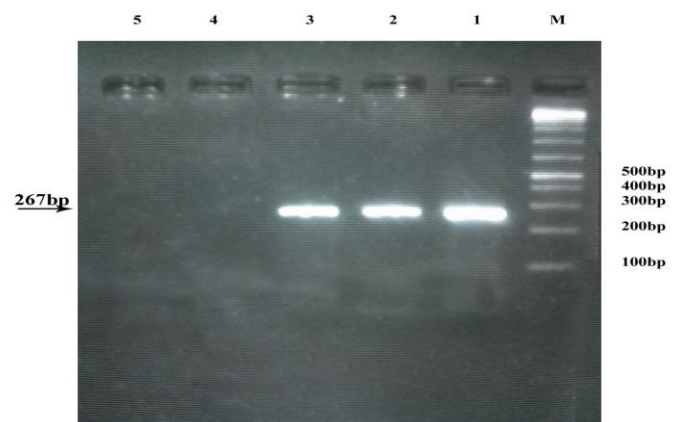


Figure 2. KSHV amplification products electrophoresed on a 1.5% agarose gel. Lane M: 100 bp molecular marker; lane 1: Positive control; lane 2 and 3: Viral DNA (+); lane 4: Viral DNA (-); lane 4 Negative control.

Regarding the infection with KSHV, the PCR amplification was positive for 6 patients with carcinoma (25%), of which 3 were at the age group of 35-55 years, and the other 3 were over 55 years old. All 6 patients infected with the virus were suffering from ductal carcinoma that 3 of them (50%) had lymph node involvement. As to the tumor size, 4 samples were larger than 2 cm in diameter, while 2 samples were below 2 cm. Of all patients infected with virus, 3 were at stage III, 2 in stage II, and 1 in stage I. Out of 24 samples of fibroadenoma, 9 (37.5%) were detected to be infected with KSHV. Among 9 patients, 7 were below 35 years old, while 2 were at the group of 35-55 years. The statistical analysis showed no significant relationship between the frequencies of this virus in the samples studied.

DISCUSSION

KSHV is not considered as a common virus as compared to all other herpes viruses. However, serological tests have shown that 5%-10% of Americans are infected by HHV8. It is proposed that this virus can be transmitted through sexual contact between homosexual men, as a high level of infection (37%) is observed among them. The infection is more common in Africa (30%-60%), where the virus is transmitted non-sexually at the early stages of life. KSHV is detectable in saliva and can be transmitted through kidney transplantation too. During the past decade, it has also been confirmed that this virus plays a role in formation of some types of tumors [20]. KSHV tumorigenic property is attributed to the production of different cytokines such as interleukin 6 (IL6).

A high level of IL6 has been found to be associated with some types of cancers such as prostate [24], ovarian [25], and lymphomas [26]. Moreover, some other studies [27-30] have shown an association between higher levels of IL6 and

tumor development as well as metastasis in breast carcinoma. As well as Kaposi's sarcoma herpes-virus encoded Small noncoding RNAs (miRNAs) may promote cancer pathogenesis. Burgeoning evidence suggests that dysregulation of miRNAs may promote cancer pathogenesis [21-23].

The current research that was conducted to determine the frequency of KSHV on the benign and malignant tumor, led to the identification of this virus in both types of tumors with a prevalence of 25% and 37.5% in ductal carcinoma and fibroadenoma, respectively. There was no significant relationship between the KSHV and tumor type, size and stage as well as age of patients. This might be due to the small sample size. Therefore further investigation in a larger cohort is necessary. Opposite to the results obtained by this study, some studies have confirmed an association between KSHV and breast fibroadenoma. Newton and his colleagues suggested a relationship between KSHV and breast cancer using immunoserology tests [20]. Also, Hsu and colleagues reported a significant association between this virus and breast cancer using PCR and Southern blotting techniques [14]. Although these results indicate a correlation between KSHV and breast cancer, however, the molecular events underlying the role of this virus in formation of breast tumors have not been defined so far.

CONCLUSION

The results obtained by this study showed the presence of KSHV in both malignant and benign tumors of breast.

Although these results do not support the hypothesis that there is a relationship between KSHV infection and breast cancer because it is a pilot study and validation is necessary in a large cohort. However, further studies are needed to confirm the role of KSHV in formation of breast tumors.

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